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Study of Plasmid-Mediated Extended-Spectrum β-Lactamase-Producing Strains of Enterobacteriaceae, Isolated from Diabetic Foot Infections in a North Indian Tertiary-Care Hospital

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Abstract

Aim: This study evaluated the incidence and factors responsible for plasmid-mediated extended-spectrum β-lactamase (ESBL) infection among patients with diabetic foot ulcer (DFU).

Subjects and Methods: A prospective study was conducted on 162 DFU inpatients treated in a multidisciplinary-based diabetes and endocrinology center at Jawaharlal Nehru Medical College of Aligarh Muslim University, Aligarh, India, during the period of December 2008–March 2011. Detailed history and patient's profile, grade of DFU, co-morbidities and complications, laboratory data, and final outcome were collected. Standard methods were used for culture identification, sensitivity testing, and ESBL detection. Polymerase chain reaction for *bla* genes was performed, and the risk factors for *bla* gene positivity were determined by univariate analysis with 95% confidence interval.

Results: In total, 127 (78.3%) Enterobacteriaceae members were isolated. The most common isolate was *Escherichia coli* (71; 55.9%), followed by *Klebsiella* sp. (33; 25.9%) and *Proteus* sp. (13; 10.2%). By phenotypic methods, 67.8% were ESBL producers. In the molecular detection of ESBLs, 81.9% were found to be positive for the *bla* gene, of which bla_{CTX-M} showed 81.8% positivity, followed by bla_{TEM} (50%) and bla_{SHV} (46.9%). In a univariate analysis, *bla* gene–positive status was associated with low-density lipoprotein-cholesterol (>100 mg/dL) ($P<0.004$, odds ratio 13.4, relative risk 8.65) and triglycerides (>200 mg/dL) ($P<0.003$, odds ratio 6.5, relative risk 4.11).

Conclusion: ESBL constitutes a major threat to currently available β-lactam therapy, leading to complications in DFUs. Aminoglycosides, cephalosporin, and β-lactam inhibitor drugs would probably be more appropriate empirical agents after establishing the patient's history of previous antibiotic use. The detection of ESBL should be done on a routine basis.

Introduction

Infected foot ulcer is a common cause of morbidity in diabetes patients, ultimately leading to dreaded complications like gangrene and amputations. The lifetime risk to a person with diabetes for developing a foot ulcer could be as high as 25%.[1] Infection is most often a consequence of foot ulceration, which typically follows trauma to a neuropathic foot.[2] These infections are usually polymicrobial and include aerobic Gram-positive cocci (*Staphylococcus aureus*), Gram-negative bacilli (*Escherichia coli*, *Klebsiella* sp., and *Proteus* sp.), and anaerobes (*Bacteroides* sp. and *Peptostreptococcus* sp.).[3–6] Enterobacteriaceae are an important pathogenic group in community and hospital-acquired infections, and resistance to antibiotics has become increasingly common. The most serious emerging problem is resistance to Gram-negative organisms, including resistance to extended-spectrum cephalosporins and penicillin, among the diabetic foot ulcer (DFU) isolates.[3,6–9]

Extended-spectrum β-lactamases (ESBLs) capable of degrading the extended-spectrum cephalosporins and monobactams are the most relevant determinants of resistance

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emerging worldwide in the Enterobacteriaceae.[10] ESBL strains exhibit multidrug resistance, which includes resistance to the aminoglycoside and fluoroquinolone groups, and the therapeutic options associated with these strains are fairly limited. In addition to this, increased morbidity, mortality, and cost of health care are also associated.[11] ESBLs are derived through one or more amino acid substitutions from the parental enzymes TEM-1, TEM-2, and SHV-1, and other enzymes, including CTX-M and PER, have reportedly been detected with increasing frequency in many regions.[10] In India, the choice of empirical antimicrobials is extrapolated from available data for Western countries, which may or may not be appropriate for Indian patients.[8] Because long-term studies from India on ESBL-producing organisms isolated from foot infections in diabetes are scarce, we planned to study the prevalence of plasmid-mediated ESBL-producing strains of Enterobacteriaceae and antimicrobial resistance with regard to bacterial species and specimens isolated from foot infections in a North Indian multidisciplinary tertiary-care hospital.

Materials and Methods

Study design

This was a prospective cohort hospital-based study. In total, 162 diabetes patients who were admitted in the Centre for Diabetes and Endocrinology, Jawaharlal Nehru Medical College Hospital, Aligarh Muslim University, Aligarh, India, and had an ulcer or ulcers in their foot during the period December 2008–March 2011 were included in this study. Infection in patients with a cefotaxime-resistant Enterobacteriaceae member (*E. coli* and *Klebsiella* sp.) was selected for the genotypic study. The number of patients included for univariate analysis was 58. Multiple ulcers during one hospitalization were considered as one ulcer episode, and the worst outcome of various ulcers was recorded as the final outcome. All the subjects gave informed consent, and clearance was obtained from the Institutional Ethics Committee.

Clinical examination

A detailed history and physical examination was carried out for every subject. Age, sex, anthropometric measurements (body mass index), duration of diabetes, glycemic control prior to and during the hospital stay, lipid profile, presence of retinopathy, nephropathy (creatinine $>1.5\,mg/100\,mL$ or presence of micro- or macroalbuminuria), neuropathy (absence of perception of the Semmes–Weinstein monofilament at two of 10 standard plantar sites on either foot), peripheral vascular disease (ischemic symptoms and intermittent claudication of rest pain, with or without absence of pedal pulses or posterior tibial pulses), hypertension, duration, site, and size of ulcer, history of smoking, history of previous amputation, and clinical outcome were noted in every patient. Clinical assessment for signs of infection (swelling, exudates, surrounding cellulitis, odor, tissue necrosis, crepitation, and pyrexia) was done by one researcher classifying the ulcers and determining the presence of clinical signs of infection. Ulcer size was determined by multiplying the longest and the widest diameters and expressed in centimeters squared. The wound was graded and staged at the time of hospitalization according to the University of Texas Wound Classification System as grade 1 (superficial wound, not involving, tendon, capsule, or bone), grade 2 (wound penetrating to tendon or capsule), and grade 3 (wound penetrating bone or joint). Grade 0 patients (pre- or postulcerative site that had healed) were excluded from the study. Diagnosis of extension to the bone was made in the majority of patients by probing with a sterile steel probe. In the absence of sinus tract or an exposed bone, a standard radiograph showing signs of osteomyelitis in the bone was considered definitive, and later on magnetic resonance imaging was done to confirm the osteomyelitis in suspected patients. Amputation was defined as the complete loss in the transverse anatomical plane of any part of the lower limb.[9]

Microbiological methods

Culture specimens were obtained at the time of admission, after the surface of the wound had been washed vigorously by normal saline, followed by debridement of superficial exudates. Specimens were then obtained by scraping the base of the ulcer or the deep portion of the wound edge with a sterile curette after cleaning the base of the ulcer with a sterile swab stick.[5,6] The specimens were promptly sent to the Microbiology Department and processed for aerobic and anaerobic bacteria. Standard methods for isolation and identification of aerobic[12,13] and anaerobic[14,15] bacteria were used.

Susceptibility testing of aerobic and anaerobic isolates was performed using the disc diffusion method as described by the Clinical and Laboratory Standards Institute.[16] Antimicrobial disk used were imepenem (10 μg), aztreonam (30 μg), amoxyclav (30 μg), cefpodoxime (10 μg), metronidazole (5 μg), ofloxacin (5 μg), cefotaxime (30 μg), cefepime (30 μg), cefixime (5 μg), cefoperazone (75 μg), cefoperazone/sulbactam (75/10 μg), oxacillin (1 μg), piperacillin (100 μg), piperacillin/tazobactum (100/10 μg), ceftazidime (30 μg), ceftazidime/clavulanic acid (30/10 μg), amikacin (30 μg), amoxicillin (20 μg), cefotaxime/clavulanic acid (30/10 μg), ceftriaxone (30 μg), cefoxitin (30 μg), chloramphenicol (30 μg), gentamicin (10 μg), gatifloxacin (5 μg), levofloxacin (5 μg), sparfloxacin (5 μg), streptomycin (10 μg), vancomycin (30 μg), clindamycin (2 μg), tobramycin (10 μg), azithromycin (15 μg), erythromycin (15 μg), and bacitracin (10 units). All discs were obtained from Hi-Media Labs, Mumbai, India. Interpretative criteria for each antimicrobial tested were those recommended by the manufacturer's guidelines.

Phenotypic methods for ESBL detection

Gram-negative bacilli were first screened for the production of ESBL by the disc diffusion method (screening test) using cefotaxime, ceftriaxone, aztreonam, cefepime, cefoxitin, and ceftazidime and later on confirmed by the cephalosporin/clavulanate combination disk (confirmatory test) (disk potential) method using ceftazidime, ceftazidime + clavulanic acid, cefotaxime, cefotaxime + clavulanic acid, piperacillin, cefoperazone + sulbactam, cefoperazone, and piperacillin + tazobactum.[17] *E. coli* ATCC 25922 (non-ESBL producer) and *Klebsiella pneumoniae* 700603 (ESBL producer) were used as control strains, respectively.

Molecular methods for β-lactamase detection

Preparation of DNA template. Template DNA was prepared from freshly cultured bacterial isolates by suspending

three to five colonies in 50 μL of molecular-grade water and then heating at 95°C for 5 min and immediately chilling at 4°C. Positive controls harboring bla_{CTX-M}, bla_{TEM}, and bla_{SHV} and the negative control (*E. coli* ATCC 25922) were processed in the same way for DNA extraction.

Detection of *bla* genes by polymerase chain reaction. Molecular detection of bla_{CTX-M}, bla_{TEM}, and bla_{SHV} was performed in cefotaxime-resistant *E. coli* and *Klebsiella* sp. isolates by using polymerase chain reaction (PCR) according to methods described previously with minor modifications.[18,19] The primers and cycling conditions for detection of *bla* genes were the same as those described by Shahid et al.[19]

Antibiotic treatment

Initially, the antibiotic regimen was selected according to published recommendations.[9] The treatment was modified in accordance with the culture results. The duration of the treatment was at least 4–6 weeks and was prolonged in cases of osteomyelitis. All patients also received an intensive insulin treatment.

Statistical analysis

Cefotaxime-resistant patients were divided into two groups: *bla* gene positive and *bla* gene negative. Quantitative variables were expressed as mean ± SD values. The odds ratio (OR) (strength of association) and risk ratio (RR) (probability of association) with 95% confidence interval were reported for independent variables associated with the outcome variables of *bla* gene positive and negative. All analyses were performed using SPSS version 19.0 software (SPSS, Inc., Chicago, IL). A P value of < 0.05 were considered significant.

Results

Men were predominant (105 patients, 64.8%) in the study subjects compared with women (57 patients, 35.1%) (Table 1). All patients had ulcers graded 1–3 in the University of Texas Wound Classification System. Type 2 diabetes mellitus occurred in 134 (82.7%) patients, whereas only 28 (17.2%) had type 1 diabetes mellitus. The mean age of the subjects was 51.1 ± 11.4 years. The mean duration of diabetes was 13.5 ± 4.6 years, and nearly 111 (68.5%) had had the condition for ≤ 10 years. Eighty-two patients (50.6%) had neuropathy, 72 (54.4%) nephropathy, 82 (50.6%) retinopathy, and 92 (56.7%) hypertension. Osteomyelitis was present in 20 (12.3%) subjects. Nearly one-third (60 patients, 37%) had had the ulcer for > 1 month before presentation at the hospital. The bacterial infection in the ulcer was superficial in 48 (29.6%) cases, subcutaneous in 94 (58%), and osteomyelitic in 20 (12.3%). Glycemic control was poor in 123 patients (69.7%) during the first 5 days of the hospital stay. The majority of the subjects (147, 90.7%) had poor glycemic control (glycated hemoglobin > 7%) at the time of hospital admission. Sixty-three (38.8%) received surgical treatment, mainly in the form of debridement. Forty-six (28.4%) patients were subject to amputation, and 18 (11.1%) died during the hospital stay (mean hospital stay, 22.9 ± 15.5 days). Most ulcers were found on interdigits (43.2%), followed by the plantar surface (28.4%), heel (29.5%), margins (12.6%), malleoli (16.7%), and leg (3.6%) and on multiple areas (two or more sites) (31.4%). The size of the ulcer being ≤ 4 cm^2 was observed in 38 (23.4%) patients, compared with > 4 cm^2 in 124 (76.5%), of which the majority of the patients were males. Grade 1 ulcer was found in 48 (29.6%), Grade 2 in 94 (58%), and Grade 3 in 20 (12.3%) patients.

Microbiological observations

In total, 272 (aerobic + anaerobic) bacteria were isolated, averaging 1.67 (1.57 aerobic, 0.10 anaerobic) species per patient. Thirty-five percent patients had monomicrobial infection, whereas polymicrobial etiology was observed in 65%.

Gram-positive cocci made up 34.5% of infections, compared with Gram-negative bacilli in 65.4%; the Gram-positive to Gram-negative ratio was 1:1.8. The frequency of bacterial isolates from DFUs is shown in Table 2. *E. coli* was the most common isolate, accounting for 27.8%, followed by *S. aureus* (23.5%), *Pseudomonas aeruginosa* (15.6%), *Klebsiella oxytoca* (7%), *K. pneumoniae* (5.8%), *Proteus vulgaris* and *Enterococcus faecalis* (3.5% each), *Acinetobacter* sp. (3.1%), coryneform sp. (2.7%), β-hemolytic *Streptococcus* spp. and coagulase-negative staphylococcal spp. (2.3% each), *Proteus mirabilis* (1.5%), and *Morganella morganii* (0.7%). Among the anaerobic bacteria isolated, Gram-positive cocci made up 58.8% of infections, Gram-positive bacilli 29.4%, and Gram-negative bacilli for 11.7%. *Peptostreptococcus* sp. was the most common isolate, accounting for 35.2%, followed by *Peptostreptococcus anaerobius* (23.5%), *Propionibacterium* sp. (17.6%), *Bacteroides ureolyticus* (11.7%), *Clostridium perfringens* (5.8%), and *Eggerthella lenta* (5.8%) (Table 2).

Antibiotic resistance profile

A higher percentage of antibiotic resistance (67.1%) was shown by coagulase-negative staphylococcal spp., followed by *P. aeruginosa* (63.7%), *P. mirabilis* (57.5%), *M. morganii* (57.5%), *E. faecalis* (55.2%), *Acinetobacter* sp. (51.9%), *P. vulgaris* (50.3%), β-hemolytic *Streptococcus* (47.6%), *E. coli* (45.9%), *K. pneumoniae* (44.8%), *S. aureus* (44.3%), *K. oxytoca* (42.9%), and coryneform sp. (37.1%). All the anaerobes were susceptible to metronidazole, amoxicillin + clavulanate, and imepenem. The detailed antibiotic resistance for each bacterium is summarized in Table 3.

Phenotypic ESBL detection

On average, 74.2% of Gram-negative DFU isolates were positive in the screening of ESBL by the disc diffusion method: 142 (85.0%) isolates were positive using cefotaxime, followed by 127 (76.0%) for cefpodoxime, 117 (70.0%) for aztreonam, and 116 (69.4%) for ceftriaxone and ceftazidime each. In the confirmatory ESBL test, 67.8% were found to be positive by the disc potential method: 132 (79%) using cefoperazone/cefoperazone + sulbactam, followed by 126 (75.4%) by piperacillin/piperacillin + tazobactum, and 114 (68.2%) by cefotaxime/cefotaxime + clavulanic, whereas ceftazidime/ceftazidime + clavulanic acid showed only 81 (48.5%) produced ESBL (Table 4).

Occurrence of bla genes

The frequency of *bla* genes in DFU isolates is shown in Table 4. Only the *E. coli* and *Klebsiella* sp. resistant to cefotaxime were subjected to Class A (CTX-M, TEM, and SHV) ESBL study. On average, 89.3% cefotaxime-resistant isolates were found positive for *bla* genes, of which CTX-M was found to be the most prevalent ESBL noted in 54 (81.8%), followed

Table 1. Baseline Characteristics of 162 Diabetic Foot Ulcer Patients and Association of Study Characteristics in Two Groups of Diabetes Patients Having Cefotaxime-Resistant Gram-Negative Bacterial Infection in Diabetic Foot Ulcers According to *bla* Gene Positivity and Negativity

	Total	bla +	bla –	P	*OR (95% CI)*	*RR (95% CI)*
n	162	51 (87.93)	7 (12.0)	—	—	—
Sex (female)	57 (35.1)	14 (27.4)	2 (28.5)	1.00	1.05 (0.18–6.09)	1.05 (0.22–4.87)
Age >40 years	90 (55.5)	37 (47.0)	6 (85.7)	1.00	1.65 (0.17–5.81)	1.54 (0.21–11.24)
Type 2 diabetes	134 (82.7)	43 (84.3)	5 (71.4)	0.591	2.15 (0.35–3.07)	1.92 (0.43–8.53)
Duration of diabetes >10 years	51 (31.4)	12 (23.5)	2 (28.5)	1.00	0.78 (0.13–4.60)	0.81 (0.17–3.73)
Ulcer duration >1 month	60 (37.0)	17 (33.3)	2 (28.5)	1.00	1.25 (0.21–7.12)	1.21 (0.25–5.71)
Ulcer size >4 cm^2	124 (76.5)	42 (82.3)	6 (85.7)	1.00	0.7 (0.07–6.48)	0.72 (0.09–5.44)
Complications						
Hypertension	92 (56.7)	33 (64.7)	6 (85.7)	0.407	0.30 (0.03–2.73)	0.34 (0.04–2.64)
Retinopathy	82 (50.6)	27 (52.9)	6 (85.7)	0.127	0.18 (0.02–1.67)	0.22 (0.02–1.71)
Neuropathy	82 (50.6)	33 (64.7)	5 (71.4)	1.00	0.73 (0.12–4.16)	0.76 (0.16–3.57)
Nephropathy	72 (54.4)	30 (58.8)	5 (71.4)	0.69	0.571 (0.10–0.22)	0.60 (0.12–2.87)
Grade of ulcer (Texas Wound System)						
1	48 (29.6)	14 (27.4)	3 (42.8)	0.661	0.504 (0.1–2.54)	0.55 (0.13–2.21)
2	94 (58.0)	31 (60.7)	3 (42.8)	0.432	2.06 (0.41–10.2)	1.88 (0.46–7.68)
3	20 (12.3)	6 (11.7)	1 (14.2)	1.00	0.8 (0.08–7.83)	0.82 (0.11–5.87)
Nature of ulcer (necrotic)	55 (33.9)	24 (47.0)	1 (14.2)	0.127	5.33 (0.59–7.52)	4.54 (0.58–35.38)
Amputation	46 (28.3)	24 (47.0)	2 (28.5)	0.44	2.22 (0.39–12.5)	2.03 (0.42–4.60)
Previous antibiotic use	65 (40.5)	27 (52.9)	1 (14.2)	0.100	6.75 (0.75–0.14)	5.6 (0.71–43.65)
Discharge status						
Alive	144 (88.8)	43 (84.3)	6 (85.7)		1.11 (0.11–0.56)	1.10 (0.15–8.09)
Died	18 (11.1)	8 (15.6)	1 (14.2)			
Hospital stay (>1 month)	76 (46.9)	46 (90.1)	4 (57.1)	0.047	6.9 (1.18–40.05)	4.68 (1.28–17.15)
Bacterial infection type						
Superficial	48 (29.6)	14 (27.4)	3 (42.8)	0.661	0.504 (0.1–2.54)	0.55 (0.13–2.21)
Subcutaneous	94 (58.0)	31 (60.7)	3 (42.8)	0.432	2.06 (0.41–10.2)	1.88 (0.46–7.68)
Osteomyelitis	20 (12.3)	6 (11.7)	1 (14.2)	1.00	0.8 (0.08–7.83)	0.82 (0.11–5.87)
HbA1c (>7%)	147 (90.7)	42 (82.3)	6 (85.7)	0.653	0.77 (0.08–7.27)	0.8 (0.10–5.9)
WBC count ($10^3/\mu L$)	120 (74.0)	14 (27.4)	4 (57.1)	0.187	0.28 (0.05–1.43)	0.33 (0.08–1.35)
Hb (g/dL)	72 (44.4)	29 (56.8)	5 (71.4)	0.687	0.52 (0.09–2.97)	0.56 (0.11–2.68)
Serum creatinine (>1.5 mg/dL)	49 (30.2)	13 (25.4)	3 (42.8)	0.31	0.45 (0.08–2.3)	0.50 (0.12–2.0)
SGOT/AST (>34 IU/L)	61 (37.6)	3 (5.8)	2 (28.5)	0.105	0.15 (0.02–1.16)	0.23 (0.06–0.91)
SGPT/AST (>35 IU/L)	95 (58.6)	5 (9.8)	3 (42.8)	0.04	0.14 (0.02–0.84)	0.21 (0.05–0.78)
LDL-C (>100 mg/dL)	91 (56.1)	43 (84.3)	2 (28.5)	<0.004	13.4 (2.20–81.7)	8.65 (1.89–39.5)
Total cholesterol (>150 mg/dL)	53 (32.7)	37 (72.5)	3 (42.8)	0.187	3.52 (0.69–17.7)	2.9 (0.73–11.8)
HDL-C (<40 mg/dL)	50 (30.8)	29 (56.8)	2 (28.5)	0.233	3.29 (0.53–18.8)	2.8 (0.60–13.6)
Triglycerides (>200 mg/dL)	51 (31.4)	34 (66.6)	4 (57.1)	<0.003	6.5 (1.8–22.9)	4.11 (1.49–11.3)

Data are mean ± SD values or *n* (%) unless otherwise indicated.

CI, confidence interval; Hb, hemoglobin; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; OR, odds ratio; RR, relative risk; SGOT/AST, serum glutamic oxaloacetic transaminase/aspartate transaminase; SGPT/AST, serum glutamate-pyruvate transaminase/aspartate transaminase; WBC, white blood cells.

by TEM in 33 (50%) isolates and SHV β-lactamases in 31 (46.9%) isolates. Twenty-three (37.2%) strains had all three genes (CTX-M + TEM + SHV), nine (15.2%) strains had CTX-M + SHV, four (6.7%) strains had CTX-M + TEM, two (3.3%) strains had TEM + SHV, 19 (32.2%) strains had CTX-M only, one (1.6%) strain had SHV only, and two (3.3%) strains had TEM only (Fig. 1a, b, and c).

In a univariate analysis (Table 1), OR and RR were calculated between the two groups of patients in which *bla* tests were performed. The predictive factors that were associated with the *bla* gene (CTX-M, TEM, and SHV) positivity in cefotaxime-resistant Enterobacteriaceae members (*E. coli* and *Klebsiella* sp.) isolated from DFU patients are summarized in Table 1. The significant factors that were more likely to have an association for *bla* gene positivity were hospital stay (>1 month) ($P = 0.047$, OR 6.9, RR 4.67), low-density lipoprotein-cholesterol (>100 mg/dL) ($P < 0.004$, OR 13.4, RR 8.65), triglycerides (>200 mg/dL) ($P < 0.003$, OR 6.5, RR 4.11), duration of infection >1 month ($P = 0.58$, OR 1.25, RR 1.21), nature of ulcer (necrotic) ($P = 0.127$, OR 5.33, RR 4.54), type 2 diabetes mellitus ($P = 0.591$, OR 2.15, RR 1.92), history of previous antibiotic use ($P = 0.100$, OR 6.75, RR 5.60), smoking history ($P = 0.618$, OR 1.098, RR 1.08), high-density lipoprotein-cholesterol (<40 mg/dL) ($P = 0.233$, OR 3.29, RR 2.80), total cholesterol (>150 mg/dL) ($P = 0.187$, OR 3.52, RR 2.9), and serum glutamate-pyruvic transaminase/aspartate aminotransferase ($P = 0.04$, OR 0.14, RR 0.21).

Discussion

This study presents a comprehensive clinical and microbiological profile of infected DFUs in hospitalized patients with

Table 2. Frequency of Distribution of Isolates from 162 Diabetic Foot Ulcer Patients in Relation to Treatment

Name of isolate	*Total*	*Growth type*		
		Single	*Two*	*Polymicrobial*
Aerobic				
Gram-positive cocci	88 (34.5)			
S. aureus	60 (23.5)	8 (13.3)	25 (41.6)	27 (45.0)
E. faecalis	9 (3.5)	3 (33.3)	4 (44.4)	2 (22.2)
β-Hemolytic *Streptococcus*	6 (2.3)	3 (50)	3 (50)	0 (0)
CONS	6 (2.3)	3 (50)	3 (50)	0 (0)
Coryneform spp.	7 (2.7)	0 (0)	5 (71.4)	2 (28.5)
Gram-negative bacilli	167 (65.4)			
E. coli	71 (27.8)	15 (21.1)	34 (47.8)	22 (30.9)
P. aeruginosa	40 (15.6)	11 (27.5)	20 (50)	9 (22.5)
K. oxytoca	18 (7.0)	0 (0)	10 (55.5)	8 (44.4)
K. pneumoniae	15 (5.8)	2 (13.3)	4 (26.6)	9 (60)
P. vulgaris	9 (3.5)	0 (0)	5 (55.5)	4 (44.4)
P. mirabilis	4 (1.5)	0 (0)	2 (50)	2 (50)
Acinetobacter spp.	8 (3.13)	2 (25)	6 (75)	0 (0)
M. morganii	2 (0.7)	0 (0)	0 (0)	2 (100)
Total aerobic	255 (93.7)			
Anaerobic				
Gram-positive cocci	10 (58.8)	—	—	—
Peptostreptococcus spp.	6 (35.2)	—	—	—
P. anaerobius	4 (23.5)	—	—	—
Gram-positive bacilli	5 (29.4)	—	—	—
Propionibacterium spp.	3 (17.6)	—	—	—
C. perfringens	1 (5.8)	—	—	—
E. lenta	1 (5.8)	—	—	—
Gram-negative bacilli	2 (11.7)	—	—	—
B. ureolyticus	2 (11.7)	—	—	—
Total anaerobic	17 (6.25)	—	—	—
Grand total	272	—	—	—

Data are number (%).
CONS, coagulase-negative staphylococcal spp.

Table 3. Antimicrobial Resistance Pattern of Bacteria Isolated from Diabetic Foot Ulcers in Diabetes Patients (N=255)

Antimicrobial agent	*Bacterial isolate*[a]												
	Ps	*Ec*	*Pv*	*Pm*	*Mm*	*Ko*	*Kp*	*Ac*	*Sa*	*Bhs*	*CONS*	*Cr*	*En*
Number of isolates	40	71	9	4	2	18	15	8	60	6	6	7	9
Penicillin	56.8	83.1	55.6	100	100	72.2	86.6	91.6	54.1	66.6	91.6	49.9	50.9
Cephalosporin	75.6	60.2	58.3	81.2	43.7	64.1	74.1	60.9	62.5	54.1	54.1	75.0	22.2
Monobactam	77.5	39.4	33.3	100	100	22.2	93.3	62.5	NT	NT	NT	NT	NT
Carbapenem	55	42.3	0.0	0.0	0.0	0.0	0.0	0.0	NT	NT	NT	NT	NT
Aminoglycosides	55.0	42.3	0.0	0.0	0.0	0.0	0.0	0.0	55.8	50.0	83.3	66.6	88.9
Chloramphenicol	62.5	38	66.7	50	100	5.6	20	50	11.7	83.3	83.3	0	88.9
Quinolones and fluoroquinolones	75.8	65.7	88.9	66.6	66.6	90.7	31.1	41.6	60.8	66.7	58.3	28.6	52.7
β-Lactam inhibitors	36.8	11.9	33.3	25.0	0.0	0.0	10	65.6	42	0.0	33.3	0.0	22.2
Macrolide	NT	NT	NT	NT	NT	NT	NT	NT	67.5	58.3	100	28.6	94.4
Lincosamides	NT	NT	NT	NT	NT	NT	NT	NT	27 (45)	3 (50)	6 (100)	6 (85.7)	7 (77.8)
Glycopeptide	NT	NT	NT	NT	NT	NT	NT	NT	0 (0)	0 (0)	0(0)	0(0)	0 (0)
Total	63.7	45.9	50.3	57.5	57.5	42.9	44.8	51.9	44.3	47.6	67.1	37.1	55.2

[a]Ps, *P. aeruginosa*; Ec, *E. coli*; Pv, *P. vulgaris;* Pm, *P. mirabilis*; Mm, *M. morganii*; Ko, *K. oxytoca*; Kp, *K. pneumoniae*; Ac, *Acinetobacter* sp; Sa, *S. aureus*; Bhs, *β*-hemolytic *Streptococcus*; CONS, coagulase-negative staphylococcal sp.; Cr, Coryneform sp. En, *E. faecalis.*
NT, not tested.

Table 4. Screening Test and Confirmatory Test Results of Extended-Spectrum β-Lactamase-Producing Gram-Negative Bacilli, $bla_{CTX\text{-}M,\ TEM,\ SHV}$ Gene Results, and $bla_{CTX\text{-}M,\ SHV,\ TEM}$ Gene Distribution Among the Bacterial Isolates from Diabetic Foot Ulcer Patients

	n *(%)*
Screening ESBL result	$n=167$
Aztreonam	117 (70)
Cefpodoxime	127 (76.0)
Ceftazidime	116 (69.4)
Cefotaxime	142 (85.0)
Ceftriaxone	116 (69.4)
Average screening positivity	74.2%
Confirmatory ESBL result	$n=167$
Ceftazidime + clavulanic acid	81 (48.5)
Cefotaxime + clavulanic acid	114 (68.2)
Piperacillin + tazobactum	126 (75.4)
Cefoperazone + sulbactam	132 (79.0)
Average confirmatory positivity	67.8%
bla gene	$n=66$
bla positivity	59 (89.3)
CTX-M	54 (81.8)
TEM	33 (50)
SHV	31 (46.9)
E. coli ($n=45$)	38 (84.4)
Klebsiella sp. ($n=21$)	21 (100)
bla gene distribution	$n=59$
CTX-M + TEM + SHV	22 (37.2)
CTX-M + TEM	4 (6.7)
CTX-M + SHV	9 (15.2)
TEM + SHV	2 (3.3)
CTX-M alone	19 (32.2)
SHV alone	1 (1.6)
TEM alone	2 (3.3)

ESBL, extended-spectrum β-lactamase.

special reference to identifying the factors associated with the risk of *bla* gene–positive cefotaxime-resistant Enterobacteriaceae members (*E. coli* and *Klebsiella* sp.) isolated from DFU patients.

In the present study polymicrobial etiology was found in 65% and monomicrobial in 35% of patients with a rate of isolation of about 1.67 bacteria per patient, which is lower than in previous studies,[6,20] which showed a rate of isolation between 2.3% and 5.8%. The major infective organisms in DFUs in our patients appear to be different. We found Gram-negative aerobic bacteria were most frequently isolated, which is in accordance with our previous report.[3] Studies from Western countries show that Gram-positive aerobes are the predominant organisms isolated from DFU.[21–25] Zubair et al.,[3] in their study on 102 DFU patients, recovered 152 aerobic bacteria, of which 63.8% were Gram-negative and only 36.1% were Gram-positive. Gadepalli et al.[6] also reported Gram-negative aerobes to be the most frequently isolated pathogens (28.7%), followed by 13.8% Gram-positive aerobes. Similar results were also reported by Shankar et al.[26] Studies from Malaysia have also reported a predominance of Gram-negative bacteria (52%) in patients with DFU, with the most common pathogens isolated being *Proteus* sp., *K. pneumoniae*, *E. coli*, and *Enterobacter cloacae*.[27] The difference in observation in the prevalence of Gram-negative bacilli in DFU between diabetes patients from Eastern and Western countries remains largely unknown. Environmental factors such as sanitary habits (e.g., use of water for perianal wash [ablution] after defecation leading to contamination of hands with fecal flora) are proposed to be responsible for increased the prevalence of Gram-negative organisms in the developing world compared with the West.[8,28] In our anaerobic study, *Peptostreptococcus* sp. was the most predominant one, which is in accordance with the previous studies.[29,30] We recovered fewer anaerobic species compared with earlier culture reports[31,32] because most of our patients did not have chronic draining wounds, and only 12% had gangrene associated with their infections. This may be an indication of fewer anaerobic species among nonthreatening lower-extremity infections, which was also reported earlier.[33]

The overall resistance in our patients was high. It is unclear why the Gram-negative isolates should manifest such high rates of resistance. One reason may include differences in the use of antimicrobial substances, in infection control practices, in different climates, and in other unrecognized factors.[34] Another possible reason could be that most of our patients received some antimicrobial treatment before presenting at our center from the referring hospitals using a combination of different antimicrobials empirically. The Enterobacteriaceae are an important group in community and hospital-acquired infections. The most serious resistance patterns now emerging among Gram-negative organisms include resistance to extended-spectrum cephalosporins and penicillins.[35] This resistance is commonly mediated by ESBLs in *E. coli* and *Klebsiella* sp. or by the hyperproduction of chromosomally mediated cephalosporinases (Bush group I ampicillin C enzymes) in *Serratia* and *Citrobacter* species.[36] The ESBL genes generally result from point mutations in the genes of broad-spectrum β-lactamase Ambler class A enzymes, such as TEM-1, TEM-2, or SHV-1. They are usually located in conjugative mega-plasmids, which often carry genes responsible for resistance to other antibacterial drugs, making it extremely difficult to treat infections caused by bacteria that produce these enzymes.[37]

Along with ESBLs, plasmid-mediated Ambler class C cephalosporinases (or Bush group 1 cephalosporinases) have been found in clinical isolates of the Enterobacteriaceae. These enzymes can produce resistance to cephamycins, extended-spectrum cephalosporins, and aztreonam, and unlike class A ESBLs, β-lactamase inhibitors do not inhibit these bacteria.[38] The resistance pattern in our study was similar to the recent studies done in India and outside.[6,8,26,27] In the present study, Gram-negative bacilli were isolated as ESBL producers in 67.8% of isolates, similar to our previous findings[3] and also in accordance with the reports of Mathur et al.[39] from India. Babypadmini et al.[40] also showed that 40% of *K. pneumoniae* isolates and 41% of *E. coli* isolates were ESBL producers in their study cohort. Gadepalli et al.[6] have reported 54.5% of *E. coli* isolates to be ESBL producers in diabetic foot infections. A study in Brazil reported the prevalence of ESBL as only 6% among *E. coli* isolates.[41] There is a paucity of data on the prevalence of ESBLs in diabetic foot infection in Gram-negative bacteria other than *E. coli* and *Klebsiella* sp. In the screening test, 74.2% of Gram-negative DFU isolates were ESBL-positive, and 67.8% of ESBL producers tested positive with β-lactam inhibitors in a confirmatory test. In a recent study Shobha et al.[42] have reported 27.3% of *K. pneumoniae*, 25.2% of *E. coli*, 21.42% of

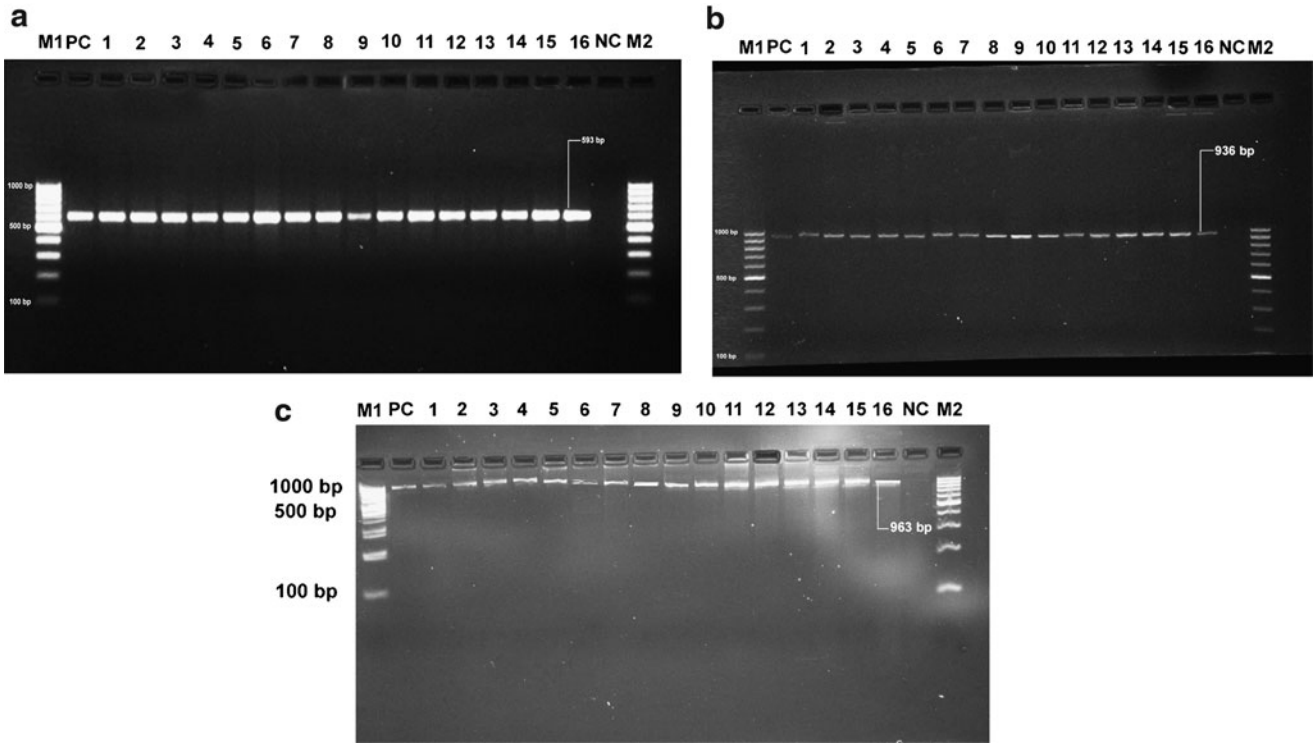

FIG. 1. Molecular detection of (**a**) $bla_{\text{CTX-M}}$, (**b**) bla_{SHV}, and (**c**) bla_{TEM} shows positive bands at 593 bp, 936 bp, and 963 bp for CTX-M, SHV, and TEM, respectively. Lanes M1 and M2, 100-bp DNA ladder (Fermentas, Thermo Scientific, Burlington, ON, Canada; supplied by Century Scientific Pvt. Ltd., Aligarh, India); lane PC, positive control; lane NC, negative control. (**a**) Lanes 1–8, positive CTX-M *E. coli* isolates; Lanes 9–16, *Klebsiella* sp. showing CTX-M positivity at 596 bp. (**b**) Lanes 1–8, positive SHV *E. coli* isolates; Lanes 9–16, *Klebsiella* sp. showing SHV positivity at 936 bp. (**c**) Lanes 1–8, positive TEM *E. coli* isolates; Lanes 9–16, *Klebsiella* sp. showing TEM positivity at 963 bp.

Pseudomonas sp., 25% of *Enterobacter* sp., and 17% of *Acinetobacter* sp. to be ESBL producers.

The correct identifications of the genes involved in ESBL-mediated resistance are necessary for the surveillance and epidemiological studies of their transmission in hospitals. In the early study of ESBLs, isoelectric focusing was the better option, but it was time consuming. Recently, several molecular methods have been proposed for the identification of TEM, SHV, and CTX-M derivatives. Multiplex PCR can be used to screen $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV}. Edelstein et al.[43] detected 15.8% of *E. coli* and 60.8% of *K. pneumoniae* isolates as CTX-M ESBL positive, of which 93% were CTX-M-1- and 7% were CTX-M-2-type enzymes. In India, a variant of CTX-M-3 enzyme, designated CTX-M-15, was reported from six unrelated members of the family Enterobacteriaceae.[44] Rodríguez-Baño et al.[45] have reported that 53% of the nosocomial Gram-negative isolates were ESBL producers. In another study, 14 of 39 selected isolates were found to be positive for the $bla_{\text{CTX-M}}$ gene by Sekar et al.[46] The prevalence of $bla_{\text{CTX-M}}$ was 72 (77.4%) of the 93 *E. coli* isolates found to be CTX-M group-1 positive by PCR in North Indian isolates.[42] Overall, CTX-M was the commonest genotype (54.3%); 97.2% of them belonged to the CTX-M-1 cluster. On sequence analysis all 20 CTX-M-1 cluster-matched with CTXM-3 subtype.[47]

In the present study, occurrence of ESBL in the bacterial isolates was substantiated by $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} in DFU using PCR to find out true resistance to antibiotics. The *bla* gene positivity was 89.3%, of which $bla_{\text{CTX-M}}$ positivity was higher (81.8%), followed by bla_{TEM} (50.0%) and bla_{SHV} (46.9%). We also found higher $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} positivity in bacteria isolated from DFU patients. The most prevalent ESBL gene was $bla_{\text{CTX-M}}$ (81.8%), followed by 50% bla_{TEM} and 46.9% bla_{SHV}, in the cefotaxime-resistant Enterobacteriaceae members (*E. coli* and *Klebsiella* sp.) isolated from DFU patients. The genotypic methods helped us to confirm the genes responsible for the production of ESBLs in a single isolate. To the best of our knowledge, no such type of study has been reported from isolates of DFUs; however, $bla_{\text{CTX-M}}$ as the most prevalent and widely disseminated gene in the DFU bacterial population has been reported in our previous findings.[3,7]

Univariate analysis for the factors that were associated with the *bla* gene (CTX-M, TEM, and SHV) positivity in cefotaxime-resistant Enterobacteriaceae members (*E. coli* and *Klebsiella* sp.) isolated from DFU patients is summarized in Table 1. Duration of infection >1 month, hospital stay (>1 month), nature of ulcer (necrotic), type 2 diabetes mellitus, history of previous antibiotic use, dyslipidemia (low-density lipoprotein-cholesterol [>100 mg/dL], high-density lipoprotein-cholesterol [<40 mg/dL], triglycerides [>200 mg/dL], and total cholesterol [>150 mg/dL]) were independent predictors of *bla* gene positivity. To the best of our knowledge, no such type of study has been reported from isolates of DFU patients, except our one report on the correlation of CTX-M with the clinical characteristics, in which the positivity of $bla_{\text{CTX-M}}$ status was associated with poor glycemic control, osteomyelitis, neuropathy, and previous antibiotic use.[7]

Resistance to antibiotics is seen when they are used for a prolonged period of time. This resistance is an acquired form rather than an intrinsic one. The former develops following a mutation in the DNA of a microorganism or by acquisition of a new DNA. Acquisition of new DNA is accomplished by genetic elements such as plasmids or transposons. Resistance plasmids may have approximately 10 resistance genes for various antibiotics. Bacteria can transmit these characteristics to other bacteria.[48] Frequent or unnecessary use of antibiotics result in a selection favoring resistant bacteria. In this study history and discharge summaries showed that an overwhelming majority (12.3%) of the diabetes patients with osteomyelitis who were referred to our center had received antibiotic treatment before. However, which antibiotics and in what doses they were given were not clear; only the duration of the therapy was evident.

Presence of vascular disease characterized by disrupted micro- and macrocirculations causes a delay in wound healing in diabetes patients.[49,50] Disruption of wound healing results from a decreased blood flow into the ulceration and an aberrant expression of growth factors and cytokines as well. These factors, which delay wound healing, cause foot ulcers. Infections of these foot ulcers require a longer duration of treatment with antibiotics and the use of an appropriate antibiotic in an appropriate dosage.[51] In fact, we found that ESBL-positive bacteria were more frequently isolated in our cases. Although this increase was statistically insignificant, we believe that a multicentric study with increased number of cases could positively affect significance. Manual minimum inhibitory concentration determination was not carried out as it was time consuming and tedious for all the ESBL-producing isolates obtained in the present study. The duration of hospital stay may also depend on the management policy of the hospital. In our hospital, patients are discharged once the healing begins and are advised to come to follow-up at the outpatient clinic every week.

Summary

In conclusion, the present study in India, unlike in Western countries, concludes that Gram-negative bacteria dominated in DFU patients, suggesting thereby that all DFU patients admitted to a tertiary-care hospital in India require empirical therapy for Gram-positive as well as Gram-negative organisms. The treatment modes can be modified based on the severity of infection and on the microbiological culture report and current first-day Gram-stained smear finding. There is also a need for periodic antibiotic resistance surveys to help orient physicians and the local population on the best treatment strategies. However, chloramphenicol and carbapenems can be used as a reserve drugs in infections refractory to DFU with conventional drugs. For ESBLs, phenotypic methods are only screening methods for detection in a routine laboratory. The genotypic methods help to confirm the genes responsible for ESBL production. Sometimes multiple genes are responsible for production of ESBLs in a single isolate. Multiplex PCRs for the detection of bla_{TEM}, bla_{SHV}, and $bla_{\mathrm{CTX\text{-}M}}$ genes in ESBL-producing bacteria provides an efficient, rapid differentiation of ESBLs in selected species of Enterobacteriaceae and can be used as a rapid tool for epidemiological studies among ESBL isolates.

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Author Disclosure Statement

Z.M. researched data, wrote the manuscript, and contributed to the discussion. M.A. contributed to the discussion and reviewed/edited the manuscript. J.A. contributed to the discussion and reviewed/edited the manuscript. No competing financial interests exist.

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A study of biofilm production by gram-negative organisms isolated from diabetic foot ulcer patients

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Abstract

The present study was undertaken to study the difference in antibiotic resistance profile and minimum antibiotic concentration (MIC) of biofilm producing and non-biofilm producing gram-negative bacilli isolated from diabetic foot ulcer (DFU) patients in a tertiary care hospital in North India. Among the diabetic foot patients, 73.6% were males and 15% were females. 77.1% had T2DM whereas only 24.4% patients had T1DM. Poor glycemic control and poor HbA1c (>8) was observed in 68.7% and 70.1% patients respectively. Among the 57 patients, 97 gram-negative bacilli were isolated in which mixed bacterial infection was found in 67.8% and monomicrobial in 32.2% only. *Escherichia coli* was the most common (42.2%) isolate followed by *Pseudomonas aeruginosa* (23.7%), *Klebsiella oxytoca* (11.3%), *Klebsiella pneumonia* (9.2%), *Proteus vulgaris* (5.1%), *Acinetobacter* sp (5.1%), *Proteus mirabilis* (2%) and *Morganella morganii* (1.0%). 77.1% DFU patients had infection by biofilm producing organisms. BFP positive status was associated with the presence of neuropathy (O.R. 7.65), osteomyelitis (O.R. 3.14), duration of ulcer (O.R. 25.7), grade of ulcer (O.R. 9.12), necrotising ulcer (O.R. 14.4) and ulcer size >4cm^2 (O.R. 3.30) but not with patients characteristic, type of diabetes and type of diabetes, or duration of hospital stay. Poor glycemic control in 56.1% patients, amputation (24.5%), hospital stay (38.5%) and age distribution were independently associated with risk of biofilm producing infection in diabetic foot patients.

Keywords: Diabetic foot ulcer; bacterial profile; antibiotic resistance; biofilm production.

Introduction

Toole et al. (2005) who observed that, the bacteria are not free floating but grow upon submerged surfaces. The basic architecture of biofilms shows that the microcolony is actually the basic structural unit of the biofilm. The exhaustive structural analysis of hundreds of monospecies *in vitro* biofilms, and of dozens of multispecies natural biofilms, has shown that microcolonies are discrete matrix-enclosed communities of bacterial cells that may include cells of one or of many species. Depending on the species involved, the microcolony may be composed of 10–25% cells and 75–90% extracellular exopolysaccharide matrix (EEM). The matrix material often appears to be most dense in the area closest to the core of the microcolony, which is characterized by their lack of Brownian movement. Costerton *et al.* (1999) showed the arrangement of micro-colonies are in horizontal array in thin biofilms, but also form a vertical arrays in very thick sessile communities. Biofilm EEM, which is also referred to as *slime* (although not everything described as slime is a biofilm), is a polymeric conglomeration generally composed of extracellular DNA, proteins, polysaccharides, adhesins (PS/A) and autolysin (encoded by *atlE* gene) are involved in regulation of biofilm production present in various configurations. The *ica* gene codes for intracellular adhesion (ICA) and may also code for PS/A and, is required for biofilm production (Toole *et al*., 2005; Donlan *et al.,* 2002; Carol *et al.,* 2005).

Biofilm which forms on living or non-living surfaces establishes a protective environment of microbial life in natural, industrial and hospital settings (Stoodley *et al.,* 2004), which are, physiologically distinct from planktonic cells of the same organism, which, by contrast, are single-cells that may float or swim in a liquid medium (Karatan *et al*., 2009; Hoffman *et al*., 2005). When a cell switches to the biofilm mode of growth, it undergoes a phenotypic shift in behavior in which large suites of genes are differentially regulated (An *et al*., 2007). Biofilms are also often the site for quorum sensing influence the availability of key nutrients for biofilm formation, chemotaxis towards surface, motility of bacteria, surface adhesion and presence of surfactants are certain factors which influence biofilm formation (Carol *et al*., 2005; Thomas *et al*., 2007). According to a recent public announcement from National Institute of Health (NIH), more than 60% of all infections are caused by biofilm (Kim *et al*., 2001). Moreover, these ulcers adversely influence the patients' quality of life, leading to decrease in

social, physical and physiological functions (Raiber *et al.*, 1998). Various factors including defects in host defense mechanisms (impaired leukocyte functions) are responsible for this increase in infection rates. Wound infection is known to impair wound healing in both acute and chronic DFUs (Robson *et al.,* 1997). That most of the infections in DFU are polymicrobial in nature have recently been documented in our studies also (Zubair *et al.,* 2010a,b). Although the numbers and type of bacteria in a wound are critical for infection to occur, recently a new concept of bacterial biofilms has emerged as a potential way to better understand how bacteria deter healing. Therefore, a better understanding of bacterial biofilms is needed, and this may ultimately result in development of novel therapeutics for the prevention and treatment of DFU infections. The biofilm producing organisms have an inherent resistance to antibiotics and in the long run they may be very damaging because of the development of immune complex diseases (Donlan *et al.,* 2002; Raad *et al.,* 1995; Souli *et al.,* 1998).

There are only scarce reports on biofilm formation by clinical isolates from DFU especially in North India. Keeping this in mind, the present study was undertaken to study the difference in their antibiotic resistance profile and minimum antibiotic concentration of biofilm producing and non-biofilm producing gram-negative bacilli isolated from diabetic foot ulcer in a tertiary care hospital in North India.

Materials and Methods

Study Design

The study was carried out prospectively at the Diabetic and Endocrinology ward, J.N. Medical College, Aligarh Muslim University. Aligarh, India, from June 2009 to February 2010. Subjects studied were all in-patients of the male and female ward who had ulcer/infection in their foot with gram-negative bacterial infection.

Clinical Examination

A detailed clinical history and physical examination was carried out for every subject, which include a record of age, sex, anthropometric measurements, duration of ulcer, duration of diabetes and glycemic control. Foot ulcers were categorized into six grades (0-5) based on Meggit Wagner Classification System (Wagner *et al*, 1981). Neuropathy was quantified in each patient assessing vibration sensation using a 128 Hz tuning fork and a 10g monofilament (absence of perception of the Semmes Weinstein monofilament at 2 of 10 standardized plantar sites on either foot).

Ulcers were assessed for signs of infection (swelling, exudates, surrounding, cellulitis, odor, tissue necrosis and crepitation) and size was determined by multiplying the longest and widest diameters expressed in cm^2. Each patient was included only once in the study. All cases were monitored until discharged from the hospital. All the subjects gave informed consent and clearance was obtained from the hospital ethics committee.

Microbiological Methods

The microbiological methods described by Gadepalli *et al.* (2006) as adopted in our previous studies (Zubair *et al.,* 2010b, c) were used. Total transfer time to the laboratory was not more than 30 minutes. Direct microscopic examination of ulcer sample was performed and all the bacterial isolates were identified to the species level using standard identification techniques (Collee *et al.*, 1996).

Susceptibility Testing

Antimicrobial susceptibility testing was performed as described by the CLSI and adopted by us elsewhere (Zubair *et al.,* 2010b,c). Antimicrobial discs used were Aztreonam (30µg), Imipenem (10µg), Amoxyclav (30µg), Cefpodoxime (10µg), Cefepime (30µg), Cefoperazone (75µg), Cefoperazone/sulbactam (75/10µg), Cefixime (5µg), Piperacillin (100µg), Ceftazidime (30µg), Piperacillin/tazobactam (100/10µg), Ceftazidime/clavulanic acid (30/10µg), Amoxycillin (20µg), Cephotaxime (30µg), Cephotaxime/clavulanic acid (30/10µg), Ceftriaxone (30µg), Cephoxitin (30µg), Amikacin (30µg), Chloramphenicol (30µg), Gentamicin (10µg), Gatifloxacin (5µg), Ofloxacin (5µg), Levofloxacin (5µg). All discs were obtained from Hi-Media Laboratory, Mumbai, India. Interpretative criteria for each antimicrobial tested were those recommended by manufacturer's guidelines (Hi-Media Labs, Mumbai, India).

***Biofilm Assay**: Tissue Culture Plate (TCP) method*

The biofilm assay described by Mathur *et al*. (2006) was adopted. Stated briefly, 10 ml of trypticase soy broth (TSB) with 1% glucose was inoculated with a loopful of test organism from overnight culture on nutrient agar. The TSB broth was incubated at 37^{o}C for 24 hours. The culture was further diluted 1:100 with fresh medium and flat bottom tissue culture plates (96 wells) were filled with 200µl of diluted cultures individually. Uninoculated

sterile broth served as blank. Similarly, control organisms were also diluted and incubated. The culture plates were incubated at 37°C for 24 hours. After incubation, gentle tapping of the plates was done. The wells were washed with 200 µl of phosphate buffer saline (pH 7.2) four times to remove free-floating bacteria. Biofilms which remained adherent to the walls and the bottoms of the wells were fixed with 2% sodium acetate and stained with 0.1% crystal violet. Excess stain was washed with deionized water and plates were dried properly. Optical densities (OD) of stained adherent biofilm were obtained with a micro ELISA auto-reader at wavelength of 570 nm. Experiments were performed in duplicate and the average of OD values of sterile medium were calculated and subtracted from all test values.

Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined in doubling dilutions from 512 µg/ml to 0.05 µg/ml (CLSI). Antibiotic powders were obtained from Hi-Media Labs, Mumbai, India, except potassium clavulanate (clavulanic acid) which was procured from the Center for Diabetes and Endocrinology, A.M.U., Aligarh.

Antibiotic Treatment

Antibiotics were selected according to published recommendation (Hartemann-Heurtier *et al*., 2009). In mild infections amoxicillin clavulanic acid was given empirically by the oral route. However, in moderate infections intravenous route was preferred taking into consideration the likelihood of osteomyelitis. Considering that the causative agent was polymicrobial, we initiated ampicillin-sulbactam plus an aminoglycoside/quinolone or piperacillin-tazobactam or ceftriaxone plus metronidazole/clindamycin. In the presence of severe infections, surgical debridement and amputation were performed immediately after admission. Metronidazole (500 mg I.V. every 8 hours) was added to the drug regimen if cellulitis or gangrene was also present. The treatment was later modified in accordance with the culture results. The duration of the treatment was at least 4-6 weeks and prolonged in cases of osteomyelitis. All patients also received an intensive insulin treatment.

Statistical Analysis

The data was analyzed using SPSS version 17.0 for descriptive statistics. Quantitative variables were expressed as mean±sd while qualitative variables were expressed as percentage (%). Continuous variables were compared using 2 sample *t tests* for independent samples. Odds ratios and 95% confidence interval (CI) were reported for independent variables associated with the outcome variable: presence of anaerobic infection.

Results

Clinical

Males were predominant 42(73.6%) in the study subjects. Majority 44(77.1%) of subjects had T2DM. The mean age of the subjects was 49.1±12.4 years. The mean duration of diabetes was 12.6±6.4 years. Thirty-four patients (59.4%) had neuropathy, 35(61.4%) nephropathy, 32(56.1%) retinopathy, and 33(57.8%) were hypertensive. Osteomyelitis was present in 18(31.5%) subjects. Majority (77.0%) of the DFU patients were from Meggit Wagner grade II to grade IV. Grade I ulcer was found in 8.7%, Grade II in 14%, Grade III in 28%, Grade IV in 35%, and Grade V in 8.7% of patients. Majority of the subjects 31(54.3%) had lesions for >1 month before presentation at the hospital. The ulcer was necrotic in 25(43.8%) cases. Glycemic control was poor in 67(65.6%). HbA1c was <7% in 12 patients (21%), 7%-8% in 5(8.7%) and >8% in 40(70.1%) subjects. More than 38(66.6%) received surgical treatment, mainly in the form of debridement. 19(33.3%) patients were subject to amputation and 3(5.3%) died during the hospital stay (mean hospital stay 19.6±12.5) (Table 1). Majority of the ulcers were found on interdigits and the plantar surface (47.3% each), followed by heels (42.1%), margins (28%), malleoli (24.5%), and legs (8.7%) and on multiple (≥2 sites) 47.3%. Size of ulcer ≤4cm^2 was observed in 21% patients and ≥4cm^2 in 64.9% patients.

Microbiological Observations

A total of 97 gram-negative bacteria were isolated from 57 DFU patients, averaging 1.7 species per patient. Monomicrobial infection was observed in 32.2% patients whereas polymicrobial etiology was observed in 67.8% patients. In the direct microscopic examination of ulcer samples, 96% findings correspond with the culture growth on next day and in 4% patients, direct smear result differed with their culture growth. The frequency of bacterial isolates from DFU is shown in Table 2. *Escherichia coli* was the most common isolate, accounting for 41(42.2%), followed by *Pseudomonas aeruginosa* 23(23.7%), *Klebsiella oxytoca* 11(11.3%), *Klebsiella pneumoniae* 9(9.2%), *Proteus vulgaris*

5(5.1%), *Acinetobacter* sp. 5(5.1%), *Proteus mirabilis* 2(2%) *and Morganella morganii* 1(1%).

Biofilm Assay

Among the 97 gram-negative bacterial isolates, 60(59.4%) were biofilm producers. A total of 80% *P. vulgaris* isolates were biofilm producers, followed by *K. pneumoniae* (77.7%), *E. coli* (63.4%), *K. oxytoca* (63.4%), *Acinetobacter* sp. (60%) and *P. aeruginosa* (52.1%). The lone isolate of *M. morganii* was a biofilm producer (Table 2).

Antibiotic Resistance Profile of BFP and BFN Isolates

The result of resistance studies are summarized in Fig. 1. High degree of antibiotic resistance was exhibited by all the BFP isolates compared with NBP. High degree of resistance by BFP isolates was observed against cefoparazone (79.6%) followed by piperacillin (68.4%), cephotaxime (67.3%), amoxyclav (64.3%), cefixime (64.3%), amoxycillin (63.3%), ofloxacin (63.3%), cefepime (59.2%), gatifloxacin (57.1%), levofloxacin (51.0%), cefpodoxime (49.0%), ceftriaxone (44.9%), ceftazidime (42.9%), amikacin and gentamicin (40.8% each), astreonam (39.8%), cephoxitin (36.7%), chloramphenicol (31.6%), imepenem (24.5%), piperacillin+tazobactum (21.4%), cefotaxime+clavulanic acid (12.2%), and Ceftazidime+clavulanic acid (9.2%).

Minimum Inhibitory Concentration (MIC)

The MIC values of the piperacillin (with/without tazobactam), cefoparazone (with/without sulbactam), ceftazidime (with/without clavulanic acid) and levofloxacin between the BFP and NBP were given in Table 3. Percentage of BFP isolates that had an MIC of ≥2µg/ml was 93.3% for cefoparazone followed 90% for piperacillin, 81.6% for ceftazidime, and 75% for levofloxacin. The isolates that had an MIC ≥2µg/ml antibiotics with inhibitor were 80% for piperacillin+tazobactum, followed by 73.3 % for cefoparazone+sulbactum and 48.3% for ceftazidime + clavulanic acid.

Correlation of Biofilm Assay and Clinical Characteristics of DFU Patients

Table 1 also shows the result of univariate analysis of factors to be associated with the presence of biofilm producing organism infections. The age distribution [O.R. 1.23, P = 0.489], Type 2 diabetes [O.R. 2.16, P<0.207], duration of ulcer >1 month [O.R. 25.7, P < 0.001] was observed in 52.6% patients having biofilm producing infection. The size of ulcer more than 4 cm^2 [O.R. 3.30, P < 0.89] was found in 64.9% patients with biofilm positive infection and in 14.0% patients having ulcer size less than 4 cm^2. The neuropathy [O.R. 7.65, P < 0.003], osteomyelitis [O.R. 3.14, P <0.136], necrotising ulcer [O.R. 14.4, P< 0.002] and poor glycemic control (HbA1c : >8%)[O.R. 1.66, P<0.32] were significantly associated with biofilm producing bacterial infection. There was a significant relation between the biofilm producing bacterial growth with Wagner's grading. Majority of the biofilm positive patients were from grade 4 [O.R. 9.12, P<0.001] followed by grade 3 [O.R. 2.56, P< 0.23], grade 2 [O.R. 2.27, P< 0.40] and grade 5 [O.R. 1.5, P< 0.68]. (Fig. 5).

Discussion

This study presents a comprehensive clinical and microbiological profile of infected diabetic foot ulcers in hospitalized patients with special reference to the study of biofilm production in the gram-negative bacterial isolates.

With the rise in the prevalence of diabetes mellitus there is increasing problem of infections, especially foot infections. According to some studies, patients with diabetic foot infections account for 20% of hospital admissions (Shankar *et al.,* 2005). India is the home for the largest number of diabetic individuals. As higher resistance is a growing problem, effort was made to study the association of different study characteristics with the presence of resistant organisms. The prevalence of diabetic foot ulcers among male subjects was found to be 73.6% against 26.3% in female i.e. a ratio of 2.3:1 which may be due to higher level of outdoor activity among males compared to females (Zubair *et al.,* 2010b,c). With increasing duration of diabetes, there is increased risk of diabetes related complications especially chronic complications like sensory neuropathy. This study also reports a high prevalence of neuropathy (59.4%). There was a marked variation of sensory neuropathy from our earlier studies (Zubair *et al.,* 2010b,c), which showed a slightly higher percentage (66.6% & 78.5%) of neuropathy in North India. Ako *et al.* (2006) in a Nigerian study, showed the increase in neuropathy to 77.8% and 56.8% in a South Indian study (Shankar *et al.,* 2005). This marked variation in the prevalence may be due to difference in the methods used for the diagnosis of these conditions (10g monofilament or biothesiometer).

In Table 1, duration of infection >1month, prior antibiotic use and ulcer size >4cm^2 were independent predictors of infection. Thus patients with a large ulcer, with

a history of prior antibiotic use and duration of infection >1month were more likely to harbor BFP organisms. In the present study, mean duration of ulcer was found to be 41.5±47.6 days with 54.3% having ulcer for more than 1 month. About 78.9% presented with a large ulcer of approximate size of >4cm^2, thereby accounting for approximately 77.1% of the patients presenting with Wagner's grade II and IV. The reasons for presentation with advanced grade and stage of ulceration could be because of lack of structured health care delivery in the country, attempted self-medication and trust in traditional healers (Boulton *et al*., 2001; Zubair *et al*., 2010b,c). Diabetic foot infections are usually polymicrobial in nature and this has been well documented in the literature. In our study also, we found polymicrobial etiology in 67.8% and monomicrobial in 32.2% patients with the rate of isolation of about 1.7 bacteria per patient which is higher than the previous reports (Zubair *et al.,* 2010a,b,c) whereas Gerding *et al*., (1995) and Gadepalli *et al*. (2006) have reported higher isolation rate of 2.0%-5.8%. The present study also confirms the high resistance among the DFU isolates which was extremely common in hospitalized patients with diabetic foot ulcers. This is in accordance with the reports of Hartemann-Heurtier *et al*. (2009) and Zubair *et al*. (2010a,b,c).

This high degree of antibiotic resistance may be due to the fact that ours is a tertiary care hospital with widespread usage of broad spectrum antibiotics leading to selective survival advantage of pathogen. Our results of antimicrobial resistance pattern were similar to the recent studies done in India and outside (Shankar *et al,* 2005; Raja *et al.,* 2007). Gram-negative bacteria that are regarded as normal flora of the skin, like *P. aeruginosa*, may cause severe tissue damage in diabetics and should never be automatically disregarded as insignificant in diabetic foot ulcers (Zubair *et al.,* 2010b).

Another reason for this high antimicrobial resistance among the BFP appears to be due to the close cell-cell contact that permits bacteria to more effectively transfer plasmids to one another than in the planktonic state. These plasmids can encode for resistance to several different antimicrobial agents (Mah and Toole, 2001). Another factor contributing to resistance is quorum sensing, which through the processes described above can force bacteria into a slow-growing state when placed in an environment with adverse growth conditions; when in this state of intermission, bacteria are less susceptible to antimicrobial attack (Mertz, 2003). The biofilm also provides a physical protection to bacteria because antimicrobial agents are also ineffective at penetrating the biofilm, decreasing the concentration acting on the bacterial cells within the biofilm and as a consequence their efficacy (Mah and Toole, 2001). In addition to the resistance to antimicrobials, biofilms also appear to have an antiphagocytic property within the biofilm, which renders leukocytes present within the matrix ineffective (Leid, 2002). Additionally, there appears to be a component within the polysaccharide that inactivates and traps both complement and host antibodies. These factors lead to an accumulation of host immune factors that can lead to host tissue damage and eventually chronic inflammation (Percival and Bowler, 2004).

The idea of disrupting a biofilm that is already formed is attractive. This could be accomplished in a number of ways, including physical methods and/or application of topical substances. Among potential physical methods, debridement, electrical stimulation, or ultrasound could be used. Debridement may not only remove the bacteria and biofilm but also may aid in the removal of necrotic tissue for which the bacteria would thrive on. Electrical stimulation has been used over the years to assist penetration of various topical agents but have a limited application (e.g., electroporation and electrophoresis have been shown to enhance the penetration of a photosensitizer) (Johnson and Oseroff, 2002).

Changing the perspective about chronic infectious disease to include biofilm enables two important insights. First, it opens new methods for detection and treatment. Second, it provides a global reconceptualization of many chronic infectious diseases as resulting from a biofilm, allowing biofilm principles to be shared across disciplines. Recent studies have investigated new methods for detecting the components of a biofilm. Several investigations have used modern molecular methods, such as denaturing gradient gel electrophoresis and denaturing high performance liquid chromatography, along with imaging techniques including fluorescent in situ hybridization. Also, molecular methods such as polymerase chain reaction (PCR) and pyrosequencing in conjunction with conventional culture methods have been used to determine the bacterial species composition of chronic infections (Dowd *et al*., 2008). Performing molecular tests as part of routine bacterial analysis is becoming a real option for clinical laboratories. These tests could include methods such as PCR, reverse transcriptase–

PCR, microarrays, antigen testing, and rapid sequencing. Only a few of these methods are being used to test for certain pathogens, but culture-free identification of all pathogens and their corresponding resistance markers may soon become routine (Espy *et al.*, 2006). A biofilm focus also provides new strategies for treatment of chronic infections. Biofilm-based treatments might block initial bacterial attachment to a surface, block or destroy EPS formation, interfere with cell-cell signalling pathways, and use bacteriostatic or bactericidal agents at the same time. Concomitant therapies that not only attempt to eradicate bacteria but also affect the biofilm's community structure and communications may prove more effective than a single or sequential strategy such as antibiotic therapy (Ehrlich *et al.*, 2005). This multimodality approach to therapy is commonly used in other areas of medicine, such as the treatment of human immunodeficiency virus for which combination antiretroviral therapy is used to achieve the best clinical outcome.

Conclusion

Diabetic foot infections are a significant burden on patients as well as a burden on the health care delivery system. It is important to quickly and effectively identify and treat these ulcers and prevent complications. Biofilm formation on these wounds may be responsible for the chronicity of these wounds and for their common infectious complications. The presence of biofilm also represents an important barrier to effective treatment. Although in vitro study of novel approaches to control or eradicate biofilm formation are being performed, *in vivo* testing is necessary because various factors (e.g., wound fluid, proteases, growth factors, and so forth) need to be taken into consideration to determine the true efficacy of these agents. Treating the DFU by shifting from the planktonic model of microbiology to the biofilm model makes available new methods for detection and treatment. Because of molecular methods, science now has the ability to detect biofilms and understand the implications of interspecies chaos that contribute to infections. With these new scientific approaches along with coordination of clinical and laboratory efforts, education, and research, it is possible to imagine overcoming much of biofilm disease.

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Table 1: Demographic presentation of DFU patients in response to biofilm assay positive and negative bacterial infections (mean±sd and n(%) of otherwise indicated).

N=57	Total	Biofilm + (n=44)	Biofilm – (n=13)	P-value	OR(95%CI)
Sex					
Male	42(73.6)	32	10	0.532	0.8(0.18-3.4)
Female	15(26.3)	12	3		
Age distribution (years)	49.1±12.4	44.6±7.3	54.3±10.2		
<40	10(17.54	7(12.2)	3(5.2)		
41-60	33(57.8)	26(45.6)	7(12.2)	0.489	**1.23(0.35-4.3)**
>61	14(24.5)	11(19.2)	3(5.2)		
Type of Diabetes					
Type 1	14(24.4)	9(15.7)	5(8.7)		
Type 2	44 (77.1)	35(61.4)	9(15.7)	0.207	**2.16(0.57-8.0)**
Duration of Ulcer	41.5 ± 47.5	39.6±2.6	22.7±1.0		
< 1month	26 (45.6)	14(24.5)	12(21.0)		
>1 month	31(54.3)	30(52.6)	1(1.7)	**0.0001**	**25.7(3.0-217.7)**
Hospital stay(days)	19.6 ± 12.5	20.6±12.3	9.2±10.2		
≤20	19(33.3)	10(17.5)	9(15.70		
20-40	24(42.1)	22(38.5)	2(3.5)	0.46	**1.22(0.42-3.5)**
>40	14(24.5)	12(21.0)	2(3.5)		
Ulcer Grade (Wagner)					
grade 0	3(5.2)	0(0)	3(5.2)	-	-
grade 1	5(8.7)	0(0)	5(8.7)	-	-
grade 2	8(14)	7(12.2)	1(1.7)	0.40	**2.27(0.25-20.3)**
grade 3	16(28)	14(24.5)	2(3.5)	0.23	**2.56(0.5-13.1)**
grade 4	20(35)	19(33.3)	1(1.7)	**0.001**	**9.12(1.08-76.3)**
grade 5	5(8.7)	4(7.0)	1(1.7)	0.68	**1.2(0.12-11.7)**
Status					
discharge	54 (94.7)	41(71.9)	12(22.2)		
Dead	3 (5.3)	2(3.5)	1(1.7)	0.656	**0.878(0.08-9.2)**
Treatment					
conservative	38(66.6)	30(52.6)	8(14.0)		
amputation	19 (33.3)	14(24.5)	5(8.7)	0.447	**0.74(0.28-2.6)**
Diabetes duration(years)	12.6 ± 6.40	14.9±2.6	7.6±2.7		
Size of ulcer	20.14 ± 44.85	19.2±3.7	9.8±2.6		
≤4 cm^2	12 (21)	7(12.2)	5(8.7)		
>4 cm^2	45 (78.9)	37(64.9)	8(14.0)	0.89	**3.30(0.83-13.1)**
Complications					
neuropathy	38(66.6)	34(89.4)	4(10.5)	**0.003**	**7.65(1.9-30.1)**
nephropathy	35(61.4)	27(77.1)	8(22.8)	0.627	0.49(0.27-3.54)
retinopathy	32(56.1)	22(68.7)	10(31.2)	0.078	0.30(0.07-1.24)
hypertension	33(57.8)	24(72.7)	9(27.2)	0.269	0.53(0.14-1.99)
osteomyelitis	18(31.5)	16(88.8)	2(11.1)	0.136	**3.14(0.61-15.9)**
Nature of Ulcer					
necrotising	25(43.8)	24(96)	1(4)	**0.002**	**14.4(1.72-120)**
non-necrotising	32(56.1)	20(62.5)	12(37.5)		
Body Mass Index	20.59±4.41	20.3±2.1	18.6±1.8		
Plasma Glucose					
fasting	174.28±85.33	184.7±24.7	142.4±2.8		
postprandial	222.72±92.18	238.4±32.7	187.4±12.7		
HbA1c %	10.11±2.50	10.7±1.7	7.1±2.5		
<7 %(good control)	12(21.0)	9(15.7)	3(5.2)		
7-8 % (fair control)	5(8.7)	3(5.2)	2(3.5)		
>8 % (poor control)	40(70.1)	32(56.1)	8(14.0)	0.32	**1.66(0.45-6.11)**

Table 2: Gram-negative bacilli isolated from 57 diabetic foot ulcers (N=97).

	Name of DFU isolates	Biofilm assay		Total
		Positive	***Negative***	
1	*Escherichia coli*	26(63.4)	15(36.5)	**41(42.2)**
2	*Pseudomonas aeruginosa*	12(52.1)	13(47.9)	**23(23.7)**
3	*Klebsiella oxytoca*	7(63.6)	4(36.4)	**11(11.3)**
4	*Klebsiella pneumoniae*	7(77.7)	2(22.3)	**9(9.2)**
5	*Proteus vulgaris*	4(80)	1(20)	**5(5.1)**
6	*Proteus mirabilis*	-	2(100)	**2(2.0)**
7	*Acinetobacter sp*	3(60)	2(40)	**5(5.1)**
8	*Morganella morganii*	1(100)	-	**1(1.0)**
	Total	**60(59.4)**	**37(38.1)**	**97**

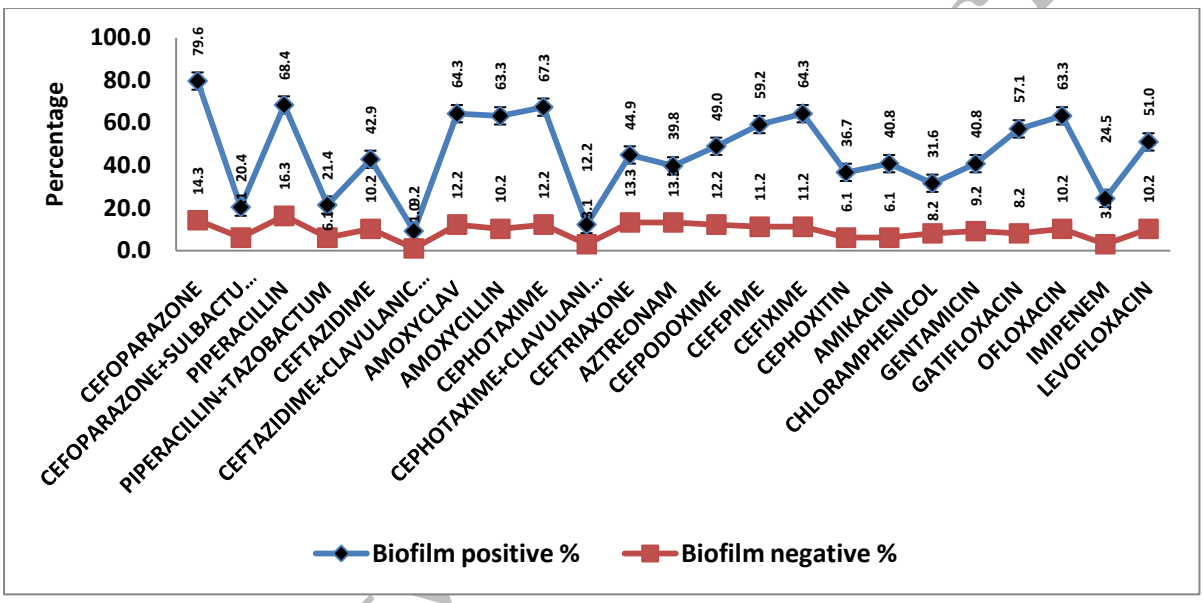


Fig. 1: Average resistance percentage of biofilm positive and negative gram-negative DFU isolates tested against various antibiotics.

Table 3: MIC of gram-negative bacilli (GNB) isolated from 57 DFU patients (N=97).

MIC	Biofilm producers	Non-biofilm producers
	≥2µg/ml	**≥2µg/ml**
Piperacillin	54(90)	6(10)
Piperacillin+Tazobactum	48(80)	12(20)
Cefoparazone	56(93.3)	1(6.7)
Cefoparazone+Sulbactum	44(73.3)	16(27)
Ceftazidime	49(81.6)	11(18.4)
Ceftazidime+Clavulanic acid	29(48.3)	31(51.6)
Levofloxacin	45(75)	15(25)

Fig. 2: Fasting and postprandial blood glucose level among DFU patients having infection with the biofilm producing and non-producing gram-negative bacterial infections at the time of admission and discharge from the hospital.

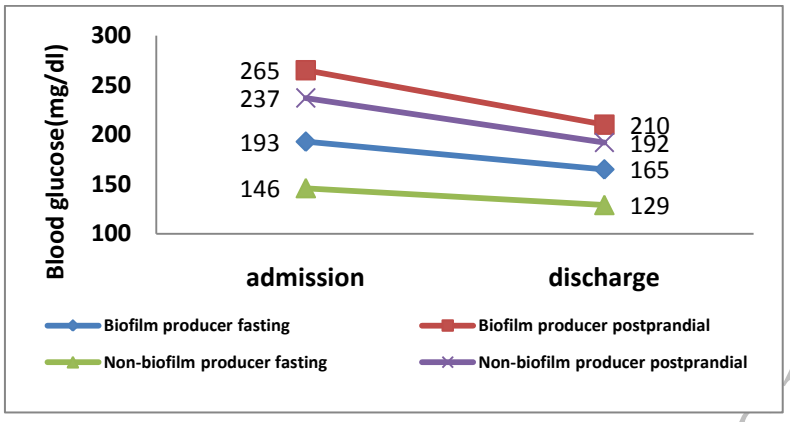


Fig. 3: HbA1c values among the DFU patients having infection with the biofilm producing and non-producing gram-negative bacterial infections at the time of admission and discharge from the hospital.

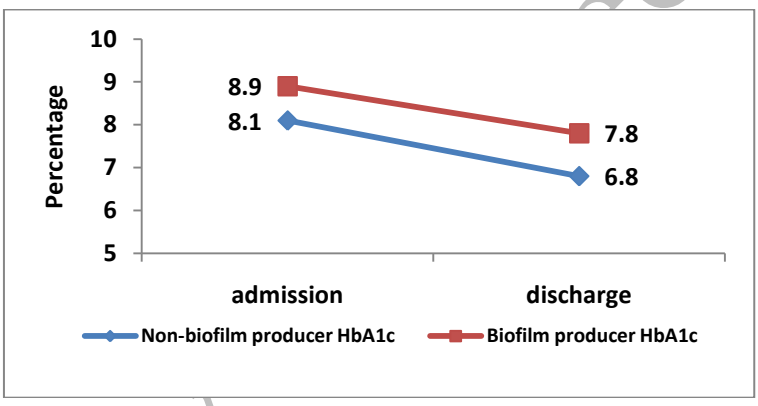
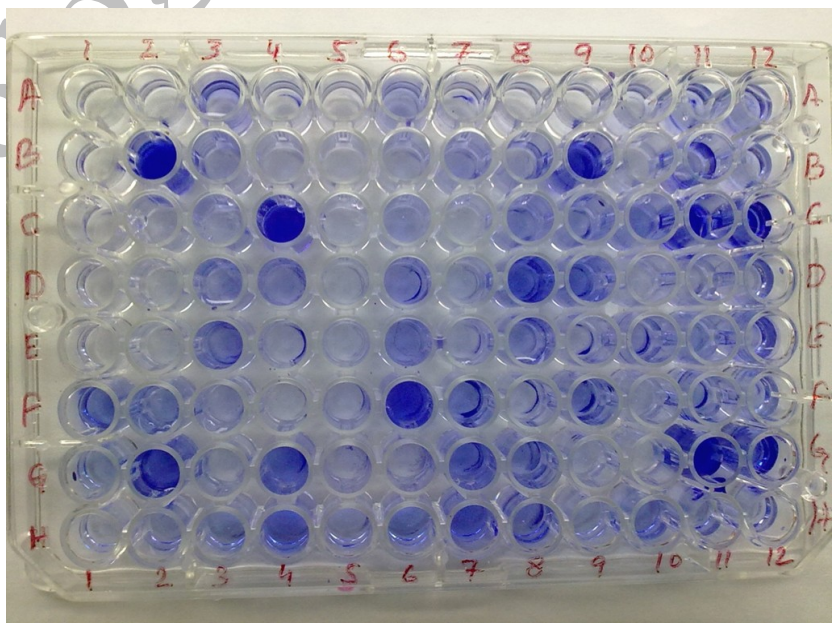


Fig. 4: Tissue culture plate showing the result of biofilm assay, A1 and B1 were blank.



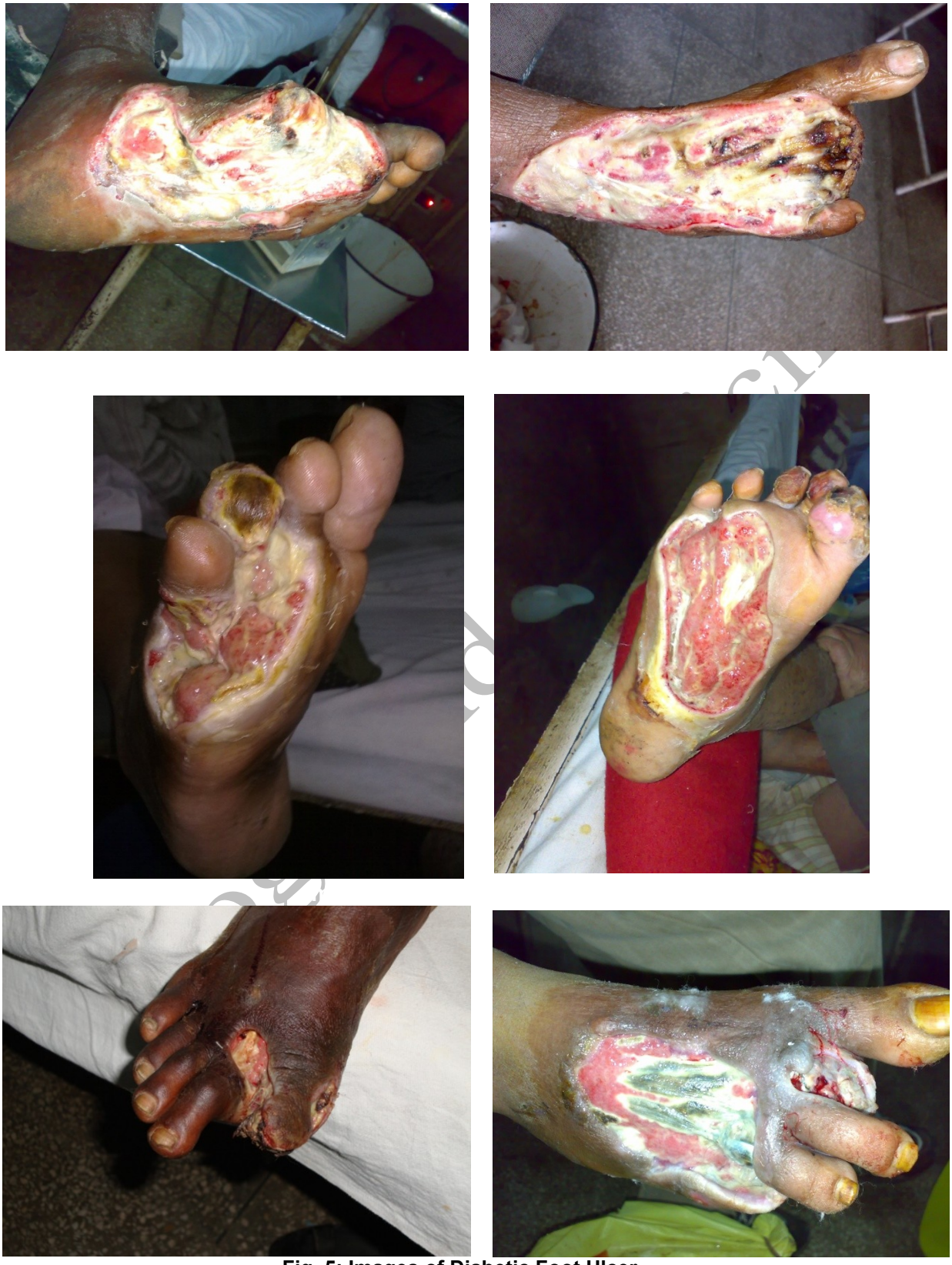


Fig. 5: Images of Diabetic Foot Ulcer.

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ABSTRACTS

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ATTD 2011 Oral Presentations

O-01

IMPROVED PHARMACOKINETIC AND PHARMACODYNAMIC PROFILE OF RAPID-ACTING INSULIN USING NEEDLE-FREE JET INJECTION

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Background: Insulin jet injectors use a high-velocity jet to deliver insulin into the subcutaneous region without penetrating the skin with a needle. The pharmacodynamic and pharmacokinetic profile of rapid-acting insulin using this method of insulin administration is unknown.

Methods: Euglycaemic glucose clamp tests were performed in 18 healthy volunteers (M/F 5/13; age 27 ± 9 years, BMI $23.6 \pm 2.8\,kg/m^2$) after subcutaneous administration of 0.2 U/kg aspart insulin, either by jet injection or by conventional pen injection, using a double-blind, double-dummy cross-over study design.

Results: The time to maximal exogenous glucose infusion rate (GIR) was 51 ± 3 min with the jet injector versus 105 ± 11 min with the insulin pen ($P = 0.0001$). There was no difference in the maximal GIR between the two modes of insulin administration (6.49 ± 0.58 versus 6.09 ± 0.56 mg/kg/min, $P = 0.50$), but the duration of the glucose lowering effect as reflected by the time of 50% glucose disposal was shorter with the jet injector (123 ± 7 versus 166 ± 6 min, $P < 0.0001$). In analogy, the time to peak insulin concentration was shorter (31 ± 3 versus 64 ± 6 min, $P < 0.0001$) and the maximal insulin concentration was higher (108 ± 13 versus 79 ± 7 mU/L, $P = 0.01$) with the jet injector than with the pen.

Conclusion: Insulin injected by jet injection resulted in faster insulin absorption and higher insulin peak levels as well as faster onset and shorter duration of glucose-lowering action than insulin injected by conventional pens. The pharmacological profile of insulin administration by jet injection better resembles the profile of endogenous insulin secretion than that of insulin injected by conventional pens.

O-02

USING THE BRAIN TO REDUCE HYPOGLYCAEMIA

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Introduction: Hypoglycaemia remains the major acute complication of insulin therapy and hypoglycaemia itself, and fear of hypoglycaemia, limit attempts to achieve normoglycaemia with exogenous insulin or insulin secretagogues. Loss of awareness of hypoglycaemia increases risk of severe hypoglycaemia 6-fold. Restoration of awareness is possible with strict hypoglycaemia avoidance. Nevertheless, hypoglycaemia unawareness is a problem for about one third of people with Type 1 diabetes and an as yet unknown proportion of people with Type 2.

Aims of presentation: In this presentation we will review the mechanisms of hypoglycaemia awareness, concentrating on the brain's response to hypoglycaemia. We will look at how the brain's responses are different in unawareness and discuss the impact of those differences on clinical behaviours. We will review the evidence that educational strategies around insulin use can restore hypoglycaemia awareness and to what extent they do not. Finally, we will look at the evidence base for developing newer ways to assist in restoration of awareness for people with insulin treated diabetes who are at particularly high risk for problematic hypoglycaemia.

O-03

THE DREAM PROJECT—AUTOMATED OVERNIGHT GLYCEMIC CONTROL UNDER MD-LOGIC ARTIFICIAL PANCREAS

T. Danne[1], O. Kordonouri[1], T. Biester[1], T. Battelino[2], N. Bratina[2], M. Avbelj[2], R. Nimri[3], **E. Atlas**[3], E.A. Grunberg[3], S. Miller[3], M. Phillip[3]

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One third of people's life is spent in sleep, therefore the overnight period is a significant proportion of the time requiring good glycaemic control. Nocturnal hypoglycaemia is a common and worrying occurrence in type 1 diabetes, even more so if patients loose their hypoglycaemic awareness. This study aims to establish good overnight glucose control and reduce the risk of nocturnal hypoglycaemias by use of the MD-Logic Artificial Pancreas (MDLAP) system. The MDLAP allows individualized automatic glucose regulation based on continuous subcutaneous glucose sensing and insulin delivery. Our ongoing CRC hospital-based pilot study is the first step towards evaluating the MDLAP study in the outpatient setting at patient's home.

The **DREAM** (**D**iabetes wi**rel**e**s**s **a**rtificial pancreas consortiu**m**) project is a multicenter, prospective, open label, randomized, cross over, pilot study being conducted in Germany, Slovenia and Israel. Fifteen eligible patients will be

recruited when each patient will participate in two overnight sessions: one session with MDLAP where insulin treatment will be fully automated and another session when the patient's usual continuous subcutaneous insulin infusion (CSII) therapy will be used. Each session will last 12 hours, during which the patient will receive dinner and remain in the clinic for overnight sleep. In both sessions a pre-prandial bolus will be given manually by the patient according to their regular insulin regimen.

Interim results of part of the patients will be presented.

O-04

A HYPOGLYCAEMIA ALARM BASED ON CONTINUOUS EEG MONITORING AND REAL-TIME DATA PROCESSING

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Hypoglycaemia is associated with low frequency waves in the electroencephalogram. We propose the development of an EEG based hypoglycaemia alarm for permanent use. The device will be small, easy to use and not depend on calibration.

Our initial studies focused on the feasibility of recording EEG from subcutaneous temporal electrodes and the development of a robust general multiparameter algorithm which could detect hypoglycemia associated EEG changes with high sensitivity and specificity. All patients developed specific EEG changes during hypoglycemia. In 12 of 15 type 1 diabetes patients (T1D) (duration 6–53 years) EEG changes occurred before severe cognitive impairment.

Due to the clinical importance of nocturnal hypoglycemia we studied T1D patients with impaired awareness of hypoglycemia during sleep. EEG was recorded continuously during insulin induced hypoglycemia and analyzed real-time by the automated algorithm. All patients developed hypoglycaemia associated EEG changes irrespective of sleep stage. In 4 of 10 experiments the patient reacted upon alarm thus avoiding impending severe hypoglycaemia. Only few events of false alarms were observed.

We have developed a miniaturized EEG recorder consisting of an internal part, implantable by a minor non-complicated surgical procedure, and an external device that conducts real-time EEG analysis, powers the internal part, and alarms in case of hypoglycaemia. Healthy subjects carried this device for five weeks with a consistent recording of EEG.

Data from conducted trials have allowed us to optimize the algorithm thereby increasing sensitivity and specificity. Large scale clinical trials in T1D patients with impaired hypoglycaemia awareness are scheduled and approved.

O-05

PENS, PUMPS, PATCHES AND SENSORS IN TYPE 2 DIABETES

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International Diabetes Center, Minneapolis, MN, USA

The definition of optimal glycemic control has evolved to include both the level of HbA1c and amount of hypoglycemia. A universal definition of optimal control which includes standardized parameters of SMBG, CGM and quality of life (QOL) is needed and would allow technology like pens, pumps, patch pumps and continuous sensors to potentially play an important role in T2D.

A major cause for clinical inertia (not taking action when above goal) in T2D is the need to start and frequently adjust insulin. Insulin pens have helped patients more consistently take insulin as prescribed and have improved patient satisfaction and QOL. In the US 30% of T1D use insulin pumps while less than 1% of T2D patients. There have only been a few RCT's of pump therapy in T2D. Large RCT's show no difference in A1c, smaller trials show a reduction in A1c and glycemic variability and improved QOL for pump vs MDI. A series of simple patch pumps (insulin reservoir and automatic pumping system) are being tested. Some deliver a fixed basal, others have only a bolus capability and finally one has a fixed basal and flexible bolus features. Pilot studies show patch pumps to be popular with insulin requiring T2D patients and they improve glucose control at least as effectively as insulin injections. Continuous glucose sensors in T2D may be an excellent tool to highlight abnormalities in glucose profiles, direct the best SMBG testing regimen, select the most appropriate diabetes mediations or help to effectively titrate insulin doses.

O-06

10 YEARS OF CSII TREATMENT IN CHILDREN WITH TYPE 1 DIABETES IN SLOVENIA

N. Bratina[1], L. Lusa[2], S. Starc[1], T. Battelino[1]

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Studies have shown that good metabolic control plays an important role in reducing the microvascular and macrovascular late complications associated with type 1 diabetes mellitus.

Insulin pumps and real-time continuous glucose monitoring systems help patients with type 1 diabetes to reach better metabolic control safely.

In the last 10 years CSII became the leading therapy for children and adolescents with type 1 diabetes in Slovenia. In the year 2010 more than 70% of children visiting University Children's Hospital in Ljubljana are using CSII as preferred kind of therapy. Among them is a group of 50 children bellow 6 years who are using RT-CGM continuously.

Recently an analysis was performed for the period of 10 years showing improved metabolic control for the whole group of patients (525 patients). The median follow-up time was 5 years. HbA1c significantly ($P < .001$) decreased over 10 years: from 9.25% in the year 2000 to 7.75% in the year 2009. BMI SDS slightly but significantly increased, while daily insulin dose decreased over the time. Duration of CSII treatment was significantly associated with a decrease in HbA1c ($P < .001$). The incidence rate of severe acute complications was very low in all patients, did not change with time, and was 1.40 per 100 patient-years for severe diabetic ketoacidosis and 0.73 per 100 patient-years for severe hypoglycemia. Metabolic control improved in subgroups such as toddlers, or eating disorder as well.

Good education for children, families and teachers as well is of great benefit.

O-07

SAFETY SUPERVISION SYSTEM: FIRST CLINICAL TRIALS

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Safety is fundamental to ongoing developments of CGM enhanced insulin pumps. We demonstrate the feasibility of a Safety Supervision System (SSS) for insulin treatment of type 1 diabetes (T1DM) and its capacity to reduce hypoglycemia and hyperglycemia risks in 14 T1DM subjects during an exercise protocol.

The SSS uses continuous glucose monitoring and insulin pump feedback to (i) attenuate insulin delivery, and (ii) detect imminent hypoglycemia. In addition, the system mitigates hyperglycemia by advising the patient on correction boluses, up to once an hour.

Fourteen T1DM patients followed a randomized cross-over protocol at Montpellier University Hospital and the University of Virginia using a CGM device (Abbott Navigator®, Dexcom 7®) and an insulin pump (Insulet Omnipod®). Admissions contained meal challenges and moderate exercise. The SSS was activated during the treatment admission from 2pm to 8am.

When the SSS was active:

(i) overall hypoglycemic events were reduced two fold 21 vs. 9 as follows: no effect during exercise (3 vs. 2), significant reduction post exercise (4 vs. 1), after dinner (3 vs. 1), and overnight (9 vs. 3);
(ii) 9 of 9 hypoglycemic events were detected on average 21min in advance. In addition, average glucose was reduced: 138 vs. 154 ($P = 0.033$); and percent in target ranges 70–180 mg/dL and 80–140 mg/dL increased: 83% vs. 64% ($P = 0.007$) and 49% vs. 36% ($P = 0.018$), respectively.

We demonstrated that the SSS is capable of significantly improving glucose control in adults with T1DM, detecting in advance all hypoglycemic events and reducing hypoglycemia two fold during an exercise protocol.

O-08

A NEW TOOL FOR EARLY DETECTION OF TYPE 2 DIABETES COMPLICATIONS: COMPARISON OF SUDOSCAN WITH STANDARD METHODS

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IMPETO MEDICAL, Paris, France

Introduction: SUDOSCAN™ is a quick, non-invasive and quantitative method to measure sudomotor dysfunction on hands and feet as indicator of autonomic neuropathy. This new electrochemical sweat conductance (ESC) test is based on electrochemical reaction between sweat chloride and electrodes in contact with hands and feet. The aim of the study was to compare this new tool for early detection of diabetic complications with the standard methods used for assessment of diabetes foot risk, cardiac autonomic neuropathy and renal complications.

Methods: 257 diabetic patients were involved in the study. The following tests were performed: vibration perception threshold (VPT) using biothesiometer, heart rate variability using Holter, Ewing's battery test for assessment of cardiac neuropathy (4 tests), estimation of the Glomerular Filtration Rate (MDRD eGFR) for assessment of renal function and measurements of feet and hands ESC by SUDOSCAN. According to ESC values patients were classified as at no risk: feet ESC $> 60\,\mu$S, moderate risk: feet ESC: 40–60 μS, high risk: feet ESC $< 40\,\mu$S.

Results: In the 66 patients classified as high risk according to the SUDOSCAN results (Feet ESC $< 40\,\mu$S) the percentage of patients with at least 1 or 2 Ewing tests disturbed and the mean VPT were higher while the mean Low Frequency components (Holter analysis) and the mean MDRD eGFR were lower when compared to other two groups (127 without risk and 64 with moderate risk).

Conclusion: SUDOSCAN appears to be a useful tool for early assessment of diabetes complications.

O-09

EFFECT OF A HYBRID CLOSED-LOOP (HCL) ON RESTORING METABOLIC CONTROL AT THE ONSET OF DIABETES

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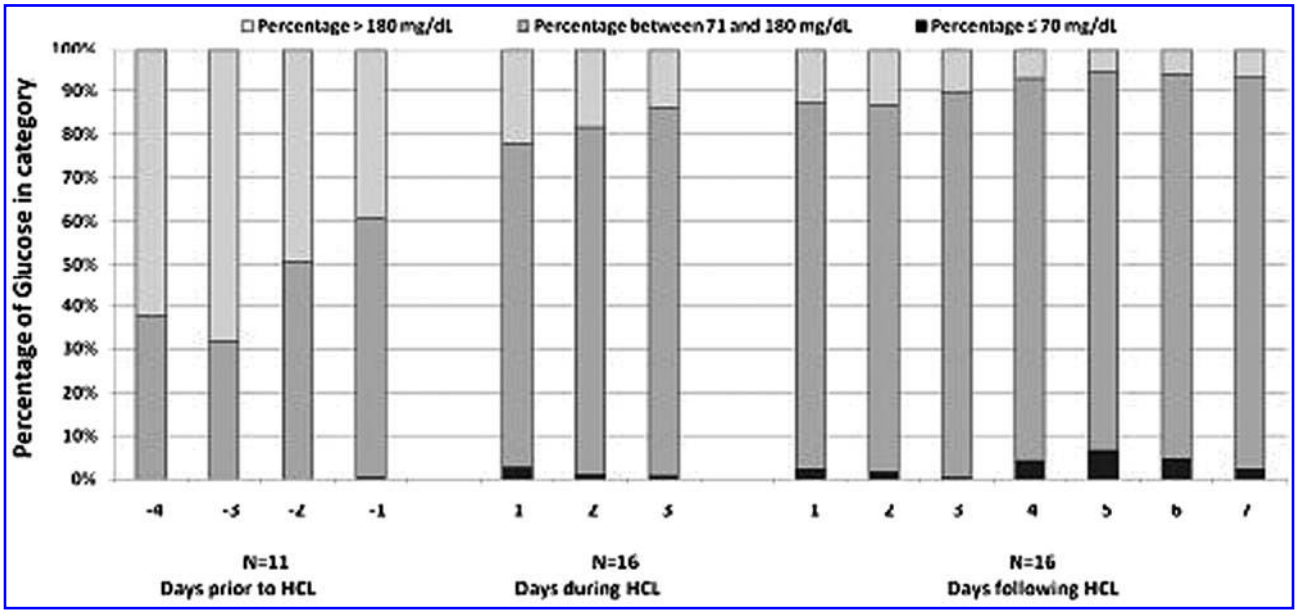
Objective: As part of a NIH-funded TrialNet and DirecNet randomized controlled trial to assess the impact of tight metabolic control at diagnosis of type 1 diabetes on islet recovery we utilized the Medtronic MiniMed ePID system. This system combines an external subcutaneous pump and sensor with a proportional-integral-derivative (PID) algorithm utilizing insulin feedback.

Methods: Subjects are admitted within 1 week of diagnosis for 3 to 4 days of Hybrid Closed-Loop (HCL) therapy. About 75% of the estimated insulin for a meal is given prior to an unrestricted meal. Reference blood glucose levels are measured every ½ hour.

Results: The system was utilized an average of 3 days for a total of 1,117 hours in 16 subjects ages 10–17 years. The mean (±SD) glucose was 140 (±10) mg/dL, and median insulin dose was 1.2 units/kg/day. There were no severe hypoglycemic or hyperglycemic events. As shown in the figure, 46% of sensor glucose values were between 71–180 mg/dL prior to HCL, 85% on the third day of HCL and 88% for the week following HCL.

Conclusion: HCL therapy can rapidly and safely restore metabolic control following diagnosis of diabetes and the effect is sustained on discontinuation of HCL therapy.



Blinded and Unblinded Sensor Glucose Data.

O-10

APPLICATION OF A MULTISENSOR DEVICE FOR NON INVASIVE CONTINUOUS GLUCOSE MONITORING

A. Caduff[1], M. Mueller[1], F. Dewarrat[1], P. Zakharov[1], A. Megej[1], M. Donath[2], J. Klisic[1], W.A. Stahel[3], M.S. Talary[1]

[1]*Solianis Monitoring,* [2]*University Hospital Zurich,* [3]*ETH Zurich, Zurich, Switzerland*

We have previously reported about the findings in clinical-experimental studies with a novel Multisensor system for non invasive continuous glucose monitoring. In this study a Multisensor version with fully integrated sensors and battery was experimentally tested.

Six T1DM patients (age 44 ± 16 y; BMI $24.1 \pm 1.3\,kg/m^2$, duration of diabetes 27 ± 12 y; HbA1c $7.3 \pm 1.0\%$) wore the same Multisensor at the upper arm, performing a total of 45 in-clinic study days. Glucose changes were induced by the administration of an oral or i.v. glucose solution. The first 22 study day's data

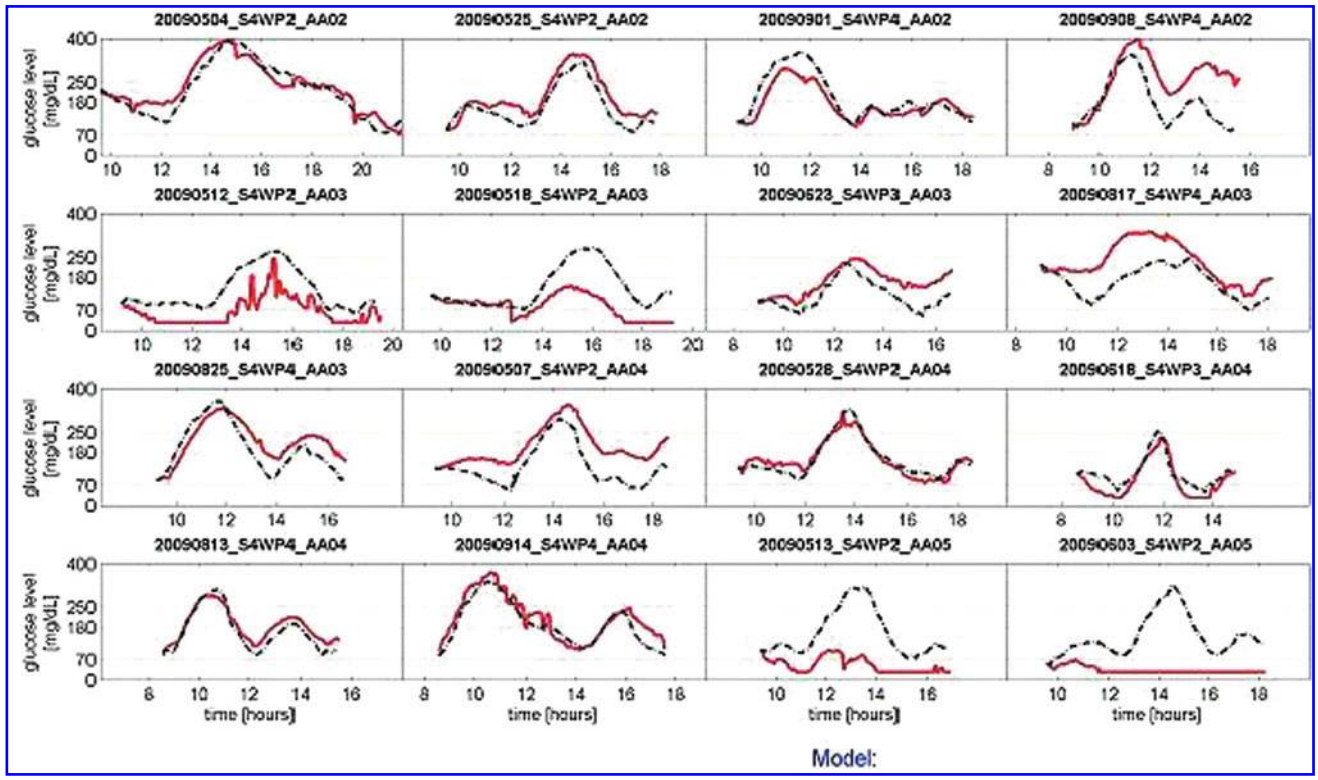


FIG. 1.

spanning all subjects were used to train a linear regression model. The global model derived was then prospectively applied to the data of the remaining 23 study days allowing for external validation. One initial baseline adjustment at the very beginning of each study day was used to adjust the level of the glucose estimate.

Figure 1 shows the time series of all 23 externally validated study days. When comparing the estimated glucose to the blood glucose reference values, the model yielded a Mean Absolute Relative Difference (MARD) of 40.8%, a Mean Absolute Difference (MAD) of 51.9 mg/dL, and an average R2 of 0.70. The Clarke error grid analyses showed 89.0% of paired glucose values in A + B, 4.5% in C, 4.6% in D and 1.9% in the E region.

This work demonstrates that glucose variations under controlled conditions can be monitored non invasively by a prospectively applied multiple regression statistical model.

O-11

EFFECT OF A NOVEL WARMING DEVICE ON PHARMACODYNAMICS (PD) OF RAPID ACTING INSULIN IN YOUTH WITH TYPE 1 DIABETES (T1D)

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Background: Post-prandial blood glucose excursions in open- and closed-loop insulin delivery systems could be mitigated by accelerating insulin absorption and action.

Methods: Glucose clamp technique was used to examine the effect of a novel infusion site warming device, InsuPatch, on the PD of a 0.2 u/kg bolus of aspart insulin in pump-treated subjects. Studies were performed on 2 separate mornings with and without the activation of InsuPatch device. To date, 8 subjects (14 ± 2 yrs, 3 female, A1c $7.3 \pm 0.6\%$) have completed both clamps.

Results: As hypothesized, time to peak insulin action (T_{maxGIR}) and time to early half maximal activity ($T_{early\ 50\%}$) occurred much earlier, and area under the time action profile during the first ninety minutes of clamp ($AUC_{GIR\ 0–90min}$) was significantly greater with the InsuPatch than without the InsuPatch. Mean GIR, GIR_{max} and $AUC_{GIR\ 0–300min}$ were unaffected by infusion site warming (data not shown).

Conclusion: Our preliminary data indicate that warming of the infusion site with InsuPatch results in earlier onset and peak action of rapid acting insulin analog which could be beneficial in limiting the post-meal plasma glucose rise in pump treated patients.

PD Parameters with & without InsuPatch

	No InsuPatch	*With InsuPatch*	P *value*
$T_{maxGIR\ (min)}$	133 ± 27	84 ± 18	0.0003
$T_{early50\%\ (min)}$	66 ± 16	41 ± 15	0.01
$GIR_{0–90\ min\ (mg/kg/min)}$	2.4 ± 1	3.7 ± 2	<0.0001
$AUC_{GIR0–90\ min}$	226 ± 100	343 ± 141	<0.0001

O-12

PREVENTION OF HYPOGLYCEMIA: NOW AND IN THE FUTURE

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Introduction: Hypoglycemia is the rate-limiting factor in attaining optimal glycemic control.

Objectives: The objectives of this presentation are to present current information on the incidence, causes and prevention of hypoglycemia in subjects with type 1 diabetes (T1D).

Methods: The use of continuous subcutaneous insulin infusion (CSII) and of continuous glucose monitoring (CGM) can now aid in the prevention of hypoglycemia. Exercise studies using features of CSII to reduce immediate and delayed hypoglycemia will be presented. Data from large randomized clinical trials using CGM will be presented to show increased time spent within glycemic targets and reduced time spent in hypoglycemia.

Prevention of hypoglycemia in the future will involve use of prediction algorithms, the closed loop pancreas and preservation of C-peptide production.

A current obstacle for hypoglycemia prevention research in the US relates to FDA-required endpoints.

Conclusion: The incidence of hypoglycemia can continue to be reduced in subjects with T1D.

O-13

DIRECT MEASUREMENT OF 3-B-HYDROXYBUTYRATE IN THE MANAGEMENT OF DIABETIC KETOACIDOSIS IN CHILDREN: A COST EFFECTIVE ENHANCEMENT IN THE MANAGEMENT OF DIABETIC KETOACIDOSIS IN CHILDREN

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Precise quantification of β-hydroxybutyrate (β-HBA) levels in capillary blood is a method that better correlates than acetoacetate with changes in acid-base status during the treatment. We performed a study in order to evaluate whether this ketone-testing method was able to reduce the duration and costs of intensive treatment. We analyzed the treatment of 33 children with severe (arterial $pH \leq 7.2$) or moderate (arterial $pH > 7.2 \leq 7.3$) DKA. All patients were treated with the same standard low-dose insulin infusion protocol. Sixteen patients were randomly monitored with blood β-HBA (group1) and seventeen patients with a commercial test for urine ketone bodies (UKB) (group2). Contrary to UKB, β-HBA levels showed good correlation with: HbA1c Values on admission ($r = 0.99$; $P = 0.0001$); number of days before diagnosis of diabetes ($r = 0.95$; $P = 0.0001$); changes in arterial pH ($r = -0.82$; $P = 0.0001$) and blood bicarbonate ($r = -0.63$; $P = 0.001$) during the DKA treatment. The duration of the treatment before the resolution of ketosis in group I has been found to be related to the values of β-HBA on admission ($r = 0.84$; $P < 0.001$). Determination of β-HBA showed that ketosis in group1 patients disappeared 4 to 9.5 hours earlier then in group2 patients. This resulted in 22 hours saved for clinical assessment and 375 laboratory investigations for a total saving of 2940 euros including costs for laboratory tests (29.8%) and clinical assessment (70.27%). Quantitative determination of β-HBA offers useful information for monitoring ketosis

in newly diagnosed diabetic children and for reducing time and cost in an intensive care unit.

O-14

WHAT HYPOGLYCEMIC LEVEL IS CLINICALLY RELEVANT? THE AMERICAN VIEW

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The American Diabetes Association (ADA) Workgroup on Hypoglycemia defined hypoglycemia in diabetes as "all episodes of an abnormally low plasma glucose concentration that expose the individual to potential harm" (*Diabetes Care* 28:1245, 2005). Because the glycemic thresholds for symptoms are dynamic, it is not possible to state a single plasma glucose concentration that defines hypoglycemia. Nonetheless, the ADA Workgroup recommended that people with diabetes at risk should become concerned about the possibility of developing hypoglycemia at a self-monitored plasma glucose concentration of $\leq$3.9 mmol/L (70 mg/dL). That alert value is both data-driven—3.9 mmol/L approximates the lower limit of the normal postabsorptive plasma glucose concentration, the normal glycemic thresholds for activation of glucose counterregulatory systems and the highest low glucose level reported to reduce counterregulatory responses to subsequent hypoglycemia—and pragmatic. People with diabetes need not always self-treat at an estimated plasma glucose concentration of $\leq$3.9 mmol/L. The options include repeating the glucose estimate in the short-term, changing behavior (e.g., avoiding exercise or driving until the glucose level is higher), ingesting carbohydrates or adjusting the treatment regimen. Criticisms of this alert value have been raised but there is actually rather little disagreement on this ostensibly contentious issue (*Diabetologia* 52:35, 2009).

O-15

TREATMENT OF CHILDREN WITH PATCH PUMPS VS. DURABLE PUMPS

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Worldwide insulin pump use in children is increasing quickly. According to the German nationwide dpv initiative the percentage pediatric CSII patients with a diabetes duration of more than 1 year was 36%, for those younger than 5 years even 66%. In our pediatric service 377 of 635 patients use CSII (60%), 44 of 53 (83%) of those up to age 6. Previously the percentage has increased continuously, however numbers have reached a plateau in the past three years. So far these pumps have been durable pumps. Recently the world's first tubing-free insulin pump, the mylife OmniPod System® (Ypsomed) has been introduced to the German market. The Pod integrates the pumping mechanism, cannula, needle and reservoir into one wearable and completely disposable unit. A Personal Diabetes Manager wirelessly programs insulin delivery, calculates suggested doses, and has a built-in blood glucose meter. It remains to be seen how many of those previously reluctant to start pumps will choose such a tubeless system and how many will switch from durable to patch pumps. An initial study with pediatric patients is underway. With multiple parts, pieces that have to be primed and attached and detached, and adhesives meant to keep a container of insulin comfortably stuck to your skin for three days at a time, these systems may be rather complex for some children. Nevertheless, many families have already voiced their opinion that the tube-free pumping is most exciting to them as long as the systems are convenient and easy to use.

O-16

THE ARTIFICIAL PANCREAS SYSTEM, A COMPREHENSIVE SYSTEM FOR THE CLINICAL EVALUATION OF CONTROL ALGORITHMS

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The objective in developing the Artificial Pancreas System (APS®) was to design a flexible platform that facilitated four-way communication between a mathematical control algorithm, an insulin pump, a glucose sensor and the end user in order to support clinical evaluation of an artificial pancreas.

The APS's modular architecture was designed so that a control algorithm, which may be written in a variety of computer languages, could be easily implemented in a clinical setting by a range of possible devices. Human machine interfaces were standardized to allow simple toggling between the different supported pumps, Insulet Omnipod® system, Roche's Accu-Chek® Spirit Combo and Animas' OneTouch® Ping® as well as two CGMs, DexCom Seven plus® and Abbott FreeStyle Navigator®.

By simplifying user interaction and streamlining communication, the APS allows clinical researchers to focus on the evaluation of control algorithms. Simple PID controllers written in C and Matlab were shown to function identically in simulations with all APS compatible devices without the need to customize the algorithm to a specific device. In addition, the newly integrated Animas and Roche pumps are capable of remote communication at a significant distance ($\sim$5 meters), allowing the possibility of closed- loop testing while patients participate in exercise away from the machine running the APS.

The APS is the most versatile tool available for the clinical evaluation of artificial pancreas control algorithms across devices and computer languages. The APS allows standardization in data management, user machine interfaces, device communication and application program interface.

O-17

THE ARTIFICIAL PANCREAS ARCHITECTURE, LAYERS AND ALGORITHMS—A SYSTEM PERSPECTIVE

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Closed-loop algorithms are an integral part of modern life. Automation and control are used constantly to enhance productivity and quality as well as to provide safety and, most

importantly, to improve the quality of life. Closed-loop systems and algorithms can be found in home appliances, automobiles, aviation and more. As in other aspects of life, the principles of automation and control can be used in medical devices and in the management of Type 1 Diabetes Mellitus (T1DM). The idea of an algorithmic/technological way to control glycemia is not new and has been researched for more than four decades. However, recent improvement in both glucose sensing technology and insulin delivery, together with advances in control and systems engineering, has made this dream of an artificial pancreas become possible.

Different attempts are being made to design algorithms for the artificial pancreas; some are targeting overnight control, while others are designed to prevent nocturnal hypoglycemia or overcome meal challenges and exercise based on bi-hormonal or uni-hormonal design. These are for the most part designed as a single algorithm that is evaluated based on the suggested protocol.

When we envision an artificial pancreas we need to remember that this is not merely an insulin pump, a glucose sensor and a mathematical formulation that, when connected, can magically control glycemia. The artificial pancreas is an autonomous system that will be tasked with glucose regulation; as such a system perspective with multiple layers is required to achieve a safe and effective design.

O-18

GLUCOSE VARIABILITY

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The importance of glucose variability has been intensively debated over the last years. Sparkled by a now withdrawn post-hoc analysis of the DCCT and a reported relation with oxidative stress, investigators have sought associations between glucose variability and various harmful conditions. There is a plethora of measures to assess glucose variability. Different measures for glucose variability give highly inter-correlated results, illustrating the absence of a 'gold' standard. To complicate things further there is a high correlation between mean glucose and glucose variability, and glucose variability is also connected to hypoglycemia. From a mathematical point of view one may even ask whether glucose variability is perhaps as important or even more important than mean glucose, but from a clinical point of view the relationship between mean glucose and outcome is so well established that the relevant question is whether glucose variability has anything to add. Intervention studies in which glucose variability is lowered without affecting mean glucose are sparse. An overview of the status in the field and results of a new post-hoc analysis of a recently reported outcome study will be presented. This will also include a newly proposed variability measure, the Mean Aboslute Glucose change (MAG), which has the potential to become the 'gold' standard for the assessment of glucose variability.

O-19

PHYSICAL ACTIVITY IS A LIKELY DETERMINANT OF CGM RATE OF CHANGE

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The assessment of the relationship between physical activity (PA) and blood glucose (BG) dynamics is of great importance to understand the glucose regulatory system and may help future research on artificial pancreas.

The aim of this study was thus to assess if, in absence of disturbances e.g. meal, PA is a determinant of BG rate of change.

Twelve healthy subjects (6 males; age 31 ± 2 yrs; BMI $25 \pm 1.0\,kg/m^2$) were studied in the Clinical Research Unit at Mayo Rochester MN for a 88 hour period during which a planned program of PA was captured with PAMS (a sytem that has double accelerometers and inclinometers for highly accurate recording of body postures and movement) and BG captured with CGM Dexcom Seven® Plus. Glucose derivative was caluculated from CGM signal using a deconvolution-based method, in six intervals per subjects (sufficiently far from meals and where PMAS calibration did not occur). The relationship between the PA and CGM derivative was assessed using cross-correlation, which takes into account the presence of delay between the signals.

Correlation was higher than 0.5 ($P < 0.0001$) in 56%, and higher than 0.7 ($P < 0.0001$) in 20% of the analyzed intervals. Eight subjects present at least one interval with correlation higher than 0.7; in the remanining four, the maximum correlation was higher than 0.59.

Present results show that PAMS technology can accurately capture physical activity in a noninvasive way and that accelerometer measurement generally well correlate with glucose fluctuations due to physical activity.

O-20

CLOSED-LOOP ARTIFICIAL PANCREAS UTILIZING PRANDIAL INHALED INSULIN

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Type 1 diabetes mellitus (T1DM) is a metabolic disorder in which an individual experiences chronic hyperglycemia due to inability to produce sufficient insulin. One of the challenges of the artificial pancreas is lowering postprandial glucose peaks. However, this task has been extremely challenging without a pre-meal insulin bolus that could imitate cephalic and first-phase insulin delivery in anticipation of a meal, due to the slow absorption rate of subcutaneous insulin.

A novel hybrid artificial pancreas that utilize Technosphere Insulin (TI) as a way to deliver first phase insulin in conjunction with a control algorithm that delivers second phase and basal insulin via CSII pump was designed. This approach provides the ability to overcome meal challenges with minimal prandial hyperglycemia and postprandial hypoglycemia. We have evaluated this system using 100 *in silico* subjects from the UVa/Padova FDA-accepted metabolic simulator following a one meal protocol of 75 g under closed-loop control with and without 10 U of TI. The controller design was based on a model predictive control with fixed model and fixed tuning for all the simulations. The results show that the subjects treated with the hybrid artificial pancreas system spent 91% less time in the hyperglycemia region

(>180 mg/dL) compared to the control group (no TI) with 47% less hypoglycemia events compared to the control group.

These encouraging results suggest that a hybrid approach that utilizes TI as a mean to overcome sharp glucose elevations with rapid acting insulin managed by an artificial pancreas may provide tight glucose regulation.

O-21

THE CHALLENGES OF ACHIEVING GOOD GLYCAEMIC CONTROL IN PRETERM INFANTS

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There remains controversy regarding the definition of normoglycaemia in the preterm infant, although in utero glucose levels are tightly regulated between 4–6 mmol/L. Hyperglycaemia is a common problem in infants requiring intensive care, partly related to prematurity and partly due to effects of critical care. As in adults, hyperglycaemia has been linked to both mortality and morbidities, including intraventricular haemorhage, retinopathy of prematurity and chronic lung disease. Although there are many parallels between critically ill adults and the preterm infant, in the neonate, hyperglycaemia may also be a marker of relative insulin deficiency, due to immature pancreatic beta cell development. The NIRTURE Trial in preterm infants undertook to test the hypothesis that early insulin replacement would impact on anabolism, improve glucose control and improve clinical outcomes. Although there was a reduction in hyperglycaemia in the intervention arm, the differences in the levels of glucose control between the study arms was not as large as in a pilot study, and 36% of control infants received insulin treatment. In addition there was an increase in episodes of hypoglycaemia, a finding which has been common across intensive care studies. The CGMs data collected as part of NIRTURE highlight the wide variation in insulin sensitivity, and rapid fluctuations in glucose levels which are not identified with intermittent blood sampling, making clinical management of glucose control challenging. Methods for refining glucose control using real time CGMS, and closed loop regulation of insulin delivery may increase the possibility of reducing hyperglycaemia without increasing the risk of hypoglycaemia.

O-22

RATIONALE DRUG DESIGN TO BLOCK BETA CELL AUTOIMMUNITY

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It is now possible to predict the development of Type 1A diabetes by determining a series of islet autoantibodies (GAD65, IA-2, insulin, and ZnT8). Individuals expressing ≥ 2 of these autoantibodies almost always progress to overt diabetes, though a few individuals take longer than a decade to progress. Levels of insulin autoantibodies correlate with rate of progression to diabetes and progressive metabolic abnormalities almost always precede onset. There is now a considerable body of evidence, particularly in the NOD mouse model that insulin is the primary target autoantigen driving beta cell destruction though multiple antigens are targeted by T lymphocytes. In addition, the NOD mouse trimolecular complexes (consisting of a class II MHC molecule presenting insulin peptide B:9–23 to specific T cell receptors) appear critical for developing disease. With this structural knowledge, we are developing small molecules able to bind specific pockets along the peptide binding groove of the class II molecule and block T cell receptor recognition or enhance production of protective cytokines. We believe targeting essential recognition structures for islet autoimmunity will provide a rationale pathway to develop preventive therapeutics.

O-23

CONTINUOUS MONITORING OF PATIENTS WITH UNCONTROLLED TYPE I DIABETES DEMONSTRATED BLOOD GLUCOSE REDUCTION UPON PREPRANDIAL ADMINISTRATION OF ORMD-0801 INSULIN CAPSULES

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The unpredictable behavior of uncontrolled diabetes often involves frequent swings in blood glucose levels that impact maintenance of a daily routine. Treatment through intensified insulin regimens is often unsuccessful, while other therapeutic options, such as amylin analog injections, use of continuous glucose sensors, or islet or pancreas transplantation are of limited clinical use. In efforts to provide patients with a more compliable treatment method, Oramed Pharmaceuticals tested the capacity of its oral insulin capsule (ORMD-0801, 8 mg insulin) in addressing this resistant clinical state. Eight Type I diabetes patients with uncontrolled diabetes (HgA1c: 8–10%) were monitored throughout a 15-day study period by means of a blind continuous glucose monitoring device. Baseline patient blood glucose behavior was monitored and recorded over a five-day pretreatment screening period. During the ensuing ten-day treatment phase, patients were asked to conduct themselves as usual and to self-administer an oral insulin capsule three times daily, just prior to meal intake. Treatment with ORMD-0801 was associated with a 20% drop in mean glucose values during waking hours and a 15% drop in their nighttime levels. Moreover, the oral insulin capsules consistently led to lower frequencies of glucose recordings above 200 mg/dL, with the greatest impact (31–37% decrease from mean pretreatment frequencies, $P < 0.01$) observed from 9 AM–6 PM. Additionally, no adverse events were reported throughout the study. In conclusion, ORMD-0801 oral insulin capsules in conjunction with subcutaneous insulin injections, were safe, well tolerated and effectively reduced glycemia throughout the day.

O-24

DECISION SUPPORT FOR GLYCEMIC CONTROL IN THE INTENSIVE CARE UNIT

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Glycemic control for critically ill patients is still one of the most discussed topics in intensive care medicine[1,2,3]. Both the American Diabetes Association as well as the American Association of Clinical Endocrinologists[4] state that uncontrolled high blood

glucose can lead to serious problems for hospitalized patients. Strategies have to be identified to help hospitals to establish safe and effective management of blood glucose in intensive care units and other hospital settings.

The B. Braun Space GC system offers decision support which facilitates glucose management in the intensive care unit. It offers two options for glucose management, intensive insulin treatment in a range of 80–110 mg/dL as well as moderate glucose control within a range of 80–150 mg/dL. Using Space GC no intuitive decision making is required. Both enteral and parenteral feeding are automatically considered for calculation of the insulin dose. Space GC predicts the required glucose sampling interval and calculates and transmits the required insulin rate directly to the infusion pump.

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O-25

TIGHT GLYCAEMIC CONTROL AND COMPUTERIZED DECISION SUPPORT SYSTEMS—FUTURE AND CHALLENGES

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An interesting IT intervention in clinical medicine is the use of computerized decision support systems (CDSS) that provide meaningful feedback to professionals in order to influence their behaviour. Glycaemic control forms an important application domain for CDSSs. However, issues pertaining to their design, implementation, critical success factors, as well as their evaluation are largely still open. This study describes six important issues for designing and implementing CDSSs for glycaemic control with several practical examples based on our literature reviews and lessons learned during our trials.

Applicability: The first step in CDSS development is selecting an area where CDSS could be effective and implemented.

Integration: To reach an optimal effect, the CDSS should be integrated into clinical workflows. The question is however what is the best place and time for presenting the information to the user.

Technical aspects: Integration with other systems and connections to the databases should be defined in the most secure way and should not reduce the performance of the host system.

Safety of safety approach: It is important to understand the risks faced by the system and generate dependability requirements to cope with these risks.

Evaluation issues: One should hence isolate the effect of the CDSS from the underlying guideline. One should also be aware that introducing the CDSS may imply a transition phase in which users go through a learning curve and/or some structural changes in the long term.

Quality indicators: Uniform and unambiguous quality indicators should be used for evaluating the effect of CDSS.

O-26

AUTOMATIC ASSESSMENT OF PHYSICAL ACTIVITY USING MULTI-AXIAL ACCELEROMETRY AND HEART RATE

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Objective: To develop a fully-automated system based on artificial intelligence schemes which detects periods of physical activity using multi-axial accelerometry and heart rate measurements. We propose an algorithm capable of distinguishing three states: rest, light activity and moderate-to-vigorous exercise.

Methods: Seven participants wore an ActiTrainer (ActiGraph, USA) accelerometer and a Polar WearLink (Polar, Finland) pulsimeter. Main -vertical- and dual axis accelerations, step count and heart rate were simultaneously registered every 10 seconds. Volunteers reported their physical activity (timing and exercise description) while wearing the devices.

The learning algorithm consisted of defining a set of time-domain features, dimensionality reduction by standard Principal Component Analysis (PCA) and unsupervised clustering, namely by k-means or Gaussian Mixture Models (GMM). Hidden Markov Model (HMM) filtering was subsequently applied to seize temporal redundancy in consecutive samples.

Results: In total, 149.35 hours were recorded: 70.57% rest, 17.69% light activity, 11.74% moderate-to-vigorous exercise.

A multi-parameter optimization was first performed to adjust the learning scheme. Cross-validation yielded 89.24% global accuracy using k-means (88.82% for GMM). Classification mismatches were brief and mostly occurred around transitions between intensity levels, being therefore of reduced overall impact.

Conclusions: The system achieves an accurate automatic physical activity monitoring. In similar models in the literature, heart rate data are not considered; although our results show that they provide valuable information when combined with accelerometry.

The proposed algorithm is suitable for the assessment of individuals' adherence to exercise programs and should also be considered as a first step in the estimation of daily energy expenditure.

O-27

THE SPRING PATCH PUMP SOLUTION

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The innovative technology already implemented in the traditional **ADI** system is currently being transformed into a "convertible" CSII.

The unique spring-based - **Intellispring**™- technology alongside with the **Total Line Control**, represents a new dimension in diabetes control.

Intellispring™ utilizes the spring energy, triggered by insulin loading, as the driving force for insulin delivery.

Differential pressure between the drug-reservoir and the patient-line, precisely determines the dose, irrespective of environmental circumstances.

Its proprietary pressure sensing and valve assembly enables reliable drug delivery. Unlike motor driven, this pump uniquely controls actual insulin output offering ultimate safety.

Among the inherent technology features, are smallest increments, immediate occlusion alert and the unique air bubble detection. The "Patch System" provides life-style flexibility by allowing a choice between tube-free skin-patch or the smallest traditional "Insulin Pump."

This IPX 8 water-tight device enables use with or without a remote management tool which also serves as BGM analyzer. The infusion set has an automatic introducer, featuring painless insertion, non-visible needle, a sharps-protector and the exceptional Detach Detect mechanism.

While featuring highest level of safety and user friendliness, the **Spring Patch system** is rather economic for use and environment friendly. The device is comprised of a multiple use, long-lasting control element that clasps on a single-use drug reservoir. No electronic elements, soldering material or batteries are being disposed of; the only waste consists of a tiny plastic cartridge. This energy-saving, motorless system may run for about thirty days on a standard AAA battery, tolerating single use or rechargeable batteries alike.

O-28

CLINICAL EVALUATION OF A SUBCONJUNCTIVAL GLUCOSE SENSOR

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Purpose: Eysesense company recently developed a method to monitor glucose concentrations by a subconjunctival implant (Diabetes 2008; 57, Suppl 1, A13). The implant contained a glucose binding lectin dispersed thoughout a hydrogel as well as competitive binding fluorophore fluorescing near infrared. The glucose dependent fluorescence is measured by a small handheld fluorophotometer. In a proof-of-principle study we investigated the correlation between glucose concentration measured by the Eyesense implant and by finger pricking as well as tolerability and safety of the implant.

Methods: Study was performed in 15 diabetic patients. The implant was inserted subconjunctival into the right eye in local anaesthesia. Correlation between capillary glucose measured by laboratory method and interstitial glucose measured by the implant was investigated by inducing an increase and decrease of glucose values in a range between 60–300 mg/dL.

Results: After implantation most patients showed postoperatively a little subconjunctival hemorrhage which disappeared within few days. Except for a minor foreign body sensation lasting about one week. The implants were well tolerated during the observation period of 4 weeks. The simultaneously measured glucose concentrations ($n = 2100$) showed a close correlation ($r = 0.93$; $P < 0.01$). The MARE of the glucose value measured by the implant compared with capillary glucose was in average 10.1%. The ISO standard 15197 was reached in 87.6% of all measurement sessions. Time lag between blood and interstitial glucose was usually 5–15 min.

Conclusion: The study shows a good tolerability of the implant with a precise measurement performance.

O-29

BIOSIMILAR INSULIN'S: HOW SIMILAR IS SIMILAR?

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Biosimilar insulin's (BI) are looked at as commercially attractive products by a number of companies. In order to obtain approval in the EU or the USA—not a single BI is on the market in these countries until now—a manufacturer needs to demonstrate that a given BI has a safety and efficacy profile that is similar to that of the 'original' insulin formulation that is already on the market. As simple as this may appear at first glance, for a number of good reasons this is not trivial at all. As during protein manufacturing modifications in the structure of the insulin molecule can take place (which can have serious consequences for the biological effects induced), a rigid and careful assessment of the safety and efficacy of BI's is absolutely necessary. The example of Marvel's failed application of their BI's with EMA provides interesting insights into the regulatory and clinical challenges surrounding the regulatory approval of BI. Although a challenging BI approval process might be regarded as a hurdle to keep companies out of certain markets, it is fair to say that the potential safety and efficacy issues surrounding BI are substantial and relevant, and do warrant a careful and evidence driven approval process.

O-30

WHAT HYPOGLYCEMIC LEVEL IS CLINICALLY RELEVANT—THE EUROPEAN VIEW

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In 2005, an ADA working party proposed that clinically relevant hypoglycaemia should be set at 70 mg/dL (3.9 mmol/L), provoking some critical comment. The main criticism was that this level is too high and would lead to reporting of clinically 'irrelevant' episodes.

A review of both the arguments and the evidence suggests that the cause of this Atlantic divide, is in part due to a failure to take into account the widespread use of SI units to report blood glucose. However the important issue to emerge from this debate is that attempts to find a single overriding glucose concentration used to define hypoglycaemia are doomed to failure.

For example, a self monitored glucose concentration which prompts patients to consider appropriate changes in their insulin dose probably needs a glucose range, perhaps 4 to 4.5 mmol/L for those using SI units and 70–80 where mg/L is in use.

A different definition could be used in trials where effects on hypoglycaemia are being measured. If round figures are needed, then 70 mg/dL is suitable but where SI units are used, a level of 3.5 mmol/L might be more applicable.

The situation is even more uncertain where CGM is being evaluated or used to monitor glucose levels, since CGM

measures interstitial values and the relationship with plasma glucose is not fixed. Given these limitations defining interstitial glucose concentrations for use in trials or the clinical situation is at present premature and awaits the results of further well-designed studies.

O-31

NORMATIVE RANGES FOR MEAN TISSUE GLUCOSE AND GLYCEMIC VARIABILITY DERIVED FROM CONTINUOUS GLUCOSE MONITORING FOR NON-DIABETIC SUBJECTS IN DIFFERENT ETHNIC GROUPS

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Background: Glycemic variability has been proposed as a contributing factor in diabetes complications. Measures exist to calculate the magnitude of glycemic variability but ranges for non-diabetic subjects have not been described. We present normative ranges for ten measures of glycemic variability in different ethnic groups.

Methods: 80 subjects, fasting plasma glucoses of less than 120 mg/dL, underwent 72-hours of continuous glucose monitoring (CGM) with a Medtronic CGMS Gold device. Glycemic variability was calculated using EasyGV software, a custom program which calculates M-value, mean amplitude of glycemic variability (MAGE), average daily risk ratio (ADRR), Lability index (LI), J-Index, low blood glucose index (LBGI), high blood glucose index (HBGI), continuous overlapping net glycemic action (CONGA), mean of daily differences (MODD), glycemic risk assessment in diabetes equation (GRADE) and mean absolute glucose (MAG).

Results: Eight CGM traces were excluded due to inadequate data. The remaining 72 traces are used to define normative ranges for glycemic variability in different ethnicities, Table 1. Mean tissue glucose ($P = 0.04$) and measures of glycemic variability were significantly higher in Asian subjects (LGBI:$P = 0.049$, CONGA:$P = 0.009$) than other ethnicities.

Conclusions: We present normative ranges for ten measures of glycemic variability in non-diabetic subjects for use in clinical care and academic research. We also show glycemic variability is significantly different between ethnicities.

O-32

SIMPLE STRATEGIES FOR DIFFICULT SITUATIONS USING IV INSULIN IN THE HOSPITAL

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Numerous intravenous insulin infusions have been published with generally good control of blood glucose in ICU patients.

A less studied topic is the best strategies for IV insulin during steroid therapy when the patient is allowed to eat or during periods of large quantities of calorie intake, such as total parenteral nutrition (TPN) or tube feeding.

The situation with the least amount of discussion in the literature is the patient receiving steroids who is eating. At the University of Washington in Seattle, we traditionally do not use IV insulin for patients eating. However, we find using subcutaneous insulin in this population less than ideal due to different levels of insulin resistance overnight compared to the postprandial period. IV insulin alone also is a poor choice in patients who are eating We have found this situation is best served by using our IV insulin protocol with SC rapid-acting analogue for the meal. Further research on this would be welcomed.

For TPN or enteral feedings, several approaches have been suggested in the literature. For the former, we prefer to use an IV insulin infusion to estimate insulin requirements and then use that approximate dose of insulin placed into the TPN bag. For 24-hour enteral feedings in a critically ill patient, use of an IV insulin infusion seems easiest. For patients not requiring an ICU bed and especially for long-term use, there is debate about which subcutaneous regimen will work best.

O-33

THE ARTIFICIAL PANCREAS—MORE TIME IN ZONE FOR A BETTER TODAY AND AN IMPROVED TOMORROW

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There is a clear relationship between fasting plasma glucose (FPG), post-meal glucose (PMG) and HbA1c. Glycaemic variability and chronic hyperglycaemia influence oxidative stress,

Table 1. Normative Ranges for the Non-Diabetic Populations (Low – High) Defined as Mean ± 2×SD or* = Exponentiated log (Mean) ± 2×SD

Ethnicity	*SD*	*CONGA*	*LI**	*JINDEX*	*LGBI*	*HGBI**	*GRADE**	*MODD*	*MAGECGM*	*ADRR**	*MVALUE*	*MAG*
Asian (n = 9)	0.0–3.2	3.9–6.1	0.0–2.0	10.1–22.3	0.0–4.0	0.0–2.7	0.0–2.2	0.2–1.2	0.6–4.0	0.1–6.8	0.0–6.7	0.1–3.5
African American (n = 8)	0.0–4.0	3.9–5.4	0.0–6.5	5.7–27.6	0.0–9.5	0.0–13.0	0.1–2.3	0.0–2.0	0.7–6.0	0.0–22.7	0.0–15.5	0.0–5.4
Caucasian (n = 44)	0.1–2.8	3.5–5.4	0.1–1.5	3.8–23.6	0.0–7.3	0.0–4.1	0.1–1.7	0.3–1.4	0.8–3.6	0.0–23.4	0.0–13.8	1.2–3.4
Hispanic (n = 13)	0.0–2.7	3.8–5.4	0.1–0.9	5.5–21.8	0.2–4.8	0.0–1.1	0.1–0.9	0.2–1.1	0.5–3.5	0.0–4.7	0.0–7.4	0.7–3.0
All (n = 72)	0.0–2.9	3.6–5.6	0.1–1.8	4.9–23.6	0.0–6.9	0.0–3.6	0.1–1.7	0.2–1.4	0.7–3.8	0.0–21.7	0.0–12.4	0.8–3.6

which is driven by FPG and PMG. The standard parameters of glycaemia (HbA1c, FPG, PPG) insufficiently reflect glycaemic variability and the levels of oxidative stress which can contribute to the development of diabetic complications. Management of oxidative stress is emerging as a key outcome in the management of diabetes. The goal of future technologies is to achieve glycaemic control, ensure safety and guide therapy.

Subjective awareness of the onset of hypoglycaemia is a critical defence against severe hypoglycaemia. When an individual loses their ability to recognise symptoms, real problems with hypoglycaemia can occur. New research reveals the brain's responses to hypoglycaemia. When an individual experiences a hypoglycaemic event with symptoms, the brain remembers it as unpleasant and forms a memory that encourages them to prevent it happening again. With hypoglycaemia unawareness, adrenalin responses start to happen later and awareness is delayed. As plasma glucose falls, there is greater cognitive impairment and an inability to recognize the symptoms, hence blood glucose falls further.

Closed-loop control is superior to open-loop control in achieving greater time in target range, with less hyperglycaemia and hypoglycaemia. Artificial pancreas technology may therefore have a significant and positive impact on hypoglycaemia, hyperglycemia and glucose variability.

O-34

OVERNIGHT CLOSED-LOOP INSULIN DELIVERY

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Research over the past decade shows that blood glucose control is the most important predictor of diabetes complications. Achieving good blood glucose control dramatically lowers the risk of serious complications, by as much as 75% for some problems. Yet recent studies reveals that even the best-controlled patients spend less than 50 percent of their day within the normal blood sugar range—especially overnight, when patients are most vulnerable to hypoglycemia.

Our group performed three randomised crossover studies evaluating overnight closed-loop in young subjects with type 1 diabetes. During 33 closed-loop nights, sensor glucose values were fed into an MPC, which calculated the insulin infusion rate and the insulin pump was adjusted manually by a research nurse every 15 min. During 22 control nights, subject's standard insulin pump settings were applied. An analysis of pooled data documented increased time in the target range between 3.9 to 8.0 mmol/L (60% vs. 40%) and reduced time that glucose levels were below 3.9 mmol/L (2.1% vs. 4.1%). Closed-loop reduced frequency of plasma glucose below 3.3 mmol/L from 7.5% to 0.7%. No events with plasma glucose concentration lower than 3.0 mmol/L were recorded during closed-loop delivery, compared with nine events during standard treatment. Studies in adults and pregnancies are also promising.

O-35

INSULIN DEGLUDEC: MULTI-HEXAMER FORMATION IS THE UNDERLYING BASIS FOR THIS NEW GENERATION ULTRA-LONG BASAL INSULIN

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Insulin degludec is a new generation ultra-long acting basal insulin in clinical development. Insulin degludec is des B30 human insulin with a 16-carbon fatty diacid attached to LysB29 via a glutamic acid spacer. The aim of this study was to demonstrate that under *in vitro* conditions mimicking the physiological injection site, insulin degludec self-associates to form large soluble multi-hexamers and that this ultimately results in an ultra-long and peak-less pharmacokinetic (PK) profile in people with type 1 diabetes.

Size exclusion chromatography (SEC) experiments were performed to characterise the molecular size of insulin degludec. Various formulations with and without phenol were examined to simulate conditions before and following sub-cutaneous injection. To examine the PK profile of insulin degludec, a clinical pharmacology study was conducted in subjects ($n = 12$) with type 1 diabetes. The steady state PK profile (24 hour) was determined after 6 consecutive days of once daily dosing with insulin degludec (5.0 nmol/kg).

SEC (phenol containing buffer) demonstrated that insulin degludec forms di-hexamers. To mimic a subcutaneous injection, SEC was conducted in the absence of phenol and it was revealed that there is a reorganisation from di-hexamers to multi-hexamers. When insulin degludec was administered to subjects with type 1 diabetes, the steady-state PK profile demonstrated a smooth and stable exposure over 24 hours. Insulin degludec was found to have a $t_{½}$ longer than 24 hours.

In summary, insulin degludec is a new generation soluble basal insulin with an ultra-long, peak-less PK profile attributed to multi-hexamer formation and slow release of monomers.

O-36

FREQUENT MONITORING OF A1C DURING PREGNANCY AS A TREATMENT TOOL TO GUIDE THERAPY

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Background: A1C, used to follow diabetes treatment effectiveness in non-pregnant patients, is routinely measured every 3 months. There are no guidelines for measurement of A1C during pregnancy and it is not recommended routinely for women with gestational diabetes mellitus. The aim of this study was to document the rate of fall during the first month of treatment in women with GDM.

Methods: All women with GDM are referred to the Santa Barbara County Clinic prenatal/endocrine clinic for management. Treatment for GDM consists of a carbohydrate restricted diet, self monitoring blood glucose pre- and one hour postprandial and insulin initiated when the diet does not achieve normoglycemia Blood was collected from a fingerstick and analyzed for A1C on the DCA2000® + analyzer.

Results: 24 Latina women with GDM whose initial A1C was $\geq$7.0% were followed during the first month of treatment. At enrollment, the age (mean $\pm$ 1SD) was 29.0 $\pm$ 7.3 years and the A1C% was 8.8 $\pm$ 1.8. A1C was measured weekly and the mean follow up was 3.2 weeks (range 1.0 to 4.0 weeks). The average A1C decrease during this time was 0.47% per week (range 0.10–1.15%). The maximum A1C drop was 4.3% in 4 weeks.

Discussion: This study documents the utility of frequent monitoring of A1C to guide therapy in GDM. The rate of fall of A1C can be used to assess the glycemia and document the success of the dietary therapy. Lack of fall of A1C is an indication for insulin therapy.

O-37

DIFFERENCES IN MEASURES OF GLYCEMIC VARIABILITY BETWEEN THE MULTIPLE DAILY INJECTION THERAPY AND SENSOR-AUGMENTED PUMP THERAPY GROUPS IN THE STAR 3 STUDY

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Objective: The STAR 3 study showed that sensor-augmented pump (SAP) therapy resulted in a significant reduction in A1C compared to multiple daily injections (MDI). In addition to improving A1C, SAP may reduce glycemic variability (GV) compared to MDI.

Methods: At entry on MDI, A1C was 7.4–9.5%. Subjects were seen quarterly to review glucose data, measure A1C and review fingerstick glucose (MDI) or sensor glucose and pump (SAP) data via the Medtronic CareLink Therapy Management System. At 12 months, the MDI group wore blinded CGM and data were used to compare GV with the data derived from CGM in the SAP group. The coefficient of variation of sensor glucose values (CV, equal to the standard deviation divided by the mean value) was used to assess GV. CV was compared at 4 different A1C levels to contrast the MDI and SAP treatment arms.

Results: The graph shows the relationship between CV and A1C values at 1 year for SAP and MDI treatment arms, along with number of subjects in each cohort. Throughout different A1C levels, there was significantly lower CV for SAP compared to MDI ($P < 0.01$). The lowest CV was found among SAP subjects with low A1C values.

Conclusions: Since a goal of T1D therapy is to reduce GV, SAP should be recommended to improve A1C and reduce glycemic excursions.

O-38

SELF-MANAGEMENT SUPPORT INTERVENTIONS THAT ARE CLINICALLY-LINKED AND TECHNOLOGY-ENABLED: CAN THEY SUCCESSFULLY PREVENT AND TREAT DIABETES?

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Patients with diabetes need a complex set of services and supports. The challenge integrating these services into the diabetes regimen can be successfully overcome through self-management support interventions that are clinically-linked and technology-enabled.

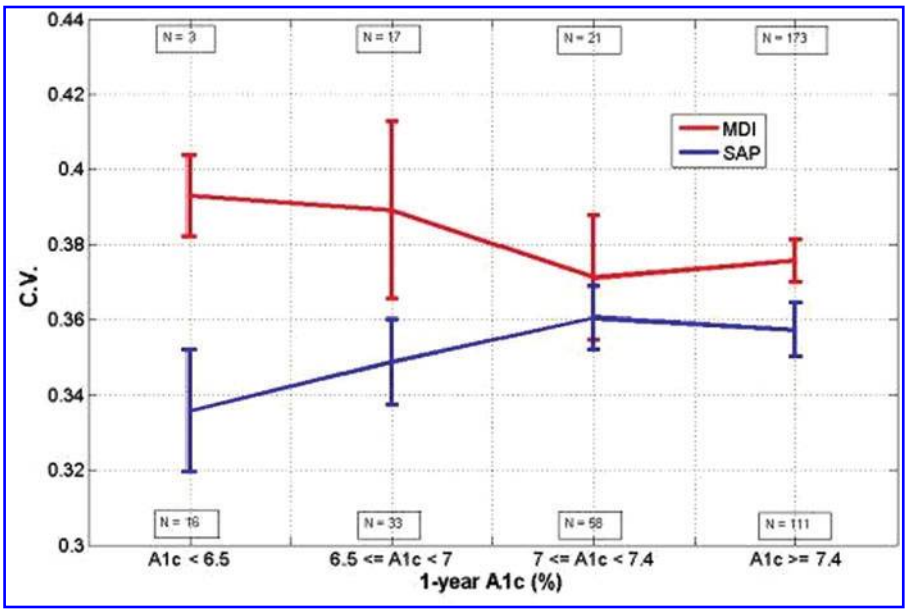
Self-management support because patients need help mastering the knowledge, attitudes, skills and behaviors so necessary for good outcomes.

Interventions because comprehensive theory-based, evidence-proven, long-term, longitudinal interventions work better than direct to consumer or non-planned health promotion approaches.

Clinically-linked because patients are more likely to adopt new behaviors when the approach is in the context of a trusted therapeutic relationship and within an effective medical care system.

And, **technology-enabled** because capitalizing on the amazing power of information technology leads to the delivery of cost-effective, scalable, engaging solutions that prevent and manage diabetes.

This presentation will provide an overview of the state-of-the-evidence for the effectiveness of Self-management support interventions that are clinically-linked and technology-enable.



GV at different A1C levels, SAP v. MDI.

O-39

SEQUENTIAL STEPS TOWARDS REGULATORY APPROVAL AND CLINICAL ACCEPTANCE OF CLOSED-LOOP CONTROL: SAFETY FIRST

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Objective: Propose systems approach, system design principles, and sequential steps for development and deployment at home of automated closed-loop control.

Methods: The engineering basis for sequential system development, approval, and acceptance is set by a modular architecture, which outlines several subsystems (modules) that can be developed and tested independently, and then sequentially integrated into a closed-loop control device.

Results:

Step 1: A safety system alone is deployed first, which can work with any mode of insulin delivery, i.e. physician-prescribed open-loop control or a control algorithm. The safety performs four functions:

(i) attenuate or discontinue insulin delivery if hypoglycemia is anticipated;
(ii) intercept potentially hazardous insulin boluses,
(iii) warn about impending hypoglycemia, and
(iv) detect and attenuate CGM errors.

Step 2: Safety is deployed together with advisory system suggesting optimal insulin dosing to the patient. Works as adjunct to standard physician-prescribed open-loop control;

Step 3: Control-to-Range - safety is deployed together with an algorithm suggesting postprandial insulin corrections. Works as adjunct to standard physician-prescribed open-loop pre-meal boluses and basal rate;

Step 4: Fully-automated closed-loop control.

All principal elements of the system have been tested in hospital-based clinical trials. Field deployment of Step 1 is anticipated.

Conclusions: A system comprised of a continuous glucose monitor (CGM) and an insulin pump can be better than the sum of its components, making the pump safer and the CGM more accurate. Sequential testing and deployment of safety, advisory, and closed-loop control features is a regulatory-acceptable route to closed-loop control at home.

O-40

SOLO MICROPUMP: THE RISE OF A NEW STAR?

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Although insulin pump therapy represents the most sophisticated form of intensified insulin treatment its application in clinical routine is restricted so far. One major reason is the limited acceptance of the technical tools and the required investment in time. Therefore, beside the advancements of technical and therapeutical options an essential goal for the development of new insulin infusion systems is to reduce limitations and incommodiousness with durable pumps.

Consequently, during the development of the SOLO insulin pump system the reliability and user friendliness as well as its acceptance has been tested comprehensively in validation and verification as well usability studies. The results of this research enabled the system's optimization and at the same time addressed the needs and requirements of users and health care professionals.

Further clinical trials planned in conjunction with the launch of this insulin pump system will prove the benefits of this treatment option for patients and evaluate the impact on metabolic control.

O-41

MILD PHYSICAL ACTIVITY PERFORMED POST MEAL LOWERS POST PRANDIAL GLUCOSE

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Objective: The effect of Physical activity (PA) on glucose variability (GV) is of great importance especially for its incorporation into Artificial endocrine pancreas (AEP) algorithms. We measured physical activity using a physical activity monitoring system (PAMS) and GVusing DexCom Seven® Plus continuous glucose monitoring system (CGM). Our aim was to quantify the effect of mild post meal physical activity on glycemic variability.

Methods: Twelve healthy subjects (6 males; age 31 ± 2 yrs; BMI $25 \pm 1.0\,kg/m^2$, fasting glucose 4.8 ± 0.1 mM; HbA1c $5.3 \pm 0.1\%$) were studied. During the study period of 4 nights and 3 days, there were three labeled and six unlabeled meals. All ingested meals had similar macronutrient composition that did not differ between meals or between days. Average physical activity following labeled meals amounted to ~1.0 METS and 1.7 METS following the unlabeled meals during the 4.5 hr postprandial period.

Results: Blood glucose was analyzed from 0.5 hours prior to meal ingestion up to 4.5 hours after meal consumption. The post meal glucose increased by 4.7 ± 0.7 mmol during the labeled meals while the increase was only 1.6 ± 0.1 mmol over basal glucose following unlabeled meals ($P = 0.016$). Pre-prandial glucose concentrations did not differ between unlabeled (5.55 ± 0.14 mM) or labeled (5.55 ± 1.25 mM) meals. Corresponding total physical activity for the 5-hr periods were 14.3 ± 4.0 A.U. and 1.4 ± 0.5 A.U. respectively.

Conclusions: Our results indicate that GV following a meal is significantly improved when low grade physical activity is undertaken compared to no physical activity. These results may have significant implications for insulin delivery in an AEP.

O-42

CLOSING THE LOOP OVERNIGHT IN ADULTS WITH TYPE 1 DIABETES FOLLOWING STANDARD MEAL AND LARGE MEAL WITH ALCOHOL

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Objective: To evaluate an overnight closed-loop (CL) system in adults with type 1 diabetes (T1D).

Methods: Two randomised, crossover studies compared CL with conventional continuous subcutaneous insulin infusion (CSII). In a feasibility study, a medium-sized evening meal containing 60 g carbohydrate (CHO) was consumed. In a follow-up study, subjects consumed a large evening meal (100 g CHO) accompanied by 0.75 g/kg ethanol as 13% white wine (mean intake 564 ± 133 ml). Twenty-four subjects (M/F 10/14; age 37.5 ± 9.1 years, T1D duration 20.6 ± 9.7 years, HbA1c $7.8 \pm 0.6\%$; mean $\pm$ SD) were studied. During CL nights (n = 24), every 15 minutes, subcutaneous continuous glucose monitoring values from the Freestyle Navigator CGM System were fed into a model predictive control algorithm, which calculated the infusion rate of Aspart to be adjusted manually for delivery via the Deltec Cozmo insulin pump. During control nights (n = 24), subjects' usual insulin pump settings were applied. Plasma glucose was measured every 15 minutes to assess CL performance.

Results: Following 60 g-CHO meal, overnight CL increased time in target plasma glucose 3.9–8.0 mmol/L (80% vs 51% CL vs CSII, $P = 0.002$), with similar outcomes following 100 g-CHO meal and alcohol (70% vs 47%, $P = 0.01$). Secondary analysis of pooled data documented increased time in target (76% vs 50%; CL vs CSII, $P < 0.001$), reduced time < 3.9 mmol/L (2.8% vs 6.7%, $P = 0.04$) and >8.0 mmol/L (18% vs 30%, $P = 0.006$), and reduced SD of glucose measuring glycaemic variability (1.4 vs 2.0 mmol/L, $P = 0.001$). There was no difference in average overnight insulin infusion (0.8 vs 0.8 U/h, $P = 0.83$).

Conclusion: Overnight CL may significantly improve glucose control and reduce risk of nocturnal hypoglycaemia in adults with T1D.

O-43

SICK DAY MANAGEMENT AND OPPORTUNITIES TO PREVENT DKA

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Self-monitoring of blood glucose (BG) is fundamental to diabetes treatment; there are new approaches to blood ketone monitoring to advance diabetes management. The critical ketone, ß-hydroxybutyrate (ß-OHB) can be tested easily and rapidly within 10 seconds at point-of-care by the patient at home or in the acute-care setting. ß-OHB is the ketone most closely linked to diabetic ketoacidosis (DKA). Traditional urine ketone dipsticks identify only acetoacetate, and occasionally acetone. Urine dipsticks provide only qualitative assessments of ketone levels based upon colorimetric change after 30–60 seconds; ß-OHB testing is quantitative.

Insulin-treated patients are at-risk for metabolic decompensation with illness, stress, or missed insulin. Without aggressive monitoring and treatment, DKA can ensue. There are >110,000 DKA episodes/year in the U.S. with >50% of hospitalizations for young patients with diabetes resulting from DKA. Most DKA cases occur in patients with established diabetes, providing opportunity for prevention through careful monitoring and early intervention.

Sick day rules include frequent BG and ketone monitoring with provision of supplemental insulin and fluids. Ketones should be checked during any intercurrent illness or when BG is consistently >250–300 mg/dL (13.9–16.7 mmol/L). ß-OHB levels rise and fall more rapidly than urine ketone levels and even reflect insulin deficiency and replacement more rapidly than BG levels, offering an opportunity to avert DKA. ß-OHB $\leq$ 0–0.5 mmol/L is normal; 0.6–1.5 mmol/L suggests need for extra insulin; $\geq$1.6 mmol/L indicates need for extra insulin and denotes risk for DKA. Patients should be encouraged to check ß-OHB levels and work with their healthcare team for sick day management support.

O-44

THE EFFECTIVENESS OF USE OF INTERNET-BASED BLOOD GLUCOSE MONITORING SYSTEM ON IMPROVING DIABETES CONTROL IN ADOLESCENTS WITH TYPE 1 DIABETES

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Objective: To determine whether the use of an internet-based blood glucose monitoring system could improve glycemic control in adolescents with type 1 diabetes. The primary end point was change from the baseline HbA1c level.

Methods: A total of 70 adolescent subjects with type 1 diabetes were recruited to this randomized, controlled clinical trial. Subjects randomized to the Intervention Group (n = 36) submitted their blood glucose levels weekly via the Medtronics internet website to the Diabetes Care Team. Subjects randomized to the Control Group (n = 34) did not submit results.

Results: Patients were 15.1 ± 2.6 years of age with mean HbA1c at baseline of $8.3 \pm 1.3\%$. Groups were similar in terms of demographic and metabolic parameters. At the 6-month follow-up period, by-group differences in change from baseline to end of treatment Hba1c were not detected. In the Intervention Group, 12/36 did not send blood glucose levels and were classified as non-compliant. In a secondary exploratory analysis in which non-compliant patients were omitted, HbA1c values in the Intervention Group declined from $8.5 \pm 1.7\%$ at baseline to $8.15 \pm 1.2\%$ at 6 months, while in the Control Group, HbA1c values increased from $8.18 \pm 1.1\%$ at baseline to $8.4 \pm 1.1\%$ 6-months, representing a relative improvement of 60% in the Intervention vs. the Control Group; nevertheless, this difference did not reach statistical significance.

Conclusion: An internet-based blood glucose monitoring system was not associated with improved glycemic control in adolescents with type 1 diabetes. Identification of a sub-group of compliant subjects who may improve metabolic control by using this tool is needed.

O-45

TYPE 2 DIABETES: PAST AND PRESENT

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The worldwide pandemic in obesity is associated with an increased rate of type 2 diabetes including young adults. As a re-

sult, there has also been a rapid rise in the number of pregnancies complicated by type 2 diabetes. The increase in type 2 diabetes requires responses to several questions: Have we achieved measurable success in the past few decades in the management of type 2 diabetes in pregnancy? Is the rate of complications in type 2 diabetes parallel to those found in type 1? Which evaluative criteria of glycemic control should be instituted? What are the targeted glucose thresholds for type 2 diabetes that will optimize pregnancy outcome? Which management protocol should be used during pregnancy?

Studies have shown risk adverse outcome, including congenital malformation and perinatal mortality, is the same or increased in type 2 diabetes compared with type 1. Although there has been improvement in glycemic control compared to that in type 1 diabetes, the rates of perinatal morbidity, including preterm birth and macrosomia, appear to be similar. Risk factors associated with poor pregnancy outcome in type 2 diabetes include obesity and negligible pre-pregnancy counseling.

The presentation will address practical aspects of type 2 diabetes management before pre-conception counseling and treatment and during pregnancy using pharmacological therapies.

O-46

INTERVENTIONAL DIABETOLOGY: LONG TERM EXPERIENCE WITH THE TANTALUS® SYSTEM

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Background: The TANTALUS System increases satiety and modulates GI hormones in Type 2 Diabetes Mellitus and Obesity.

Methods: Fifty T2DM patients (26F) inadequately controlled by oral anti-diabetic medications (Baseline A1c $\geq$ 7.0%) were implanted in Europe and the US. Subjects were under a stable medication regimen and without any specific lifestyle or dietary instructions.

Results: Thirty-two subjects completed 2 years (Group A). Eighteen patients were either explanted (n = 12) or lost to follow up (n = 6), (Group B). Using the LOCF technique, significant reductions of 0.7% in A1c and 4 kg in weight (n = 50) were observed. Group A shows equal benefit in glycemic control and weight. Comparison between groups at 6 months, reveals a similar benefit in glycemic control but significantly less pronounced reduction in weight in Group B compared to Group A.

Conclusions: The results show stable reduction in weight and improved glycemia, supporting the clinical benefit of the TANTALUS System. Sustainable results, coupled with a low level of risk, positions TANTALUS as a valid option for Interventional Diabetology.

O-47

CONTINUOUS GLUCOSE MONITORING IN TYPE 1 DIABETES, A SYSTEMATIC REVIEW

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Aim: Assess efficacy of CGM compared to self measurement of blood glucose (SMBG).

Methods: Medline, CENTRAL, EMBASE and Cinahl databases were searched for randomised controlled trials comparing CGM with SMBG in patients with type 1 diabetes.

Preliminary results: 24 studies were included. Six studies reporting HbA1c decline at 6 months were pooled, mean difference in HbA1c decline in the CGM group compared to the SMBG group was −0.42% [95% CI − 0.59%, −0.26%]. Occurrence of severe hypoglycaemia, in trials with a follow-up $\geq$ 6 months (7 studies) was pooled, an overall odds ratio of 1.27 [95% CI 0.77, 2.11] was found when using CGM. Five studies reporting incidence of ketoacidosis were pooled, an odds ratio of 0.96 [95% CI 0.59, 1.56] was found. Studies reporting HbA1c level at 3 months were pooled (4 studies), a mean difference of −0.21% [95% CI − 0.81%, 0.40%] was found in favour of CGM. Analysis of studies reporting occurrence of minor hypoglycaemia (4 studies) showed a mean difference of −0.00 [-0.26, 0.25]. When CGM was only worn briefly to subsequently make treatment changes (5 studies), mean difference of HbA1c at 3 months was −0.00% [95% CI − 0.24%, 0.24%].

Conclusion: Continuous CGM use, compared to SMBG, was associated with HbA1c reduction at 6 months. Reduction in the risk of severe hypoglycaemia could not be demonstrated. When CGM was only used briefly, HbA1c levels at 3 months did not differ from those of controls.

O-48

RISK, REASON AND RESPONSIBILITY. SAFE DESIGN OF A HOME USE ARTIFICIAL PANCREAS SYSTEM

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		Baseline	*6 months*	*Change*	*2 Years*	*Change*
A1c (%)	All, n = 50	8.4 ± 0.1	7.4 ± 0.2*	1	7.7 ± 0.2*	0.7
	Group A, n = 32	8.2 ± 0.1	7.1 ± 0.2*	1.1	7.5 ± 0.2*	0.7
	Group B, n = 18	8.7 ± 0.2	7.9 ± 0.3*	0.8	N/A	N/A
Weight (Kg)	All	109 ± 3	105 ± 3*	4	105 ± 3*	4
	Group A	111 ± 4	105 ± 4*	6	105 ± 4*	6
	Group B	107 ± 5	104 ± 5*	3**	N/A	N/A

(*) $P < 0.05$ vs. baseline, (**) $P < 0.05$ vs. change in group A.

Introduction: The potential patient benefits of an artificial pancreas is well publicised in scientific literature. Clinical studies reported to date have been conducted in an environment in which the participating subjects are closely monitored by clinical staff. This surveillance offers the safety reassurance subjects deserve, but is artificial in respect of their comfort. In the absence of adequate evidence, safety concerns surround the prospect of unsupervised operation of prototype artificial pancreas system within the home environment.

Aim: This paper aims to describe the process followed, and results obtained from a hazard analysis conducted for a prototype 'home use' artificial pancreas system - 'Florence'.

Method: Few will argue that hazard analysis is an integral part of the medical device design cycle. This process begins with the identification of hazards relating to device use, followed by the quantification of risk. This often results in the need to identify appropriate mitigations to reduce the risk to acceptable levels, or As_Low_As_Reasonably_Practicable. If residual ALARP risks remain, risk/benefit analysis is required.

Results: Ninety-six system level hazards associated with unsupervised operation of 'Florence' have been identified. These range from high risk events such as incorrect calibration, to low risk events such as tripping on trailing cables. Mitigations have been defined which reduce risk level to acceptable/ALARP levels.

Conclusions: Hazard analysis has been conducted for unsupervised operation of 'Florence' within the home environment. Although inherent risk remains, with implementation of identified mitigations, we strongly consider that the potential patient benefit of 'Florence' shall far outweigh the risk.

O-49

REAL-TIME CONTINUOUS GLUCOSE MONITORING IN TODDLERS WITH DIABETES

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Background: Controlling diabetes in toddlers is a challenge, especially because of fear of recurrent hypoglycemic events. The aim of this study was to assess the impact of real-time continuous glucose monitoring (RT-CGM) on diabetes control and parents' satisfaction in this age group.

Subjects and methods: Seven children aged 3.9–6 years who used an insulin pump were enrolled. The children used a blinded glucose monitoring system (Ipro, Medtronic Minimed) for 6 days and then the RT-CGM (Guardian RT Medtronic MiniMed) for 18–21 days. Area under the curve (AUC > 180 and AUC < 70 mg%) were compared while using CGMS and RT-CGM. Quality of life (QOL) and degree of parental anxiety before and after using the sensor was assessed by questionnaires. HbA1C was measured before using the sensor and 3 and 6 months of follow-up visits.

Results: AUC < 70 mg% decreased significantly from 0.7 on Ipro to 0.07 ($P = 0.04$) while using RT-CGM. AUC > 180 mg% decreased from 44 (23–86) to 32 (6–58) but didn't reach statistical significance. HbA1C at 3 and 6 months did not differ significantly from baseline. Parents' degree of anxiety and QOL didn't change after using the sensor, but parents reported that they felt safer with the sensor and could finally leave their children with older siblings and family friends.

Conclusions: Real-time continuous glucose monitoring is an important tool in the treatment of toddlers with diabetes, especially in reducing hypoglycemias. We believe that constant use of RT-CGM will also enable us to improve metabolic control and parent's anxiety in the future.

O-50

SETTING THE STAGE FOR INTERVENTIONAL DIABETOLOGY

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Up until the late 1970s, cardiologists used to treat patients with drugs; and, when that failed, they sent them to cardiac surgeons for open heart procedure. That changed in 1977 when Grüntzig performed the first coronary angioplasty procedure and initiated a new medical discipline - Interventional Cardiology.

Placing a stent has now become a much more popular alternative than performing a coronary bypass procedure; valve replacement via catheterization will soon be available. Morbidity and mortality are significantly reduced, and patients enjoy a better quality of life.

Are we are facing a similar situation with Type 2 Diabetes Mellitus management?

The pioneers are already here. TANTALUS, commercially available in Europe, provides non-pacing electrical stimulation during food intake, enhancing the body's natural response. Data shows it reduces HbA1c by 1% while also reducing weight by 4–5 Kg, improving liver enzyme levels, lipids and blood pressure. These effects, once obtained, seem to be sustained without the side effects associated with diabetes medications.

Other products include: the Balance System (stimulating the duodenum), and the Endobarrier, a passive device, limiting food absorption in the upper part of the small intestine.

These emerging products are pioneering the field of interventional Diabetology and making it a reality. They could provide a real alternative for patients unable to control their blood sugar by medications, and unwilling or not indicated for bariatric surgery. These fascinating alternatives may significantly reduce the compliance challenges, and alleviate safety concerns associated with any pharmaceutical treatment.

O-51

CONTINUOUS POSTOPERATIVE BLOOD GLUCOSE MONITORING AND CONTROL BY ARTIFICIAL PANCREAS IN PATIENTS HAVING HEPATIC OR PANCREATIC RESECTION

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Background and aims: Optimal blood glucose range to reduce postoperative infection (POI) remains unclear. The aim of this study was to evaluate a closed-loop system providing

continuous monitoring and strict control of perioperative blood glucose following hepatic and pancreatic resection.

Patients and methods: We performed two prospective randomized clinical studies in patients undergoing hepatic (n = 88) or pancreatic (n = 30) resection. Perioperative blood glucose levels were monitoring using an artificial endocrine pancreas (STG-22™, Nikkiso, Tokyo, Japan). Glucose levels were controlled using either the sliding scale method (SS group) or the artificial pancreas (AP group). Targeted blood glucose zone in SS or AP group was 150–200 mg/dL or 80–110 mg/dL, respectively.

Results: There were not statistically significant differences of preoperative parameters between SS and AP groups. Neither group in two studies showed hypoglycemia. In these studies, surgical site infection (SSI) in AP group was significantly lower than that in SS group. In study of hepatic resection, postoperative hospital stay in AP group was significantly shorter than that in SS group.

Another prospective randomized clinical study is now on going in patients undergoing hepatic or pancreatic resection. Glucose levels are controlled using STG-22™. Targeted blood glucose zone in intensive insulin therapy (IIT) group or control group is 80–110 mg/dL or 140–160 mg/dL, respectively. At present, 42 hepatectomized patients and 20 pancreatectomized patients are enrolled.

Conclusions: IIT using STG-22™ was safe and effective method to reduce SSI without hypoglycemia. Optimal blood glucose range will come out.

O-52

CLOSED-LOOP INSULIN DELIVERY DURING PREGNANCY COMPLICATED BY TYPE 1 DIABETES

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Objective: To evaluate closed-loop insulin delivery with model predictive control algorithm during early (12–16 weeks) and late gestation (28–32 weeks) in pregnant women with type 1 diabetes.

Research design and methods: 10 women with type 1 diabetes (age 31 years, diabetes duration 19 years, BMI 24.1 kg/m^2, Booking HbA1c 6.9%) were studied over 24 hours during early (14.8 weeks) and late pregnancy (28.0 weeks). A nurse adjusted the basal insulin infusion rate from continuous glucose measurements (CGM), fed into a model predictive control (MPC) algorithm every 15 minutes. Mean glucose and time spent in target (63–140 mg/dL), hyperglycaemic (>140 mg/dL, ≥ 180 mg/dL) and hypoglycaemic (<63 mg/dL, ≤ 50 mg/dL) were calculated using plasma and sensor glucose measurements. Linear mixed effects models were used to compare glucose control during early and late gestation.

Results: During closed loop insulin delivery plasma glucose levels were 117 mg/dL (100.8–154.8) in early and 126 mg/dL (109.8–140.4) in late gestation, [median (interquartile range); $P = 0.72$]. The overnight plasma glucose time in target was 84% (50–100) in early and 100% (94–100) in late pregnancy ($P = 0.09$). Overnight time spent hyperglycaemic (>140 mg/dL) was 7% (0–40) in early and 0% (0–6) in late pregnancy ($P = 0.25$) and hypoglycaemic (<63 mg/dL) was 0% (0–3) and 0% (0–0) respectively ($P = 0.18$). Postprandial glucose control, glucose variability, insulin infusion rates and CGM sensor accuracy were no different in early or late pregnancy.

Conclusions: MPC algorithm performance was maintained throughout pregnancy suggesting that overnight closed loop insulin delivery could be used safely during pregnancy. More work is needed to achieve optimal post prandial glucose control.

O-53

MICRODIALYSIS AS A HIGHLY PRECISE OPTION FOR CONTINUOUS GLUCOSE MONITORING

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For over 30 years microdialysis has been used extensively in research for monitoring changes in tissue chemistry. A key attribute is its ability to continuously sample without unduly perturbing the tissue under investigation. The development of online sensor systems for use in combination with intravascular microdialysis enables continuous, automated and real-time monitoring without blood withdrawal.

Many clinical studies report the incidence of dysglycaemia but without clear evidence that such events can be reliably identified when typically blood levels are measured only once every 1–4 hours. Attention has focussed on the inaccuracy of existing technologies to measure blood glucose. Discrepancies between results have been attributed to the use of different sources of blood (i.e. capillary, arterial, venous) and the different analysers used. However, it is importance to establish that the frequency of measurement is appropriate in defining the true picture of the patients' condition, particularly when measurements are used to change therapy, for example, insulin administration rates. Researchers conducting euglycaemic clamp studies on healthy individuals typically use more frequent sampling (1 to 20 minute frequency).

A continuous blood glucose monitoring system using the MicroEye® intravenous microdialysis device has been developed and initial studies demonstrated good agreement with reference blood glucose concentrations. This presentation describes the key attributes, methods and techniques that enable intravascular microdialysis to be used as a highly precise option for continuous glucose monitoring with the potential to become a valuable tool for clinicians seeking to avert dysglycaemia in critically ill patients.

O-54

TECHNOLOGY FOR OVERCOMING THE BARRIERS FOR PEOPLE WITH TYPE 2 DIABETES TO INTENSIVE INSULIN THERAPY

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The large scale UK population study of patients with type 2 diabetes (UKPDS) included a conclusion that in order to have optimal glucose values, 15% should have insulin within the first year of diagnosis, and 50% with the first 5 years. Intensive insulin treatment in type 2 diabetic patients (Kumamoto study) shows a considerable reduction in late complications with an improvement in HbA1c. Multiple daily injections are a treatment

modality is indispensible in treatment of Type 2 diabetes in its advanced stages. As demonstrated in the Peyrot and colleagues study in Diabetes Care of February 2010, the obstacles to MDI are injection pain, interference with daily activities and embarrassment of the injections in a public venue. Traditional insulin pumps overcome this to some extend, but pumps are complicated to use and the up-front cost as well as the tubing and maintenance expenditures are high.

Bruce Bode, MD writes in his review of June 2010: "to overcome these barriers, CSII regimens and technologies for people with T2DM that effectively reduce glucose levels, must s be simple and unobtrusive, and be cost-effective' (Diabetes Technology & Therapeutics). Further studies have shown that a simple CSII insulin regimen with fixed basal rates can contribute to reaching glycemic control in patients with T2DM. The simple-to-use PaQ system with no tubes is easily applied and sits for three days. It eliminates the obstacles that often prevent people with T2DM from reaching their glycemic goals.

O-55

THE EVIDENCE BASE FOR CGM: MAKING SENSE OF GLUCOSE SENSORS

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Though CGM has been available in clinical practice for a decade or so, it is only recently that randomised controlled trials (RCTs) have compared glycaemic control vs. self monitoring of blood glucose (SMBG). In long-term RCTs of CGM vs. SMBG, mean HbA1c is reduced by a variable amount on CGM (from no significant change to about 0.6%), depending on subject age and sensor usage. In most RCTs published to date, mean hypoglycaemia frequency seems to be unchanged by CGM, though none used patients with a high initial hypoglycaemia rate.

We hypothesised that the efficacy of CGM (and therefore the cost effectiveness) is greatest in those with the worst baseline glycaemic control, and in those who used the sensor most often. To test this and quantify the efficacy of CGM, we performed an individual patient data meta-analysis of all RCTs of long-term real-time CMG vs. SMBG where insulin treatment was the same in both arms. Using data from 892 patients (449 randomized to CGM and 443 to SMBG) we developed models that related final HbA1c to baseline HbA1c, sensor usage (days per week) and age. We also investigated change in area under the curve (AUC) of hypoglycaemia for blood glucose concentrations < 3.9 mmol/L during CGM and SBG and its relationship to baseline hypoglycaemia, age and sensor usage. AUC hypoglycaemia was obtained in each study from a period of blinded CGM at the start and completion of the trial. The results of the meta-analysis will be presented.

O-56

PATIENT- OR PHYSICIAN-DRIVEN CONTINUOUS GLUCOSE MONITORING IMPROVES CONTROL FOR ONE YEAR IN POORLY-CONTROLLED TYPE 1 DIABETES UNDER BASAL-BOLUS INSULIN REGIMEN

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The benefits of continuous glucose monitoring (CGM) in type 1 diabetes (T1D) remain unknown in real life. Our multi-centre study assessed the impact on diabetes control: A1c, SD of glucose levels, hypoglycaemia, as well as quality of life (QoL) in the conditions of patient- or physician-led CGM use.

We randomized 197 T1D patients with sustained A1c levels >8% for self-driven use of CGM (group 1) or physician-prescribed CGM (group 2) or no CGM (group 3) on 12 months. Used device was the FreeStyle Navigator CGM system. Data were assessable in 178 patients; age: 36 ± 14, duration of T1D: 17 ± 10 years, A1c: 8.9 ± 0.9%, SD glucose: 70 [52; 84] mg/dL (mean $\pm$ SD, or mean [95% CI]).

After 12 months, A1c was reduced by 0.50 [0; 0.29]% ($P < 0.0001$), 0.52 [0; 0.28] ($P < 0.0006$) and 0.47 [0; 0.23] ($P < 0.0018$) in groups $1 + 2$, 1 and 2 vs. 3, respectively. Percent patients with A1c < 7.5% after 12 months were 9.7, 14.8 and 1.6 in groups 1, 2 and 3 respectively ($P = 0.026$ for group 1 or 2 vs. 3). SD glucose was also reduced by 11.9 [0; 2.6] mg/dL in groups $1 + 2$ vs. 3 ($P = 0.018$) and by 15.1 [0; 3.5] mg/dL in group 2 vs.3 ($P = 0.049$). Occurrence of hypoglycaemia and QoL were similar in the 3 groups.

From our data, patient- or physician-led CGM use allows significant and sustained improvement of poorly-controlled T1D. The factors associated with the highest benefit need further analysis.

O-57

NEW TECHNOLOGIES FOR IN-PATIENTS DM MANAGEMENT

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Glucose control has been demonstrated as a key element to improve in-patient outcomes in intensive care units, post-surgical conditions and acute coronary syndromes, both for patients with or without diabetes mellitus when admitted.

Continuous intravenous insulin delivery adapted according to frequent blood glucose measurements from capillary or peripheral arterial/venous samples is the reference procedure to maintain glucose levels in a near-normal range. Simple 'static' algorithms based on iterative glucose measurements are still widely used to tune insulin delivery rate. However, more elaborated 'dynamic' algorithms based on the peripheral-integral-derivative (PID) concept or model predictive control (MPC) have been developed during the recent years that allow more stable glucose control. Whether continuous glucose monitoring in interstitial space by 'needle-type' sensors or external sensing devices connected to microdialysis probes can be used as an alternative to direct blood measurements remains to be further investigated according to the patient condition. The development of intravenous sensors would better fulfill accuracy needs for glucose assessment in patients with impaired hemodynamic conditions. Current works dedicated to the elaboration of artificial pancreas models are expected to provide improvements in glucose management of in-patients

through safety supervision modules that minimize the risk of hypoglycemia.

O-58

PATIENTS AFFECTED BY TYPE 1 DIABETES WITH A HIGH RISK OF KETOACIDOSIS: WHO ARE THEY?

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Ketoacidosis remains a severe, eventually lethal, complication of type 1 diabetes. It also represents a significant burden for healthcare costs related to type 1 diabetes. While still present in about 30% of cases who reveal type 1 diabetes, its occurrence and its recurrence have been well identified in specific cases already diagnosed. Female adolescents who poorly adhere to the basic rules of insulin therapy represent a specific subset of patients prone to recurrent ketoacidosis. Weight concerns combined with eating disorders are common roots for behavioural troubles, including omissions of insulin injections and lack of self monitoring of blood glucose (SMBG). Family conflicts, low healthcare insurance coverage, school problems and psychiatric disorders promote these deleterious behaviours. Sustained high levels of HbA1c characterize this high risk population for recurrent ketoacidosis. Patients treated by insulin pumps who poorly adhere to educational recommendations associated with this mode of therapy are also candidates for ketoacidosis. Due to the limited subcutaneous insulin depot in continuous subcutaneous insulin infusion, any failure in the recommended performance of SMBG and in the requested attention given to the infusion site may easily result in undetected hyperglycaemia due to insulin underdelivery, leading to ketoacidosis in a few hours. Diabetic pregnancy is associated with an early appearance of ketone production at blood glucose levels which are much lower than those associated with ketone excess in non pregnant conditions. If willing to perform it, an early detection of ketone excess in plasma could dramatically reduce the incidence of ketoacidosis in these patients.

O-59

O-60

CAN THE INDIVIDUAL GLUCOSE LOWERING EFFECT OF INCRETINS BE PREDICTED PRIOR THERAPEUTIC APPLICATION?

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Objectives and aims: Incretin analogues and DPP4 inhibitors are a new class of anti-hyperglycaemic agents that have the possibility to improve glycaemic control similar to natural incretin hormones. Initial application of incretin analogues and DPP4 inhibitors have demonstrated that some patients meet the expected effects on glycaemic control but others failed. At present, no pre-clinical method is available to predict low or high incretin responders prior therapeutic application.

Materials and methods: The Karlsburg Diabetes-Management System KADIS® which allows personalized prediction of daily glucose profiles in relation to different therapeutic measures was used to develop a model-based method for outcome prediction of therapeutic application of the incretins and to validate the method by *in silico* testing the incretin analogue Exenatide in 58 non-insulin treated type 2 diabetic patients. For this purpose, KADIS® was adapted to the special requirements to meet the aim of this study.

Results: The overall glycaemic lowering effect of 20 μg Exenatide was estimated to be 0.81 ± 0.50 mmol/L and equals an insulin dose of 12.6 ± 4.8 IU. 41% of the study patients could be identified to be high responders to an Exenatide therapy and 59% were low responders. In the group of high responders a higher BMI (31.4 vs. 28.5 kg/m^2), an enhanced endogenous insulin supply (62.2 vs. 49.7 IU/day), and a higher HbA1c (6.7 vs. 6.2%) at baseline were observed.

Conclusions: The individual metabolic effect of an incretin therapy can be predicted prior to therapeutic application by using CGM in combination with KADIS®-based *in silico* simulation strategy.

O-61

EFFECTS OF 2-H AEROBIC/ANAEROBIC EXERCISE ON INSULIN PUMP THERAPY IN CHILDREN WITH TYPE 1 DIABETES: A RANDOMISED CONTROLLED TRIAL. EVALUATION OF CGM DATA

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Background: Few studies evaluated the effect of physical activity on insulin pump therapy in children with type 1 diabetes. The most effective strategy with insulin pump therapy in children with type 1 diabetes undergoing 2 hours of aerobic-anaerobic exercise has been evaluated.

Methods: Fifteen children, aged 10–18 yrs., with type 1 diabetes for 7.2 ± 3.3 yrs, (BMI $20.05 \pm 3.05\,m/kg^2$, insulin requirement $0.85 \pm 0.15\,U/kg/day$, HbA1c $7.66 \pm 0.81\%$), who were using a sensor-augmented insulin pump, were enrolled. Exercise has been maintained at the same level during each session ($P = 0.339$), and replicated for 3 consecutive days, with a randomly assigned insulin pump scheme: pump kept active; pump suspended; pump suspended + 'correction' bolus (equal to the basal insulin the patient would have injected during the 2h-exercise, reduced by 30%).

Results: All strategies ensured a general good controlling of the glycaemic values (all $P > 0.05$). CONGA1 index showed best control immediately after the exercise ($P = 0.035$) and during the night ($P = 0.018$) keeping pump active. These results were confirmed by CGM data in term of time in hyperglycemia (cut-off: 180 mg/dL or 250 mg/dL, $P < 0.05$ pump on compared to the two other schemes). Even time in hypoglycemia were less when pump was on than off ($P = 0.007$) or off + correction bolus ($P = 0.002$).

Conclusions: Keeping pump active during exercise seems the best option to properly manage exercise in children with type 1 diabetes, while for those sports that do not allow the use of insulin pump, suspending the pump might be a good option. A practical flow-chart has been drawn.

O-62

MAKING HOSPITALS DIABETES FRIENDLY: ONE BARRIER AT A TIME

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Introduction: As the number of people with diabetes in the United States grows in epidemic proportions, so does the percentage of hospitalized patients with diabetes and "new" hyperglycemia. Aggressive treatment has proven to lower the risk of serious complications in acute care, yet many patients do not achieve recognized goals. One major challenge is that many hospital processes have not been adapted to facilitate following diabetes regimens as we would recommend in the outpatient setting.

Objective: To discuss barriers to following diabetes regimens during hospitalization and possible solutions.

Methods: System-wide changes hold the key to reducing barriers and facilitating successful implementation of evidence-based practice recommendations to achieve glycemic targets in the inpatient setting. We assessed care processes such as menus, meal tray delivery, timing of blood glucose monitoring, treatment of hypoglycemia and insulin initiation and titration. Road blocks to providing optimal care were identified and possible solutions were brainstormed and piloted. Policies and procedures related to blood glucose monitoring, hypoglycemia treatment and insulin delivery were reviewed, revised and implemented. Ultimately, five comprehensive glycemic control electronic order sets were created and implemented to make it easy to enter the "right" orders for a variety of common patient types.

Results: Clinicians were educated in the need for consistent carbohydrate meal planning, importance of A1c in the hospitalized patient and how to place orders in the new order sets.

Conclusions: Comprehensive Glycemic Control Electronic Orders can facilitate more timely initiation of insulin therapy and reduce incidences of hypo and hyperglycemia.

O-63

SURGICAL SOLUTIONS FOR TYPE 2 DIABETES

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It has been observed since the 1950's, that gastrointestinal surgery for conditions such as peptic ulcer disease, can have striking beneficial effects on type 2 diabetes mellitus.

Since the 1980's, its has been demonstrated that bariatric surgery for intractable morbid obesity (generally gastrointestinal procedures) also can result in dramatic improvement and even resolution of type 2 diabetes mellitus. The current popularity of bariatric surgery has brought this phenomenon to the forefront and has led to efforts to understand the mechanisms of action. Research is also underway to evaluate the effects of "bariatric-like" operative procedures in non-morbidly obese type 2 diabetics and to investigate novel procedures that are less invasive. This presentation will review the latest research and published outcome results for both conventional and novel operative procedures.

O-64

DATA TRANSFORMS FOR STABLE AND EFFECTIVE NOCTURNAL HYPOGLYCAEMIA ALARMS: RESULTS FROM HYPOMON® CLINICAL TRIAL DATA

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Nocturnal hypoglycaemia in persons with type 1 diabetes mellitus (T1DM) can be detected by using data transforms to enhance measurement of physiological changes triggered by the autonomic nervous system. The HypoMon® is a real-time multi-parameter physiologically-based alarm for nocturnal hypoglycaemia. It has been validated on over 300 patients with reference venous blood glucose levels measured using Yellow Springs Instrument (YSI).

A key physiological response of heart rate and transformed heart rate (HypoMon algorithm) in persons with T1DM are examined during hyperinsulinemic clamps (HC) and natural nocturnal hypoglycaemia (NNH) protocols. Changes in mean

heart rate, and mean transformed heart rate from baseline (euglycaemic control YSI > 5.0 mmol/L) to hypoglycaemia (YSI ≤ 3.8 mmol/L until nadir/treatment) are compared in both HC (n = 8) and NNH (n = 26) protocols. Baseline to hypoglycaemia comparisons are made using 2-tailed paired Student's t-tests.

A non-significant increase in mean heart rate (71 ± 12 vs. 76 ± 12 beats/minute, $P = 0.13$) under HC protocol, and non significant changes during NNH protocol (73 ± 10 vs. 71 ± 9, $P = 0.18$) were observed. Transformed heart rate enhances changes during both HC and NNH protocols (−1.96 ± 1.82 vs. 2.43 ± 2.34 $P = 0.01$) and (−1.88 ± 3.96 vs. 0.48 ± 2.74 $P = 0.0083$) respectively.

Heart rate changes during hypoglycaemia can be enhanced via HypoMon data transforms. The transformed data shows stable and detectable changes not present in raw heart rate data, which appear swamped by circadian fluctuations. Data transforms permit changes in physiological parameters to be translated into an effective alarm for nocturnal hypoglycaemia using the HypoMon system.

O-65

EXPERIENCE WITH CONTINUAL GLUCOSE MONITORING DURING AND AFTER THE CARDIAL SURGERY WITH EXTRACORPORAL OXYGENATION AND HYPOTHERMIA

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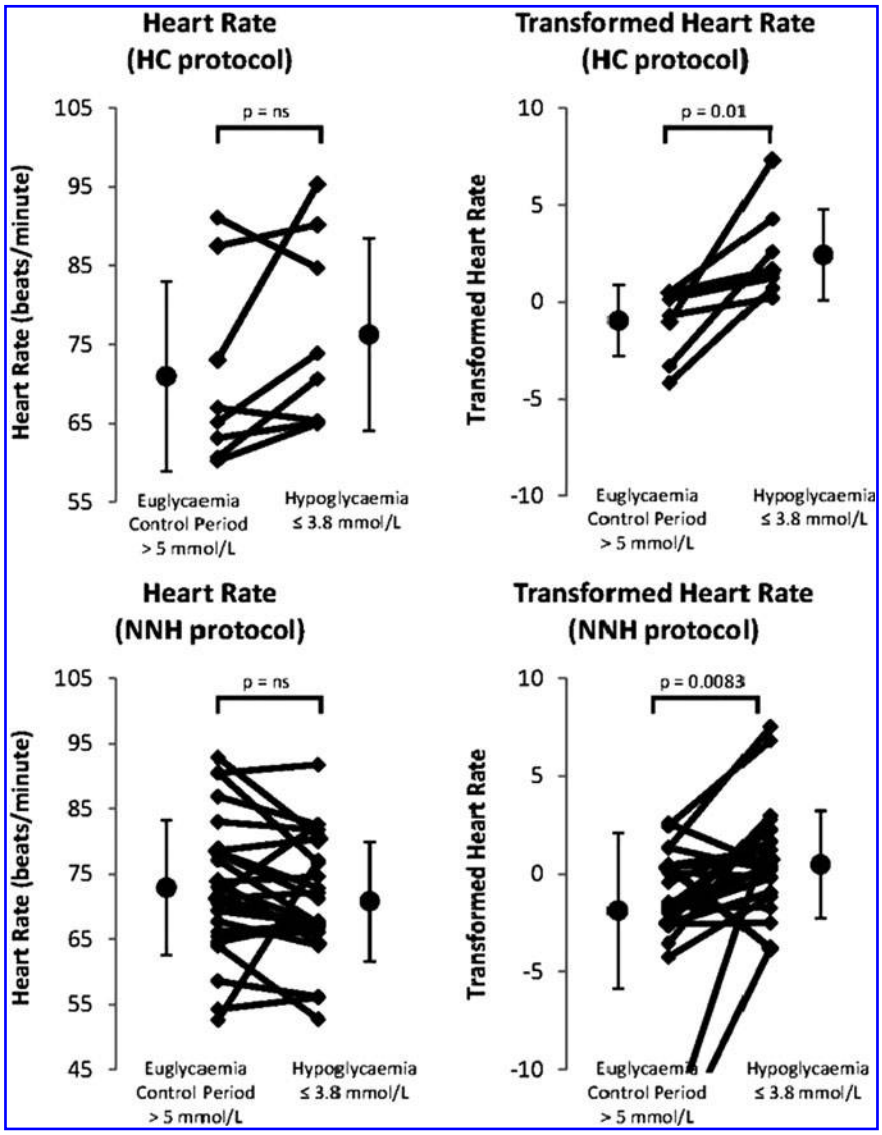
Our experience with the application of continual glucose monitoring system (CGMS) during invasive surgery and following intensive care is reported.

Seven-year old girl has been treated one year for diabetes. Her mother suffers from diabetes as well. Probably as a consequence of maternal diabetes the girl developed a heart atrial defect (16×16 mm with the dilatation of right cardial compartments). Due to the progression of cardial complications she was recommended to the surgical intervention with the necessity of



Physiological change from control to hypoglycaemia.

extracorporal oxygenation and hypothermia. Prior the surgery she was treated by insulin.

(0.5 IU/kg) in four injections daily (HbA1c 7.8% according IFCC). During the surgery and 4 days later she received insulin intravenously. During this period Guardian Real-Time was used (the sensor was situated to left subclavicular area because we expected better perfusion there). The total asystolic time was 24 minutes. During the surgery and after it glycaemia was stable (median 9.8 mmol/L, range 9.3–11.3 mmol/L), the insulin dose was adjusted according to glycaemia and according to trends displayed by CGMS. Values from CGMS correlated well with laboratory measurements ($r = 0.7$, $P = 0.017$). After the conversion to multiple daily injections the girl had short period of hyperglycaemia when the insulin dose had to be increased to 1.8 IU/kg but in general her recovery was without important complications and she is now in very good physical condition (HbA1c 6.5%) with the same insulin dose as formerly.

Conclusion: CGMS was successfully applied during quite complicated surgery as well as during postoperative period.

O-66

NOVEL APPROACHES TO ACHIEVING LONG ACTING INSULIN FORMULATIONS

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When administered by subcutaneous injections, the animal derived insulins first used to treat diabetes in the 1920s, lasted between 4 and 6 hours requiring multiple doses per day. As a result they were called "fast acting" insulin. In 1936, a low molecular weight protein, protamine, along with zinc was added to animal derived insulin to produce a formulation that released the precipitated insulin slowly from its subcutaneous depot, lasting from 24–36 hours. So began the quest for long acting basal insulin formulations that would last at least 24 hours and whose pharmacokinetic profile was both flat and reproducible within a given patient from day to day as well as between different patients. Different approaches taken to achieve this goal, such as co-precipitation, development of analog insulins with different pH solubility (insulin glargine) and structural manipulations causing the analog to bind to endogenous proteins (insulin detemir and insulin degludec) will be examined. Newer attempts which employ novel analogs and those that combine the long acting analog insulins with both precipitation techniques and hydrogen binding techniques will be presented. Finally, progress in making basal insulin formulations that release insulin into systemic circulation in proportion to subcutaneous glucose concentration; the "smart basal insulins" will be discussed.

O-67

COMPARISON OF INSULIN PUMP SETTINGS AND INSULIN USAGE PATTERNS IN ADULT AND PEDIATRIC SUBJECTS IN THE STAR 3 STUDY

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Objective: The STAR 3 study showed that mean A1C reduction after switching from multiple daily injections to sensor-augmented pump (SAP) therapy is larger in adults than in children. Comparison of pump and insulin usage patterns may help explain this difference.

Method: The SAP arm of STAR 3 included 78 pediatric (age 7–18) and 166 adult (age 19–70) subjects with baseline A1C values of 7.4–9.5%. Quarterly A1C values were obtained. Pump and CGM data were collected with Medtronic CareLink Therapy Management System for Diabetes - Clinical. Analyses were performed on 4th quarter data.

Results: For both age cohorts, greater decreases in A1C were positively associated with the percentage of glucose values in the target range (%TR) of 70–180 mg/dL ($P < 0.001$) and inversely associated with SD of sensor glucose values at 1 year ($P < 0.05$). Favorable associations were found with sensor wear (SW, $P < 0.001$) and Bolus Wizard boluses/day (BW, $P < 0.05$), but not bolus/basal ratio (B/B) or total daily dose of insulin/kg (TDD). Among children, there was an association between the number daily boluses (#B, $P = 0.0568$) and basal rates (BR, $P = 0.0498$). Given baseline A1c and A1c decrease at 1 year, adults had greater SW than children ($P = 0.0003$), while children had greater BW ($P = 0.0341$), BR ($P = 0.092$), #B ($P = 0.0702$), B/B ($P = 0.0065$), SD ($P < 0.0001$) and TDD ($P < 0.0001$) than adults.

Conclusions: STAR 3 results suggest that sensor data and bolus calculator features enable both adults and children to improve glycemic control, but with some differences in outcomes and insulin utilization patterns.

O-68

GO LOW, DON'T FOLLOW THE FLOW: LOW BASAL RATES IN CSII PREDICT BETTER HBA1C

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Objective: Most patients who use 'typical' protocol values of 50% of TDD as bolus and 50% basal were not able to reach good control defined as reaching the HbA1c target. We previously reported data on the role of basal insulin rate (BIR) in outcome of CSII and showed that a lower BIR (at similar total daily dose (TDD)) resulted in better HbA1c's. To strengthen our observations we evaluated historical data in a statistical analysis and in a prediction model.

Method: A historical cohort with data from 230 CSII (Paradigm, Medtronic; constant use of whizard) patients with T1DM patients over a twelve-month period was analyzed for predictors of better outcome of HbA1c.

Results: Average basal insulin was 32.8% of the TDD. Fifteen of the eighteen parameters explored, strongly correlated with HbA1c including BIR/kg ($P < 0.001$). This effect remains when analyzed in different 4 different age groups (0–6 years, >6–12 years, >12–18 years and adults) all improving at lower BIR's.

Conclusions: 25.4% of HbA1c value is explained by BIR per kg bodyweight, total daily insulin units/kg, frequency of daily boluses and age group. These data strongly support that lower basal insulin per kg bodyweight will lead to a better glycaemic control. Based on same TDD this requires more bolus and subsequently

better knowledge and application of carbs and food-effects. These data prompt for a substantial change in pump protocols and education and pose the question if application of CGM and (semi) closed loops should't include or be proceeded by a low BIR approach.

O-69

DIALBETICS: A NOVEL SMARTPHONE-BASED SELF-MANAGEMENT SUPPORT SYSTEM FOR TYPE 2 DIABETIC PATIENTS

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Introduction: Web-based management of patients' data improves glycemic control but increases the healthcare providers' workloads.

Objectives: We developed and studied a semi-automated system to interpret patients' data—with interactive communication of findings—achieving diabetes management without increasing the physician's workload.

Methods: This semi-computerized system, DialBetics (Figure), is composed of three modules:

(1) Data transmission: patients' data are sent to the server twice a day by Bluetooth.
(2) Evaluation: data are automatically evaluated following the Japan Diabetes Society guideline's targeted values; DialBetics determines if each reading satisfies guideline requirements, then sends apposite questions on diet and exercise to each patient's smartphone.
(3) Communication:
   (a) the patient's voice message in response to DialBetics is converted to text by speech-recognition device and ontologically matched with text in the DialBetics database;
   (b) advice on life-style modification, matched to the patient's answer, is fed back to each patient by e-mail.

Results: DialBetics was tested with five type 2 diabetic patients and found accuracy and safety in data transmission, evaluation. There was, however, about a 5% discrepancy in voice-text conversion, resulting in imperfectly matched answers. Nevertheless, all subjects pronounced themselves well satisfied with DialBetics; they were enthusiastic about using it.

Conclusions: DialBetics may improve patient satisfaction and self-management. The discrepancy in voice-text conversion may be reduced-over time, case-by-case-by updating the database for more sensitive conversion. This novel system supported by the guideline-based-evaluation engine provides a new medical ICT model-contributing better management of diabetes without significantly increasing health professionals' workloads.

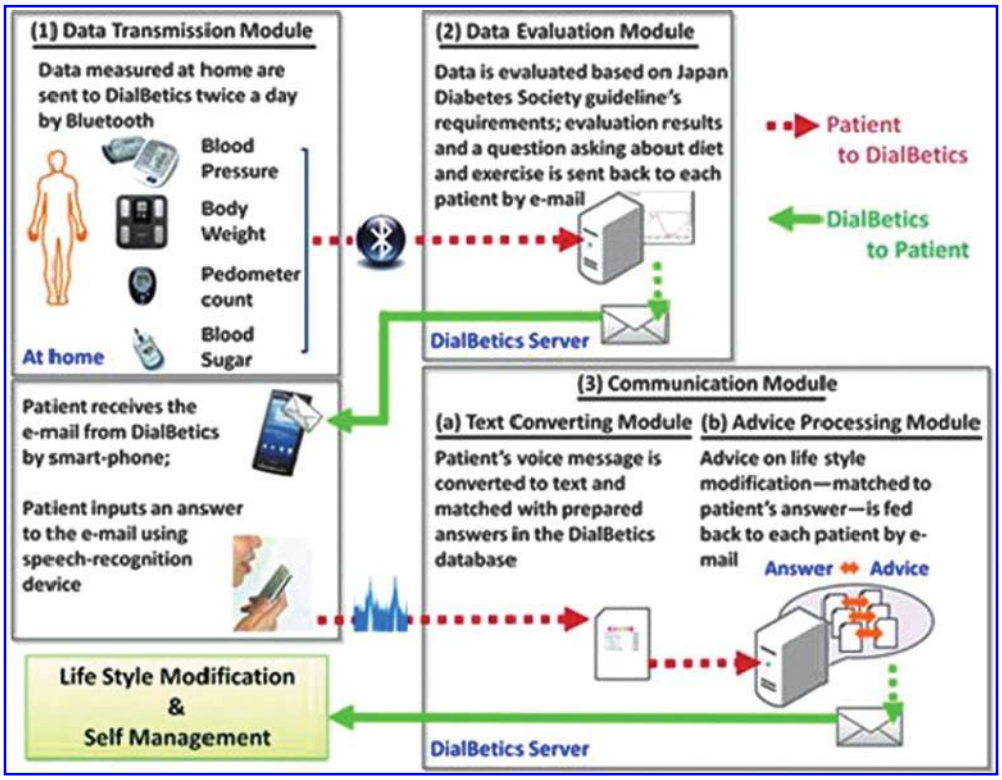

FIG. DialBetics.

O-70

CLOSED LOOP INSULIN DELIVERY REDUCES NOCTURNAL HYPOGLYCEMIA DURING NIGHTS WITH OR WITHOUT ANTECEDENT AFTERNOON EXERCISE

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Objective: To evaluate whether a closed-loop (CL) system utilizing subcutaneous glucose sensing and insulin delivery reduces the frequency of nocturnal hypoglycemia following standardized daytime exercise compared to usual open-loop (OL) pump use in adolescents and young adults with type 1 diabetes (T1D).

Methods: Subjects completed two separate 48-hour inpatient periods of glucose control, in random order: usual open-loop pump therapy (OL), and a CL system using a PID-IFB algorithm. Each admission included a sedentary day and an "exercise day", with a standardized protocol of four 15-minute periods of brisk treadmill walking to 65–70% HR_{max} starting at 3PM. During CL control, target glucose was set at 120 mg/dL; nocturnal hypoglycemia (NH) was defined as reference blood glucose level <60 mg/dL between 10PM-6AM.

Results: Twelve subjects (7 female, age 12.4–26.3 y, A1C = 7.4 ± 0.6%) completed both admissions. One episode of NH after daytime exercise occurred during CL compared to 15 during OL admission ($P = 0.06$); NH following sedentary day occurred twice during CL vs 8 during OL ($P = 0.09$). Total hypoglycemia episodes (day and night) were 19 during CL vs 63 during OL ($P = 0.05$). Mean nocturnal glucose was 119 ± 27 mg/dL during CL and 124 ± 45 mg/dL during OL ($P = 0.07$).

Conclusion: The CL system was associated with fewer episodes of nocturnal hypoglycemia following both sedentary and exercise days and a reduction of total hypoglycemia, without a deterioration in mean glucose. Such systems, even if used at night only, may be useful to minimize the risk of NH in people with T1D in the home setting.

O-71

EFFECT OF ADJUVANT INJECTED PRAMLINTIDE ON CLOSED-LOOP INSULIN DELIVERY: PRELIMINARY FINDINGS

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Background: Even under closed-loop conditions, meal-related blood glucose excursions frequently exceed target levels due to delays in insulin absorption. We hypothesized the addition of pramlintide would improve post-prandial glycemia by allowing better match-up between glucose and insulin absorption.

Methods: Using a PID + IFB algorithm, subjects were studied for 24 h on CL control alone and 24 h on CL plus pre-meal pramlintide, 30 mcg injections. Target glucose was set at 120 mg/dL; meals were served at 8 AM, 1 PM, and 6 PM, and were identical for both days of study. No pre-meal manual boluses were given. Reference blood glucose excursions, defined as incremental glucose rise from pre-meal to peak, were compared between conditions for each meal.

Results: Among the first six subjects (4 female, age 16.2–27.8 y, A1c 7.5 ± 0.8%), time to glucose peak was markedly delayed by pramlintide after each meal ($P < 0.03$). Pramlintide did not alter the magnitude of the post-breakfast glucose excursion but did lower the increments in BG after lunch and dinner ($P < 0.03$ for lunch). Mean glucose levels were identical in both conditions (145 ± 51 mg/dL).

Conclusion: Pramlintide delayed the time to peak post-prandial BG but had a less consistent effect on the magnitude of prandial BG excursions. Further refinements to the CL algorithm may be necessary to optimize prandial glucose control.

	Breakfast BG Excursion (mg/dL)	*Lunch BG Excursion (mg/dL)*	*Dinner BG Excursion (mg/dL)*	*Breakfast Time to peak (hr)*	*Lunch Time to peak (hr)*	*Dinner Time to peak (hr)*
CL only	123 ± 10	113 ± 32	82 ± 31	1.5 ± 0.7	1.4 ± 0.6	1.4 ± 1.2
CL + Pramlintide	122 ± 45	73 ± 21	62 ± 37	2.4 ± 0.5	2.6 ± 0.5	2.7 ± 0.5

O-72

REMOTE MONITORING OF THE ARTIFICIAL PANCREAS: IMPLICATIONS FOR THE FUTURE CLINICAL MANAGEMENT OF DIABETES

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In the last few years researchers have made tremendous strides in the quest towards a fully automated closed-loop artificial pancreas. They are designing systems combining insulin pumps and continuous glucose monitors with advanced control theory and wireless technology. Much of the current research is focused on basic building blocks that will be used to construct an optimal functional system. One under-represented area of investigation is how to monitor the overall performance and safety of a system once it is ready to be used in the real world. There will need to be redundant safety layers and the ability to trouble shoot.

Fifty years ago, patients with diabetes relied on urine glucose testing and had infrequent laboratory blood glucose determinations. Now they have access to SMBG and CGM, which gathers a continuous stream of valuable glucose information. Unfortunately, most of that information stays in the patient's hip pocket. Existing technology, initially developed for the artificial

pancreas, can be harnessed to allow almost instant access to pump and sensor information to allow parents to know what is happening with the child during the school day and to allow data transfer to health care providers to allow optimization of insulin delivery and glucose control. The artificial pancreas will probably be introduced in stages: remote monitoring and adviser mode, pump shut-off, overnight control, meal recognition, etc. Hopefully, soon we will be able to implement these stratagies designed to monitor the health of the artificial pancreas to improve the health of patients with diabetes.

ATTD 2011 Poster Presentations

P-01

CONTINUOUS GLUCOSE MONITORING (CGM) IN CRITICALLY DIABETIC PATIENTS HOSPITALIZED: OUR EXPERIENCE

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Obtain strictly glycemic control in hospitalized critically diabetic patients is widespread accepted: is undoubted the relationship between mortality inside unit care department and high glycemic levels. CGM represent an interesting tool to obtain a better glycemic control, to improve insulin therapy and finally to get good prognosis. We present our experience about the use of CGM in 25 critically diabetic patients hospitalized: 14 pz in CCU, 6 pz in Surgery division and 5 pz in ICU. We investigated the accuracy/applicability of the CGM in critically diabetic patients, the percent of time of glycemic control on target (140–180 mg/dL) and the glycemic variability (MAGE, SD, LBGI, HBGI). 25 diabetic patients (men/women 14/11, age 63 ± 16 years, intravenous/subcutaneous insulin 9/16) were recruited. 48 hour CGM was performed using a "real time" subcutaneous glucose sensor (Glucoday S) and compared with capillary BG or arterial BG (in ICU). CGM use improve glycemic control on target by means of titrated insulin dosage: during 48-h CGM glycemia reached target (140–180 mg/dL) in $42 \pm 16\%$, was 110–140 mg/dL $22 \pm 12\%$, was 80–110 mg/dL in $16 \pm 9\%$, was >180 mg/dL $13 \pm 7\%$, was <80 mg/dL in $5 \pm 4\%$ of the time and patients with subcutaneous versus intravenous insulin had more glycemia readings >180 mg/dL. The CGM values correlated well with capillary BG and arterial BG. The patients who received subcutaneous insulin had more glycemia readings, higher (180 mg/dL) than patients who received intravenous insulin. CGM values reading correlated with capillary blood glucose as well as arterial blood glucose.

P-02

DIETARY SARDINE PROTEIN LOWERS INSULIN RESISTANCE AND IMPROVES ERYTHROCYTES OXIDATIVE STRESS INDUCED BY A HIGH-FRUCTOSE DIET IN RATS

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This investigation examines whether sardine protein feeding ameliorates insulin resistance, lipid profile and erythrocytes oxidative stress, in rats fed a high fructose diet.

Male Wistar rats were fed casein (C) or sardine protein (S) with or without fructose (64%) (F) for 2 months. Glucose tolerance test, plasma glucose, insulin, lipids and erythrocytes lipid and protein oxidation and antioxidant enzymes were determined.

The results indicated that SF intake resulted in a significant reduction of plasma glucose (21%), insulin (35%), HbA_1C (37%) and glucose intolerance (51%) than CF. HOMA-IR was significantly higher in fructose groups than in control groups. In addition, HOMA-IR was 1.72- and 1.91-fold lower in SF and S rats than in CF and C rats, respectively. Plasma cholesterol, triglycerides and free fatty acids were greater in fructose rats and lower in SF. TBARS, hydroperoxide and carbonyl concentrations were significantly higher in fructose-fed rats as compared to control rats. Moreover, feeding sardine diet resulted in low TBARS (21%), hydroperoxides (17%) and carbonyls (64%) than feeding casein diet. The consumption of fructose diet decreased significantly SOD, CAT and GSH-Px activities relative to casein. Rats fed sardine protein had higher SOD (54%) and CAT (18%) activities. Erythrocytes ascorbic acid levels was 57% greater in SF group than in CF group.

In conclusion, sardine protein consumption exerts beneficial effects on insulin resistance, lipid profile and erythrocytes oxidative stress in high-fructose-induced metabolic syndrome. This protein may be a safe strategy in a number of high-risk subjects.

P-03

RELATIONSHIP OF WEIGHT SUBGROUPS AND DIABETES IN A SAMPLE POPULATION OF CENTRAL PART OF IRAN (BASED ON ISFAHAN HEALTY HEART PROGRAM)

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Aims: Atherosclerosis is one of the leading causes of mortality all around the world. Diabetes is known as a leading cause for Atherosclerosis diseases. Obesity and overweight is an independent risk factor for atherosclerosis and diabetes. So in this paper we wanted to examine the effect of subgroups of weight on risk factors for diabetes.

Methods: This cross-sectional study was done in 2006 with data of Iranian Healthy Heart Program based on classification of obesity that performed by the World Health Organization and laboratory measuring testes with SPSS software tasted the samples.

Results: 12,514 persons participated in this study with mean age about 38 years old. Women overweight subgroups and obesity were more than men (56.4% of women & 40% of men had BMI $\geq$25 Kg/m^2, 13% of samples FBS >110). We found that all of risk factors for diabetes raised with increasing weights.

Conclusions: One of every two American persons in all ages and both sexes were obese. Overweight and obesity combined with CVD, and also diabetes risk factors. Many studies in different countries with different way done to find the relationship between overweights and obesity with diabetes risk factors. For example one study that was done in China shows that increasing BMI has an indirect effect on diabetes risk factors. This data was same as our results. But in China they found that this relationship in men is stronger than women, but we find of opposite effect.

P-04

ASSOCIATION OF URINARY THIAMINE LEVELS VIA HPLC DETERMINATION TO GLYCEMIC PARAMETERS TO PATIENTS WITH TYPE 1 AND 2 DIABETES MELLITUS VERSUS CONTROLS

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Microvascular complications of diabetes are an insidious chronic morbidity leading to impairment of quality of life and, for nephropathy, contribute to increased risk of cardiovascular disease and mortality. Experimental diabetes was associated with thiamine deficiency, caused by increased renal clearance of thiamine, that exacerbated biochemical dysfunction linked to complications. In this on-going study, we will attempt to present urinary thiamine levels and their association to glycemic parameters among patients with type 1 and 2 diabetes mellitus, and compare them to non-diabetic controls. A total of 120 consenting adult Saudi subjects (60 type 1 DM and 60 type 2 DM) and an additional 20 non-diabetic controls were recruited. Patients with diabetes were further subdivided according to degree of microalbuminuria. Glycemic parameters including HBA1c will be determined and urinary thiamine will be quantified using high performance liquid chromatography. We expect an association between glycemic parameters and urinary thiamine levels to further strengthen the hypothesis that urinary thiamine levels are good prognostic indicators of diabetes complications.

P-05

AUTOMATED OVERNIGHT CLOSED-LOOP GLUCOSE CONTROL IN YOUNG CHILDREN WITH TYPE 1 DIABETES

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Objective: We evaluated automated overnight closed-loop (AOCL) in young children with type 1 diabetes (T1D).

Methods: Eight children with T1D (M 4; age 9.4 $\pm$ 2.7 years; BMI 18.3 $\pm$ 2.3 kg/m^2; duration of diabetes 3.9 $\pm$ 2.5 years; total daily insulin dose 0.7 $\pm$ 0.1 U/kg/day; A1C 7.9 $\pm$ 0.9%; mean $\pm$ SD) were studied at a clinical research facility on two occasions. Subjects had meal at 18:00 (77 $\pm$ 8 g CHO) and snack at 21:00 (21 $\pm$ 6 g CHO) both accompanied by insulin bolus. In random order, AOCL started at 18:00 or 21:00 and ran until 08:00 next day. Subcutaneous (sc) continuous glucose monitoring (CGM) data was fed automatically into model predictive control algorithm. Calculated sc insulin infusion rates were sent wirelessly to an insulin pump. Overnight CGM and insulin delivery recorded at home before each study occasion were evaluated for comparison.

Results: No rescue carbohydrates were administered. Time spent with plasma glucose in the target range 3.9–8.0 mmol/L was 50.7(29.0,72.2)% and it did not differ on the two occasions [42(18,64) vs 58(32,79)%; median(IQR); $P = 0.161$]. Time above 8.0 mmol/L [42(25,82) vs 29(14,64)%, $P = 0.093$], time below 3.9 mmol/L [0(0,11) vs 8(0,17)%, $P = 0.500$], low blood glucose index [0.1(0.0,2.5) vs 1.7(0.4,3.3), $P = 0.380$], plasma glucose at the start of AOCL [12.5 $\pm$ 2.7 vs 11.6 $\pm$ 4.2 mmol/L, mean $\pm$ SD, $P = 0.562$] and mean overnight plasma glucose [8.3 $\pm$ 2.1 vs 7.5 $\pm$ 2.2 mmol/L, $P = 0.246$] were also similar. Mean overnight CGM (8.8 $\pm$ 2.0 vs 10.5 $\pm$ 3.6 mmol/L, $P = 0.081$) and time in target [61(24,73) vs 38(4,52)%, median(IQR), $P = 0.099$], though improved during AOCL, weren't statistically different from standard treatment at home. Insulin infusion rates were higher during AOCL (0.7 $\pm$ 0.3 vs 0.5 $\pm$ 0.3U/h, $P = 0.005$).

Conclusions: Automated overnight closed-loop is feasible in young children with type 1 diabetes. Comparable results were obtained when closed-loop was initiated at 18:00 or 21:00.

P-06

EFFECTS OF SOME PLANT OILS ON PHYSIOLOGICAL RESPONSES IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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The effects of some plant oils on some physiological parameters were examined in streptozotocin (STZ)-induced diabetic and non-diabetic male Wistar rats. STZ-induced diabetic rats given the control diet had the lowest body weight change, thyroid-stimulating hormone (TSH), triiodothyronine (T3) and thyroxine (T4) levels after 7 weeks. Diabetic rats given diets containing the oils of neem, sesame, sunflower, evening primrose, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower had higher body weight change, TSH, T3 and T4 levels than diabetic rats given the control diet. No significant differences were observed in the above physiological parameters of normal rats fed on the examined oils when compared with those rats fed on the control diet after 7 weeks. There were no significant differences in body weight change of diabetic rats fed on the diets containing the different oils when compared with normal rats fed on the same diets after 7 weeks. These data indicate that the diets containing the oils improve the examined physiological parameters in STZ-induced diabetic rats. From the present new findings, it was suggested that neem, sesame, sunflower, evening primrose, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower oils supplementation may act as antioxidant agents and these oils could be an excellent adjuvant support in the therapy of diabetic mellitus and its complications.

P-07

DIABETIC KETOACIDOSIS AS INITAL PRESENTATION OF TYPE 1 DIABETES MELLITUS IN SOUTHERN REGION OF SAUDI ARABIA

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The prevalance of type 1 DM is much less than type 2 DM. This leads to less knowledge about type 1 DM in general populations, or even some times withe junior doctors.

Here we are presenting the percentage of DKA as initial presentation of type 1 DM in Aseer Region over a period of ten years. A total of 614 patients with type 1 DM were registerd. Among them 487 patients with completed data, 228 patients were seen in DKA as initial presentation (47%), whereas 259 patients were discovered befor reaching DKA (53%).

This percentage is higher than what have been published from United States (25%) and in between if compared to other countries (16–80%). In relation to age, we found that around 80% of patients who are less than 1 year of age had DKA as initial presentation. Beyond that age there was no much difference.

Follow up study is planned to evaluate what can be thecause of this relatively high percentage.

P-08

CARDIOPROTECTIVE EFFECTS OF ZINC SUPPLEMENTATION IN STREPTOZOTOCIN-INDUCED DIABETES IN RATS

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Zinc is an essential mineral that is required for various cellular functions. Its abnormal metabolism is related to certain disorders such as diabetic complications. This study aimed to investigate whether combination of zinc sulfate with glibenclamide prevent or delay cardiovascular complications. Diabetes was induced in rats by a single intraperitoneal injection of streptozotocin (STZ, 40 mg kg^{-1}). After 45 days, diabetic group showed cardiovascular complications manifested by significant elevations of troponin-I (0.24 ± 0.03 ng ml^{-1} Vs. 0.023 ± 0.001, $P < 0.001$) as well as cardiac enzymes. Total cholesterol, LDL-C and triglycerides were significantly increased compared to non-diabetic group. Diabetic group showed impaired oxidative status manifested by a significant elevation of lipid peroxides, and reductions of glutathione and L-ascorbic acid. Serum levels of TNF-α were significantly increased compared to non-diabetic group (236.5 ± 16.2 ng ml^{-1} Vs. 169.7 ± 2.9, $P < 0.001$). Nitric oxide (NO) and vascular endothelial growth factor (VEGF) were markedly higher indicating endothelial dysfunction. Treatment with glibenclamide (600 μg kg^{-1}, i.p) resulted in an improvement in most of the deviated biochemical parameters. However, combination of glibenclamide with zinc sulfate (20 mg kg^{-1}, i.p) showed more pronounced hypoglycemic effect and a significant improvement in cardiac function as compared to glibenclamide alone treated group. LDL-C was significantly decreased by the combination (34.46 ± 2.7 mg dL^{-1} Vs. 49.35 ± 4.1, $P < 0.01$). Oxidative stress markers were improved by the combination. Serum levels of TNF-α, NO and VEGF were significantly reduced by the combination. These findings indicate that combination of zinc with glibenclamide may limit the diabetic complications.

P-09

EXPLOITING THE DVB-T TECHNOLOGY AND JAVA-TV FOR THE DEVELOPMENT OF A TELE-HOME CARE SYSTEM FOR THE REMOTE MONITORING OF DIABETIC PATIENTS

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For health reasons, diabetic patients need to be frequently monitored and in some cases they could easily perform at home the requested physiological measurements (i.e. glycemia and body weight) sending the measured data to the care staff in the hospital.

The aim of this work is the development of a low-cost tele-home care system based on the Digital Video Broadcast Terrestrial (DVB-T) technology for the remote monitoring of patients with diabetes, even exploitable by elderly people.

The proposed system is composed of two main parts, hosted respectively in a remote center care (RCC) and at the patient's home.

In the RCC there is only a PC with a database, a USB connected GSM module and running a HTTPS server. Through the GSM module, the PC receives the patient's data via SMS (that the PC will store in the database) that the secure web server will show in the internet browser of an authotized physician for patient monitoring and threatment corrections.

On the patient side, only a TV with an interactive DVB-T set-top box and a custom smart card are needed. The custom smart card is needed for patient identification, data acquisition and data communication. It embeds a GSM module for patient's SMS delivery to the RCC and a bluetooth radio for the acquisition of the patient data via simple commercial bluetooth glucose meters and body scale. The entire process is managed by an interactive application (Java Xlet) loaded on the patient's set-top box through an ether broadcast DVB-T transmission.

P-10

QUANTITATIVE ANALYSIS OF BOLUS DELIVERY SPEED AMONGST INSULIN INFUSION PUMPS

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Background: The chief benefit of insulin pump therapy is customized, flexible basal and bolus dosing to meet patients' individual insulin requirements while reducing the risk of severe hypoglycemia. Despite the advantages of insulin pump therapy, optimal control is often times not achieved. PK/PD of current insulin analogues are not rapid enough to match physiologic needs and carbohydrate absorption. Delays in the actual delivery of meal-time boluses may postpone peak insulin concentration. More rapid infusion of a bolus of insulin may avoid further unnecessary delays.

Method: The insulin bolus delivery volume and the speed of delivery were measured with a time stamped gravimetric measurement system. Preset bolus deliveries using the Animas One Touch Ping, the Medtronic Revel and Insulet OmniPod system were measured.

Results: Animas One Touch Ping bolus delivery was at least 8.5 times faster than Revel or Omnipod.

Conclusions: Highlighting the challenges of using current insulin analogues, are recommendations to dose significantly ahead of a meal in order to compensate for their relatively slow PK. Adding delays in the actual delivery of a pre-meal bolus only exacerbates the problem. Looking towards the future, successful development of closed loop systems will be aided by more rapidly acting insulins or devices and infusion sets that increase absorption of insulin. Delivering a bolus of insulin more quickly may partly offset the slow PK and improve glycemic control. Further research in this area is warranted.

P-11

MOBILE DIABETES SELF-MANAGEMENT TOOLS—WHAT'S THE ROLE OF CLINICIANS?

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People living with Type 1 diabetes require support for their self-management efforts. We developed non-obtrusive diabetes self-management support tools that leverage a widely-adopted technology platform - mobile "smartphones".

Our system, called the Few Touch application, was originally developed to support the needs and preferences of people with Type 2 diabetes, who have been involved throughout all phases of its design. We are now modifying the Few Touch to also support users with Type 1 diabetes. For example, we have added functionality that provides easy entry of insulin data via the phone's touch-sensitive screen, resulting in a complete system for monitoring blood glucose in relation to nutrition, physical activity, and medication.

Blood glucose and step count data are automatically transferred to the patient's phone from glucose meters and pedometers via a wireless Bluetooth interface. Using the phone's touch screen, patients manually enter data about their eating and other behaviors with only a few touches to the relevant user interface screens. The accumulated data set is used to build statistical predictive models of patients' blood glucose levels. In addition to providing support directly to patients, the Few Touch application is also designed for collaborative use with their physicians or other health care providers.

We are currently exploring patients' and providers' perspectives on the design of Few Touch, emphasizing elicitation of their perspectives on design elements related to patient/provider collaborative use cases. Clinicians' view of how tools like these can be part of future health care, both from Seattle, USA and Tromsø, Norway, will be presented and discussed.

P-12

BRH SYSTEM—INCREASING BLOOD FLOW ULTRASOUND AND ELECTRIC CURRENT FIELDS (IBFUSEC) IMPROVES CLINICAL OUTCOME OF DIABETIC FOOT ULCERS

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Introduction: Diabetic ulcers are the most common cause of foot and leg amputation. 25% of diabetic individuals will develop chronic ulcers throughout their life. Approximately 14–24% of these individual's condition will lead to amputation.

The BRH system uses the combination of Low Intensity Ultrasound and Low Frequency Electric current fields. Thermal and non-thermal physical effects (resonance) of the combination of US and ECF increases blood flow, reduces muscle spasm and increases extensibility of collagen fibers and a pro-inflammatory response.

Methods: 8 patients with severe diabetic ulcers were included in this preliminary study. All patients were treated prior to affiliation for at least 6 months with systemic and local treatments such as debridement, antibiotics, hyperbaric oxygenation, vacuum systems and ozone therapy. During these months their wounds did not close. In the study, patients were treated, a one hour treatment, 3–4 times a week. Systemic and local ozone therapies were performed in conjunction with the study's treatments. Wounds parameters were photographed and measured by depth and surface area.

Results: The wounds of all 8 patients closed within 3–12 weeks. In two patients with a fistula from post-operative osteomyelitic lesions the wounds closed, fistula stopped draining, and no collection was detected with Ultrasound. 7 patients experienced dramatic pain improvement.

Conclusion: In this initial study the use of ***IBFUSEC-BRH system enabled rapid closure of complicated wounds which had previously failed to heal. Further prospective, randomized studies should be performed for assessing this method of wound healing.***

P-13

EXPERIENCE OF APPLICATION OF PUMP THERAPIES AND INSULIN ANALOGS IN HIGH-MOUNTAINOUS CONDITIONS OF EXPEDITION TO ELBRUS AT PATIENTS WITH T1D FROM UKRAINE (JULY–AUGUST 2010)

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Aims: Our experience of conducting patients with T1D in the conditions at altitude vs. intensive physical activities during expedition to mountain Elbrus (5642ì) has shown necessity rigidly to supervise glycemia and quickly in due time to correct a mode of introduction of insulin in need of. It successfully managed to achieve thanking uses insulin analogs and PUMP therapies.

Methods: In expedition have taken part 3 patients with T1D (age 21–25 years). The experience of diabetes are from 7 till 18 years. There were 2 patients on Pump of therapy Minimed 722 (0,5–2,5 years) and 1 patient used insulin analogs Lantus and Apidra.

Results: The period of preparation for expedition proceeded 6 months. Medical examination has been spent before the beginning of a stage of preparation and in 6 months. Indicators of ÍâÀ1ñ in January 2010 were within 7.0–8.5%, before a campaign of 6.2–7.7%. Intensive decrease in the expense of insulin at patients with T1D in the conditions of high mountains vs. intensive physical activities during expedition on mountain Elbrus was marked. So temp basal the mode on transitions decreased to 2–5%. Moderate signs of mountain illness in kind dyspepsia, psychological instability, lability glycemia were marked.

Conclusions: Conducting patients with T1D using insulin analogs and PUMP therapies has allowed to obtain good proof compensation T1D vs. an intensive mode of physical activities, successful carrying out of expedition to Elbrus at altitude. Our experience shows possibility of a healthy active way of life at patients with T1D.

P-14

OUR EXPERIENCE OF OMNIPOD SYSTEM COMPARED TO INSULIN INJECTIONS IN DIABETIC CHILDREN

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Background: OmniPod System used innovative design and materials and overcame a number of technological challenges to create a system that has all the functionality of a conventional insulin pump with no tubing. In pump therapy, a system such as the OmniPod delivers a constant stream of rapid-acting insulin through a tiny, flexible catheter called a cannula.

Aims: Because the optimal delivery rate changes from child to child, pediatrician can program the OmniPod System to deliver insulin at the basal rate that best matches insulin needs.

Methods: A prospective, open-label study measured the impact of short-term infusion set disconnects on glucose levels. It shows that even short-term interruption of insulin delivery can cause blood glucose levels to rise and remain elevated for hours post-interruption.

Results: Studies in children have demonstrated the advantages of insulin pump therapy over multiple daily insulin injections. These advantages include better glycemic control, fewer hypoglycemic events, reduced glycemic variability and improved quality of life in diabetic children.

Discussion: Even short-term interruption of insulin delivery can cause blood glucose levels to rise and remain elevated for hours post-interruption.

Conclusions: The PDM (Personal Diabetes Manager) wirelessly programs personalized insulin delivery, calculates suggested doses, and has a convenient, built-in FreeStyle blood glucose meter. The study showed that the OmniPod system demonstrated improvements in glycemic control with kids patients previously on MDI. The main advantage of pump therapy is the reduced instance of highs and lows in sick children.

P-15

SIGNAL-TO-NOISE CONSIDERATIONS FOR NON-INVASIVE GLUCOSE MEASUREMENT DEVICES

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Objective: Non-invasive glucose monitoring systems are a goal of diabetic treatment providers, and success in this area would significantly improve treatment options. These devices often reveal plausibly useful results in controlled laboratory testing conditions, but in practicing clinical environments do not perform satisfactorily well for treatment purposes. The challenge to measure in-vivo glucose concentrations with sufficient accuracy and precision is complicated by underlying physics, chemistry and physiology.

We present here our investigations to solving this problem from a physical chemical and physiological perspective with both direct (near-infrared spectroscopy) and indirect (impedance and dielectric spectroscopy) methodologies, in comparison to strip testing techniques.

Methods: We compare the major factors of variance in these sensing devices, including: environmental, physiological, chemical physical and computational factors, and measure their robustness in terms of downstream error and susceptibility to signal-to-noise. We analyse the capability of measuring glucose in terms of signal-to-noise relating to the relative abundance of glucose within the blood, including all other blood components.

Conclusions: We show that improvements in signal-to-noise in both direct and indirect measurements are necessary to robustly detect in-vivo glucose levels.

We also show that both direct and indirect techniques require an improvement in analyte measurement selectivity if performance matching test strips is to be made. Further we show that direct measurements have greater potential for achieving this in the near term.

We present a methodology for applying this principal to direct optical techniques, and show the capability to increase glucose measurement robustness and reduce algorithm complexity.

P-16

CONTINUOUS SUBCUTANEOUS INSULIN INFUSION IN TYPE 1 DIABETES MELLITUS PATIENTS WITH KIDNEY TRANSPLANT

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Introduction: The benefits of intensive management of diabetes have been well established in several studies. CSII is a treatment option in some type 1 diabetic patient. It is associated with A1c reduction, lower glicemic excursion, less hypoglicemia and better quality of life.

Objective: To evaluate the use of CSII in type 1 diabetic patients with kidney transplant.

Patients and methods: Patient I- male; 27 years old; diabetes duration: 22 years; kidney transplant duration: 50 months; intensive therapy with multiple daily insulin; A1c: 8.2%; CGMS identified "down phenomenom"; Double imunossupression (CyA and MMF). Patient II- female; 38 years old; diabetes duration: 34 years; kidney transplant duration: 111 months; intensive therapy with multiple daily insulin; A1c: 11.5%; CGMS identified important glicemic excursion. Frequent severe hypoglicemias. Double imunossupression (CyA and MMF). CSII was used in both cases.

Results: After six months of treatment, both patients present an excellent compliance to the device and a significant A1c decrease (patient I- 6.9%; patient II- 8.1%). No mechanical problem or local infection were reported. None had severe hypoglicemia or ketoacidosis. No weight gain was seen.

Conclusions: CSII is an effective therapy in intensive management of diabetes. It improves glicemic control and it is safe in imunusuppessed patients.

P-17

SELF MONITORING OF BLOOD GLUCOSE—A SURVEY OF DIABETES UK MEMBERS WITH TYPE 2 DIABETES WHO USE SMBG

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Background: Aim - to survey members of Diabetes UK who had Type 2 diabetes, and who used self monitoring of blood glucose (SMBG), to elicit their views on its usefulness in the management of their diabetes, and how they used the results. A questionnaire was developed for the DUK website. The questionnaire was posted on the Diabetes UK website until over 500 people had responded. Questions asked users to specify the benefits gained from SMBG, and how these benefits were achieved. We carried out both quantitative analysis and a thematic analysis for the open ended free-text questions.

Findings: 554 participants completed the survey, of whom 289 (52.2%) were male. 20% of respondents were recently diagnosed (<6 months). Frequency of SMBG varied, with 43% of participants testing between once and four times a day and 22% testing less than once a month or for occasional periods.

80% of respondents reported high satisfaction with SMBG, and reported feeling more 'in control' of their diabetes management using it. The most frequently reported use of SMBG was to make adjustments to food intake or confirm a hyperglycaemic episode.

Women were significantly more likely to report feelings of guilt or self-chastisement associated with out of range readings ($P \leq .001$).

Conclusion: SMBG was clearly of benefit to this group of confirmed users, who used the results to adjust diet, physical activity or medications. However many individuals (particularly women) reported feelings of anxiety and depression associated with its use.

P-18

CLINICAL PROFILE OF IMPAIRED FASTING GLUCOSE PATIENTS AT NORTHERN MINDANAO MEDICAL CENTER, PHILIPPINES: IMPLICATIONS FOR FILIPINOS

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Introduction: Diabetic patients go through a prediabetic stage characterized by IGT or IFG. The clinical profile of IFG among Filipinos has not been established.

Objectives: This study aims to describe the clinical profile of patients with impaired fasting glucose at Northern Mindanao Medical Center, Cagayan de Oro City, Philippines from January 2004 to December 2007 and to determine associations between the levels of fasting blood sugar and body mass index, hypertension, and family history of diabetes.

Methods: This study uses chart review in the out patient section of the Family Medicine clinic. This is a retrospective study using percentages, mean and its standard deviation, and χ^2 ($P = 0.05$) to detect associations.

Results: There were 302 patients diagnosed with impaired fasting glucose. All were Filipinos between 18 to 84years old (55.94 SD $\pm$ 13years). There were more females than males. Mean BMI was 23.87 SD $\pm$ 4.44 kg/m^2 while the mean fasting blood sugar was 111.6 SD $\pm$ 7.23 mg/dL. There was no association between fasting blood sugar levels and obesity ($\chi^2 = 0.73$) and hypertension ($\chi^2 = 1.83$) in this study. However, FBS > 113 mg/dL was strongly associated with the presence of family history of diabetes in this population ($\chi^2 = 6.98$).

Conclusions: IFG can occur to young, non-obese patients with family history of diabetes. This study recommends that screening for impaired fasting glucose among Filipinos should be done to all patients with strong family history of diabetes regardless of age, gender, BMI status and hypertension. In this manner, there is earlier detection of this illness hence, early lifestyle intervention and management can be pursued.

P-19

THE EFFECT OF LOW-LEVEL LASER THERAPY ON BONE IN DIABETIC AND NON-DIABETIC RATS

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Low-level laser therapy(LLLT) has been found to accelerate fracture healing in animals. Diabetes mellitus decreases bone volume and its biomechanical parameters.

The aim of the present study was to examine the effects of LLLT on the tibia of streptozotocin-induced diabetic (STZ-D) rats.

Twenty rats were divided randomly into four groups. Rats in the first two groups were administrated a single injection of STZ to induce diabetes, while animals in groups 3 and 4 were given a sham injection of distilled water. The right tibia in groups 1 and 2 was treated with a He-Ne laser (632.8 nm, 10 mW) of 28.6 and 382.2 J/cm^2, respectively. LLLT was performed daily for 14 consecutive days. The right tibia of rats in group 3 was treated with LLLT the same as group 2. The right tibia of rats in group 4 was used for based line studies. After 14 d, right tibiae and left tibiae (control bone) were extracted and subjected to the three-point bending test and histological study.

Maximum force (N) was significantly greater in laser-treated bones of groups 2 and 3 compared with their relevant control groups (paired Student t test, $P = 0.05$ and $P = 0.007$, respectively). Density of the bone lamella meshwork of compact bone in group 2 was significantly greater in comparison with its control group (paired Student t test, $P = 0.005$).

LLLT on tibia of STZ-D rats increased the bone lamella meshwork density of compact bone and also increased its strength.

P-20

MODULATION OF GLUCOSE UPTAKE MECHANISM OF METFORMIN

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Diabetes represents a spectrum of metabolic disorders, which has become a major health challenge worldwide. Metformin has shorter half-life and rapid clearance from kidney and may results into lactic acidosis. It is reported that metformin is mainly metabolised by CYP3A4 and rabeprazole is inhibitor of CYP3A4. In the present study, the influence of rabeprazole on the glucose uptake mechanism in presence of metformin in alloxan induced diabetic rat was studied by everted intestinal sac model. Rabeprazole (0.062–0.25 mg/mL) + metformin (0.25 mg/mL) in combination and metformin (0.25 mg/mL) alone added to the mucosal solution. Glucose concentrations of mucosal disappearance, serosal appearance and gut wall content were determined before and after incubation. Results showed significant decrease in glucose transport across gut membrane in presence of rabeprazole (0.13 mg/mL) + metformin (0.25 mg/mL) in combination in comparison to metformin (0.25 mg/mL) alone. The study indicated that rabeprazole sodium has significantly enhanced the glucose uptake effect of metformin hydrochloride.

P-21

A NEW CONTROL MODEL FOR CLOSED-LOOP ALGORITHMS IN TYPE 1 DIABETES

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Introduction: In this contribution, we propose a new control model representing accurately the plasma glucose-insulin dynamics in T1DM.

Methods: The proposed model is in the form of a nonlinear system of three time-continuous state equations and is not expressed in terms of variations around basal values. Its design is defined by two features. First, two successive remote compartments for insulin are introduced in order to account for slow and fast dynamics. Second, the insulin action in glucose disappearance is modeled through an original nonlinear form. This form is chosen so that the mathematical equilibrium relation of the control model is consistent with observed equilibrium points.

Results: The ten adults of the UVa T1DM simulator are used to generate our data and three experiments are performed in order to cross validate the estimated models. The table Results present the mean fit obtained for each adult for the parametric identification (global mean 96%) and the validation (global mean 82%). This indicates clearly satisfying results. The proposed model seems accurate.

Results of the New Control Model

Subjects	*(Identification) Mean fit*	*(Identification) Std Dev fit*	*(Cross Validation) Mean fit*	*(Cross Validation) Std Dev fit*
Adult 1	97%	1%	82%	8%
Adult 2	97%	1%	80%	7%
Adult 4	96%	4%	86%	8%
Adult 6	97%	3%	65%	11%
Adult 7	96%	4%	85%	7%
Adult 8	94%	3%	78%	8%
All Ten Adults	96%	1%	82%	7%

P-22

DUODENAL ELECTRICAL STIMULATION FOR THE TREATMENT OF TYPE 2 DIABETES—PRELIMINARY SAFETY AND EFFICACY RESULTS IN HUMAN

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Diabetes Mellitus is an increasingly global epidemic incidence from which millions of people suffer worldwide. Gastrointestinal electrical stimulation utilized to treat gastroparesis and obesity, has been also suggested for the treatment of diabetes mellitus. Therefore, the aim of the current study has been to investigate the effect of electrical stimulation on human intestine and the following effect on glycemic control in type 2 diabetic patients. Safety and efficacy of such therapeutic procedures are being evaluated also in obese type 2 diabetic patients. Submucosal electrodes implanted in the duodenum wall, proximal to the pylorus with minimal surgical procedure were connected to pulse generator implanted subcutaneously. Following each meal, the pulse generator sends programmed electrical impulses to the electrodes within the duodenal wall. Preclinical studies have demonstrated that duodenal electrical stimulation decreases postprandial blood glucose levels which probably induced due to delayed gastric emptying and increased duodenal flow rate. Preliminary results indicate that duodenal electrical stimulation is safe and had no serious adverse events. Moreover, duodenal electrical stimulation does improve glycemic control in type 2 diabetic patients, as measured by fasting blood glucose, HbA1c and continuous glucose monitoring technique. Weight loss in these patients was also noted as a major phenomenon.

P-23

METFORMIN AND GESTATIONAL DIABETES MELLITUS: MATERNAL FACTORS RELATED TO SUCCESSFUL TREATMENT

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Objective: To identify maternal characteristics related to successful treatment of gestational diabetes mellitus (GDM) with metformin.

Methods: Retrospective descriptive study, conducted from July 2008 until September 2010, which included 104 pregnant

women with GDM who required adjunctive therapy to diet and physical activity. Metformin was maintained until delivery if glycemic control was obtained and fetal abdominal circumference (FAC) < 90% and was thus considered a therapeutic success. In the absence of glucose control or FAC ≥ 90% was added to insulin therapy. The women were divided into two groups: one that succeeded with the initial therapy (n = 82) and another failure (n = 22). The epidemiological characteristics of the two groups were compared.

Results: No difference was found ($P > 0.05$) in the two groups regarding maternal age (32.42 vs. 32.38, $P = 0.96$), number of previous pregnancies (2.73 vs. 3.19, $P = 0.13$), weight gain during pregnancy (7.30 vs. 5.38, $P = 0.24$), 2-hour 75 g oral glucose tolerance test (OGTT) (164.15 vs. 167.15, $P = 0.56$). Differences was found ($P \leq 0.05$) in the gestational age at inclusion (27.57 vs. 24.33, $P = 0.04$), maternal body mass index (BMI) (27.64 vs. 31.62, $P < 0.01$) and fasting glucose in the OGTT (91.56 vs. 108.09, $P < 0.01$) among the patients who were successful and those who required insulin supplement.

Conclusions: Pregnant women who have achieved success with metformin had a gestational age later at enrollment, lower body mass index and lower fasting glucose in oral tolerance test.

P-24

COMPARATIVE STUDY BETWEEN METFORMIN AND GLIBENCLAMIDE IN THE TREATMENT OF GESTATIONAL DIABETES MELLITUS

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Objective: To evaluate the perinatal impact of metformin and glibenclamide in the treatment of gestational diabetes mellitus (GDM).

Methods: Randomized clinical trial conducted from July 2008 until September 2010, including 200 pregnant women with GDM who required adjunctive therapy to diet and physical activity. Patients were randomized to use metformin (n = 104) or glibenclamide (n = 96). The drugs were replaced by insulin when they reached the maximum dose without glycemic control. Assessed outcomes: weight and neonatal blood glucose.

Results: No difference was found ($P > 0.05$) in the groups regarding maternal age, gestational age at inclusion, body mass index, glucose levels in OGTT 75 g and glycemic control. Difference was found in the number of previous pregnancies (2.84 vs. 2.47 $P = 0.04$) and weight gain during pregnancy (7.78 vs. 9.84 $P = 0.04$) in the metformin group and glibenclamide respectively. The perinatal results showed no difference ($P > 0.05$) in the percentage of cesarean delivery, gestational age at delivery, number of LGA newborns, neonatal hypoglycemia, admission to intensive care unit and perinatal death. We found differences in weight (3193 g vs 3387 g $P = 0.01$) and ponderal index (2.87 vs 2.96 $P = 0.05$) of newborns, as well as in neonatal blood glucose levels in the 1st (59.78 vs 54.08 $P = 0.01$) and 3rd hour (61.53 vs 55.89 $P = 0.01$) after birth among metformin and glibenclamide respectively.

Conclusions: Weight, ponderal index and glucose levels (1st and 3rd hours after birth) were lower in the newborns of the metformin group.

P-25

A COMPARATIVE STUDY OF METHANOL EXTRACTS OF *ALLIUM CEPA* AND *ALLIUM SATIVUM* IN DIABETIC NEUROPATHY IN MICE

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Diabetic Neuropathy (DN) is the major complication of uncontrolled diabetes. The exact pathophysiological mechanism by which nerves are damaged in DN is controversial but prolonged hyperglycaemia is an accepted primary causative mechanism. One of the mechanisms by which hyperglycemia causes neural degeneration is via the increased oxidative stress that accompanies diabetes. Hence plants with strong antioxidant constituents play an important role in improvement of diabetes and its complications. The aim of the present study is to evaluate the effect methanolic extracts of outer scales and edible portion of *Allium cepa* and *Allium sativum* in diabetic neuropathy in mice. Tail immersion assay was employed for the assessment of DN. STZ-diabetic mice exhibited significant hyperalgesia along with increased plasma glucose and decreased body weight as compared with control group. Treatment with methanolic extracts of outer scales and edible portion of *A. cepa* and *A. sativum* (200 mg/kg, p.o.) once daily for 14 days after onset of DN, demonstrated significant attenuation of tail immersion latency time, prevented loss in body weight, decreased plasma glucose level, increased GSH level, decreased serum nitrite and TBARS level as compared with diabetic control group. Of the four extracts, methanolic extract of outer scales of onion has shown most significant improvement. This may be due to higher phenolic compounds present in outer scales of *A. cepa*. Results indicate that *A. cepa* may be employed for effective management of diabetic neuropathy.

P-26

COMPENSATION FOR HAEMATOCRIT INTERFERENCE EFFECTS USING THE ONE TOUCH VERIO BLOOD GLUCOSE MEASUREMENT SYSTEM

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Self monitoring of blood glucose (SMBG) is a valuable tool for individuals with diabetes. Haematocrit is a confounding factor in accurate monitoring, where low and high haematocrit can give rise to falsely elevated and lowered readings respectively.

Many approaches have been developed to counteract haematocrit induced error: On-strip blood filtration, sample chamber fill time, on strip capacitance measurements or, in electrochemical systems, use of additional electrodes. Such approaches add cost and complexity.

The electrochemical OneTouch Verio system employs an alternative approach making use of specific features of the transient response profile (current-time relationship). Increasing levels of haematocrit can lead to measurable changes in the transient (Fig. 1) that can be analysed by on-meter software to

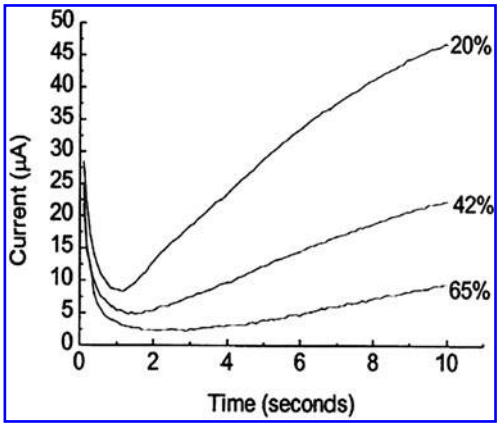

FIG. 1. Effect of haematocrit on transients.

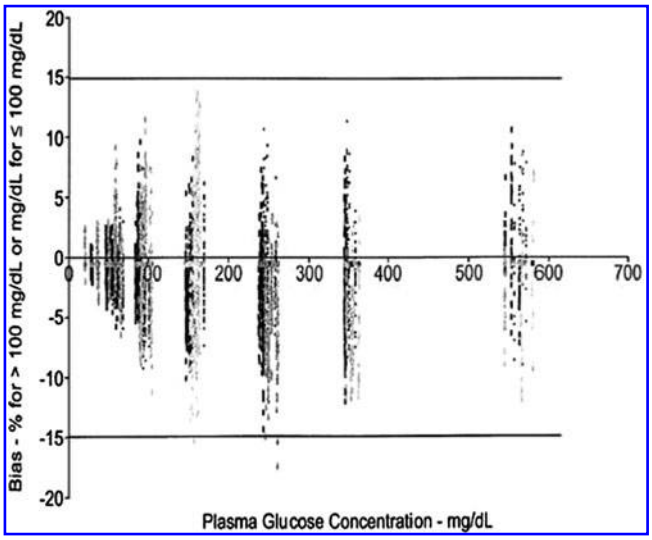

FIG. 2. Haematocrit-insensitive results.

provide a 'haematocrit corrected' glucose value. Performance may be enhanced by application of "complex waveforms" that vary electrode potentials at different times in the measurement process.

The One Touch Verio system employs a novel SMBG strip architecture and potential-time waveform that allows sophisticated compensation for haematocrit. The sensor assembly comprises co-facial thin film gold and palladium electrodes separated by a thin spacer material, forming a small volume sample chamber that fills by capillarity. The applied waveform consists of three phases in which positive and negative voltages are applied. Several current measurements are combined within an algorithm, to produce meter results that are accurate measurements of plasma glucose concentrations, and are insensitive to the haematocrit effect (Fig. 2).

P-27

AN INNOVATIVE TOOL OF DECISION SUPPORT SYSTEM IN DEVELOPMENT PROGRAM OF FOOD PRODUCTS FOR DIABETES TREATMENT

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Thai herbs and local vegetables have traditionally been used as medicine and consumed in local meals for diabetes risk reduction and treatment. The informative data, particularly, their uses and properties related with health benefits can lead to application of such herbs and local vegetables in food product development for diabetes treatment. Therefore, this study was aimed to create the decision support system in development of Thai food product for diabetes treatment program (DFDSS) incorporated with database system of indigenous knowledge on uses and nutritional values of Thai herbs and local vegetables. The specified models were then designed for predicting the correlation and changes in inhibitory activity against carbohydrate digestive enzymes, consequently blood glucose absorption delay by food products produced from Thai herbs and local vegetables. All data provided in the database, including physico-chemical properties of Thai herbs and local vegetables and designed models were used to develop the DFDSS. The DFDSS software can assist users in optimal selection of feasible vegetable and herbs associated with its potential in carbohydrate digestive enzyme inhibition and appropriate production process in development of food product. It can be an effective tool widely applied for food product developer, diabetes patients, persons at risk of diabetes, and others with optimal and cost-effective. Additionally, this DFDSS is an innovative tool that exploits the indigenous knowledge to drive the development of food product for diabetes risk reduction.

P-28

CHANGING THERAPY FROM MDII TO CSII IN CHILDREN WTIH TYPE 1 DIABETES— EVALUATION OF OWN STRUCTURED EDUCATIONAL PROGRAMME IN THE THREE MONTHS OBSERVATION PERIOD

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Background: Pump therapy in diabetes requires versatile education, because the rules of nutrition and dosing insulin are different and complicated as compared to MDII method.

The aim of this study was to assess our own educational programme for new pump users by means of analyzing patients satisfaction and to check practical knowledge directly after the training and three months later.

Material and methods: We evaluated 46 diabetic children, aged 9.7 yrs. Children were admitted to hospital in the aim of changing therapy from MDII to CSII, combined with 5 days educational programme. We analysed three parts questionnaire that concerned: I - period before pump therapy, II - evaluation of the educational programme and family knowledge just after the hospitalization, III - evaluation of family knowledge three months later.

Results: While being on MDII therapy majority of patients consumed regularly 6 meals daily, and to eating "forbidden" meals admitted 36%. Practical test directly after the course re-

vealed satisfactory skills of the families as regards all aspects of new method. However, test repeated three months later demonstrated improper counting of food units (34% vs 19%). 62% patients admitted to completely irregular eating, and 100% admitted frequently eating "forbidden" food. HbA1c level did not change.

Conclusions: The study revealed the vast need to increase educational time for nutrition rules, and the great need of methodical, early reeducation. Diet rules, applied too freely, together with poor, and forgotten knowledge of adjusting insulin doses resulted in the lack of improvement of metabolic control.

P-29

INSULIN THERAPY WITH INSULIN PUMP IN 1 MONTH OLD BOY WITH PERMANENT NEONATAL DIABETES MELLITUS

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Background: Insulin pump therapy is alternative method to insulin therapy with pens in children with diabetes mellitus. In infants and newborn with diabetes mellitus it is the only acceptable method of treatment with insulin.

Aim of the study was to present insulin therapy with continuous subcutaneous insulin infusion by personal insulin pump in 1 month old infant with permanent newborn diabetes mellitus.

Material: Newborn in 28 day of his life was admitted to Department of Paediatric Diabetology in Medical University of Gdansk with newly diagnosed of diabetes mellitus. He was in bad general condition with severe ketoacidosis. After three days of intravenous therapy with insulin and fluids, insulin pump was connected. In first three days of insulin pump theraphy requirement for insulin was over 1 unit/kg/day and it was difficult to achieve normoglycemia. From fourth day, insulin dose decreased to 0.8–0.9 unit/kg/day and good glycemia control was achieved. After one month of treatment daily insulin dose was 0.7 unit /kg. Baby is developing normally and gaining weight properly. Genetic tests were done and mutation in gene of Kir 6.2 was diagnosed.

In near future sulfonylurea will be given to the infant.

Conclusion: In this case therapy with insulin pump was the only possible and acceptable therapy after diagnosis of diabetes mellitus in infant.

The pump ensured the proper development of the child.

P-30

THE ACCURACY OF REAL-TIME CONTINUOUS GLUCOSE MONITORING SENSOR IN PATIENTS WITH TYPE 1 DIABETES DURING A PHYSICAL ACTIVITY

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Introduction: The aim of the study was to determine accuracy of Medtronic Diabetes Real-Time (RT)-Continuous Glucose Monitoring (CGM) sensor during a physical activity.

Methods: 9 patients with Type 1 DM used CGM sensor for 4 days. Physical exercise was performed in the second day using an electrically braked ergometer (Ergometrics 800s; Ergoline) at the target heart rate according to 50% of individual heart rate reserve, calculated following the Karvonen equation. Exercise was terminated when hypoglycemia or it's symptoms occured. The sensor data were compared to contemporaneous glycemic values of arterialized blood obtained in approximately 5 minute intervals.

Results: We evaluated interval defined by patient's physical load. The mean absolute relative difference for all sensors was 28.39%. Maximum absolute difference was 5.66 mmol/L. Paired glucose values from RT-CGM and arterialized blood demonstrated that 84.2% of paired points fell in zones A and B, 14.8% in Zone D, none in zone C and E of Clarke Error Grid.

Conclusion: Continuous glucose monitoring during a physical activity has limited accuracy and ability to detect hypoglycemia. This should be mentioned in CGM education.

P-31

THE RISK OF HYPOGLYCEMIA AND HYPOGLYCEMIA PREVENTION IN TYPE 1 DIABETICS TREATED WITH CSII DURING DRIVING—THE RESULTS OF CGM MONITORING AND GRAVELLING'S QUESTIONAIRE

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Introduction: Hypoglycaemic episode during driving could cause a fatal incident. Using a continuos glucose monitoring in type 1 diabetic patients we wanted to determine the glycaemic excursions during periods of driving.

Methods: We monitored 22 patients with type 1 diabetes mellitus treated with CSII (6 female, 16 men), duration of disease 11.8 ± 5.2 year, duration of treatment 4.5 ± 1.72 year. Each patient wore CGMS for 3–5 working days during his normal activity and was not alowed to see actual glycaemic values. Patients were asked to record all important events (such as insulin injection, exercise, meals, working periods) including periods of a car driving. After CMGS use, continuous glucose profiles were reviewed to identify glycaemic excursion during periods of car driving with a special interest in hypoglycemic episodes (values under 3.5 mmol/L) and periods of glycaemias under 4.5 mmol/L with considerable risk of hypoglycaemia.

Results: We evaluated 4882 min (81 hours 22 min) of driving, an average 73.9 ± 33.6 per day and patient. Patients recorded 4 symptomatic episodes while driving. We also found 11 episodes of asymptomatic hypoglycaemias, all in 4 patients. Total duration of driving with asymptomatic hpoglycaemia was 42 min (0.88% of total driving time). Total duration of period with glycaemia between 3.5–4.5 mmol/L was 196 min (4.1% of total driving time).

Conclusion: Risk of hypoglycemia even in well experienced patients with type 1 diabetes mellitus treated with CSII during driving is considerable and this theme should be regulary focused in the education.

P-32

UTILITY OF "DOWNLOAD SYSTEMS" IN A PEDIATRIC DIABETES CENTRE

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Background: Having a 'good' control measured by glycated hemoglobin (HbA1c $< 7.5\%$) seems to be insufficient to reduce cardiovascular risk in T1DM patients. Recently also in pediatric age it appears to be interesting to investigate the variability of glucose values in each day by means of modern technologies.

Purpose: To evaluate the usefulness of download of the glucose profile by the modern technologies in the management of T1DM pediatric patients.

Methods: We evaluated 52 patients (treated with insulin pump) at 3 and 6 months. In 31 we considered only the diary glucose levels (Group A); in 21 also the glucose levels data are downloaded (Smart pix, Care Link, Diasend, Ez Manager) during to the control visit (Group B). Weight, height, BMI, insulin dose/kg, HbA1c, blood glucose levels (mean and standard deviation) were evaluated at 3 and 6 months.

Results: in group A HbA1c ($7{,}7 \pm 0{,}84$) remained unchanged (7.82 ± 0.54 NS; 7.75 ± 0.79 NS, respectively). On the contrary Group B showed a significant reduction of HbA1c and of blood glucose (mean and standard deviation) both at 3 and 6 months (table).

Conclusion: data download could improve metabolic control and glucose variability.

	T0	*3 months*	*6 months*	
BMI Z-score	0.52 ± 0.71	0.59 ± 0.8	0.56 ± 0.61	NS
Ins UI/Kg	0.78 ± 0.2	0.82 ± 0.26	0.80 ± 0.25	NS
HbA1c (%)	8.42 ± 0.82	8.26 ± 0.51	7.87 ± 0.54	$P < 0.005$
Average glucose	189 ± 40	161 ± 25	155 ± 20	$P = 0.008$
SD glucose	83 ± 20	78 ± 18	71 ± 18	$P < 0.005$

P-33

C-REACTIVE PROTEINS—NON INVASIVE METHOD FOR DETECTION OF EARLY-STAGE ATHEROSCLEROSIS RISK IN RECENTLY DIAGNOSED TYPE 1 DIABETIC CHILDREN

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Introduction: Cardiovascular (CVD) disease is a leading cause of mortality and morbidity in diabetes mellitus. Recent studies indicate that the atherosclerosis slowly but surely altered from a model of chronic degenerative disease entirely affecting patients with advanced age to a model of subclinical chronic inflammatory disease even present in childhood. Diabetes mellitus is a risk factor for atherosclerosis and asymptomatic low grade inflammation occurs prior to unconcealed vascular lesions in these patients. A low grade inflammation can be determined by serum C-Reactive Proteins.

Objective: C-reactive proteins (CRP) has been a widely used inflammatory marker for cardiovascular disease because its plasma concentration is easy to determine. The aim of this study was to evaluate serum CRP levels in recently diagnosed (duration of diabetes <two years) type 1 diabetic children to foresee early cardiovascular complications without employ any invasive procedure.

Methodology: This was a prospective study. The serum CRP levels were determined in 39 diabetic children and 40 age-sex matched healthy volunteers as control. CRP in concentrations was determined by ELISA by an automated ELISA analyzer. The values were expressed as mean $\pm$ standard deviation and data from patients and controls was compared using Student's-t test.

Results: Serum CRP levels were significantly elevated in diabetic children as compared to controls ($P < 0.001$).

Conclusion: Assessment of serum CRP can be used as a potent non invasive method in addition to other traditional risk factors like dyslipidemia, hypertension, obesity and smoking to detect high risk diabetic patients for subclinical atherosclerosis.

P-34

SIMULTANEOUS USE OF TWO MULTISENSORS ON THE LEFT AND RIGHT ARM FOR NON INVASIVE CONTINUOUS GLUCOSE MONITORING

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We have previously reported about the findings in clinical-experimental studies with a novel Multisensor system for non invasive continuous glucose monitoring. In this study a fully integrated Multisensor version was experimentally tested, investigating location related measurement characteristics.

Four T1DM patients (age 43 ± 9 y; BMI $24.5 \pm 3.7\,kg/m^2$, duration of diabetes 22 ± 11 y; HbA1c $7.7 \pm 0.5\%$) performed 4 in-clinic study days with a Multisensor attached to the left and right upper arm. As a result, 32 datasets from 16 study days were obtained. Different data evaluation routines were applied to the Multisensor data to obtain global (identical coefficients) and personal (personal coefficients) models (Table 1).

Figure 1 shows all 32 glucose profiles obtained during the 16 study days, using the global model with a prospective initial baseline calibration (CEG A 44.9, B 48.0, C 3.6, D 3.0, E 0.5%).

Dynamics of glucose time courses estimated by the Multisensors from the two arms are repeatably comparable, even with a global model with one initial baseline calibration only. This indicates that the sensor signal characteristics are robust enough to allow changing from one arm to the other using the same device settings and calibration.

Table 1. Different Models and Evaluation Routines

Model & type of Baseline adjustment	*Average R^2*	*MAD [mg/dL]*	*MARD [%]*
Global model initial Baseline	0.76	47	32.3
Global model full Baseline	0.75	29.9	21.3
Personal model initial Baseline	0.85	43.3	30.7
Personal model full Baseline	0.84	24.1	17.6

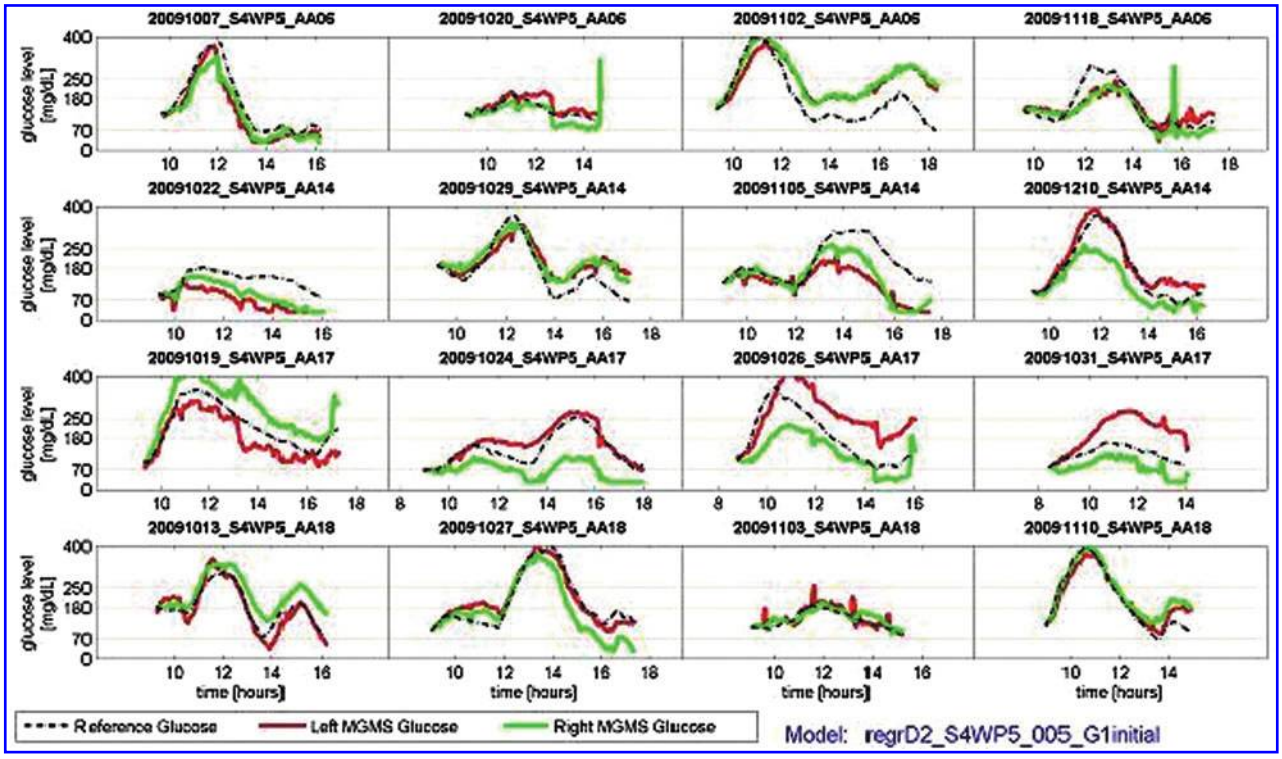

FIG. 1. All 32 predicted glucose profiles.

P-35

A TELEMEDICINE SYSTEM FOR EVALUATING ARTIFICIAL PANCREAS SYSTEMS AND TRAINING PATIENTS

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The latest achievements in bio-technologies are making available Continuous Glucose Monitoring (CGM) sensors, and let envision a true closed-loop therapeutic scheme, known as an Artificial Pancreas (AP), whose control replicates the process occurring in healthy individuals.

Besides CGM sensors and pumps, APs also require a control unit for processing BGL readings and driving the pump according to a suitable model of glucose absorption. Given the criticalities related with using those systems, extensive stress tests are required before they can be applied to ordinary human treatment. But even when APs will be in regular use, kickoff periods will be advisable for properly tuning the unit and training patients.

To support the treating staff in accomplishing those tasks we exploited a generic telemedicine architecture previously designed by us, based on the separation between services and devices, for implementing customizable real-time monitoring on patient data.

More specifically, the possibility of assembling different services upon various devices has been exploited for the integration of monitoring services on top of AP units provided by others. Those enhanced AP units are able to send data to a Hospital Agent building up the patient EHR. Additional services have been deployed on the Hospital Agent for the immediate detection of criticalities, and their notifications to the relevant actor devices. The platform is complemented by a service allowing the presentation of data through a web interface. This can be used by the treating staff to monitor the closed-loop sessions of their patients through data visualizations and charting.

P-36

INDIVIDUALS WITH TYPE 2 DIABETES ARE ABLE AND WILLING TO PERFORM AND ACCURATELY DOCUMENT GLUCOSE MONITORING USING A 7-POINT PROFILE REGIMEN

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Aim: The Accu-Chek® 360° View Blood Glucose Analysis System (Tool) is an easy-to-use paper form that facilitates collection/interpretation of 7-point glucose profiles over 3 consecutive days. We assessed the willingness/ability of type 2 diabetes patients to accurately complete the Tool within a clinical setting.

Methods: We analyzed data from completed Tools generated by type 2 diabetic subjects who used the Tool over 12 months as part of a large clinical trial. We assessed patient accuracy within key parameters: overall error rate; documentation of date of testing, time of testing and blood glucose (BG) value; and placement of "X" in Tool grid. Per protocol (PP) analyses were performed. Median error rate for each subject across all five study visits was used for the analyses.

Results: Data from 102 randomly selected subjects were analyzed. PP analysis showed a significantly greater error rate in non-adherent subjects ($n = 37$) in median total errors compared

with adherent subjects (n = 65): 23.6% (29.4) vs. 4.5% (12.9); $P < 0.0001$. Incorrect entries and entries left blank were significantly higher among non-adherent subjects compared with adherent subjects: 3.2% (5.5) vs. 1.5% (3.0); $P = 0.0056$ and 15.0% (29.0) vs. 1.5% (6.1); $P < 0.0001$, respectively.

Conclusions: Our analyses suggest that the majority of patients with poorly-controlled type 2 diabetes can accurately complete the Tool as prescribed and are willing to perform 7-point profile BG testing over 3 consecutive days. Patients who are motivated to perform this type of structured testing tend to be more accurate and complete in documenting their test results.

P-37

A MODEL OF GLUCOSE KINETICS WITH IMPROVED PERFORMANCE IN HYPOGLYCEMIA

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We propose a model of glucose kinetics in type 1 diabetes mellitus (T1DM) that accounts for the nonlinear insulin action across the blood glucose (BG) range previously reported in (Chan 2010).

Fifty hyperinsulinemic euglycemic and hypoglycemic clamps were performed in T1DM subjects. BG, insulin and dextrose injections were collected every 5 min. The proposed model is partially based on an accepted glucose-insulin model (Dalla Man 2007) and added with an exponential term to account for the hypoglycemic effect. It includes a linear insulin-independent glucose utilization and a nonlinear insulin-dependent glucose utilization represented by the sum of an exponential and a Michaelis-Menten equation. Using the Akaike Information Criterion (AIC) and mean squared error (MSE), we compared the "full model" with a "reduced model" (exponential term removed).

Both models were successfully fitted to the clamps. The full model outperformed the reduced model overall (AIC: 121.9 ± 33.2 vs 126.5 ± 29.3, $P = 0.052$), and showed dramatic improvement for BG < 80 mg/dL (MSE: 9.9 ± 10.9 vs 15.5 ± 19.6, $P = 0.029$) (Figure 1).

Accounting for the nonlinearity of insulin action improves the modeling of glucose kinetics in T1DM. Although not fitted in hyperglycemia, the model is based on a model validated in hyperglycemia. Improved accuracy in hypoglycemia will greatly benefit CGM-pump safety systems.

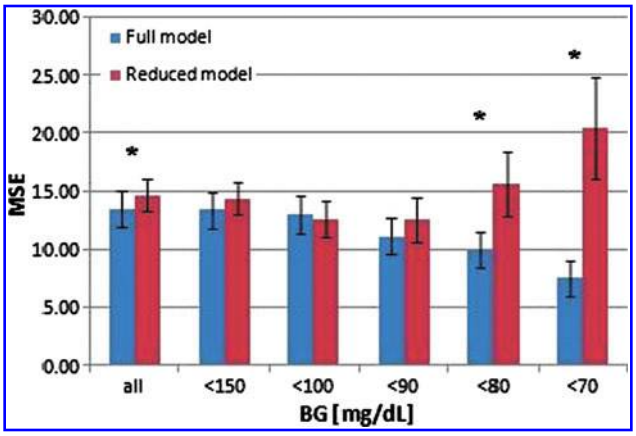

FIG. 1.

P-38

OPTICAL MUELLER MATRIX METHOD OF DETECTING GLUCOSE LEVEL

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Diabetes Mellitus has probably been known to medical science longer than any other hereditary metabolic disease. It is a complex group of syndromes that results in an elevated blood sugar which causes severe complications including blindness, cardiac and kidney failure. Intensive therapy and frequent glucose testing is the best possible approach to control the devastating complications like heart disease, blindness, kidney failure or amputations arises due to the disease. With the advances in diagnostic technology next generation diagnostics of bloodless, painless, accurate glucose instruments are replacing the present day detection techniques which are painful time consuming and expensive. This paper presents a blood less, painless optical scheme for blood glucose sensing. Optical Mueller matrix is an approach to analyze the sugar concentration in solution having different concentration which in turn may be a all optical approach for detecting blood sugar level of human body. The basis of this optical approach is that the when a highly coherent monochromatic linearly polarized light is allowed to fall on a solution having optical active substance the plane of polarization is rotated and the rotation is proportional to the concentration of the optical substance in the solution.

P-39

METHOD OF MYOCARDIAL PRECONDITIONING DURING REPEATED EXERCISE TESTS IN PATIENTS WITH MYOCARDIAL INFARCTION AND TYPE 2 DIABETES MELLITUS

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Study aim: To study the effect of early started repeated Treadmil exercise tests in patients with repeated exercise tests.

Design: Open, randomized study on 124 MI and type 2 DM patients, 52.3 ± 3.6 years old. Patients were on 5–7 days of MI and received a basic cardiovascular and dietary therapy were randomized to two treatment groups at 1:1 ratio. Control group (CG) - received a standard therapy and standard physical rehabilitations; Training group (TG) - received a standard therapy and 10 repeated exercise tests twice a day.

Study results: ST depression did not differ significantly in both groups at the beginning. However after 10 days in TG times for onset of ischemic changes (228 ± 94 sec vs 365 ± 103 sec, $P = 0.01$) and appearance of angina (282 ± 153 sec vs 428 ± 177 sec, $P = 0.04$) were observed. In CG ST depression characteristics were not significantly changed (238 ± 96 sec vs 265 ± 99 sec) and appearance of angina (276 ± 148 sec vs 293 ± 153 sec) were observed.

Physical activity tolerance were increase significantly in TG (42%), and increase not significantly in CG (8%).

Patients in TG had significantly less duration hospitalizations 15.1 ± 2.3 days and 18.6 ± 2.2 days in the comparison group.

Total of 48 hospitalizations occurred during the follow-up period, 15 in the TG and 33 in the CG. TG had significantly less hospitalizations due to cardiovascular cause (7.5% vs 37%).

Conclusion: Repeated exercise tests in patients with type 2 DM and MI decrease myocardial ischemia, duration of hospi-

talizations and number of cardiovascular events during 12 month after MI.

P-40

ENHANCING COMPLIANCE, QUALITY OF LIFE AND BMI IN PATIENTS WITH ARTERIAL HYPERTENSION, OBESITY AND COGNITIVE DISORDERS

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Aim: To evaluate the efficiency of pronoran as a part of cardiovascular therapy including methods of interactive education, in patients with mild cognitive disorders, arterial hypertension and obesity.

Design: Open, randomized study in 58 patients with arterial hypertension, obesity and mild cognitive disorders. Patients received a basic cardiovascular and dietary therapy and were randomized to two groups at 1:1 ratio. Group 1 underwent the interactive education in combination with pronoran 50 mg per day; group 2 underwent the interactive education.

Study results: Total of 62 hospitalizations occurred during t12 month, 16 in the pronoran group and 46 in the comparison group. Hospitalizations for CV conditions made up 34% of the total number of hospitalizations in the pronoran group and 72% in the comparison group. Incidence of calls for medical aid due to exacerbations CV disease was 37 cases in the pronoral group and 87 cases in the comparison group.

After 12 months of follow-up, compliance to medicines was 85% in the pronoral group and 60% in the comparison group. Compliance to the non-drug treatment was 60% and 9% in groups 1 and 2, respectively.

At baseline BMI level was $35.4 \pm 1.3\,kg/m^2$ and $35.3 \pm 1.5\,kg/m^2$ in groups 1 and 2, respectively. After 12 months of follow-up, BMI was $28.4 \pm 2.3\,kg/m^2$ and $32.4 \pm 2.2\,kg/m^2$ in groups 1 and 2, respectively.

Conclusion: Education of patients with mild cognitive impairment associated with arterial hypertension and obesity receiving pronoran resulted in improvements of cardiovascular disease.

P-41

PLASMA GLUCOSE CONCENTRATIONS ON NEW GLUCOMETER CALLA STRONGLY CORRELATE WITH LABORATORY ANALYSER COBAS INTEGRA 400 PLUS UNDER VARIOUS CLINICAL CONDITIONS

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Background and aims: Recently, the accuracy and precision of the glucometers CALLA, Wellion, Austria, was demonstrated under standardized laboratory conditions. The purpose of this clinical trial was to assess the correlation of results and relative variability of differences in glucose concentrations estimated from fingerprick plasma (1) on glucometer CALLA and (2) on the analyzer COBAS INTEGRA 400 PLUS using hexokinase reference method.

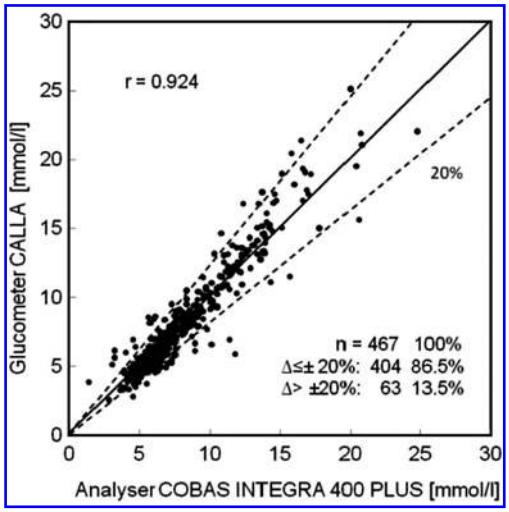

FIG. 1. Correlation between P-glucose on CALLA and INTEGRA.

Methods: Four glucometers Calla Light were used by 40 nurses in four wards (Group 1 to 4) at different times of the day in 37 in-patients. One glucometer was operated in laboratory

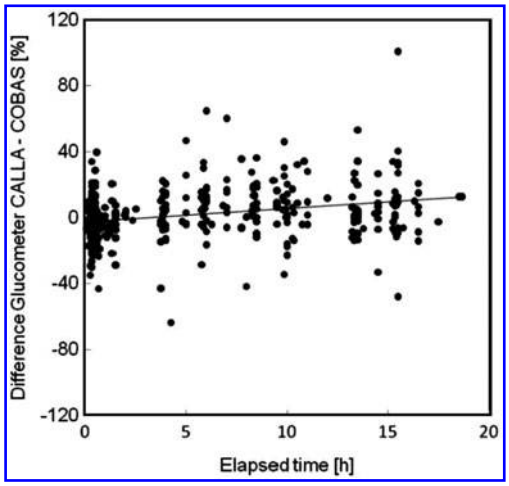

FIG. 2.

Table 1. Differences and Time from Collection to Analysis

Group	All groups (1–5)	Group 1 (WARD 1)	Group 2 (WARD 2)	Group 3 (WARD 3)	Group 4 (WARD 4)	Group 5 (LAB)
Number of pairs	467	100	93	82	91	101
ΔMedian [%]	6.6	6.4	9.6	7.6	9.2	4.5
ΔMean [%]	10.2	10.2	11.1	10.2	13.9	6.1
ΔSD [%]	11.0	11.3	8.6	9.6	16.2	5.5

Relative differences between CALLA and INTEGRA.

by one professional in 68 out-patients (Group 5). Within 2 minutes following the glucometer reading, 200 uL blood from the same fingerprick was collected in a heparinized tube with fluoride and centrifuged. Overnight collections were stored at 2 to 8°C.

Results: There were 54/521 paired-samples excluded from statistical analysis due to hemolysis etc. Correlations and differences see Fig. 1, Fig. 2, Table 1.

Conclusions: CALLA appears to be an acceptable means for routine clinical monitoring. Variability of results may also be related to transportation and laboratory processing.

P-42

CGMS ENABLES ROUTINE DETERMINATION OF GLYCAEMIC INDEXES OF FOODS AT DIFFERENT TIMES OF DAY

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Backgrounds and aims: Glycaemic index of foods is routinely determined after an overnight fast using fingerprick blood. The purpose of this prospective open lable study was to assess the differences of GI for 6 popular foods determined by means of continuous glucose monitoring system (CGMS) at breakfast- lunch- snack- and dinner times.

Methods: Nineteen healthy persons aged 20–33 y, BMI $24.1 \pm 0.8\,kg/m^2$ (mean ± SE), 5 men, CGMS-trained, completed the study. Each subject consumed 6 different foods and glucose standard on four separate occasions at four different times of day according to a defined meal plan over an 8-day period (Table 1).

Results: See Table 2.

Conclusions: Only the difference between GI for wheat-cookies at lunch vs dinner was significant.

P-43

GLUCOMETERS (CALLA) APPEAR TO BE BETTER FOR ROUTINE P-GLUCOSE MONITORING IN PATIENTS ADMITTED TO HOSPITAL THAN A LABORATORY ANALYZER (COBAS INTEGRA)

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Background and aims: Relative differences between P-glucose concentrations estimated in wards on glucometer CALLA and in laboratory were 6.4% to 9.6%. The relative difference in the laboratory was only 4.5%. The purpose of this trial was to compare the correlations of glucose concentrations estimated in laboratory

Table 1. Defined Meal Plan (8 Days)

Day	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Breakfast (7.00 h)		Yogurt vanilla	Wheat-cookies with cheese	Chocolate wafers dia	Chocolate wafers orig.	Diabetic biscuits	Gluten free biscuits	Glucose standard
Lunch (11.00 h)	Wheat-cookies with cheese	Chocolate wafers dia	Chocolate wafers orig.	Diabetic biscuits	Gluten free biscuits	Glucose standard	Yogurt vanilla	
Snack (15.00 h)	Chocolate wafers orig.	Diabetic biscuits	Gluten free biscuits	Glucose standard	Yogurt vanilla	Wheat-cookies with cheese	Chocolate wafers dia	
Dinner (19.00 h)	Gluten free biscuits	Glucose standard	Yogurt vanilla	Wheat-cookies with cheese	Chocolate wafers dia	Chocolate wafers orig.	Diabetic biscuits	

Table 2. GI of Foods (Medians); N = 19

Test	*Breakfast 7.00 h*	*Lunch 11.00 h*	*Snack 15.00 h*	*Dinner 19.00 h*	*Median (all tests)*
Glucose 50 g	120	77	118	77	100
Wheat-cookies with cheese	85	42	93	88	75
Chocolate wafers Orig.	60	45	63	54	66
Gluten free biscuits	66	38	73	75	63
Chocolate wafers Dia	32	36	44	45	44
Yogurt vanilla	34	46	49	48	43
Diabetic biscuits	72	78	43	39	58

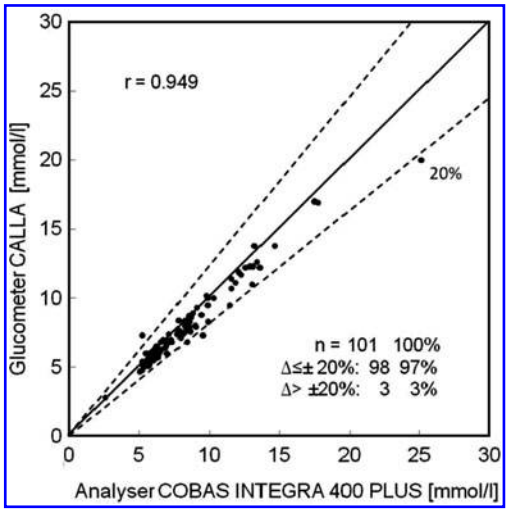

FIG. 1. CALLA vs COBAS in laboratory.

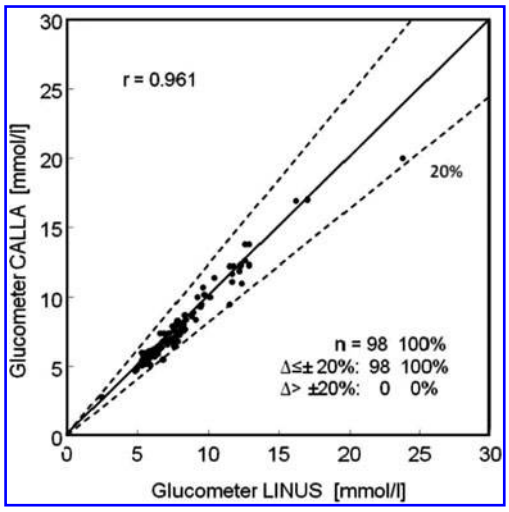

FIG. 3. LINUS vs CALLA in laboratory.

by one professional from fingerprick plasma (1) on glucometer CALLA, Wellion, (2) on glucometer LINUS, Agamatrix and (3) on the analyzer COBAS INTEGRA.

Methods: One glucometer CALLA Light and one glucometer LINUS operated by 1 laboratory professional were used in the course of 5 weeks in 68 out-patients. Within 2 following minutes 200 uL blood from the same fingerprick was also collected in a heparinized tube with fluoride and centrifuged. Plasma was investigated by means of the COBAS INTEGRA analyser using hexokinase method.

Results: There were 101 CALLA vs COBAS (A), 98 LINUS vs COBAS (B) and 98 LINUS vs. CALLA (C) paired-samples collected. Spearman correlations and differences see Fig. 1, Fig. 2 and Fig. 3. There was no significant difference between correlation coefficients A vs B, B vs C and A vs C.

Conclusions: Glucometer CALLA appears to be suitable for routine P-glucose monitoring in wards. Strong correlations between CALLA, LINUS and INTEGRA analyzer in laboratory paired-samples demonstrate its high accuracy. Supported by IGA MZCR NR 7825-3 and MSM 6891959216, Czech Republic.

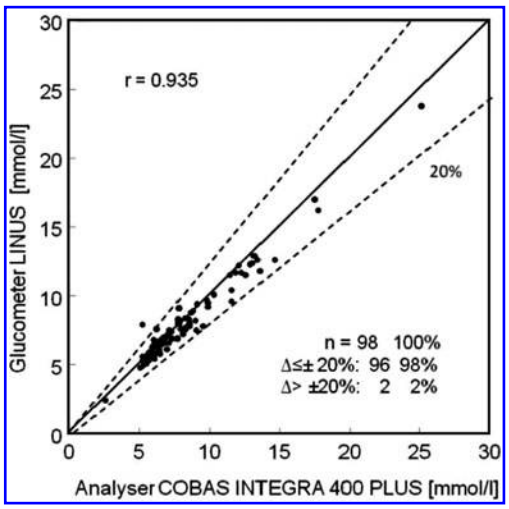

FIG. 2. LINUS vs COBAS in laboratory.

P-44

INITIAL GLYCATED HAEMOGLOBIN A1C (HBA1C) AS A PREDICTIVE FACTOR FOR FURTHER GLYCAEMIC CONTROL IN TYPE 1 DIABETES (T1DM) CHILDREN

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Objectives: HbA1c is a gold standard of glycaemic control assessment in diabetes. The aim was to estimate whether HbA1c of the initial period of T1DM duration influences patients' further glycaemic control.

Methods: From the database of the Upper Silesian Center of Child's Health, Poland for the study we selected children with T1DM duration >2yrs with ≥4 HbA1c measurements (mean 12.2 ± 5.2/patient) until 10yrs of disease duration (471pts, 218♀). First HbA1c result during initial 6 months (HbA1c I), mean HbA1c ≤ 6 months (HbA1c II) after diagnosis, 0.5–2yrs (HbA1c III) and for the following 8 years of disease duration were estimated (HbA1c IV).

Results: HbA1c I and II was available for 330 (148♀) patients - age at onset 8.7 ± 3.6yrs, T1DM duration 6.8 ± 2.0yrs; HbA1c III for 422 (196♀) children - age at onset 8.5 ± 3.6yrs, T1DM duration 6.8 ± 2.3yrs. HbA1c IV was assessed separately for both groups. HbA1c I, II and III correlated positively with respective HbA1c IV ($P < 0.01$, $P <$ 10e-6, $P <$ 10e-9 respectively). Linear prediction models could be created: HbA1c IV = ai + bi•HbA1c i, i = I, II or III). They showed that the impact of HbA1c I, II and III on HbA1c IV increased with time (bI = 0.06, 95%CI 0.01 ÷ 0.11; bII = 0.14, 95%CI 0.07 ÷ 0.20; bIII = 0.73, 95%CI 0.67 ÷ 0.80).

Conclusions: Initial HbA1c influences further glycaemic control in T1DM children. Its values 0.5–2yrs after diagnosis seem to have greater influence than the ones from the first months after diagnosis. Further mean HbA1c values for the patient can be predicted based on his initial results. Work partially financed by MNiSW grant NN519579938.

P-45

KEY ELEMENTS FOR SUCCESSFUL INTENSIVE INSULIN PUMP THERAPY IN INDIVIDUALS WITH TYPE 1 DIABETES

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Objective: Clinical trials have demonstrated that in individuals with type 1 diabetes the use of CSII pump resulted in better glucose control. Advantages of pumps therapy include many features such as the bolus calculators (wizard). These features are cost and time consuming it is important therefore to determine weather their use is associated with better glucose control. Thus, the aim of this analysis was to assess which features and parameters of insulin pump use are associated with better glucose control.

Methods: Data regarding consecutive patients with type 1 diabetes treated with an insulin pump and attending a tertiary referral for intensive glucose control was included in this analysis. The relationship between glycemic indices and treatment parameters (number of insulin units, number of glucose readings, bolus calculator use etc.) was assessed.

Results: A statistically significant relationship was found between the glycemic indices and wizard use. Thus, individuals that used the wizard function in 50% of their boluses had an A1C, mean blood glucose values that were 0.6% ($P = 0.008$) and 25.45 mg/dL ($P = 0.000$) lower respectively.

Conclusion: The use of the bolus calculator feature was associated with better glucose control. Larger prospective clinical trials are needed in order to further validate this finding.

P-46

MANY BENEFITS OF CROSSING FROM DAILY MULTIPLE INJECTIONS TO INSULIN PUMP

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Background: Insulin pumps have changed diabetes treatment in the last decade. The aim of our study was to compare the frequency of hypoglycemia which represents the main risk of intensive insulin regimen before and after insulin-pump therapy and improvements in longterm diabetes control.

Material and methods: We treated now 60 patients with the type 1 DM with insulin pump therapy (27 women, 33 men) after intensive insulin regimen, 26 new in last two years, 5 of them were pregnant.

Results: In the intensive regimen 19 patients (73%) have serious hypoglycemia. After using insulin-pump therapy there were only 5 patients with hypoglycemia (19.2%) and reduction of 73.7%. Reduction in glycosylated hemoglobin (HbA1c) was 0.46% (7.38% to 6.92%) in average duration insulin-pump therapy for 15 months. The frequence of hypoglycemia is significantly decrease in patients treated with insulin-pump therapy and glycemic control was improved, weight gain was only 2% (1.46 kg), daily insulin dosage decreased by 14.6% (50.9 to 43.5 IU).

Conclusion: Insulin pump is a modern tool for insulin therapy with proved advantages over intensive insulin therapy with multiple daily injections. This demonstrates that usage of insulin-pump therapy improves HbA1c in target range glycemia without increasing hypoglycemia. Upon analysis we can conclude that insulin pump therapy is an effective type of treatment in type 1 DM patients after intensive insulin regimen. All patients without an exception have noted convenience of using insulin-pump. Insulin pumps have many advantages for diabetic patients and should be used widely or think more about their introduction.

P-47

ARTIFICIAL PANCREAS BASED ON ERROR DYNAMICS SHAPING: *IN SILICO* TRIAL WITH THE FULL ADULT TRAINING DATABASE OF THE UVA T1DM SIMULATOR

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Objective: The present study evaluates Error Dynamics Shaping (EDS) as an alternative closed-loop strategy to control blood glucose for type 1 diabetes. This original methodology allows to control strongly disturbed nonlinear systems. The controller is designed using SC route for both insulin delivery and glucose measurement.

Method: We tested EDS *in silico* with the 10 adults from the training database of the UVa T1DM simulator. A three minutes sampling time was set for glucose measurement. Two scenarios have been investigated:

1. Nominal scenario: each subject consumes three meals per day, with prescribed amounts of CHO (50 g at 7 am, 70 g at 1 pm and 80 g at 7 pm).
2. Test against meal variability: the virtual patients are subject to 21 consecutive meals over seven days, deviating from nominal conditions, in terms of both timing (maximum variation of 1 h) and CHO contents (amount variation up to 50%). A uniformly distributed random variable was added to each factor, providing the final time and CHO amount for each meal.

Results: The mean time spent within 70–150 mg/dL was around 90%. No severe hypoglycemia was observed. The lowest glycemia, (69 mg/dL), was observed in adult 1 and lasted 30 minutes. The highest reading (267 mg/dL) occurred in adult 4.

Conclusion: The EDS controller is able to maintain glucose values close to their target level despite meal profile variability and without meal announcement. No hypoglycemia (<60 mg/dL) was recorded. *In silico*, EDS controller is effective in achieving normoglycemia and robust to meal disturbances.

P-48

A RANDOMIZED CLINICAL TRIAL ON THE EFFECT OF REFLEXOLOGY IN THE MANAGEMENT OF PATIENTS SUFFERING FROM DIABETIC NEUROPATHY

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Introduction: Reflexology, the subject of treating and detecting ailing body parts through the respective reflex areas, was used with the hypothesis of rectifying the internal organ functions by applying external stimuli on feet.

Aim: The aim of this trial was to determine the efficacy of reflexology in managing patients suffering from diabetic neuropathy.

Design: A sample size of 58 patients was enrolled and randomly distributed into study (ongoing conventional drugs + Reflexology) and control (ongoing conventional drugs) groups following the selection criteria with a follow-up period of 3 months.

Methods: Observing the different kinds of marks on reflex areas, the functional status of organs was detected. It assisted in determining the prognosis of ailment, quality assurance of therapy application and treating patients holistically. The technique was taught to the caregivers and patients. Responses in terms of Nerve Conduction Velocity, vibration-, thermal-sensitivities and other parameters were recorded at 2 stages.

Results: Reductions in mean pain scores in control-arm and study-arm subjects are 21.5% and 74.05% respectively with a *P*-value of 0.001. The quality of life in DM neuropathy, (using neuroQOL instrument) was found to be 53% improved with a *P*-value of 0.001. Other symptoms were also relieved in study group subjects as compared with control group ones with statistical significance ($P < 0.002$). The abnormal features related to dysfunction organs as observed on corresponding reflex areas were improved and data is found to be statistically highly significant ($P < 0.0001$).

Conclusions: Reflexology may offer an efficient and holistic complementary, natural therapy to treat diabetic neuropathy.

P-49

PREVENTION OF HYPOGLYCAEMIA BY USING LOW GLUCOSE SUSPEND (LGS) FUNCTION IN SENSOR-AUGMENTED PUMP THERAPY

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The sensor-augmented insulin pump (SAP) "Paradigm®VEO" offers a novel automatic insulin shut-off mechanism (LGS) possibly preventing severe hypoglycaemia. The present two-phase study (SAP only (2 weeks) vs. SAP plus LGS (6 weeks)) investigated a potential reduction in the frequency of hypoglycemic episodes by using the "low glucose suspend" function.

Data analysis was possible in 21 of 24 patients from 3 paediatric centers (age 10.8 ± 3.8 years, diabetes duration 5.9 ± 3.0 years, CSII experience 3.7 ± 1.7 years). Baseline A1C level was $7.8 \pm 1.1\%$ (DCA 2000). A total of 1298 LGS alerts occurred, 853 were shorter than 5 minutes as patients reacted immediately and no interruption of insulin delivery took place. The frequency of LGS alerts was 2.56 ± 1.86 per patient/day (6 am–10 pm: 76%). Of all LGS episodes, 42% lasted less than 30 min while 24% took more than 120 min, respectively. LGS > 120 min was more frequent in the night (84%). The AUC < 70 mg/dL was decreased by using LGS (SAP vs. SAP + LGS: 0.76 mg/dL × day vs. 0.53 mg/dL × day, $P = 0.05$) as well as the time spent in hypoglycemia (average minutes/day: 101 ± 68 min vs. 58 ± 33 min, $P = 0.002$). Also, the number of hypoglycemic excursions was significantly reduced during SAP + LGS (excursions < 70 mg/day 1.27 ± 0.75 vs. 0.95 ± 0.49, $P = 0.01$, excursions ≤ 40 mg/dL: 0.28 ± 0.18 vs. 0.13 ± 0.14 mg/dL/day, $P = 0.005$) with no difference in the mean glucose level (145 ± 23 vs. 148 ± 19 mg/dL). Regarding safety, no episodes of severe hyperglycemias or DKA were observed following LGS.

The present study provides evidence for reducing the risk for hypoglycemia with LGS without compromising the safety of CSII therapy.

P-50

ONLINE NEAR FUTURE PREDICTION OF GLUCOSE PROFILE BASED ON PERSONALIZED DATA DRIVEN MODELS

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The prediction of glucose fluctuations and the early recognition of acute hypo- and hyperglycemic events are of high importance for the prevention of abnormal metabolic stages and/or the realization of an artificial pancreas.

Data-driven models able to be auto-tuned online in order to provide personalized predictions of the glucose profile have been developed. The models use information related to glucose concentration measured by continuous glucose monitors and past insulin infusions delivered through insulin pumps. The design and evaluation of the models has been based on *in silico* and real Type 1 diabetic patient data. The data from the first four days have been used for the selection of the optimal orders and the initialization of the parameters for each model. After designed, the models have been evaluated for their ability to online predict glucose in prediction horizons of 30 and 45 minutes. During the evaluation the models' parameters were adaptively adjusted.

For both *in silico* and real data the proposed approach resulted in models with root mean square error in the order of 9.0, correlation coefficient of approximately 0.98, and time lags around 2 min. The majority of the glucose predictions were in the A + B zones of the Clarke's Error Grid Analysis for the different time horizons.

The presented methodology seems to be appropriate for the personalized prediction of glucose profile since the resulted models are able to adapt to the inter- and intra-patient variability.

P-51

CHARACTERIZATION OF A POPULATION'S BLOOD GLUCOSE DISTRIBUTION AND ITS CORRELATION WITH METRICS ASSESSING QUALITY OF GLYCEMIC CONTROL

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The most widely used metric of glycemic control is A_{1c}, but it captures only one aspect of the blood glucose (BG) distribution. Quantifying glycemic variability has been of interest but no single metric has emerged as the standard.

The BG distribution of a population was characterized from 4,000 patient-months of sensor data from Medtronic's CareLink database. The population can be characterized by the mean and standard deviations of the lognormal mean and standard

deviation for each individual patient, with both distributions having normal distributions. Due to the low correlation ($R^2 = 0.0076$) between lognormal mean and standard deviation, these four parameters can be independently sampled to construct a Monte Carlo simulation. These distributions can then be used to calculate and compare various metrics of glycemic control.

For a simulated population of 10,000 individual BG distributions, the following metrics were calculated: mean, standard deviation, coefficient of variation, A_{1c} (estimated), J-index, GRADE, M-value (M100), index of glycemic control (IGC1), IGC2, and IGC3. Pearson's correlation coefficients and Spearman's rank correlation coefficients were calculated. With the exception of J-index/IGC1 ($P = 0.278$) all other Pearson's correlations are significantly different from zero ($P < 0.002$). Spearman's correlations are all significantly different from zero ($P < 0.001$).

The metrics are all highly correlated; no combination provides any additional information than using only one metric. Glycemic control metrics are useful in assessing individual patients and for comparing the performance of different automatic control algorithms. Further research to better quantify glycemic variability is still needed.

P-52

THE USE OF NEW TECHNOLOGIES (CSII&RTCGM) IN DIFFERENT TYPES OF DELIVERY IN TYPE 1 DIABETIC WOMEN

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An optimized metabolic control during partum is mandatory to prevent maternal-neonatal complications.

Aim: We retrospectively evaluated the efficacy & safety of CSII in 59 type1diabetic women delivering far from our hospital. **Patients and Methods:** All patients (age: 30 ± 4 yrs diabetes duration:14 ± 7yr; **Pregestational BMI**: 24.6 ± 3.5 kg/m^2; pregnancy planning: 34%; %pre-pregnancy CSII use: 42.4; **pregestational HbA1c** 7.2 ± 1.3) were educated, together to their partner & obstetric team, to use CSII throughout pregnancy and delivery, following a standardised protocol. 22/59 (37%) used also RTCGM. **CSII-Protocol,** consisted of **3'basal-rate':**

A, maintaining the last basal-rate;
B, preventive 50%–60% reduction of 'A';
C, 0.1–0.2 U/h for CBG < 60 mg/dL), singly activated just before anaesthesia or at the beginning of labour.

An alternative intravenous protocol (I.V.P) was given in case of complications (CBG > 200 mg/dL or non-compliant obstetric team). 5–10 gr/h of i.v-glucose were added. Capillary Blood Glucose (CBG) targets: 70–140 mg/dL.

Statistics: 2-tU or Pt-test, c2 as appropriate; significant *$P < 0.05$.

Results: Delivery at 37 weeks; none shifted to I.Vprotocol. 52/59 (88%) caesarean sections(CS); 3/59 (5%) spontaneous labour (SP); 4/59 (6.7%) inducted labour (IL). 11/22 (50%) used RTCGM during delivery. **LGA at birth**: 39%. **Metabolic control:** LastHbA1c: 6.5 ± 1.8% (*$5.8 + 0.2$% in RTCGMgroup); Pre-partumCBG: 95 ± 25 mg/dL (85 ± 12.5 in RTCGMgroup); **PostpartumCBG:** $102 \pm$ mg/dL (95 ± 15 mg/dL in RTCGM); Neonatal hypos (30 mg/dL): 13.5%(4.5% in RTCGM). No neonatal hypos in deliveries (6 CS; 1 SP; 4 IL) using RTCGM. IntrapartumCBG values (98 ± 43 mg/dL), obtained with a 57% basal-rate reduction in CSgroup (84.7 ± 6.0 mg/dL in RTCGM). NewbornCBG at birth: 50 ± 11 mg/dL. Vaginal deliveries: n°7; mean hours of labour: 5; 1 preterm delivery; LGA 14%; LastHbA1c $6 + 0.7$%; preCBG $102 + 26$ mg/dL; postCBG $100 + 8$ mg/dL; neonatal hypos 0/6; fetalCBG 57 mg/dL.

Conclusions: CSII is possible and safe in different types of delivery in selected and educated women. RTCGM improves their metabolic management.

P-53

ESTIMATION OF GLOMERULAR FILTRATION RATE IN TYPE 2 DIABETIC PATIENTS USING THE NEW CKD-EPI EQUATION

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Aim: To compare the Modification of Diet in Renal Disease (MDRD) equation to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation in aged caucasians patients with Diabetes Mellitus type 2 (DMT2).

Patients and methods: We studied 368 caucasians participants with DMT2, of whom 168 (45.7%) were men, with mean (SD): age 65 (10) years,. GFR was measured using plasma clearance of 51Cr-EDTA (mGFR). In parallel, GFR was estimated twice, using the MDRD.

Results: MGFR was 72.0 (22.3) mL/min per 1.73 m^2, MDRDGFR was 84.6 (25.0) mL/min per 1.73 m^2 and CKD-EPIGFR was 83.0 (20.3) mL/min per 1.73 m^2 ($P < 0.05$ for difference from mGFR). Bland-Altman plots showed that 95.1% and 95.1% of estimations for MDRDGFR and CKD-EPIGFR respectively, lie within the ± 1.96SD of the mean difference. Bias was 12.1 and 10.5 mL/min per 1.73 m^2 for MDRDGFR and CKD-EPIGFR respectively ($P < 0.05$ for difference in bias between MDRDGFR and CKD-EPIGFR). Precision was 16.5 and 13.8 mL/min per 1.73 m^2 for MDRDGFR and CKD-EPIGFR respectively ($P < 0.05$ for difference in precision between MDRDGFR and CKD-EPIGFR). Accuracy 10% was 28.5% and 34.8% for MDRDGFR and CKD-EPIGFR respectively (NS). Accuracy 30% was 69.1% and 72.4% for MDRDGFR and CKD-EPIGFR respectively (NS).

Conclusions: The new CKD-EPI formula was less biased, more precise but with the same accuracy to within 10% and 30% of isotopic GFR than MDRD formula. These results support further evaluation of CKD-EPI formula for estimation of GFR in patients with DMT2.

P-54

ESTIMATION OF GLOMERULAR FILTRATION RATE IN TYPE 2 DIABETIC PATIENTS: COMPARISON OF CKD-EPI EQUATION AND CYSTATIN C-BASED FORMULA

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Aim: To compare the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation to a cystatin C (Scyst) based formula for GFR estimation in patients with type 2 diabetes (DMT2).

Patients and methods: We studied 368 caucasians participants with DMT2, of whom 168 (45.7%) were men, with the following characteristics, mean (SD): age 65 (10) years. GFR was measured using plasma clearance of 51Cr-EDTA (mGFR). In parallel, GFR was estimated twice, using the CKD-EPI.

Results: MGFR was 72.0 (22.3) mL/min per 1.73 m^2, CKD-EPIGFR was 83.0 (20.3) mL/min per 1.73 m^2 ($P < 0.05$ for difference from mGFR) and cystCGFR was 72.5 (27.9) mL/min per 1.73 m^2 (NS difference between mGFR and cystCGFR). Bland-Altman plots showed that 95.1% and 93.9% of estimations for CKD-EPIGFR and cystCGFR respectively, lie within the ± 1.96SD of the mean difference. Bias (mean difference between estimated GFR and mGFR) was 10.5 and 0.45 mL/min per 1.73 m^2 for CKD-EPIGFR and cystCGFR respectively ($P < 0.05$). Precision was 13.8 and 21.96 mL/min per 1.73 m^2 for CKD-EPIGFR and cystCGFR respectively ($P < 0.05$). Accuracy 10% was 34.8% and 33.2% for CKD-EPIGFR and cystCGFR respectively (NS). Accuracy 30% was 72.4% and 72.6% for CKD-EPIGFR and cystCGFR respectively (NS).

Conclusions: Stevens cystatin C based formula was less biased than and CKD-EPI equation. On the other hand CKD-EPI equation was more precise and presented higher agreement with measured GFR. These results support the superiority of CKD-EPI equation over Stevens cystatin C based formula for estimation of GFR in patients with type 2 diabetes.

P-55

EXENATIDE TREAMENT IN TYPE 2 DIABETES AND OBESITY

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Aim: To assess the effectiveness, safety, and tolerability of Exenatide twice daily treatment of type 2 diabetes in obese patients.

Material and methods: Retrospective review of obese type 2 diabetic patients initiated on Exenatide therapy in our Endocrine clinics between December 2008 and December 2009. We did not include any modifications in previous lifestyle plans.

Results: We included 85 patients, 54 women (63.5%) and 31 men (36.5%), mean (±s.d.) aged 53 ± 10 years and 8.7 ± 7 years diabetes evolution. At baseline, mean weight was 105 ± 17 kg and body mass index (BMI) 40 ± 6.5 Kg/m^2, 48% of them being morbidly obese patients. Baseline HbA1c was 7.8 ± 1.4%. 69 patients were on oral antidiabetic therapy and 16 were receiving insulin therapy, stopped at the moment of Exenatide initiation.

After an average follow-up of 6 months (range 3–12), mean HbA1c reduction was $0.69 \pm 1{,}2$% ($P < 0{,}05$) with 50.6% of patients reaching HbA1c < 7%. Mean weight loss was 5.1 ± 5 kg; 3.5 ± 3 Kg, 6.1 ± 5 Kg and 8.2 ± 7 Kg at 3, 6 and 12 months respectively ($P < 0{,}05$). Insulin therapy withdrawn patients experimented a greater weight loss of 7.2 ± 5.8 Kg ($P =$ ns). We have not found any significant change in blood pressure, lipid profile or renal function. Adverse effects occurred in 20% of patients, mainly mild to moderate gastrointestinal ones. Hypoglycaemia was reported in 3 patients, all of them on concomitant sulfonylurea treatment.

Conclusions: In our experience, Exenatide provides glycemic control and a substantial weight loss, being an excellent option in diabetic patients from whom weight is a main concern.

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CLINICAL USEFULNESS OF A BOLUS CALCULATOR IN PATIENTS WITH TYPE 1 DIABETES MELLITUS TREATED WITH CONTINUOUS SUBCUTANEOUS INSULIN INFUSION (CSII)

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Objective: Calculation of accurate insulin boluses is one of the major problems related to intensive insulin regimens. A bolus calculator(BC) incorporated into the insulin pump estimates the dose of insulin to be administered at the meal and makes calculation easier and more precise.

The aim of the present study was to assess the efficacy of a BC on glycaemic control of patients with DM1 on CSII.

Material and methods: We enrolled 20 DM1 patients >18 y and treated for >12 months with CSII (Minimed 722, Medtronic). They received an infrared-linked glucometer (Contour Bayer), being glycemic values directly transmitted to the pump to be used by the BC, with possibility to download all the recorded data.

Data evaluated baseline and after 3 months using the BC were: HbA1c, daily insulin dose(Basal and bolus), number of bolus/day and acute complications. Glycemic lability was evaluated using MAGE and SD from SMBG information, and quality of life (DQOL). A satisfaction questionnaire was also evaluated.

Results: After 3 months, significant changes were observed ($P < 0.05$) in weight ($71{,}8 \pm 8$ vs 72,9 Kg) and metabolic control (HbA1C $7{,}7 \pm 1{,}1$% vs $7{,}5 \pm 1$%), with no differences found in parameters related to glycemic lability. Patients were satisfied with the BC and found this option easy to use and accurate.

Conclusions: In a group of patients treated with CSII, the addition of a BC achieved an improvement in glycemic control with decrease of HbA1c levels and weight, confirming the patients a high level of satisfaction.

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EVALUATION OF ADOLESCENTS WITH TYPE 1 DIABETES AFTER TRANSITION FROM PAEDIATRIC TO ADULT CARE

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Objective: Our objective in this study was to identify the type of clinical care treatment received by young type 1 diabetic patients who have made the transition from paediatric to adult care, and to assess the metabolic and self care status of the patients. The research aimed to develop a sustainable and coordinated approach to facilitating the transition between diabetes services for adolescents and reveal from the perspective of the adolescents living with Type 1 Diabetes their experiences surrounding the process of transition.

Material and methods: We evaluate all the patients transferred to our adult unit during the last year. 23 type 1 diabetic patients were analysed. A questionnaire was used to evaluate and opinion of the patients concerning the transitional process (6 months).

Results: 23 type 1 diabetic patients (63%F/36%M) with mean evolution of diabetes 9.5 years (6–14 years). Mean BMI was 23.68 Kg/m^2, having 44,4% of the patients BMI higher than 25 kg/m^2. Mean HbA1c was 7.58%(6.5–8.6%). The patients were treated with MDI (18.18% with NPH and analogue rapid insulin and 81.8% with glargine/Detemir and analogue rapid insulin. 9% had incipient nephropathy. No other chronic complications were found. The patients were quite satisfied with the transitional process.

Conclusions: Transition marks a critical phase for young, diabetic patients as they may frequently switch from one physician or centre to another. It is important to prepare, coordinate and evaluate transitional processes between paediatric and adult units.

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SIMPLIFIED ZONE MODEL PREDICTIVE CONTROL FOR THE ARTIFICIAL PANCREATIC BETA-CELL: AN AVERAGE POPULATION MODEL APPROACH

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Objective: Normalizing glucose concentration to the near normal range can be compared to walking on a high wire. Avoiding both hypo- and hyperglycemia is extremely burdensome process for people with type 1 diabetes mellitus. An automatic control algorithm for a future artificial pancreatic β cell (AP) was designed to control glycemia to a predefined zone. The design features a universal model for each age group.

Method: A universal population ARX model for the controller was identified based on 10 *in silico* subjects from each age group (adults and adolescents) from the UVa/Padova FDA accepted metabolic simulator. Zone-MPC was evaluated on 100 *in silico* subjects from each age group following a protocol of 24 h observation where closed-loop was engaged two hours into the day. The controller was then challenged with an unannounced meal of 75 g carbohydrate given after six hours.

Results: Zone-MPC demonstrated excellent glycemic control for both age groups and successfully overcome an unannounced meal challenge. The adult population presented 57% time in range (80−180 mg/dL), mean glucose value of 146 mg/dL with an average STD of 45 mg/dL. The adolescent population presented 63% time in range, mean glucose value of 149 mg/dL with an average STD 61 mg/dL.

Conclusions: A single population model combined with the Zone-MPC algorithm demonstrated excellent glycemic control for both adults and adolescents. This novel design allows a fully automated AP that will normalize glycemic control to a predefined zone without the need for any user intervention.

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QUANTITATIVE ANALYSIS OF PATHOGENETIC EFFICIENCY OF METFORMIN IN EARLY GLUCOSE METABOLISM ABNORMALITIES AND TYPE 2 DIABETES MELLITUS

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Background: The quantitative estimation of metformin effect on hepatic glucose production in early glucose metabolism abnormalities (EGMA) (impaired glucose tolerance (IGT) and impaired fasting glycemia (IFG)) and Type 2 diabetes (T2D) is lacking.

Materials and methods: 57 persons with EGMA and 34 patients with the first time revealed T2D were randomized in two groups: 1) treatment (metformin + lifestyle modification) and 2) control group (lifestyle modification only). Intravenous glucose tolerance test (IVGTT) was performed (0.75 g glucose per kg of body mass) at time 0 and 24 weeks of intervention. Glucose production index ((the *H*-index, mmol/L) was calculated using the program (accessible in Internet: www.diabet.ru/ivgtt). Statistical analyses was performed using SPSS 16.0.

Results: In persons with EGMA the decrease of a *H*-index (from 4.8[4.0–5.8] mmol/L to 4.0 [2.1–5.5] mmol/L, ($P < 0.05$)) in treatment group and increase of a *H*-index (from 3.6 [3.3–5.0] mmol/L to 3.8 [2.1–5.1] mmol/L) in control group was revealed. Only in T2D treatment group was found out significant ($P < 0.05$) decrease of *H*-index from 5.8 [5.2–7.4] mmol/L to 5.1 [4.3–5.5] mmol/L, but was not revealed in control group (5.2 [4.2–6.1] mmol/L to 4.7 [4.6–5.4]) mmol/L ($P > 0.05$).

Conclusions: Metformin treatment during 6 months significantly decreases of hepatic glucose production in comparison to lifestyle modification in patients with T2D (to 12%) and in persons with EGMA (to 20%).

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EXENATIDE INFLUENCE ON GLUCOSE VARIABILITY IN TYPE 2 DIABETES PATIENTS

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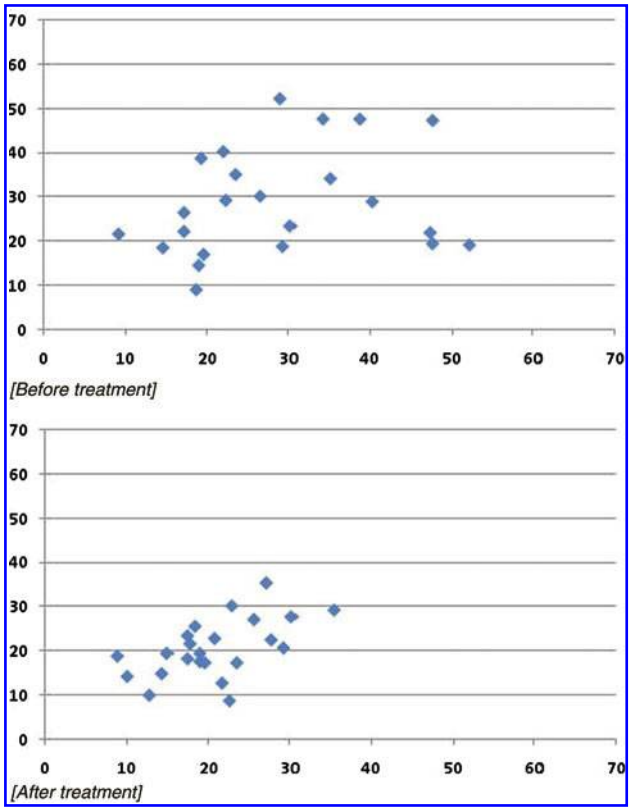
Aim was to evaluate glucose variability in exenatide treated type 2 diabetes (T2D) patients using method of symmetrization of glycemia in continuous glucose monitoring.

Material and methods: 8 T2DM patients were included. At baseline all patients was treated by metformin and/or sulfonylureas. Exenatide 5mcg per day was prescribed. CGMS (72 hours) data were recorded at baseline and 14days after exena-



tide treatment. Complex analysis of continuous glycemic curve included symmetrization of continuous glycemia scale, calculation of risk indexes of hypoglycemia (LBGI) and hyperglycemia (HBGI), hourly risk dynamics of hyperglycemia (hHBGI) and hypoglycemia (hLBGI) within 24 hours, glycemia variations using the method of Poincare. CGMS results were processed using method suggested by Kovatchev and coauthors. Statistic parameters were evaluated using SPSS16.0 computer program.

Results: After 14 days of exenatide treatment statistically significant lowering of mean HBGI was detected (baseline 16.08 ± 15.4; after 14days treatment 1.49 ± 2.2)($P < 0.05$). In three cases HBGI decreased Mean hHBGI was decreased from 17.33 ± 3.0 to 0.52 ± 0.5($P < 0.005$). Statistically significant increase in LBGI was determined (baseline 0.18 ± 0.2; after 14days treatment- 4.74 ± 6.1)($P < 0.05$). Mean hLBGI increased from 0.07 ± 0.03 to 4.3 ± 0.96($P < 0.005$). Analysis of CGMS results using Poincare method indicates that glycemia fluctuations decreased substantially after therapy which is indicated by substantial decrease of average value of glycemia range - from 28.39 ± 11.99 to 20.6 ± 6.42 ($P < 0{,}005$)(Fig. 1).

Conclusions: Our results showed a major effect of exenatide to decrease glucose variability in T2DM.

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CONTINUOUS SUBCUTANEOUS INSULIN INFUSION (CSII) STARTED IN DIABETIC CHILDREN UNDER TWO YEARS OF AGE

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Background: Children under the age of 2 with type 1 diabetes (T1DM) present a unique set of problems to health care provider and their families. The aim of the study was to evaluate the management the insulin administration using insulin pump in the youngest diabetic patients.

Material and methods: Retrospective analysis of medical charts of 50 patients under 2 years of age with mean duration of diabetes at starting CSII 0.1 ± 0.2 years (range 0–0.5 year) who have been admitted regularly, at least 4 times/year, to the outpatient clinic. Every 3 months weight, height, glycated haemoglobin (HbA1c), total daily insulin dose (TDD) and basal insulin dose defined as percentage of TDD, the frequency of severe hypoglycemia (SH) and diabetic ketoacidosis (DKA) were registered. The basal insulin pattern and insulin-to-carbohydrate (I:C) ratio were collected at the insulin pump initiation after stabilization of glycemic control.

Results: There was no statistical difference between the annual average HbA1c during the 4-years follow-up $6.9 \pm 0.7\%$ vs. $6.9 \pm 0.7\%$ vs. $7.1 \pm 0.9\%$ vs. $7.3 \pm 0.8\%$, $P = 0.184$. Daily insulin requirement increased during the study period from 0.7 to 0.8 IU/kg/d, $P = 0.008$. The mean I:C ratio was higher at breakfast than at the next meals 0.8(range 0.3–1) vs. 0.4(range 0.1–0.5) units/10 g of carbohydrates. There was no statistically significant changes in BMIsds. There were 2 episodes of DKA and 4 incidences of SH.

Conclusions: CSII is safe and effective method of therapy and should be presented as a choice of treatment for patients under 2 years of age just after diabetes onset.

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HARDWARE-IN-THE-LOOP TESTING OF A BIO-INSPIRED ARTIFICIAL PANCREAS

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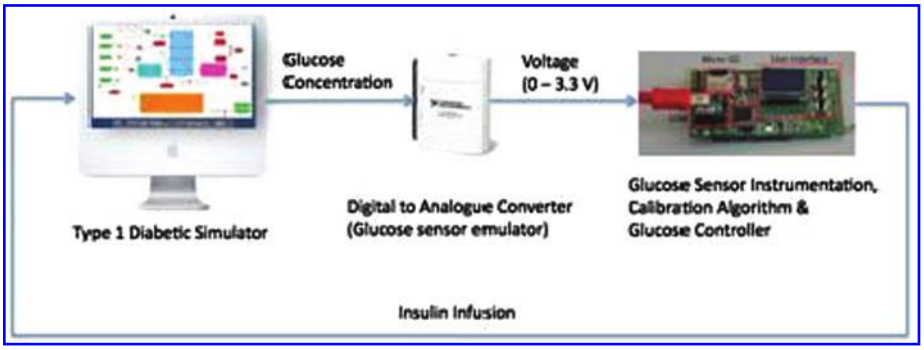
Objective: Type 1 diabetes mellitus simulators are a useful tool for the design and testing of glucose controllers for use in an artificial pancreas. However, these simulators do not allow testing of the hardware components and interfaces (i.e. sensors) used in such systems. In this work we present a new platform which allows hardware in the loop testing of artificial pancreas controllers.

Method: The presented hardware-in-the-loop platform is composed of:

- A PCB based system, incorporating a USB port, a user interface, a microcontroller and electrochemical sensor instrumentation. The microcontroller unit can be programmed with a calibration and control algorithm.
- A digital to analogue converter (NI-6212 DAC), which models the functioning of an electrochemical continuous glucose sensor.
- A commercially available MATLAB simulator of a T1DM subject.

The simulator generates a glucose concentration value that is sent through USB to the DAC. This is mapped from a range 0–20 mM to a representative sensor current. This current is sampled by the sensor instrumentation, and calibrated on the microcontroller to convert it into a glucose concentration level.



Hardware in the loop platform.

The calibrated glucose concentration is then used as an input to a glucose control algorithm that computes the required insulin dosage to be delivered. This data is then sent back to TD1M simulator through another USB port completing the loop.

Results: A recently developed bio-inspired glucose controller was successfully tested using the presented hardware-in-the-loop platform.

Conclusion: A new hardware-in-the-loop framework has been presented and tested.

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DAY-AND-NIGHT CLOSED-LOOP (CL) GLUCOSE CONTROL IN ADOLESCENTS WITH TYPE 1 DIABETES (T1D)

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Objective: To evaluate closed-loop (CL) insulin delivery in adolescents with T1D during a 36 h period, simulating normal daily activity.

Methods: Six adolescents with T1D (M 3; age 14.8 ± 1.9years; A1C 8.0 ± 0.9%; BMI 21.5 ± 1.8 kg/m^2; duration of diabetes 5.7 ± 3.5years; total daily dose 1.0 ± 0.4U/kg/day; mean ± SD) were studied at a clinical research facility on two occasions. Subjects were randomly allocated to receive either CL or open-loop (OL) for 36h from 18:30 to 08:00 two days later. During CL, subcutaneous (sc) continuous glucose monitoring data was fed manually into a model predictive control algorithm, which calculated sc insulin infusion rates for manually adjusted insulin pump. During OL, conventional continuous subcutaneous insulin infusion was applied. On each occasion, subjects engaged in normal daily activities (e.g. playing computer games, low intensity walks). They consumed meals (50–80 gCHO) accompanied by insulin boluses, and snacks (15–30 gCHO). Moderate-intensity exercise on a bicycle at 140 bpm heart-rate was performed at 10:40 (40min) and at 17:30 (20min).

Results: Overall mean plasma glucose levels were 7.2 ± 1.2 mmol/L during CL versus 8.6 ± 3.3 mmol/L in OL ($P = 0.28$, paired t-test). Time spent in target glucose range 3.9–10.0 mmol/L was 81.9 ± 12.5% vs 50.5 ± 27.2% ($P = 0.03$). Time above 10 mmol/L was 13.0 ± 13.6% vs 38.1 ± 36.0% ($P = 0.10$) and corresponding time spent hypoglycaemic < 3.9 mmol/L was 5.1 ± 5.1% vs 11.4 ± 15.7% ($P = 0.44$). Overnight, plasma glucose levels were in target for 94.6 ± 9.9% of time during CL vs 31.4 ± 29.3% in OL (first night) ($P = 0.03$) and for 90.9 ± 4.5% vs 49.0 ± 45.1% (second night) ($P = 0.05$).

Episodes of hypoglycaemia were 7 during OL versus 3 during CL.

Conclusions: Day-and-night CL may improve glucose control and reduce incidence of hypoglycaemia during normal daily activity.

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A NEW NEURAL NETWORK APPROACH TO IMPROVE EFFECTIVENESS OF SHORT-TERM GLUCOSE PREDICTION

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Aim: Recently, several time-series modeling approaches have been developed to forecast future glucose levels from CGM data. However, they do not usually allow incorporating other information such as meals consumption and insulin dosages. This knowledge is exploited by the new Neural Network Prediction Algorithm (NNPA) presented in this work with the aim of improving the effectiveness of short-term glucose prediction.

Methods: Ten *in silico* subjects were extracted from the UVA/Padova Type-1 Diabetic Simulator [US2008/067725]. The simulation scenario consisted of 7 days, 3 meals/day with random variability on value/time of meal carbohydrates and insulin dosage. A 30 min prediction horizon was considered. The NNPA inputs are CGM, the prediction error of a Linear Prediction Algorithm (LPA) taken from the literature [Sparacino et al., IEEE Trans Biomed Eng, 2007], meal carbohydrates and insulin delivery, the output is an estimation of the future error of LPA.

Results: The performance of LPA + NNPA is compared to LPA and to the feed-forward neural network (FFNN) proposed by Pérez-Gandía et al. [Diabetes Technol Ther, 2010] in terms of root mean square error (RMSE) and temporal gain (TG).

Table 1. Average RMSE and TG Results

	LPA + NNPA	*FFNN*	*LPA*
RMSE [mg/dL]	9.17	10.66	15.73
TG [min]	24.5	17.0	11.0

Conclusions: NNPA can be used to compensate the prediction error of linear models and to take into account information of meal/insulin, which further improves the accuracy of prediction. The application of NNPA on real data is currently under investigation.

P-65

ON-LINE CGM DENOISING IMPROVES HYPO/HYPER-ALERT GENERATION

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Aim: CGM signals are affected by random measurement noise, with a signal-to-noise ratio (SNR) varying from subject to subject (inter-individual variability) and even during a given recording (intra-individual variability). The purpose of the present work is to quantitatively assess how much a denoising procedure can improve the performance of hypo/hyperglycemic alert generators.

Methods: Two denoising approaches D1 and D2, with different degree of complexity, have been employed. D1 implements the Bayesian method presented by Facchinetti et al. (IEEE Trans Biomed Eng, 2010). D2 (unpublished) is a modification of D1 able to cope also with intra-individual variability of SNR. With both methods, alert generation is performed by comparing the confidence interval of the filtered level with the hypo/hyper-threshold.

Results: We considered 24 CGM time series obtained with the Menarini Glucoday® system. A total of 64 and 122 hypo/hyper-events have been detected. Results of sensitivity and specificity (in percentage) are shown below.

Sensitivity and Specificity Resutls

	Hypoglycemia		*Hyperglycemia*	
CGM	SENS (%)	SPEC (%)	SENS (%)	SPEC (%)
Raw	82.8	72.5	68.3	57.8
D1	88.9	84.1	68.3	75.0
D2	93.6	88.0	85.7	78.2

D1 improves the performance of CGM alert generation, and the improvement is even larger with D2.

Conclusions: On-line denoising algorithm significantly improves sensitivity and specificity of real-time hypo/hyper-glycemic alert generation from CGM time-series, especially if the intra-individual variability of the SNR is properly dealt with.

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IS THERE ANY IMPACT OF PSYCHOLOGICAL PARAMETERS ON THE OUTCOMES OF THE FIRST CONTINUAL GLUCOSE MONITORING?

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The first continuous glucose monitoring (CGM) is a stressful matter and it's results could be influenced by psychological characteristics.

The **aim** of our study was to evaluate the effect of psychological characteristics on the first CGM outcomes in diabetic patients.

Methods: 21 patients with diabetes mellitus Type 1 (mean age 35.2 ± 12.3 years, diabetes duration 15.5 ± 9.1 years, HbA1c according to IFCC $6.4 \pm 1.2\%$) who underwent first CGM from 1/2010–7/2010, were included into our study. Psychological characteristics such as depression rate, stress load, grades of satisfaction and negative experiences, risk types of behavior and quality of life represented by 6 domains (e.g., physical health, experiences) were assessed by psychological questionnaires. Psychological features were then correlated with parameters of CGM records (time spent in hypo-, hyperglycemia, high or low glucose alerts, etc.).

Results: Time spent in hyperglycemia correlated positively with the depression scale ($r = 0.47$; $P < 0.05$), negatively with domain of physical health ($r = -0.49$; $P < 0.05$). Time spent in hypoglycemia correlated positively with thy type of behavior ($r = 0.46$; $P < 0.05$) and the domain of experiences ($r = 0.58$; $P < 0.01$). There were also found positive correlations between the depression stage and low ($r = 0.76$; $P < 0.01$) or high glucose alerts ($r = 0.65$; $P < 0.05$). The rest of psychological parameters did not correlate significantly with CGM records.

Conclusions: Depression stage, risk type of behavior and some parameters of quality of life could influence the outcomes of first CGM. Therefore in patients revealing pathological findings during CGM is recommended to perform psychological assessment with at least psychological questionnaires.

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SIGNIFICANT IMPROVEMENT IN METABOLIC STATUS 6 MONTHS AFTER BARIATRIC SURGERY

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Background: Bariatric surgery proved to be an important support on weight loss, but also carries great advantages on metabolic disorders.

The **aim** of our study was to identify changes in metabolic profile and inflammatory profile of patients following bariatric surgery procedures.

Patients and methods: 125 severely obese patients (65 F, 60) were evaluated before and 6 months after bariatric surgery (laparoscopic gastric by-pass - 41 patients and gastric sleeve - 79 patients). Anthropometric measurements were obtained and fasting serum glucose, insulin, lipids (triglycerides, HDL, LDL and total cholesterol) and inflammatory profile (C reactive protein, TNF-alpha, IL6) were determined.

Results: Patients who underwent bariatric surgery obtained substantial weight loss with an improvement in waist hip ratio (0.87 ± 0.08 versus 0.92 ± 0.1) suggesting a redistribution of adipose tissue. There were also important changes in lipids levels (triglycerides $= 110.56 \pm 30.7$ versus 161.4 ± 42.7 mg/dL, total cholesterol $= 195.8 \pm 42.01$ versus 218.71 ± 41.92 mg/dL, $P < 0.05$)

as well as an ameliorated inflammatory profile (CRP = 0.73 ± 0.34 mg/dL versus 1.51 ± 0.65 mg/dL; IL6 = 2.86 ± 0.98 versus 4.13 ± 2.34 pg/mL $P < 0.05$). There were 12.7% patients with diabetes mellitus and all had significant improvement in metabolic status, with no need for medication in 86% of them. In non-diabetic patients we observed a dramatic improvement in insulin sensitivity assessed by HOMA -IR (mean difference = 4.85, CI [2.25 - 7.32]).

Conclusions: Weight loss achieved following bariatric surgery involves preferentially visceral adipose tissue and is accompanied by an impressive improvement in insulin sensitivity as well as a more favourable inflammatory and lipid profile.

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MXS_EDIABETO, A NOVEL WEB APPLICATION TO PROMOTE NUTRITIONAL SUPPORT AND PHYSICAL ACTIVITY PRACTICE IN SUBJECTS WITH TYPE 2 DIABETES

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In type 2 diabetes (T2D), reducing caloric intake and carbohydrates is the most effective way to reduce hyperglycemia, hyperinsulinemia and insulin resistance. Insulinsensitivity can also be improved through physical activity. These lifestyle guidelines are difficult to implement and to maintain over time. The MXS_eDiabeto web program has been developed to enable sustainable nutritional support and physical activity in T2D patients. On line nutritional and physical self-assessment are provided to patients as well as an interactive menu builder. This system allows the caregiver to set nutritional (proteins, lipids, carbohydrates, calories) and physical activity objectives and to monitor the follow-up of these patients. A feasibility study was conducted in 13 patients with T2D, age 52.2 yrs, diabetes duration 4yrs, mean baseline HbA1c 7.5%. These patients considered the MXS system easy (n = 7) or very easy (n = 3) to use. Only 3 of them were a bit more reserved. The dietary survey was reliable (difference in caloric intake assessed with MXS_eDiabeto *vs.* an experienced dietitian using reference tables, below 25%). Recommendations given by the system were considered useful or very useful by the patients, both on a nutritional (n = 13) and physical activity (n = 13) point of view. In most cases, patients stated they wanted to continue with the system.

A randomized controlled study is about to be launched, testing MXS_eDiabeto with web follow-up and dietetic consultations dedicated to motivational support (but no data collection) *vs.* a conventional management (face-to-face consultations) in T2D patients, inadequately controlled with their treatment and likely to benefit from a dietary management.

P-69

EVALUATION OF EVAPORATION AS A SOURCE OF ERROR IN SELF MONITORING OF BLOOD GLUCOSE

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Table 1. Relative BG difference Compared to Immediate Measurement

BG Monitoring System	*10 seconds*	*25 seconds*
A1	$1.4 \pm 5.9\%$	5.0 ± 7.4*
A2	$2.6 \pm 4.9\%$*	$5.0 \pm 5.7\%$*
B1	$0.4 \pm 4.2\%$	$0.9 \pm 4.2\%$*
B2	$0.8 \pm 4.5\%$	$1.5 \pm 4.0\%$*
C1	$1.9 \pm 8.2\%$	$-0.3 \pm 9.4\%$
C2	$0.4 \pm 8.4\%$	$0.0 \pm 7.1\%$

(Mean ± SD, 101 to 106 subjects, *$P < 0.05$).

Objective: Various patient informations dealing with blood glucose (BG) measurement advise patients to measure BG quickly after finger pricking. Occasionally it is reasoned, that whilst the blood stays on the skin surface, evaporation might cause an elevation of glucose concentration. Modern BG monitoring systems which require small blood drops may be prone to this evaporation effect, which we intended to verify and quantify in this study.

Methods: Capillary blood samples with BG concentrations ~50–~500 mg/dL were obtained from 108 subjects. Four drops of blood with a diameter of about 1 mm were measured consecutively from each subject. The first and fourth drops were measured immediately. The second and third drops remained on the skin surface for 10 and 25 seconds, respectively, before being measured. The experiment was carried out with three different BG monitoring systems (A/B/C; two devices each).

Results: See Table 1.

Conclusion: In two of three systems the incubation time of 25 seconds created a statistically significant effect which seems to be system-dependent. The relative difference did not exceed 5% and thus is clinically not relevant. Relevance may arise with smaller blood drops or longer incubation time, so measuring BG quickly is advisable.

P-70

VALIDITY OF CALIBRATION AS A FUNCTION OF INTERVALS BETWEEN RE-CALIBRATION IN NON-INVASIVE SELF-MONITORING OF BLOOD GLUCOSE

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Generally, non-invasive (NI) glucose monitors require performance of calibration using invasive reference, prior to glucose measurements. In home-use application, the duration of calibration procedure, as well as the period between re-calibrations play an important role in the device adherence and utilization.

GlucoTrack® requires calibration procedure that takes about 1.5–2 hours and valid for long term. 91 subjects were tested. Each individual trial was performed in two different days. During Day 1, calibration was individually performed using HemoCue® as invasive reference, followed by 6 measurement pairs. On Day 2, a full-day measurements' session was conducted. The intervals between Days 1 and 2 were 1 to 22 days, according to subjects' availability, with a median of 6 days.

Clarke Error Grid (CEG) analysis shows 96% of the points in A + B zone, of which 60% in zone A. $MARD_{mean}$ is 22.4% and

$MARD_{median}$ is 15.9%. The CEG analysis for Day 2 shows 96% of the points in the A and B zones, with 57% of the readings in A zone. $MARD_{mean}$ and $MARD_{median}$ for Day 2 are 23.4% and 16.5%, respectively. No increment in the degree of error was observed as a function of number of days after calibration.

Clinical trials show clearly that the calibration is valid for long term. The long duration between re-calibrations and the ability to perform frequent spot measurements without the need to continuously wear the device, support the use of GlucoTrack as a beneficial device for a diverse population of diabetics.

P-71

INFLUENCE OF REFERENCE DEVICE ON CALIBRATION QUALITY IN NON-INVASIVE SELF-MONITORING OF BLOOD GLUCOSE

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GlucoTrack®, a Non-Invasive (NI) home glucose monitor combines three independent methods: Ultrasound, Electromagnetic and Thermal. Like other NI devices, GlucoTrack requires calibration prior to conducting glucose measurements, to minimize the individual's tissue quasi-stable factors influence. Calibration is performed by using personal, clinic or laboratory invasive reference. However, invasive home devices differ from laboratory ones in performance, e.g. accuracy and precision. Thus, calibration based on less accurate reference is questionable and may cause subsequent inaccuracies in NI readings.

47 subjects were tested: 4 T1DM and 43 T2DM. Calibration was performed with two invasive reference devices: HemoCue® and FreeStyle®. Following calibration, simultaneous measurement triplets by HemoCue, FreeStyle and GlucoTrack were performed. We analyzed conducting measurements and calibration with same invasive device; then calibration and measurements' references were crossed.

Clarke Error Grid analysis using HemoCue as reference for calibration and measurements shows 97% of the points in the clinically accepted zones A + B, of which 63% in zone A. $MARD_{mean}$ and $MARD_{median}$ are 21.1% and 14.4%, respectively. Using FreeStyle as calibration and measurement reference produces 95% of the points in zones A + B, 63% in zone A, with $MARD_{mean}$ and $MARD_{median}$ of 21.1% and 14.4%, respectively. Calibration using Freestyle and comparison of measurements with HemoCue shows 58% in zone A, with 95% in A + B zones while $MARD_{mean}$ and $MARD_{median}$ are 25.4% and 16.8%, respectively.

The results suggest that higher reference performance lead to better NI readings. Further study should be conducted to evaluate impact of calibration by different home devices on NI readings' accuracy level.

P-72

PROPOLIS CAN CONTROL DIABETES AND PREVENTS DIABETIC OSTEOPATHY IN RATS WITH STREPTOZOTOCIN-INDUCED DIABETES

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Propolis has been shown to produce various beneficial biological and pharmacological effects in animals as well as in humans. In the present study we investigated the possible protective effects of either propolis alone in 2 doses 0.3, 0.6 gm/kgm or with insulin against the metabolic and destructive changes in streptozotocin-induced diabetes in rats leading to osteopathy. propolis intake alone or with insulin caused a significant decrease in the blood glucose and glucagon with increased levels of insulin and insulin/glucagon ratio leading to significant control of hyperglycemia. Moreover treatment with propolis in both doses with or without insulin caused a significant decrease in the lipid peroxidation activity than non treated diabetic (positive) group to approach near that of the control group. Also treatment of diabetic rats with propolis and propolis plus insulin caused normalization in the calcitonin levels while parathyroid hormone concentrations in the non treateddiabetic (positive) group were significantly increased compared to the negative controls as well as to all the treated groups. Administration of propolis with insulin caused a significant increase in femur ash (FA)/femur weight (FW) and restored ash calcium, phosphorus and magnesium concentration to approach near that of the normal controls, we reported that the best results were seen in the group treated with double dose of propolis (0.6 gm/kgm) plus insulin. It could be concluded that, propolis administration especially the double dose with the traditional treatment of type-1 diabetes (insulin) is beneficial in the control of diabetes and prevention of diabetic osteopathy.

P-73

MEDIOCALCINOSIS—MARKER FOR INCREASED CARDIOVASCULAR RISK

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Introduction: Many studies have shown, that decreased ankle-brachial pressure index (ABI) is a reliable warning sign of increased cardiovascular risk. But also values over 1.4 in mediocalcinosis are followed by increased mortality from cardiovascular reasons.

Aim of the study: To investigate the occurrence of cardiac arrhythmias and ischemia during a 24 hour Holter ECG monitoring in a group of patients with mediocalcinosis.

Methods: We investigated 22 persons (10 men and 12 women). The age range was from 50 to 85 years, with the average age of 59 years. 16 of them have been treated for diabetes mellitus type 2. We performed Doppler examination for ABI estimation. For longitudinal ECG monitoring a Marquette-Hellige device with MARS software was used. The average duration of ECG monitoring was 22.16 hour.

Results: According to Holter ECG monitoring analysis, only 2 records (9%) were with normal findings. In all the other records (91%) arrhythmia or ischemia was present. Complex form of cardiac arrhythmia was found in 11 patients. 3 patients died during 12 months follow-up.

Conclusions: Our results show a frequent occurrence of diabetes mellitus (72%), cardiac arrhythmias (63%) and myocardial ischemia (27%) in the group of patients with mediocalcinosis. Patients suffering from mediocalcinosis represent a group with higher risk of serious cardiovascular complications, including sudden cardiac death. ABI investigation is capable in detecting

persons with increased cardiovascular risk. It should be therefore extended to daily routine hospital practice especially in risk groups.

P-74

EFFECT OF LOW LEVEL HE-NE LASER IRRADIATION IN DIABETIC RATS SKIN WOUNDS

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The aim of present investigation was to determine the effects of low- level He-Ne laser therapy on biomechanical property of skin wound of healthy and streptozotocin induced diabetic(STZ-D) rats.

The study was performed by experimental method. 36 male adult Wistar rats weighing above 250 gr. were used. Rats were divided equally into control and experimental groups. Blood glucose of rats in the beginning of study was recorded and rats with more than 120 mg/dL were excluded from study. Diabetes was induced by one time intra peritoneal injection of 55 mg/kg STZ. After one month, hyperglycemia was established in experimental group. Two 15-mm, vertical incision wounds were made on the dorsum of rats. Three groups of healthy and diabetic rats were received 22.4 J/cm^2, 1.2 J/cm^2 and 4 J/cm^2 energy densities He-Ne laser for two weeks. At the end of study, rats were killed and skin sample were extracted and were submitted to a biomechanical evaluation (maximum force) examination. Data was analyzed by paired Student *t* test methods.

Mean value of blood glucose of diabetic rats was 518.37 ± 23.3. Laser-treated healthy rats with 1.2 J/cm^2 energy density showed significant increase of maximum force ($P = 0.05$). Laser-treated diabetic rats with 4 J/cm^2 energy density showed significant increase of maximum force ($P = 0.05$).

It seems ideal parameters for effectiveness of Low-Level He-Ne laser in healthy and diabetic rats are different. Wounds of diabetic rats should be radiated with more energy density of low-level laser for accelerating wound healing process in comparison with healthy rats.

P-75

SUPPORT VECTOR REGRESSION FOR PREDICTIVE MODELLING OF GLUCOSE CONCENTRATION: A STUDY ON TYPE 1 DIABETIC PATIENTS

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Objective: This study investigates the ability to accurately predict the subcutaneous glucose concentrations in type 1 diabetic patients using data-driven techniques applied to a multi-parametric set of data recorded under free-living conditions.

Materials and methods: Fifteen patients with type 1 diabetes participated in this study. All patients wore the Guardian Real-Time CGMS (Medtronic) and were also equipped with a physical activity monitoring system (SenseWear, BodyMedia). Information regarding the food intake and the insulin injections was recorded by the patients using a specially designed paper diary.

Compartmental models are employed to simulate the absorption of the subcutaneously injected insulin, the intestinal glucose absorption following a meal and the effects of exercise on plasma glucose and insulin dynamics. The glucose predictions are made using the output of compartmental models along with past glucose measurements and a set of physical activity related variables recorded by the SenseWear armband. The glucose metabolism of each patient is learned by support vector machines for regression trained individually using a V-fold cross validation algorithm.

Results: The average value of root mean square error for 15 min and 30 min prediction lengths was found 9.60 mg/dL and 16.23 mg/dL, respectively. In both cases, the predicted glucose concentrations exhibit a strong linear correlation with the measured values ($r = 0.95$ and 0.88, respectively). Clarke's Error Grid Analysis shows that the prediction results are clinically acceptable (>95% in zones A and B), even if a higher prediction length is considered (i.e. for 60 and 120 min).

P-76

A GLUCOSE BIOSENSOR BASED ON GLUCOSE OXIDASE IMMOBILIZED ON MAGNETIC AMINE-FUNCTIONALIZED CARBON NANOTUBES

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Carbon nanotube (CNT) is usually used as a highly conductive support material to immobilize biological molecules for electrochemical bio-sensing. Also magnetic nano-particles provide an alternative approach to load various biological materials on electrodes. In the present work, glucose oxidase (GOx) was immobilized on magnetic amine-functionalized multi-wall carbon nanotubes. The prepared magnetic nanotubes were fixed on a gold plate by means of a permanent magnet. Using this system the direct electrochemistry of GOx was achieved. The nanocomposite bearing GOx on gold plane electrode, displayed a quasi-reversible redox peaks. The experimental results revealed that the magnetic nano-composite could effectively mediate the electron transferring of GOx to the gold electrode and efficiently catalyze the oxidation of glucose, as well. The modified electrode exhibited an excellent performance towards glucose with a high sensitivity of about 60 μA μM^{-1} cm^{-2} and a very low detection limit of 5.4 nM.

P-77

THE EFFECTS OF NOVEL NEUROMUSCULAR ELECTRICAL STIMULATION EXERCISE IN TYPE 2 DIABETES: A PILOT INVESTIGATION

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Introduction: Exercise is a vital component in the management of type 2 diabetes (T2D); however, many individuals with T2D are unable to partake in exercise owing to associated secondary complications and/or musculoskeletal problems. Neuromuscular electrical stimulation (NMES) exercise is

a potential novel technology for those facing barriers to exercise.

Patients and methods: Six males with T2D (Age 47.5 ± 9.7 years, Height 1.79 metres (m) ± 0.67 m, Weight 103.06 kilograms (kg) ± 18.72 kg) participated. Outcome measures performed at baseline and following an 8 week NMES intervention included a DEXA scan, sub-maximal VO_2 test and venous blood sample. NMES exercise was produced by a muscle stimulator (NT2010, BioMedical Research Ltd) which delivered impulses to the proximal and distal quadriceps and hamstring muscles via custom made garments. Six 60 minute exercise sessions per week were completed.

Results: Venous blood markers demonstrated improved glycaemic control following the intervention. HbA_{1c}, a key marker of long-term glucose control, reduced from 7.5% to 7.3%, while fasting glucose demonstrated a 16% improvement (8.7 mmol/L to 7.3 mmol/L). An 11% increase (32.37 to 35.80 mL/min/kg) in predicted VO_2 max was observed following training. Percentage body fat showed a 1.28% reduction.

Conclusion: These results present preliminary evidence for the potential use of this technology in T2D. They suggest that NMES exercise can result in improvements in fitness, glycaemic control and body composition. Studies with larger participant numbers are warranted.

P-78

IMPROVED CARDIOVASCULAR RISK FACTORS PROFILE IN CHILDREN WITH DIABETES TYPE 1 TREATED WITH CONTINUOUS SUBCUTANEOUS INSULIN INFUSION (CSII) COMPARED TO MULTIPLE INSULIN INJECTIONS (MDII)

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Introduction: There have been no studies so far concerning the possible influence of the insulin delivery method on the occurrence of cardiovascular risk factors in children with diabetes type 1. The aim of the study was to compare selected traditional risk factors and new markers of the atherosclerosis in children with diabetes type 1 treated either with CSII or MDII with control group of healthy children.

Patients and methods: The study was performed in the group of 50 children with type 1 diabetes, aged 15 yrs, suffering from diabetes mean - 6.6 yrs, mean HBA1c level - 8.2. 32 children were treated with CSII and 18 with MDII. Control group consisted of 30 healthy peers. All studied children had assessed: BMI, lipids and blood pressure together with plasma level of: ADMA (asymmetric dimethyloarginine), sTM (thrombomodulin), adiponectin, hsCRP and sICAM-1.

Results: In children divided according to the method of insulin delivery we found in those treated with MDII higher level of TG (115 mg/dL vs. 92 mg/dL, $P = 0{,}02$), sTM (4.2 ng/mL vs. 3.7 ng/mL, $P = 0.03$) and sICAM-1 (293 ng/mL vs. 181 ng/mL, $P = 0.04$) compared to children treated with CSII. MDII group had higher SBP, cholesterol, triglicerides, sICAM-1, sTM and hsCRP compared to controls, while in CSII vs control groups only differences in SBP (115 mmHg vs. 109 mmHg, $P = 0.02$) and TC (173 mg/dL vs. 150 mg/dL, $P = 0.01$) were found.

Conclusion: The use of CSII method may have favourable effects on the cardiovascular risk profile in children with type 1 diabetes.

P-79

INSULIN ANALOGUE MANAGEMENT—SUBJECTIVE AND OBJECTIVE QUALITY OF LIFE IN PATIENTS PREVIOUSLY MANAGED WITH HUMAN INSULIN

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Introduction: There is an expectation that by using insulin analogue, glycemic regulation in poorly controlled type 2 diabetes (t2DM) will be improved. The aim of the study was to examine the quality of glycemic control and quality of life (QoL) of patients managed with insulin analogues, previously treated with human insulin.

Materials and methods: The pilot study included 30 t2DM outpatients, divided into two groups (15 subjects in each group). The first group was treated with human insulin, while the second group was managed with insulin analogues for at least one year, but previously treated with human insulin. The following parameters were observed: sex, age, occupation, t2DM and insulin use duration, examinees estimation of insulin therapy effects, GHQ_{12} score, and HbA1c levels. Data were analyzed by SPSS 12.0.

Results: The mean t2DM duration and duration of insulin therapy management was 5.5 ± 3.5 and 3.5 ± 3 years respectively. The mean HbA1c levels were 9.1% and 7.2% respectively and differed between groups ($t = 4.869$, $P < 0.01$). Twenty (66.6%) subjects assessed overall effects of insulin therapy as grades 3/4. HbA1c levels and subjective grades of insulin effects negatively correlated ($\rho = -0.568$, $P < 0.01$). GHQ_{12} score was 9 ± 3.5 and did not statistically differ between groups ($Z = -1.481$, $P > 0.05$). GHQ 12 score and subjective grades of insulin-analogue effects negatively correlated ($\rho = -0.730$; $P < 0.01$).

Conclusion: Insulin analogue improves glycemic control of t2DM patients. The subjective assessment of the insulin therapy effects correlated with the objective QoL parameter - GHQ_{12} score.

P-80

EVALUATIVE ANALYSIS FOR GOINO PROCEDURE AS POSSIBLE PROPHYLACTIC AGENT AGAINST DIABETES

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Background: Our previous study showed that GOINO Procedure (GOP), combination mixture prescribed by specific ratio of Ganoderma Lucidum (GL), Coriolus Versicolor (CV)

and Panax Ginseng (PG), lowered systolic blood pressure and LDL-cholesterol in the NIDDM patient with no side effects rather than any of three components as single therapy, and GOP may be utilized as a possible prophylactic agent against Diabetes.

Aim: This study was to evaluate property of GOP compositions and GOP extracted solution.

Method: 6 g of GL, CV, PG were added to 1l hot water and boiled for 90 minutes. After boiling, filtered solution was centrifuged (2000/min for 10 min.). Protein contained in the centrifuged GOP solution was measured by SDS polyacrylamide gel slab electrophoresis. SDS-PAGE Molecular Weight Standards, High Rang, Prestained SDS-PAGE Standards, Low Range, Molecular Weight marker for SDS-PAGE, Ultra-low Range were used for measurements of molecular weight. Moreover, oxidation reduction potential was measured in order to specify biocompatibility on property of GOP extracted solution.

Result: SDS resulted that protein contained in GOP was found in 14KDa-66KDa migration. It also confirmed that extracted solution has an oxidation-reduction potential less than 330 mV and an average of pH less than 6.5 pH.

Conclusion: GOP extracted solution has a low value of oxidation-reduction potential and is a weak acid solution. GOP contains low-molecular-weight protein as one of its various compositions that may result to give impacts on blood pressure and cholesterol. Present data suggest that GOP is a composition agent with a high biocompatibility.

P-81

THE PUMP TREATMENT (CSII) IN TYPE 2 DIABETES COMPARED TO INTENSIFIED INSULIN ANALOG (MDI) TREATMENT: STUDY DESIGN AND THE FIRST RESULTS

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Background: The insulin pump treatment is considered as the most effective agent for the treatment of the Type 1 diabetes. In Type 2 Diabetes is the CSII treatment potentially very contributive since a CSII could reduce the insulin resistance.

Objective: To assess the CSII treatment efficiency compared to the MDI using the analog in Type 2 diabetes by monitoring various parameters of compensation (HbA_{1c}, weight, lipids, total daily insulin) and insulin sensitivity.

Methods: It is a prospective, randomized, controlled, cross-over 2 years follow up study. Up to now 34 subjects has been randomized with a bad control of the disease ($HbA_{1c} > 7\%$), treated with MDI. Eligible subjects will be randomized into one of the following treatment groups in a 1:1 ratio. Group A: First year MDI treatment, second year CSII treatment. Group B: First year CSII treatment, second year MDI treatment.

Results: The data from 14 patients were analysed after 1 year. The improvement in HbA_{1c} (Group A 8.0; vs. 7.4; Group B 9.4 vs.8.6) was determined, but not statistically significant in both groups. The total daily insulin was significantly reduced in the Group B (Group A 79 vs. 78 UI; Group B 78 vs. 53 UI, $P < 0.01$). Insulin sensitivity was not changed in both groups.

Results:

		Group A	*Group B*	P
Total daily insulin	Baseline	79 (55;118)	78 (62;107)	0.75
	1 Year	78 (55;123.5)	53 (47;77)	0.28
	Change	4 (−0.5;8)	−23 (−36;−10)	<0.01*
BMI	Baseline	30.64 (29.44;33.81)	32.39 (28.22;33.05)	0.91
	1 Year	31.28 (29.95;34.49)	30.82 (28.31;32.43)	0.91
	Change	0.64 (0.22;0.97)	−0.37 (−0.61;0.53)	0.12
HbA1c	Baseline	8.0 (7.4;8.3)	9.4 (7.9;9.7)	0.14
	1 Year	7.4 (7.2;9.0)	8.6 (7.5;9.8)	0.34
	Change	0 (−0.2;0.3)	−0.4 (−0.8;−0.2)	0.43

Conclusions: From the CSII treatment motivated, cooperative type 2 diabetes patients could profit. The CSII treatment leads to a significant reduction of the total daily insulin and the reduction of the body weight (but not in statistical significance).

P-82

DYNAMIC RISK SPACE OF CGM TIME-SERIES: ASSESSMENT OF QUALITY OF GLUCOSE CONTROL

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Background: A transformation of blood glucose measurements into a risk-scale was originally proposed by Kovatchev et al. (Diabetes Care, 1997) and recently further developed by Guerra et al. (ATTD Proceedings, 2010) in the so-called dynamic risk space (DRS) which accounts for information present in glucose trends. Here we show that DRS is useful to retrospectively assess, both qualitatively and quantitatively, the quality of glucose control in diabetics.

Methods and results: Trajectories from 10 CGM time-series (http://glucosecontrol.ucsd.edu/data.html) were analyzed in DRS. Figure 1 shows the CGM time-series (top) and trajectory in DRS (bottom, darker regions represent higher risk) for two representative diabetic subjects. In DRS, several geometrical indexes can be obtained to characterize the quality of glycemic control. For instance, the 95% confidence ellipsis (yellow) immediately highlights the less spread trajectory and hence tighter control of Subject1 with respect to Subject2. The blue whiskers represent the weighted hypo/hyper centers of trajectories which characterize the different tendency towards hypo/hyper in the two subjects.

Conclusions: Geometrical indexes in DRS help the characterization of patients and may be used to tune their therapy by highlighting particular features, e.g. evaluating the probability of overnight hypoglycemia as dispersion of the endpoints of dinner trajectories.

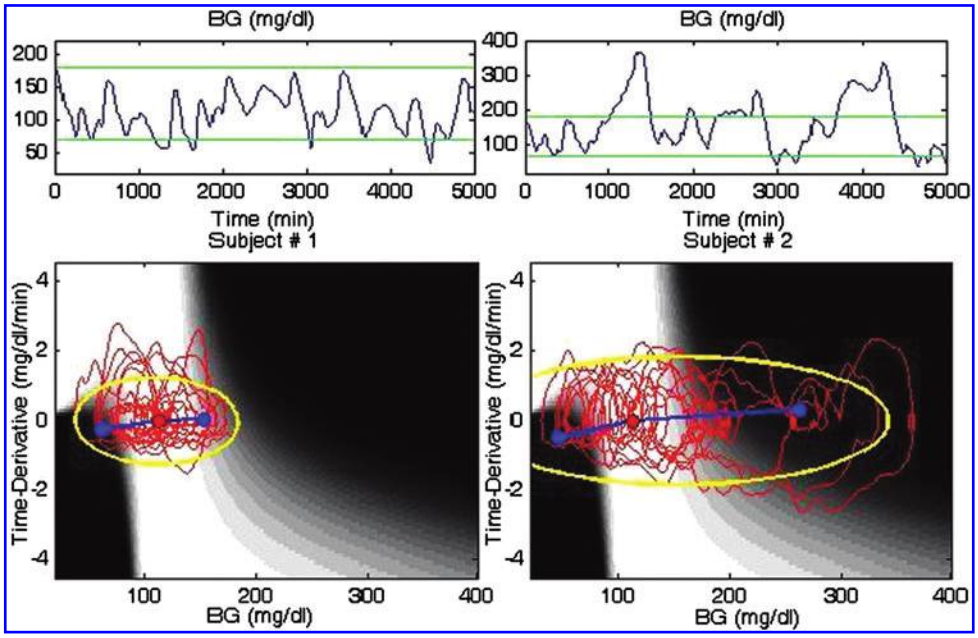

FIG. 1.

P-83

EFFECT OF OMEGA3 ON VISFATIN SERUM CONCENTRATION IN PATIENTS AFFECTED TO TYPE 2 DIABETES

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Background: Visfatin is a adipocytokin that is secreted from adipose tissue and it can be effective on the occurrence of diabetes, inflammatory reactions and on the level of serum lipids as well. From one side, Omega3 causes to decrease lipids and prevents from insulin resistance. In this study, the effect of Omega3 has been studied in comparison with placebo on Visfatin serum concentration and mean for changes of its concentration as well as other effective factors on Type 2 Diabetes.

Method of study: 71 females suffering from Type 2 Diabetes were divided to two groups under treatment with Omega3 capsule and control group with placebo capsule. In the taken blood sample, considered factors including Visfatin and lipid profiles, sugar and HbA1c were measured. Also age, height, weight, waist were taken through observation. The patients were evaluated regarding mean for Visfatin serum concentration. The obtained results were analyzed by SPSS.

Findings: Mean for Visfatin serum level had no significant difference before intervention in the two groups ($P = 0.14$) and after intervention mean for Visfatin serum level was significant in the two groups ($P < 0.001$). Also difference mean before and after Visfatin serum level showed significant difference ($P < 0.001$). Meanwhile it indicated that there is no significant correlation between Visfatin blood level and other variables under study such as Cholesterol, TG, LDL, HDL, and FBS ($P > 0.05$).

Conclusion: Considering study results, Omega3 can affect on Visfatin serum level and increase that and also cause to improve insulin effect in diabetic individuals.

P-84

COMPLICATIONS OF DIABETES MELLITUS TYPE 2 AMONG LONG-TERM PATIENTS IN RELATION TO KNOWLEDGE, ATTITUDES, AND PRACTICES AT CLINIC ALZAHRA, ISFAHAN

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This cross sectional study was carried out at clinic involving 60 DM2 outpatients 65% of females, mean age was $52.77 + 1.63$ years. 60.0% had an education level of up to secondary school, more than 50.0% were not working. The biochemical and clinical readings were taken from the patients' records during the study. 25.0% BMI of $27.52 + 0.62$ kg/m2, 69.5% had FBG of $8.86 + 1.76$ m mol/L, 56.5% having HbA1c $8.55 + 1.83$%, 64.5% had BP exceeding 140/90 mm/Hg.65% had complications due to DM2 with 45.6% of them had at least one type of complication while 8.3% and 6.6% had two-three types of complication respectively. 79.9% had low level knowledge on diabetes. 79.3% had positive attitudes towards their disease and 56.5% practiced good diabetic management. Only 20.0% exercised every day, 40.5% exercised 20–30 minutes a day. Pearson Correlation Test showed that there was a significant correlation ($P < 0.05$) between level of knowledge of respondents with age ($r = -0.302$), attitudes ($r = -0.275$) and BMI ($r = 0.254$). There was a significant correlation between attitudes with BMI ($r = 292$) and BS ($r = -202$) and between number of complications with HbA1c ($r = 0.360$), duration of having DM2 ($r = 270$). Diabetic education programs should be done by using effective language and medium of communications which should focus on the implementation of knowledge towards behavioral changes especially among the long-term diabetic patients in order to avoid and reduced serious complications.

P-85

CLINICAL AND ECONOMIC OUTCOMES OF CONTINUOUS GLUCOSE MONITORING AMONG SWEDISH RESIDENTS WITH POORLY CONTROLLED TYPE 1 DIABETES

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Introduction: According to data from the Swedish National Diabetes Register (NDR), among 40,000 Swedish residents with type 1 diabetes (T1DM), 7,200 (18%) remain poorly controlled (HbA1c $\geq$ 9%, DCCT; $\geq$ 75 mmol/mol, IFCC) despite intensive diabetes therapy. These individuals are at high risk for diabetes-related complications including serious retinopathy, nephropathy, peripheral neuropathy, hypoglycemia, and cardiovascular disease.

Methods: We used decision-tree modeling to compare anticipated rates and direct healthcare costs of diabetes-related complications among 7,200 Swedish residents with poorly-controlled T1DM who received continuous glucose monitoring with intensive diabetes therapy (CGM/IDT) versus those who received intensive diabetes therapy (IDT) alone. Clinical outcomes were obtained from the Sweden National Board of Health and Welfare; NDR; the Stockholm Diabetes Intervention Study; and published research. Economic data were obtained from published literature, and converted to 2010 USD.

Results: Assuming all 7,200 Sweden residents with T1DM and HbA1c $\geq$ 9% (DCCT) or $\geq$ 75 mmol/mol (IFCC) received CGM/IDT over one year, an expected 26% would achieve a 2-point HbA1c reduction, 50% a 1-point HbA1c reduction, and 24% no HbA1c improvement. CGM/IDT would confer 1,205 fewer cases of serious retinopathy, 514 fewer nephropathy cases, 1 less case of lower limb amputation due to peripheral neuropathy, 711 fewer cases of hypoglycemia, and 7 fewer cardiovascular disease-related events.

Conclusion: Although reductions in complication risk are not likely to occur immediately, and do not take into account the effects of temporal HbA1c changes, the ultimate benefits of CGM/IDT would account for a total annual cost savings in diabetes-related complications of $41.7 million.

P-86

ALPHA-AMYLASE INHIBITORS FROM THAI MEDICINAL HERBS FOR TREATMENT OF DIABETES

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Alpha-amylase inhibitors have been known as one of potent therapeutic drugs for treatment of non-insulin dependent diabetes mellitus (NIDDM). Thai medicinal herbs samples were extracted with aqueous methanol (10 mL/g fr.wt.) for 24 h at room temperature and subjected to porcine pancreatic a-amylase (PPA) inhibitory activity determination. Twenty-one of the 37 tested herb samples showed PPA inhibitory activity more than 50% and among these, 4 herb samples gave 90–100% PPA inhibition including leaves of guava, *Psidium guajava* L., (91.10%). The ethyl acetate partitioned fraction of guava leaves extract was purified using HPLC column: Inertsil PREP-ODS and the active fractions were structurally identified by FD-MS and ^{1}H-NMR and proven to be tannins and catechin. In addition, 3-arabinopyranoside was also obtained from HPLC column: Develosil C-30 and its structure was identified by FAB-MS and ^{1}H-NMR. The PPA inhibitory activity of tannin isolated from guava leaves extract was the most effective PPA inhibitor (100% PPA inhibition) while less activities were obtained from catechin (8% PPA inhibition) and 3-arabinopyranoside, respectively. This leads to potential possibility to develop the pharmaceutical products from guava leaves for treatment of NIDDM.

P-87

INSULIN BOLUS DELIVERY IN PAEDIATRIC PATIENTS USING INSULIN PUMP (IP) - OUR EXPERIENCE

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Background: TYPE 1 diabetes (T1DM) pediatric patients with IP therapy use different number and various types of boluses. Beside common normal and dual bolus our patients use combined bolus (normal + dual) for meal time, which imitate physiologic bolus delivered in non diabetic persons.

Methods: Paediatric patients who use insulin pump therapy (Medtronic Minimed 712 and 722) were divided into 3 groups according their HbA1c level : 1. HbA1c $\leq$ 7,0%; 2. HbA1c 7,1–8,0%; 3. HbA1c > 8,0%. After downloading data from IP memory we analysed daily number and frequency of boluses' type during one usual week.

Results: Data from 55 T1DM patients (M 34 /F24) treated by insulin pump for $\geq$ 6 months were downloaded. 1. group (20 patients): mean daily delivered boluses 5,8; 68% were combined boluses used on meal time; normal were 20% and dual 12% of all used boluses. 2. group (21 patient); average daily delivered boluses 6,9; 45% were combined; normal were 35%, dual were 20%. 3. group (14 patients): average daily delivered 4,5 boluses; 35% were combined boluses, normal were 55% and dual were 10%.

Conclusion: Most efficient type of bolus in our patients was combined bolus, which covered meal. Bad regulated patients used less boluses generally, and mostly used normal bolus for frequent snacks and corrections. Education for using appropriate number and type of bolus must be repeated in all unsatisfied regulated T1DM children with IP therapy.

P-88

PDX1 GENE MODIFIED HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS INDUCING TO DIFFERENTIATE INTO ISLET B-LIKE CELLS IN VITRO

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To explore pancreatic and duodenal homeobox factor 1(PDX1) Gene modified mesenchymal stem cells (MSCs) from human umbilical cord to differentiate into insulin-producing cells in vitro. Recombined adenovirus vectors inserted with PDX1

transfected MSCs for 7 days. After infected, cells were induced by cytokines. The genes' expression related to islet β cells such as PDX1, Neurogenin3 (NGN3), insulin, glucose transporter-2 (Glut2) and NK6 transcription factor related, locus 1 (NKX6.1) were detected by RT-PCR, immunocytochemistry and immumofluorescence, and Western blot. The levels of insulin secretion and C peptide secretion were examined by chemiluminescence immunoassay. The levels of insulin secretion and C peptide secretiont were examined with 25 mmol/L glucose stimulation after one hour. After infected by recombined adenovirus Adxsi-CMV-Pdx1 7 days and combined with cytokines induced 3 days, MSCs were aggregated and islet-like cell clusters formed. Dithizone staining of these cells was positive. After induction 10 days, the genes' expression related to islet β cells, such as Pdx1, ngn3, insulin, Glut2 and NKX6.1 could be detected by RT-PCR, immunocytochemistry and immumofluorescence and Western blot. After induction 17 days, the levels of insulin secretion and C peptide secretion were (473.11 ± 51.52) mu/L, (1.61 ± 0.41) ng/mL respectively. The levels of insulin secretion and C peptide secretion were (964.42 ± 68.19) mu/L, (3.72 ± 1.52) ng/mL respectively with 25 mmol/L glucose stimulation after one hour. We Conclude that: Adxsi-CMV-Pdx1 combined with cytokines can induce MSCs from human umbilical cord to differentiate into islet β-like cells. They can secret insulin and C peptide, and have the sensitivity to the stimulation of glucose.

P-89

ANTIDIABETIC ACTIVITY OF METHANOLIC EXTRACT OF SALVIA MIRZAYANII IN RATS

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In folk medicine, there are many uses of useful effects of some medicinal plants for treatment of disease. In traditional medicine of Kerman province, **Salvia mirzayanii** Rech. & Esfand. (Lamiaceae) or Mor-e-Porzo applies for treatment of diabetes mellitus. Literature reviews showed another species of **Salvia** had antidiabetic effects. In this study the male white rats were used. Diabetes was induced by IP injection of streptozocin (STZ) (64 mg/kg). Diabetes was allowed to develop in these STZ treated rat over of 24 h, and animals treated by 50, 100, 200, 400 mg/kg of the dried methanolic extract by IP injection for 24 hours and 400 mg/kg for 12 days. Finally surgery carried out on these animals and liver and pancreas were studied histopathologically. The maximal reduction in the blood glucose concentration of the fasted rats occurred with doses of 400 mg/kg of extract ($P < 0.01$). Histopathological studies showed the extract could repair and also increase blood supply of pancreatic cells of these diabetic animals. It is concluded that Methanolic extract of **S. mirzayanii** could reduce blood glucose of these diabetic animals and its considerable effects on damaged pancreatic tissue, persuade the complemented studies and investigation on this plant.

P-90

A GLUCAGON-EXTENDED MINIMAL MODEL FOR IN-SILICO TESTING OF BI-HORMONAL GLUCOSE CONTROLLERS

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Objective: In-silico validation of glucose controllers has been proven to be a valuable procedure for the development of an artificial pancreas. Current available simulators only incorporate insulin as a regulatory hormone, and do not include the effects of counter-regulatory hormones required for glucose homestasis. In this work, we propose an extension to the classic glucose-insulin minimal model in order to incorporate the effects of glucagon on glucose regulation. This new model now enables the testing of bi-hormonal controllers, a step towards a fully physiological artificial pancreas.

Method: In order to deal with subcutaneous insulin infusion and carbohydrates ingestion, existing models of subcutaneous insulin absorption and gastro-intestinal absorption were incorporated to the original minimal model. The same insulin absorption model was adapted for modeling subcutaneous glucagon absorption. A model of the currently available continuous glucose sensors was employed to represent the glucose measurements errors. An additional compartment was added to the minimal model in order to incorporate the glucagon effect on the glucose regulation. Clinical data were employed to identify the model parameters.

Result: The current glucagon-extended minimal model was used for testing a novel bio-inspired glucose controller. The obtained results show that tighter glycemic control can be achieved by using a bi-hormonal approach.

Conclusion: An extended minimal model incorporating glucagon is presented and validated. The new model allows the testing of bi-hormonal controllers in the context of an artificial pancreas. A cohort of virtual type 1 diabetic subjects is currently being implemented in order to incorporate the inter-patient variability.

P-91

IMPROVEMENT OF METABOLIC CONTROL BY USE OF NEWER PUMP MODELS IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES—A SINGLE CENTER EXPERIENCE

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Introduction: CSII is frequently used in pediatric patients. Several meta-analyses showed improvement in metabolic control with pump treatment. One possible cause could be the use of calculator programs for more accurate dosing of bolus amount and correction dose. After 4 years of CSII pediatric patients in Germany can change to newer pump models with more accurate calculator programs. To find out whether metabolic control improves after changing to newer pump models we reviewed the data in our patients.

Method: 17 patients; mean age: 12.9, diabetes duration 7.9, total CSII therapy 5,1, with newer pump model 1,0 years. All patients used either Medtronic or Roche pumps and chose newer pump models from the same company. Only one patient switched to Roche. Mean HbA1c were analyzed and divided into two groups: from beginning of CSII until pump changing and since newer pump model. Mean HbA1c from pubertal patients were adjusted to puberty.

Results: In all patients mean HbA1c from beginning of CSII until pump changing was 7.8% (6.7 - 9.8%), with newer pump models 7.6% (6.3 - 9.0%). Pubertal patients (n = 11) showed mean HbA1c from CSII start of 7.7% and 7.6% with newer pump models.

Summary: Using newer pump models after 4 years of CSII therapy led to mild improvement in glycemic control in our pediatric type1 diabetes patients. One possible cause could be the use of more accurate calculator programs. Pediatric patients should be better trained and encouraged to use calculator programs of newer pump models.

P-92

ANTIOXIDANTS ATTENUATED AGE-INDUCED RENAL TUBULAR HYPERTROPHY PARTLY THROUGH INHIBITION OF THE AKT/GSK/SGK SIGNALING

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The tubulointerstitium comprises approximately 90% of kidney volume and undergoes major pathologic changes in diabetes. Two key mediators implicated in the development of diabetic nephropathy include reactive oxygen species and advanced glycation end products (AGE). In this study, the role of the Akt/GSK/SGK signaling in AGE-induced renal tubular hypertrophy and the molecular mechanisms of antioxidants responsible for inhibition of renal tubulointerstitial fibrosis were examined. We found that AGE significantly increased Akt, GSK and SGK activation in human renal proximal tubular epithelial cells. These effects were not observed when cells were treated with nonglycated BSA. The Akt inhibitor (SH-5), the GSK inhibitor (A014418), and antioxidants N-acetylcysteine (NAC) and taurine treatments significantly attenuated AGE-inhibited cellular growth and AGE-induced hypertrophy. It seems that apoptosis was not observed in these treatments. There were no changes in Bcl-2 and poly(ADP-ribose) polymerase expression, caspase 3 activity, and mitochondrial cytochrome c release. However, SH-5, A014418, NAC or taurine markedly inhibited the stimulation by AGE of $p27^{Kip1}$ and α-smooth muscle actin protein levels. It is concluded that the Akt/GSK/SGK pathway may play a role in AGE-induced tubulointerstitial fibrosis. Antioxidants significantly attenuated AGE-enhanced cellular hypertrophy partly through inhibition of the Akt/GSK/SGK signaling.

P-93

INTERCEPTING INSULIN BOLUS REQUESTS—A KEY INGREDIENT OF INSULIN PUMP SAFETY SUPERVISION

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Introduction: Typically, the insulin boluses intended for delivery via insulin pump are pre-programmed and executed without further analysis, i.e. there is no safety judgment made between insulin request and insulin delivery. We propose a bolus interceptor as a *preventative* component of a safety supervision system (SSS) that will intercept potentially excessive insulin requests before delivery. This intermediary action alerts the user of insulin amounts that could result in hypoglycemia, or of other unwarranted insulin requests, such as errors in a closed-loop control algorithm.

Methods: The interceptor algorithm works by considering insulin-on-board information, the current and projected glycemic state of the patient, and the patient's biometric parameters. This information is used to determine if the requested insulin may cause hypoglycemia if no carbohydrates are ingested.

Results: In silico experiments indicate that the interceptor detected and issued warnings for 100% of pre-meal insulin boluses. Analysis of SSS activity in ten closed-loop control trials indicated that 100% of meal boluses were intercepted in vivo as well. As an example, in one patient, the bolus interceptor additionally prevented the delivery of 6 correction boluses, four of them posing a definite hypoglycemic risk.

Conclusion: We offer a preventative algorithm which, as part of a SSS, assists the prevention of hypoglycemia occurring with open- or closed-loop insulin delivery. The bolus interceptor communicates with the user to request approval before delivery of potentially dangerous insulin amounts, giving the user the opportunity to verify blood glucose, modify insulin amounts, and be advised about the need for carbohydrates.

P-94

EFFECTIVENESS OF STRUCTURED PROGRAM FOR GROUP TRAINING OF T1D PATIENTS USING CSII AND REAL-TIME CGM

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Aim: To assess how the structured program for group training influence glycemic control and quality of life (QoL) in patients with T1D using insulin pump therapy and real-time CGM.

Patients and methods: This 16-week pilot observation study included T1D patients (n = 12). Subjects were randomized to two study groups (6/6). Patients were educated by structured program at "diabetes school" and have passed technical training of insulin pump therapy. 1st group used combined insulin pump and real-time CGM and 2nd one used insulin pump therapy with SMBG only. Glycemic control was assessed by HbA_{1c}. QoL was assessed by "SF-36 health status survey".

Results: Both groups didn't differ significantly in HbA_{1c} ($8.1\% \pm 1.1$ vs $8.3\% \pm 3.3$, $P = 0.75$) and in data of QoL ($P > 0.05$) at baseline. Only RP middle scores were significantly higher in 2nd group ($P = 0{,}037$).

After 4months HbA_{1c} was significantly lower ($8.60\% \pm 2.4$ vs $7.36\% \pm 1.08$, $P = 0.0076$) and wasn't differ between groups ($7.3 \pm 0.6\%$ and $7.6 \pm 1.4\%$ respectively). SF index has significantly increased ($P = 0.028$) in 1st group and RP index has significantly increased in the 2nd one ($P = 0.043$). Developments of other QoL indexes weren't differ significantly. After 4 months of the research other QoL indexes didn't differ significantly in compared groups, but were better than before the research.

Conclusions: Glycemyc control and QoL are increased after group training by structured program in T1D patients using CSII and Real-Time CGM. Use of CSII with Real-time CGM primary elevate psychical component of QoL (SF index). Use of CSII with SMBG only primary elevate physical component of QoL (RP index).

Data of 1th Group Before and After Rsearch

Parameter	*Median (before research)*	*25–75% quartiles*	*Median (after research)*	*25–75% quartiles*
Physical Functioning (PF)	85	75–95	92.5	85–95
Role-Physical (RP)	25	25–50	50	25–75
Bodily Pain (BP)	30	20–50	35	10–50
General Health (GH)	42.5	25–65	40	35–70
Vitality (VT)	35	25–50	62.5	55–75
Social Functioning (SF)	56.25	50–75	56.25	50–62.5
Role-Emotional (RE)	33.3	33.3–100	50	33.3–100
Mental Health (MH)	48	28–60	52	44–68
HbA1c	8.1	7.7–9.0	7.3	7.1–7.5

Data of 2th Group Before and After Research

Parameter	*Median (before research)*	*25–75 quartiles*	*Median (after research)*	*25–75 quartiles*
Physical Functioning (PF)	95	95–95	90	75–95
Role-Physical (RP)	100	50–100	47.5	25–65
Bodily Pain (BP)	0	0–20	32.5	20–40
General Health (GH)	60	35–75	60	35–75
Vitality (VT)	55	45–55	57.5	50–70
Social Functioning (SF)	50	50	50	45–55
Role-Emotional (RE)	67	33.3–100	44.15	33.3–66.7
Mental Health (MH)	54	52–72	52	44–64
HbA1c	8.3	6.7–9.5	7.4	6.6–7.9

P-95

FREQUENCY OF METABOLIC SYNDROME AMONG TYPE 2 DIABETIC PATIENTS

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Objective: To determine the frequency of the metabolic syndrome in patients with type 2 diabetes mellitus who attended the diabetes clinic of Nishtar Medical College and Hospital, Multan.

Methods: A cross sectional study was conducted at the diabetes clinic of Nishtar Medical College and Hospital, Multan from June 2010 to August 2010. 187 patients with type 2 diabetes were enrolled in the study. Frequency of metabolic syndrome was estimated according to the criteria proposed by American Heart Association/National Heart, Lung and Blood Institute.

Results: Total number of patients enrolled was 187 out of which, there were 67 male patients and 120 female patients. The mean age of the patients was 48 years and the range was 28 to 80 years. By applying American Heart Association criteria, metabolic syndrome was found in 65.2% type 2 diabetics, (43.31% women and 21.92% men).

Conclusion: The frequency of the metabolic syndrome was found very high in our study. This was especially high in type 2 diabetic women at a frequency of 43.31%.

P-96

MINING THE UNDERLYING MOLECULAR MECHANISM OF TYPE 2 DIABETES (T2D) AND HYPERTENSION (HT)

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Background and aims: The prevalence of diabetes is increasing worldwide. Mounting evidence indicates that obesity along with increased rates of other chronic inflammatory diseases, such as hypertension (HT) and cardiovascular diseases (CVD) are highly associated with T2D. How all theses complications arise and manifest together is yet to be solved. Thus, understanding the underlying molecular mechanisms of T2D is essential for developing more targeted and effective therapies and preventive approaches against diabetes-related complications.

Methods: We applied a systems biology approach to build the gene interaction network models, comprised of high throughput genomic and PPI data for T2D and HT. The genes differentially regulated through T2D and HT were 'mined' and their 'connections' were studied to get a more complete understanding of the overall gene network topology and their role in disease progression.

Results: By analyzing the genes related to T2D, HT and other closely associated diseases a highly regulated gene-disease integrated network model has been developed that provides useful functional linkages among groups of genes and thus addressing how different inflammatory diseases are connected and propagated at genetic level. Based on the investigations around the 'hubs' that provided more meaningful insights about the cross-talk within gene-disease networks in terms of disease phenotype association with oxidative stress and inflammation, a hypothetical co-regulation disease mechanism model been proposed.

Conclusion: The results from this study revealed that the oxidative stress mediated regulation cascade is the common mechanistic link among the pathogenesis of T2D, HT and other inflammatory diseases such as OBS.

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CARELINK™ PRO AND DIASEND CLINIC™— TWO SYSTEMS TO SIMPLIFY DIABETES DATA MANAGEMENT BUT ALSO WITH DIFFERENCES IN APPROACH AND SPEED

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Introduction: CareLink™ Pro and Diasend Clinic™ allow data from glucose meters and insulin pumps to be downloaded to a web site. This could simplify data management.

Objective: Differences are seen regarding the need of software and compatibility, but also the way data is presented, which could affect the speed of the downloading process and active analysis.

Aims: To compare CareLink™ Pro and Diasend Clinic™ regarding the time to download different glucose meters and the perform an active analysis on screen.

Method: Different glucose meters from four major glucose meter companies were downloaded with CareLink™ Pro and Diasend Clinic™ in the same place and reviewed on the same computer. Time was measured from start of download until completion. The time it took to visualize identical views during active analysis was also measured. Views of tables, graphs and statistics, together with two different time intervals were chosen as targets.

Results: Fourteen different glucose meters were downloaded. The mean number of glucose values in the meters was 434 ± 292 values. Diasend Clinic™ was significantly faster completing the download, 55.7 ± 16.1 sec vs. 71.9 ± 22.6 sec, $P < 0.05$. Comparison of time for active analysis on screen also showed that Diasend Clinic™ was clearly faster, 68.1 ± 16.8 sec (1min 8 sec) vs. 162.9 ± 30.6 sec (2 min 43 sec), $P < 0.005$.

Conclusion: Diasend Clinic™ is faster than CareLink™ Pro when comparing both the time it takes to complete a download of glucose meters and to find key target views during active analysis on screen.

P-98

CELLPHONE USE TO IMPROVE GLUCOSE CONTROL IN A BUSY HOSPITAL PRACTICE

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Both type 1 and type 2 diabetes with a HbA1c > 8.0% where randomized to only education or education and a monthly sms for 4 months. The sms to the patient stated the following: **Remember to check bloodglucose at wake up and 2 hours after meals. If the wake up value is >5.5 mmol or 2 hours after meals >8.0 mmol on 3 occasions please phone your diabetes educator**. The study population included all diabetics presenting at the clinic during a 3 month period. Age range between 13 and 70 years. Both caucasian and non-caucasians. All pregnant diabetics were excluded as well as diabetes of less than 6 months duration or presenting with a complication. Baseline HbA1c was done and again after 4 months. The preliminary results of this study of 300 patients showed a 62.5% decrease in the sms group. There was an average 2% lowering of the HbA1c in the sms group. This was done with more active involvement of the patient into the disease with the use of easy cellphone technology that did not increase the work load of the practise and gave a good HbA1c reduction.

P-99

CLOSED-LOOP AND SEMI CLOSED-LOOP STRATEGIES FOR CONTROL OF BLOOD GLUCOSE IN PEOPLE WITH TYPE 1 DIABETES

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We compare 4 closed-loop and semi closed-loop sc-sc strategies for control of blood glucose in people with type 1 diabetes. These strategies represent idealized controllers with different information about meals and elucidate fundamental control performance limitations. These limitations apply to Model Predictive Control (MPC), PID-control, and any type of control algorithm. All 4 control strategies are evaluated and compared using virtual clinics.

The 3 first control strategies are based on Nonlinear Model Predictive Control (NMPC) and represent an upper limit on the achievable control performance. The first control strategy is based on meal announcement in advance of the meal. The second control strategy announces meals at meal time. The third control strategy is based purely on a combination of blood glucose feedback and meal announcements. For this controller we address cases with correct, incorrect and no meal information. In these cases, the ideal insulin administration profile consists of a bolus like part at meal time and residual part that we call the basal part. The fourth control strategy is a novel combination of a model based bolus calculator and a feedback controller that adjusts the basal insulin rate. This controller receives feedback from a combination of meal announcements and continuous glucose sensors (as in strategy 3). The bolus calculator also computes a blood-glucose concentration profile that is used as setpoints by the feedback controller that adjusts the basal rate. To improve performance, we also consider pre-meal lowering of glucose setpoints to robustly reduce post-prandial peaks.

P-100

POSSIBLE NEUROPROTECTIVE EFFECT OF THYMOQUINONE (ACTIVE INGREDIENT OF NIGELLA SATIVA) IN THE TREATMENT OF EXPERIMENTAL DIABETIC NEUROPATHY

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Background/aims: Neuropathy is most common debilitating complication of diabetes. Activated pathways that alter the metabolic and redox state of the cell are major source of damage and are potential therapeutic targets in diabetic neuropathy (D.N.). This work aimed to explore the protective effect of Thymoquinone (TQ), an active ingredient of Nigella sativa, against nerve damage in D.N. model. Furthermore, the role of inflammatory signaling pathways; NF-κB and p38 M.A.P.K in the pathogenesis of D.N. and their modulation by TQ were investigated.

Methods: 60 rats were divided into the following groups: Control, TQ (20 mg/kg/day for 8 weeks), D.N. (streptozotocin 65 mg/kg), D.N. treated with either TQ, insulin (12 IU/kg/day)

or combination of insulin and TQ. Functional electrophysiological nerve parameters (tail flick latency, maximum compound action potential (M.C.A.P.), conduction velocity (C.V.), relative refractory period (R.R.P.), chronaxie and gene expression of NF-κB and p38 MAPK were assessed in sciatic nerve.

Results: D.N. rats treated with T.Q. showed improved nerve parameters than in D.N. group evidenced by significant prolongation of tail flick latency, increase in M.C.A.P. amplitude and C.V as well as significant shortening of R.R.P. & chronaxie. Significant reduction in NF-κB and p38 M.A.P.K expression were also observed. Combination therapy with insulin and TQ offered the best protection against the measured nerve parameters and resulted in more significant decrease in NF-κB than either monotherapies.

Conclusions: TQ exerted a partial protective effect against nerve damage in experimental D.N. through targeting NF-κB & p38 M.A.P.K. It provides promising therapeutic strategy in combination therapy with insulin.

P-101

APPLICATION OF IN SILICO TECHNOLOGY IN DRUG DISCOVERY

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Strategy and especially the role of computer-aided drug design in drug discovery strategy of KRICT, are introduced. New drug development project in KRICT was specifically targeted for a few selected disease areas such as stroke, obesity, diabetes, those are prevalent and burdensome to ever expanding aging populations. The goal of drug discovery program KRICT is to develop new drug candidate compounds in the aforementioned therapeutic areas by enabling and securing at the global competitive level. As infra technological platform, in silico technology plays an important role in the process of development of KRICT's new drug candidate. From the early stage, discovering hit compounds from large library, to lately stage, confirming physicochemical properties and ADME/T properties of candidate compounds, in silico technologies have carried out many project including diabetes, obesity, etc. One of them, the development of DPP 4 inhibitor was taken as an example study. DPP 4 is a serine protease which cleaves the N-terminal dipeptide with a preference for Xaa-Pro or Xaa-Ala. Inhibition of DPP 4 increases the level of circulating glucagons-like peptide 1 (GLP-1), an incretin hormone stimulating glucose-dependent insulin biosynthesis and secretion, and thus increases insulin secretion, which can ameliorate hyperglycemia in Type 2 diabetes. Januvia (MK-0431) was launched into market several years ago, firstly. To develop DPP 4 inhibitor, we combined the established infra technologies and cooperated many scientists of different research areas, and so we found out a drug candidate for DPP 4 inhibition.

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ROLE OF CONTINUOUS SUBCUTANEOUS INSULIN INFUSION (INSULIN PUMP) IN REDUCING BLOOD GLUCOSE IN FOUR PATIENTS WITH TYPE 2 DIABETES AND CIRRHOSIS: A CASE SERIES

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Background: Some people with type 2 diabetes mellitus (T2DM) and cirrhosis tend to have fluctuating glucose values usually uncontrolled with conventional therapy. The aim of this case series was to retrospectively analyze the effect of continuous subcutaneous insulin infusion (CSII) on four patients with cirrhosis and poorly controlled T2DM.

Methods: The four patients in this case series presented with chronic cirrhosis with no ascitis, preexisting T2DM, and inadequate blood glucose (BG) control with conventional insulin therapy. After initiation of CSII, patients' BG values were monitored at regular intervals, and basal and bolus doses were adjusted. Fasting BG, postprandial BG, and glycated hemoglobin A1c (HbA1c) values were monitored while the patient was in the hospital, upon discharge, and at one follow-up visit.

Results: The daily dose of insulin was reduced in three patients. Fasting and postprandial BG values returned to normal ranges for all four patients. HbA1c was reduced in all four patients and reduced to normal ranges in two patients. There were no recorded incidents of severe hypoglycemia, diabetic ketoacidosis, or weight gain associated with the use of CSII.

Conclusion: Initiation of CSII in patients with T2DM and cirrhosis was beneficial in controlling BG values in the four patients studied in this case series.

P-103

EARLY ASSESSMENT OF A NEW RF PROTOCOL FOR FUTURE MEDTRONIC SENSOR AUGMENTED PUMP SYSTEMS

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Objective: To support continued advancements in sensor-augmented pump (SAP) systems, Medtronic is developing and testing new RF protocols for suitability for use in future product systems. Primary objectives for new RF systems include improving communication distance, power usage, network reliability, data transfer time (bit rate), and network security.

Methods: The RF protocol tested is a low-power, bidirectional protocol based on IEEE 802.15.4 which operates in the 2.4GHz ISM band. The network functions as a star network with a coordinator and the possibility of many endnodes. Patients wore devices which communicated over RF periodically for up to six days. The network performance was tested for feasibility in next generation SAP systems. Real time data loss and alarm frequency due to network issues were examined.

Results: Thirty subjects wore the new system to test performance. On average, there were 0.91 instances per day where real-time communication was lost due to RF performance. The average time out of network was 14.3 minutes. Many of these network losses were short in duration and resolved automatically by the system without needing any user interaction. On average, there were 0.16 RF related user alerts per day requiring user interaction to resolve.

Conclusion: As SAP systems increase in complexity and use CGM to control insulin dosing, reliable and secure data transfer among components in the system become increasingly crucial. A new RF protocol with bi-directional communication, reliable, secure, and long distance communication, and worldwide frequency acceptance will be crucial to the development of next generation SAP systems.

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DPP-IV INHIBITORS IMPROVED GLYCEMIC CONTROL IN TYPE 2 DIABETES PATIENTS

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Objectives: Estimate efficacy of vildagliptin therapy on glycemic control and glucose variability in different combination regimens of Type 2 Diabetes.

Materials and methods: 60 patients (9 M/51F) with Type 2 Diabetes participated in this 12-week study. Baseline therapy for the 1st group was metformin, for the 2nd group - basal insulin and for the 3rd group - metformin + basal insulin. Vildagliptin 50 mg was added on baseline therapy. Primary outcome parameters were the following: fasting (FPG) and postprandial (PPG) plasma glucose, HbA1c, fasting insulin and C-peptide, 72-h Continuous Glucose Monitoring.

Results: Dynamic of primary outcomes in the 1st group: FPG decreased from 10.03 ± 0.66 to 6.12 ± 0.22 mM/L ($P < 0.001$), PPG from 12.26 ± 0.87 to 7.53 ± 0.31 mM/L ($P < 0.001$), HbA1c from 8.59 ± 0.25 to $6.71 \pm 0.18\%$ ($P < 0.001$), GAAHL (glucose area above high limit) from 2.26 ± 0.62 to 0.94 ± 0.48 Mm/L*Day ($P < 0.001$), HOMA-IR from 7.31 ± 1.3 to 3.18 ± 0.48 ($P < 0.001$). In the 2nd group: FPG decreased from 10.71 ± 0.84 to 6.41 ± 0.35 mM/L ($P < 0.001$), PPG from 15.04 ± 0.71 to 8.87 ± 0.52 mM/L ($P < 0.001$), HbA1c from 9.52 ± 0.30 to $6.97 \pm 0.43\%$ ($P < 0.001$), GAAHL from 6.66 ± 0.97 to 1.50 ± 0.47 Mm/L*Day ($P < 0.001$), HOMA-IR increase from 8.11 ± 2.89 to 9.3 ± 5.33 ($P > 0.001$). In the 3rd group: FPG decreased from 8.92 ± 0.48 to 5.99 ± 0.40 mM/L ($P < 0.001$), PPG from 12.95 ± 0.89 to 8.02 ± 0.52 mM/L ($P < 0.001$), HbA1c from 8.37 ± 0.30 to $6.66 \pm 0.25\%$ ($P < 0.001$), GAAHL from 2.55 ± 0.71 to 103 ± 0.60 Mm/L*Day ($P > 0.001$), HOMA-IR from 8.02 ± 1.28 to 5.55 ± 1.27 ($P > 0.001$).

Conclusion: Vildagliptin added to metformin and insulin proved its high efficacy and safety, particularly in improvement of daily glycemic control and glucose variability.

P-105

THE ENGAGEPREDICT TECHNOLOGY TO PREDICT ONGOING PROGRAM ENGAGEMENT

A. Kaufman

DPS Health, Los Angeles, CA, USA

Many patient education and health promotion programs—particularly those delivered through information technology—expect patients to remain engaged with the program over a period of time. These programs expect a patient to perform some action—attend a class, logon to an application, track behavior, etc—over multiple weeks, months and even years.

Requisite for the program's desired outcomes, whether improved education or behavior change, is that patients remain engaged with the program. EngagePredict represents a novel technology and statistical algorithm to assess the likelihood that a patient, in a particular program will remain engaged with that program into the future. EngagePredict uses patient demographic characteristics, prior history and accounts for unobserved heterogeneity to predict future expected engagement.

EngagePredict provides valuable information to program operators and developers. The deeper understanding and prediction about current patient engagement, allows clinicians to target interventions to those who need them most. Program developers use the knowledge of successful program engagement to enhance and modify program, improving overall patient experience.

DPS Health has applied EngagePredict to a number of intervention programs. This has yielded valuable insight about how to best utilize program resources, which patients to target for intervention and how to evolve this program.

This poster presents the EngagePredict model and the results of its application to actual patient education and behavior change programs.

P-106

TECHNOLOGY ENABLED MONITORING OF PATIENTS' PERFORMANCE AND HEALTH STATUS: ONE KEY TO HIGH QUALITY PATIENT CENTERED CARE AND EFFECTIVE CARE COORDINATION

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Efficient monitoring of patients' health behaviors and well-being are essential for clinicians to effectively coordinate care and improve diabetes outcomes. Since long-term outcomes for patients with diabetes are in large part determined by patient behaviors, clinicians need a full picture of their patients' actions and health status.

For clinicians to provide and effectively coordinate high quality, patient-centered care, they need patients who are able to effectively monitor and independently act upon their health status including biologic parameters (e.g., blood glucose, weight, blood pressure), behaviors (e.g., diet, physical activity, sleep patterns), and mental health (e.g., mood, sense-of-well-being,). They need to have informed patients who are engaged with self-monitoring and motivated to practice health-promoting behaviors.

In the technology age this is becoming increasingly easier as more sophisticated devices are developed (e.g., continuous glucose sensors, accelerometers measuring physical activity intensity, scales that can be uploaded to the internet), and improved methods allow the assessment of a variety of measures reported by the patient based on experiences and perceptions during a particular time period (called Ecological Momentary Assessment).

This poster will describe ways to effectively help patients monitor and track behaviors and provide user friendly data and information displays so patients will have the wisdom and motivation to make and implement wise decisions. It will also describe the requirements for displaying the results so clinicians can easily use information in clinical decision making.

As health care evolves paying for outcomes rather than payment per service provided, monitoring patient behaviors will become even more important.

P-107

COMPARISON OF CONTINUOUS SUBCUTANEOUS INSULIN INFUSION AND MULTIPLE DAILY INSULIN INJECTIONS DURING PREGNANCY COMPLICATED BY TYPE 1 DIABETES MELLITUS

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CSII treatment in T1DM women during pregnancy seems to be non-inferior compared to MDI.

Aims: To prove it we analyzed retrospectively glycaemic control, pregnancy outcome and changes in insulin requirement, comparing data of 19 CSII- to 13 MDI-treated T1DM pregnancies.

Patients and methods: Age of women in CSII and MDI groups did not differ (29.9 ± 3.0 vs. 30.3 ± 4.2 yrs), the CSII group's diabetes duration was longer (15.4 ± 7.4 vs. 8.3 ± 6.8 yrs), starting weight (62.9 ± 6.9 vs. 68 ± 12 kg) and BMI were smaller (22.2 ± 2.2 vs. $24.1 \pm 4.1\,kg/m^2$), diabetic retinopathy was more frequent (10/19 vs. 1/13); ($P < 0.05$). Duration of pump therapy was 18.9 ± 21 months. There was no difference in starting HbA1c and fructosamine.

Results: HbA1c values in the three trimesters (6.1 ± 0.5 vs. 6.2 ± 0.6, 5.5 ± 0.4 vs. 5.5 ± 0.7, 5.9 ± 0.5 vs. $5.7 \pm 0.6\%$) did not differ. No differences in starting daily insulin dose (35.4 ± 7.4 vs. 40.2 ± 14.4 IU) and in maximal increase in insulin requirement (2.1x vs. 2.5x)($P = NS$). Similar weight gain during pregnancy (16.2 ± 5 vs. 15.3 ± 4.7 kg), severe hypoglycaemia: 2 vs. 1. Outcomes of pregnancies: in CSII group: 4, in MDI group: 2 early abortions. Time of delivery and rate of Caesarean sections did not differ, no stillbirth, 1 congenital malformation in MDI group. Birth weight (3548 ± 528 vs. 3698 ± 503 g), birth weight >90 percentile (6 vs. 4 cases) did not differ.

Conclusions: Despite longer diabetes duration and more complications in CSII group glycaemic control and pregnancy outcomes were identical with MDI group, demonstrating good efficiency of pump therapy during pregnancy even in patient population with higher risk.

P-108

CGM AS A TOOL FOR THERAPEUTIC DECISION MAKING (TDM) IN TYPE 2 DIABETES MELLITUS

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CGM use is more with T1DM and pumps.

However, there is growing evidence of glycemic variability and pathological significance thereof. We sought to explore use of CGM in T2DM on OHA and OHA + insulin in TDM.

From patients on regular treatment, 24 were chosen by convenience sampling. They were asked to keep diary on diet, exercise timing, duration, medications etc., Out of 7 patients on OHA, 4 had A1c < 7.0. Despite this, 2 of 4 showed trends usually missed by SMBG and were given tailored counselling and treatment.

One with low trends followed by rebounds in early morning was started on gliptin.

Of 2 with poorly controlled diabetes, advantages over SMBG were less obvious, but one patient showed times of prolonged hyperglycemia, impossible to obtain with SMBG (e).

Of 17 on insulin and OHA, 8 showed outcomes similar to SMBG.

9 given individualized treatment.

- changing basal insulin shots from evening to morning to avoid early morning lows (a);
- adding bolus insulin to basal regimen, timed to meal preference (b);
- changing to rapid analogue following poor control postprandially, followed by low sugar after few hours (c);
- subject had irregular meal times & late lunches due to job nature, leading to low sugars afternoon (d). CSII advised to enable variable basal rates & desired boluses;

The visual impact of CGM was powerful to change attitudes and lifestyles (f) and in selective T2DM, CGM is superior to SMBG in "TDM".

P-109

PREGNANCY, WOLFRAM SYNDROME (DIABETES INSIPIDUS, DIABETES MELLITUS, OPTIC ATROPHY, DEAFNESS) AND INSULIN PUMP: BENEFITS BEYOND GLYCEMIC CONTROL

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Wolfram Syndrome (WS) occurs 1 in 770,000 live births. Till date only 5 cases of successful pregnancy have been reported. This is the 6th & 1st WS on insulin pump. WS is a rare progressive neurodegenerative disorder with more than 65% dying before 35 years. Benefits of CSII in prevention of abortions and fetal malformations are well proven. Here the effects beyond glycemic control are demonstrated in a 31 y.o with WS, born of consanguineous marriage.

She was diagnosed of DM at age 5, optic atrophy at 14 and basal HbA1c was 8.3% when she first visited us 5 years back. DI was well controlled with desmopressin. She married from outside the family. The presence of uncontrolled diabetes and WS with limited longevity made her baby precious. She was started on pump a year prior to pregnancy. This considerably enhanced her QOL. Throughout pregnancy, sugars were intensively managed with frequent SMBG, CGM and utilising basic and advanced functions of pump. Correction boluses were administered whenever SMBG values were >125 mg%, thus maintaining an A1c below 6%. She delivered a healthy male baby of 3.1 kg via caesarean section. CSII is still continued in the subject (F.C-peptide 0.03 ng/mL) who has presently diffuse cerebral and cerebellar atrophy (MRI Scan March 2010), bilateral hydroureteronephrosis etc. Recent studies throw light into profound benefits of CSII beyond glycemic control reported as 87% reduction in peripheral neuropathic pain and 83% improvement in sexual function. WS being a progressive neurodegenerative disorder will probably help understand positive impact of CSII in enhancing longevity and retarding neuronal damage.

P-110

HYPO- AND HYPERGLYCEMIA EPISODES DURING RAMADAN IN PATIENTS WITH TYPE 2 DIABETES

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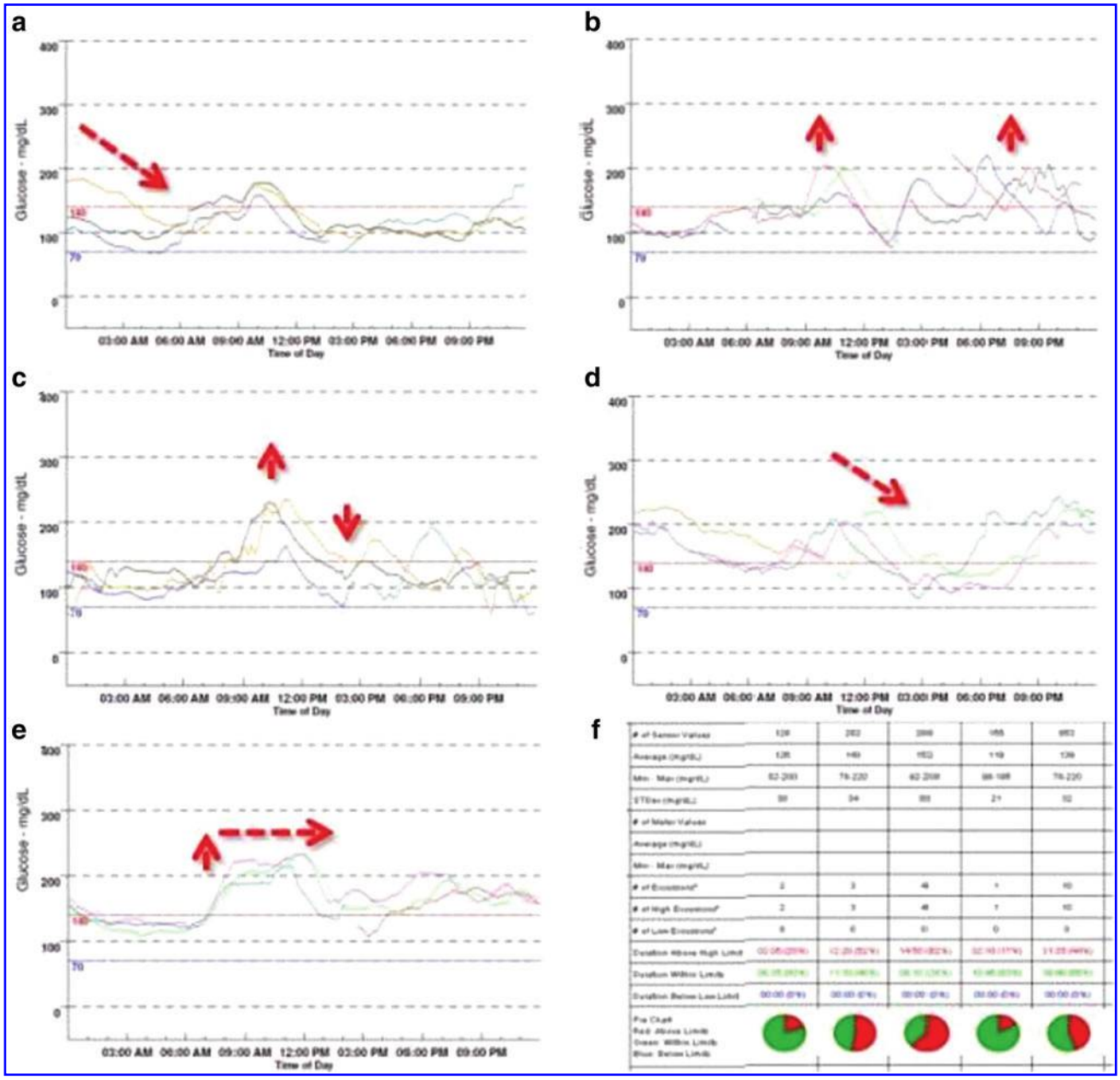
Aim: The study was aimed to record the frequency of hyper/hypoglycemia during 2009's Ramadan fasting month among patients with diabetes type 2 (DT2).



CGM dev. (see P-108 on page 237).

Methods: The study took place in the University Hospital of Sidi-Bel-Abbes and the volunteer outpatients who wanted to fast during Ramadan consisted of 44 diabetic patients aged of 56 ± 10 years, receiving oral antidiabetic drugs (OADs), followed no specific diet, on medications, and presenting no degenerative complications.

Results: showed that 88.68% of diabetic patients fast deliberately without taking advice from physicians. More hyperglycemia episodes were recorded (96%): averaged 300 ± 85 mg/dL, than hypoglycemia (4%). The change in OADs dose by diabetics and the high calories intake were the main factors contributed to increase the frequency of hyperglycemia episodes.

Conclusion: Ramadan fasting is not forbidden for patients with DT2 who are balanced; however, for those who want to fast, they need a particular attention and education in order to avoid hypo- or hyperglycemia episodes.

P-111

ROLE OF DIET IN MODULATION OF POSTPRANDIAL LIPEMIA AMONG 51 SUBJECTS WITH TYPE 2 DIABETES

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Background: Postprandial lipemia is characterized by increased triglyceride-riche lipoproteins after eating. There are several lines of evidence suggesting that postprandial lipemia remains an important cause of atherogenesis, especially in patients with diabetes type 2. Clinical data showed a correlation between postprandial lipoproteins concentration and the set or progression of coronary artery diseases.

Aim: The purpose of this study, which took place in the Diabetes Centre (Ex Gambetta) and in the University hospital (Hassani Abdelkader) of Sidi Bel Abbès city, was to show the role of diet in the modulation of postprandial lipemia level and furthermore to demonstrate the importance of this parameter in the control of 51 patients with diabetes type 2.

Methods: The daily food intake, during the course of 3 days, was recorded and then the weight, height, waist and hip circumferences, Body Mass Index and Waist to Hip Ratio were measured. Blood samples were analyzed for fasting glucose, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol in fasting state and 2 hours postprandially.

Results: Results determined that postprandial lipemia was more linked to the diet, particularly to saturated fatty acids which were correlated ($r = 0.22$) with postprandial triglyceridemia and postprandial cholesterolemia ($r = 0.30$). A correlation was also found between postprandial triglycerides and the BMI, especially in obese individuals.

Conclusions: In order to prevent the diabetes' complications, the quality of diet plays an important role in the modulation of postprandial lipemia level that should be monitored and treated in patients with diabetes type 2.

P-112

HYPO- AND HYPERGLYCEMIA EPISODES DURING RAMADAN IN PATIENTS WITH TYPE 2 DIABETES

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[1]*Department of Biology,* [2]*Endocrinology Diabetes, Djillali Liabes University, Sidi Bel Abbes, Algeria*

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Methods: The study took place in the University Hospital of Sidi-Bel-Abbes and the volunteer outpatients who wanted to fast during Ramadan consisted of 44 diabetic patients aged of 56 ± 10 years, receiving oral antidiabetic drugs (OADs), followed no specific diet, on medications, and presenting no degenerative complications.

Results: showed that 88.68% of diabetic patients fast deliberately without taking advice fromphysicians. More hyperglycemia episodes were recorded (96%): averaged 300 ± 85 mg/dL, than hypoglycemia (4%). The change in OADs dose by diabetics and the high calories intake were the main factors contributed to increase the frequency of hyperglycemia episodes.

Conclusion: Ramadan fasting is not forbidden for patients with DT2 who are balanced; however, for those who want to fast, they need a particular attention and education in order to avoid hypo- or hyperglycemia episodes.

P-113

EVALUATION OF VENLAFAXINE ON GLUCOSE HOMEOSTASIS AND OXIDATIVE STRESS IN DIABETIC MICE

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Rationale: Depression occurs frequently with diabetes affecting the quality of life. All major classes of antidepressants have been shown to have a direct pharmacologic effect on metabolic function, which further worsens glycemic control. There were no reports of effects of venlafaxine on glucose levels and oxidative stress in diabetic animals.

Objectives: The present study evaluated the effects of venlafaxine (8 and 16 mg/kg/day) on glucose homeostasis along with oxidative stress in brain in diabetic mice [streptozotocin (STZ), 40 mg/kg/day × 5 days].

Results: We observed that 21 days administration of venlafaxine (8 and 16 mg/kg/day) in diabetic mice significantly enhanced swimming in normal and STZ treated mice with a corresponding reduction in immobility. No significant difference in blood glucose levels were observed in diabetic and normal mice following venlafaxine treatment. Venlafaxine (16 mg/kg) reversed STZ induced elevated thiobarbituric acid reactive substance (TBARS) levels and also restored the glutathione (GSH) levels of diabetic mice. Venlafaxine (8 and 16 mg/kg) *per se* does not produced any significant effect in normal animals.

Conclusion: This study demonstrated dose-dependent antidepressant action of venlafaxine in diabetes induced depressive mice. It does not interfered with the blood glucose levels in normal and diabetic mice. It also reversed STZ induced oxidative stress in mice brain as evident by decrease in TBARS and elevation in GSH levels. Venlafaxine treatment appears to be a desirable drug in depression associated with diabetes.

P-114

PREDICTORS OF DIABETIC FOOT AMONG DIABETIC PATIENTS ACCORDING TO WAGNER'S CLASSIFICATION IN A RURAL TEACHING HOSPITAL

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Diabetic foot is one of the common and most devastating preventable complications of diabetes mellitus.

Objectives: To determine the proportion and risk factors for diabetic foot patients.

Methodology: 500 symptomatic diabetic patients were examined out of which 55 patients had diabetic foot lesions according to Wagner's grading. The association & comparison of risk factors with diabetic foot were highlighted. Since this was a descriptive study, therefore no inferential tests were applied.

Observation: Wagner's grade 2 (34.5%) diabetic foot were the most common clinical findings followed by Wagner's grade 3 (23.6%). Foot pain (69.1%) was commonest symptom and commonly occurs in (100%) cases of Wagner's grade 2 & 5. (61.5%) foot ulcer were on weight bearing point, (72.7%) of them occur in Wagner's grade 4. (78.2%) foot ulcer were deep seated, commonly occur in (100%) cases of Wagner's grade 3, 4 & 5. Callosities (78.2%) were commonest sign and commonly occur in (100%) cases of Wagner's grade 5. Hallus valgus/varvarus/Limitus (50.9%) were commonest deformity and most commonly (63.2%) occurred in Wagner's grade 2. Impaired S-W monofilament response (69.1%), abnormal achilis tendon reflex (50.9%), impalpable dorsalis pedis artery (40.0%), Ankle Brachial Index (<0.8) ($=16.4\%$) and per-cutaneous Oxygen saturation ($<90\%$) ($=43.6\%$) were most common examination findings. On average (64.5%) had negative response towards foot health care practices.

Conclusion: Significant risk factors for diabetic foot were: age (41–60yrs), male gender, increased duration, type 2 & glycaemic control of DM, smoking, occupation, presence of peripheral neuropathy, vasculopathy and improper foot care.

P-115

MICRO-CT SCANNING AS A TOOL FOR VISUALIZATION OF SUBCUTANEOUS INSULIN INJECTIONS

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Previously, it has been difficult to qualitatively and quantitatively estimate the actual localisation of a subcutaneously injected volume of a drug in vivo. Micro-CT scanning is here shown to be a new and useful method for visualizing and analysing the spatial distribution of drug following a subcutaneously injection in pigs.

We present a validated method, describing the impact of injection techniques and tissue handling prior to CT scanning as well as various parameters such as X-ray exposure time and energy and subsequent image processing and analysis.

All experiments have been performed by in vivo injections in pigs followed by termination of the pigs and fixation of various injection sites. In order to validate the CT method both pharmacokinetic studies and conventional histological methods have been used as reference. Different tissue handling techniques have been investigated in order to define the optimal tissue fixation method.

We show that the contrast fluid necessary for CT scanning does not have an impact on the drug's pharmacokinetic profile and that the spatial distribution visualised by CT and by antibody-stained insulin is well correlated, indicating a very good comparison between these two visualization methods.

We propose that CT scanning can be used as a new fast and reliable tool for visualising the subcutaneous depot and thus be used for evaluating the local tissue-related effect of pharmaceutical drugs. Moreover CT scanning may be a novel analytical method for drug delivery issues that may facilitate the development of improved medical devices for injection and infusion.

P-116

ANALYSIS OF HEART RATE VARIABILITY BETWEEN PEOPLE WITH DIABETES AND HEALTHY SUBJECTS IN KOREA

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Background and aims: We investigated whether differences in heart rate variability in people with diabetes and healthy controls by analysis of heart rate variability.

Materials and methods: 207 people with diabetes and 94 age- and sex-matched controls were included in the general and university hospital. We measured the time and frequency domains in the lying and standing position.

Results: There was a significant differences in the MHR, SDNN and RMSSD in people with diabetes when compared to the normal subjects in the time domain ($P < 0.01$). Also total power, LF and HF parameters was decreased in people with diabetes when compared to the normal subjects in the frequency domain ($P < 0.01$). LF/HF ratio was significantly reduced in people with diabetes in the standing position ($P = 0.001$). Both LF and HF of power spectral analysis (reflecting mainly sympathetic parasympathetic activity respectively) were reduced, indicating that sympathetic as well as parasympathetic functional impairment may be assumed. The time-domain (MHR, SDNN and RMSSD) and frequency-domain (LF, HF and LF/HF ratio) parameters showed a significant negative correlation with glucose, HbA1c and duration of diabetes respectively ($P < 0.05$).

Conclusion: People with diabetes had lower values of power spectral analysis of heart rate variability than diabetes.

P-117

CELLULAR PHONE AND INTERNET-BASED INDIVIDUAL INTERVENTION ON FASTING PLASMA GLUCOSE, BLOOD PRESSURE, AND WAIST CIRCUMFERENCE FOR PEOPLE WITH METABOLIC SYNDROME

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Purpose: The purpose of this study was to investigate the effectiveness of an educational intervention that used both the cellular phone and the Internet to provide a short messaging service (SMS) relating to fasting plasma glucose (FPG), blood pressure (BP), and waist circumference (WC) in adult people with metabolic syndrome.

Methods: Twenty-one patients were assigned to an intervention group and 19 to a control group. The goal of the intervention was to keep FPG, BP, and WC levels close to the normal range for 12 weeks. Patients in the intervention group were asked to access a web site by using a cellular phone or to wiring the Internet and input their FPG, BP, and WC levels weekly. Participants were sent the optimal recommendations by both cellular phone and the Internet weekly. The control group did not receive the intervention.

Results: Patients in the intervention group had a mean decrease in systolic blood pressure (SBP) level of 7.0 mmHg and those in the control group had a mean increase of 4.4 mmHg. There was a significant mean change in the diastolic blood pressure (DBP) level for the intervention group with a mean change of −6.7 mmHg. Patients in the intervention group had a mean decrease in WC level of 2.3 Cm and those in the control group had a mean increase of 1.6 Cm.

Conclusion: This web-based individual education can improve the control of BP and waist circumference in the patients with metabolic syndrome.

P-118

COMPARISON OF METABOLIC SYNDROME RISK FACTOR BY BLOOD GLUCOSE LEVEL AND AGE IN THE MENOPAUSE WOMEN

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Purpose: The purpose of this study was to comparison of the metabolic syndrome (MS) risk factor prevalence by blood glucose level and age in the **menopause** women.

Method: 251 subjects were recruited from the health promotion center of a tertiary care hospital in an urban city. MS was defined by third report of the national cholesterol education program expert panel on detection, evaluation, and treatment of high blood cholesterol in adults(Adult Treatment Panel III), and impaired fasting glucose (IFG) was determined by $100\,mg/dL \leq$ fasting blood glucose $\leq 125\,mg/dL$.

Results: The body mass index and diastolic blood pressure were significantly higher in the IFG group than in the normal plasma glucose (NPG) group. The prevalence of MS, obesity, and hypertriglycemia were significantly higher in the IFG group than in the NPG group. In the age of forties, MS risk facts and prevalence were not significantly difference between the IFG and NPG groups. In the age of fifties, body mass index, body fat, and MS prevalence were significantly higher in the IFG group than in the NPG group. However, HDL-cholesterol was lower in the IFG group than in the NPG group.

Conclusions: These results show that the diabetic educator should focus on the IFG fifties obese female patients for improvement of the MS risk factors.

P-119

CHANGES IN ATMOSPHERIC PRESSURE ALTERS INSULIN DELIVERY FROM INSULIN PUMPS. THIS CAUSES SHORT HAUL AIRPORT CARROUSEL HYPOGLYCAEMIA

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Case 1: A 9 year old girl with T1DM on Insulin Pump Therapy (IPT) was regularly travelling on 65 minute flights. She reported hypoglycemia while waiting for her luggage at the airport baggage carrousel. A 70% temporary basal rate did not help. She also suffered a hypoglycemic episode following a rapid ascent in a snowfield cable car.

Case 2: A 6 year old boy with T1DM on IPT travelled regularly on a 2 hour flight. Every time he flew, he had a hypoglycemic episode while waiting for his luggage at the airport baggage carrousel.

We have become aware of adults with T1DM managed with IPT who also experience hypoglycemia following air travel. Commercial aircraft environmental control systems set cabin pressure at 0.7 atmospheres (564 mmHg) which is 196 mmHg less than sea level.

The Medtronic Paradigm and Animas waterproof insulin pumps were studied. We studied insulin delivery from pumps in a hyperbaric chamber to 2 atmospheres. There was decreased insulin delivery during pressurization and a rapid delivery of insulin during depressurization. We then constructed a hypobaric chamber to mimic flight and confirmed that insulin delivery increased with reduced atmospheric pressure. Insulin delivery has also been studied in pumps taken in an elevator to 300 meters and on commercial aircraft flights.

Conclusion: Change in atmospheric pressure alters insulin delivery from waterproof insulin pumps. We discuss the mechanism behind this phenomenon and how to prevent this form of hypoglycemia. Other factors may contribute to flight related hypoglycemia.

P-120

PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES OF SOME MEDICINAL PLANTS IN CENTRAL AFRICAN ANTIDIABETIC PROPERTIES

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For thirty years, diabetes is a real public health problem worldwide. It results in abnormally high blood sugar measured in the blood several months apart, at a concentration greater than 1.4 gr. per liter at fasting and it affects all age groups.

It is a chronic metabolic disease that occurs when the pancreas does not secrete insulin, insulin-dependent (type I) usually affects young individuals age 30 or when the pancreas does not produce enough insulin secretion and that it is in deficit; form of diabetes found in adults and obese: diabetes non-insulin-dependent (type II). Besides these two forms of diabetes are primitive, there are diabetes secondary to other diseases, diabetes and gestational diabetes Mady.

Indeed, given the dissatisfaction found in modern medicine, traditional herbal tracks seem to reinforce potential interest, including the process of development, from plant to phytomedicine through appropriate scientific methods, could offer a credible alternative, for communities.

P-121

RESTORE THE BETA-CELL ISLET STRUCTURE AND DOWN-REGULATION OF PPARα/γ EXPRESSION IN TYPE II DIABETES

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The regulation of peroxisome proliferator activated receptor (PPARα/γ) expression is important for β-cell viability and function. PPARα is found primarily in the liver and regulates genes involved with fatty acid utilization and PPARγ is an important regulator of adipocyte differentiation and β-cell function. The combination of PPARα/γ is a key activator in β-cell pancreatic islet structure and increasing concentrations of glucose in β-cells. The current investigation focuses attention on regulation of PPARα/γ *expression* and the pancreatic islet β-cells dysfunction in Type II diabetes.

The competitive RT-PCR method used for expressions of PPARα/γ and immunohistochemical approaches were used to resolve structure of pancreatic islet β-cells in untreated diabetic and *G.sylvestre* treated diabetic mice. The optimum doses of *G.sylvestre* were orally administrated regularly for 30 days in STZ diabetic induced mice. There were a significant ($P < 0.05$)

decreased in blood glucose 110.73 ± 58.32. A comparative increased ($P < 0.01$) in body weight was observed in intermediated time respectively in experiment period. The morphometric analysis of the mice islets revealed that the mean diameters of the native and isolated islets were $198.5 \pm 6.63\,\mu$m and $190.23 \pm 7.11\,\mu$m. The islet β-cells structure was restored in *G.sylvestre* treated diabetic mice.

The main conclusion to be drawn from the present study is that the *G.sylvestre* oral administrations of aqueous extract regulating the blood glucose levels and weight, which control the report of hypoglycemic actions. The expression of mRNA of PPARα/γ were also significantly increase and restore the islet β-cells structure in *G.sylvestre* treated diabetic mice respectively.

P-122

QUASI IN-SILICO SIMULATION RESULTS OF A ROBUST BLOOD GLUCOSE CONTROL ALGORITHM

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Introduction: The quest for artificial pancreas can be structured in three tasks: glucose sensor, insulin pump, control algorithm. The Biomedical Engineering Laboratory of the BME investigates automatic blood glucose control possibilities from several years.

Aim: Our aim is to develop a robust control framework able to be applied on a general population and handling the diversity caused by insulin sensitivity or patient variability.

Methods: The robust control algorithm developed was tested on literature's most important models (Hovorka-, Dalla Man-, Sorensen-models), and trained and refined on a small dataset. Results were presented at the last year's ATTD conference. The current work summarizes quasi in silico results. Min. 1 week's real data of 54 type 1 diabetic patients (aged between 6–52 years) equipped with Medtronic insulin pump were compared with simulation results of the robust control algorithm.

Results: The developed framework kept blood glucose level more than 90% of the time inside the 4–8 mmol/L interval (without any recalibration of the algorithm) proving its robustness. Hypoglycaemia (not caused from physical activity) is efficiently avoided, switching off the algorithm if this event occurs.

Conclusions: Use of hard constraints proved their efficiency keeping blood glucose level inside the defined interval; moreover, it is not sensitive to meal intakes or patient variability. Hence, it could efficiently support individualized control (ex. MPC) protocols appeared in the literature.

Further steps: Extension of the robust framework regarding physical activity or nocturnal hypoglycaemia constraints are planned, as well as increasing the number of simulated cases on real data.

P-123

THE EFFECT OF METFORMIN ON MYOCARDIAL TOLERANCE TO ISCHEMIA IN RATS WITH DIABETES MELLITUS TYPE 2

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Background and aims: It's well known, that obesity and metabolic syndrome, leading to type 2 diabetes mellitus (T2DM), makes cardio-vascular risk extremally high. That's why one of the main courses in prevention of cardio-vascular deseases is to find farmacological agent, wich helps not only in correction of obesity and T2DM, but also has cardio- and vaso protective effects. Last years metformin is considered to be such drug. Several experimental studies showed that metformin may have a direct antiischemic effect in both type 1 diabetes mellitus and T2DM. But the mechanism of such action is not completely clear. The aim of our study was to find out the action of metformin on ishemic heart in the experiment.

Material and methods: In our study, myocardial sensitivity to ischemia in rats with neonatal streptozotocin T2DM was investigated using the model of global ischemia-reperfusion in the isolated perfused heart. Metformin was administered to animals intraperitoneally at a dose of 200 mg/kg/day for 3 days prior to isolated heart perfusion.

Results: Infarct size and postischemic recovery of left ventricular function were not different between controls and metformin-treated animals. Infarct size in T2DM was significantly lower than in controls ($24.4 \pm 7.6\%$ vs. $45.0 \pm 10.4\%$, respectively, $P < 0.01$), which is indicative of the phenomenon of metabolic preconditioning in T2DM.

Conclusions: Therefore, the protocol of metformin administration used in this study had not afforded a significant cardioprotective effect in animals with T2DM. The mechanisms of beneficial effect of metformin on cardiovascular complications in patients with T2DM require further investigation.

P-124

OPTICAL GLUCOSE SENSORS FOR IN VIVO USE; ONE TECHNOLOGY YIELDING THREE PRODUCT OPPORTUNITIES

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Glycemic control can be monitored by several methods. Available for the diabetic patient are a variety of traditional blood glucose meters (BGM) and a few continuous glucose sensors (CGM). Most available methods are based on electrochemistry which for the CGM type of sensors calls for a tethering between the inserted sensor and the instrumentation.

Optical glucose sensors for in vivo monitoring circumvent this need for tethering since photons can travel through the skin and hence the interrogation of the optical sensor does not require direct connection between the sensor and the instrumentation.

Medtronic is developing different uses of optical glucose sensing technology allowing the patient to choose between an invasive CGM solution, a minimal invasive CGM solution, and a discrete meter concept. The two CGM solutions will provide the patient with continuous glucose data whereas the discrete meter concept will allow the patient to obtain glucose measurements at any time without the need for drawing blood.

The presentation will show both in vitro and in vivo data supporting the feasibility of all three solutions.

P-125

COMPARATIVE STUDY BETWEEN OGTT AND HOMA-IR INDEX AS INDICATORS OF GDM IN SOUTH-INDIA

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Introduction: About 2–3% of all pregnancies are complicated by diabetes and 90% of them are Gestational Diabetes mellitus (GDM). This emphasizes the importance of an accurate diagnostic technique with more patient compliance.

Purpose: To compare the sensitivity between Oral Glucose Tolerance Test (OGTT) and Homeostatic model assessment of insulin resistance (HOMA-IR) index as indicators of GDM.

Material: One hundred high risk pregnant women for GDM between 24 to 28 weeks in our region were subjected for the test. High risk factors included in the study were family history of DM, prior birth of a macrosomic/malformed baby, previous stillbirth, unexplained perinatal loss, polyhydramnios/ recurrent vaginal candidiasis/persistent glycosuria in present pregnancy, age >30 yrs, obesity, hypertension. Overt diabetic pregnant women and GDM cases already under treatment were excluded.

Methods: Fasting insulin and plasma glucose values were measured by Elisa and Hexokinase methods respectively. Both OGTT (100 gm glucose load) and HOMA-IR index were done for all the cases under study. Cases with OGTT positive were treated as study group and cases with OGTT negative were treated as control group. HOMA-IR indexes were compared with OGTT values and efficiency of HOMA-IR index accessed using independent samples T-tests and annova.

Results: HOMA-IR Index value >2 were considered as GDM. This value was compared with OGTT values by O'Sullivan and Mahan modified by Carpentar and Coustan.

Conclusion: HOMA-IR is more accurate with better patient compliance than OGTT in diagnosing DGM. It is less cumbersome as oral administration of glucose solution is avoided.

P-126

DESIGNING OPTIMAL AMBULATORY CONDITIONS TO IMPROVE PREDICTION CAPABILITIES OF INDIVIDUAL POST-PRANDIAL GLUCOSE MODELS

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Optimal experiment design for improving identification of post-prandial models was carried out for type 1 diabetic subjects (T1DM) under ambulatory conditions, based on continuous glucose monitoring (CGM) data. Design parameters were the insulin bolus size, the meal size and the delay between them. Two meal configurations were used for identification during three different days at lunchtime. In the first configuration, a high carbohydrate (CH) meal (100 g CH) was eaten after a proportional bolus given 30 minutes in advance. In the second configuration, a low CH meal (40g CH) was administered delaying bolus for 120 minutes. Three extra days were monitored for model validation, during which subjects followed their usual therapy. In all cases, the patients chose among three menus with the same relative nutritional composition, available in low or high CH content variants. Identification was performed with a set of models of increasing complexity, and their prediction capability analysed and compared. Validation was performed considering uncertainty in the parameters identified, as well as the amount of carbohydrates ingested, by means of interval analysis. This approach results into interval models where the glucose prediction is represented as a band that accounts for intra-patient variability. Preliminary results indicate that glucose prediction bands enclose most of the observed post-prandial glucose excursions, with some differences depending on the models used. In conclusion, separating insulin and meal effects improves post-prandial model identification of T1DM subjects. Interval analysis allows for consideration of physiological variations of post-prandial glucose response within the same patient.

P-127

NUMERICAL-ACCURACY ASSESSMENT OF A REAL-TIME CONTINUOUS GLUCOSE MONITORING SYSTEM IN MEDICAL, SURGICAL, AND TRAUMA CRITICALLY-ILL PATIENTS

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Objective: Assess the accuracy of the Guardian® REAL-Time system (RT-CGMS) (Medtronic) in an intensive care unit (ICU) and determine if accuracy varies depending on the type of patient or the presence of hemodynamic shock.

Methods: 41 patients with insulin therapy at Dr. Josep Trueta Hospital's ICU (Girona, Spain) were included (APACHE II 18.0(7.5), SOFA 8.5(4.0), 14/21/6 medical/surgical/trauma). Patients were monitored for 72h using RT-CGMS. Arterial blood glucose (ABG) samples were obtained following the glycemic control protocol established at the ICU and determined using HemoCue® 201DM (HemoCue AB). ABG measurements (3–4 per day) were used for calibration. Results were evaluated using paired values (ABG/RT-CGMS), excluding the calibration set. Accuracy was assessed using MdRAD and ISO criteria.

Results: 956 ABG/RT-CGMS pairs were analyzed (370/433/153 medical/surgical/trauma). Overall MdRAD was 13.5(18.1)% and ISO criteria were 68.1%. The MdRAD in medical and surgical patients were 14.5(18.4)% and 11.7(17.0)%,

respectively; no significant differences were found ($P = 0.06$). By ISO criteria, 67.0% of medical data and 71.1% of surgical data were accurate. Trauma patients were not compared due to the insufficient size of this cohort. MdRAD of 14.8(20.5)%/11.7(15.3)% and ISO criteria of 63.8%/74.2% were reported for patients with good and poor tissue perfusion, respectively ($P = 0.01$). For patients without and with inotropic support, MdRAD of 15.3(19.5)%/12.3(16.6)% and ISO criteria of 64.4%/71.2% were reported, respectively ($P = 0.02$).

Conclusions: RT-CGMS accuracy was similar to that reported in studies in type 1 diabetes patients. Accuracy in medical and surgical patients was not significantly different. Accuracy was significantly different in patients with hemodynamic shock.

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EFFICACY OF LOW GLUCOSE SUSPEND AND LOW PREDICTIVE ALERT: *DATA ANALYSIS USING THE MEDTRONIC CARELINK® THERAPY MANAGEMENT SOFTWARE DATABASE*

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Background: We examined the extent to which the Low Glucose Suspend (LGS) and Low Predictive Alert (LPA) features of the MiniMed Paradigm® Veo™ System influence hypoglycemic exposure.

Method: Data from 338 patients were separated into groups based on LGS and LPA settings. T-tests compared the average time per week these patients spent with sensor glucose values <70 or <60 mg/dL. Additional analysis was conducted on data from 86 patients who began with either the LGS or LPA activated and concluded with both features activated.

Results: Results for the 338-patient cohort are shown in Table 1. Compared to subjects with neither feature activated, those with both features activated realized a 36.6% reduction in time (h/week) spent <70 mg/dL and a 43.4% decrease in time spent <60 mg/dL.

Data from the 86-patient cohort are shown in Table 2. Addition of the second feature (either LGS or LPA) was associated with significantly lower hypoglycemia <70 mg/dL durations.

Conclusion: Activation of LGS and LPA features reduces the duration of hypoglycemia. Devices with predictive and automatic shut-off capabilities may be useful technologies for mitigating hypoglycemia.

Table 1. Average Duration of Hypoglycemia (h/week)

	LGS OFF, LPA OFF (N = 248)	*LGS ON, LPA ON (N = 90)*	Δ	P
<70 mg/dL	12.27 h	7.78 h	−4.49 h	<0.02
<60 mg/dL	6.2 h	3.51 h	−2.69 h	<0.04

Table 2. Hypoglycemia (mean h/week (%))

LPA only	*LPA AND LGS (N = 15)*	P	*LGS only*	*LGS AND LPA (N = 71)*	P
9.24 (5.5%)	4.95 (2.94%)	<0.001	10.44 (6.21%)	9.57 (5.69%)	<0.005

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CHARACTERISTICS OF INSULIN DELIVERY AND GLYCEMIC CONTROL IN SUBJECTS WITH TYPE 2 DIABETES USING CARELINK® THERAPY MANAGEMENT SOFTWARE

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Objective: Insulin delivery patterns and blood glucose values in type 2 diabetes (T2D) were examined by mining the CareLink® Therapy Management Software database.

Methods: Data were retrospectively analyzed from individuals with T2D who uploaded ≥120 days of data regarding insulin pump usage and glucose values. Group A had mean meter blood glucose (MBG) < 155 mg/dL ***and*** low glycemic variability (GV) (SD < 56 mg/dL). Group B had mean MBG > 182 mg/dL ***or*** SD > 84 mg/dL.

Results: As a result of selection criteria, individuals in Group A (n = 195) had much lower MBG and GV values than Group B (n = 179). The Table compares insulin delivery patterns and meter glucose values in the groups.

Insulin Delivery and Glycemic Control (Means)

Property	*Group A*	*Group B*	P
TDD (U)	74.7	73.9	0.872
Infusion Set Change Interval (d)	5.2	6.4	0.015
Boluses/d	4.6	4.3	0.092
Average Bolus Size (U)	7.2	7.1	0.979
Bolus Calculator Used for CHO (%)	81.8	66.5	<0.001
Bolus Doses (% of TDD)	47.6	44.0	0.013
MBG Readings/d	4.9	4.3	0.003
MBG Values ≤70 mg/dL (%)	3.7	3.4	0.071
MBG Values ≥240 mg/dL (%)	2.5	31.2	<0.001

Conclusion: T2D subjects on pumps achieved glycemic control with appropriate use of insulin pump and BG monitoring technology, without increasing hypoglycemia.

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INTENSIVE DIABETES MANAGEMENT IN TYPE 1 DIABETIC PATIENTS IN POOR GLYCEMIC CONTROL TREATED WITH INSULIN PUMP THERAPY OR MULTIPLE DAILY INJECTIONS: TWO YEARS FOLLOW-UP

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Background and aims: Poor glycemic control is the rain problem in type 1 diabetes. Multiple daily injection (MDI) and

continuous subcutaneous insulin infusion (CSII) are two methods of intensive insulin therapy for diabetes. The aim of our study was to compare efficacy in improving glycemic control of CSII versus MDI in patients with longstanding poor glycemic control.

Materials and methods: In our diabetes center, all patients with type 1 diabetes received *intensive management.*

We identified 35 patients with persistent poor glycemic control defined as HbA1c $\geq$ 8.0% in the last 6 months before initiation of CSII (N = 19) or MDI using rapid- and long-acting insulin analogs (N = 16). Glycemic control was the primary outcome. Secondary outcomes were: hypoglycemic events, episodes of diabetic ketoacidosis and change in Body Mass Index (BMI).

Results: A decrease in HbA1c at 12 and 24 months was shown for both groups but there was no significant difference between HbA1c in CSII group compared to MDI group (7.7 $\pm$ 1.3% vs 7.6 $\pm$ 1.4 $P = 0.85$) no statistically significant differences between groups for the overall number of hypoglycemic events and episodes of diabetic ketoacidosis or change in BMI.

Conclusion: No statistically significant differences in glycemic control and hypoglycaemic events were found in our two groups of patients. It could be explaind by an intensive educational programme: infact all our patients on submitted to intensive management based on intensive insulin therapy, high frequency of clinic visit and CHO counting. This approach could provide rare advatages rather than the choice between CSII and MDI therapy.

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RAMADAN FASTING AMONG PATIENTS WITH DIABETES—ASSESSING CHANGES IN GLUCOSE PROFILES USING CONTINUOUS GLUCOSE MONITORING (CGM)

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Introduction: Fasting during the Muslim holy month of Ramadan alters glycaemic profile and control among patients with diabetes. We have used Continuous Glucose Monitoring (CGM) to study these changes.

Methods: Seventeen patients (15 male, 2 female) with diabetes mellitus had CGM for 3 days prior to and during Ramadan fasting. For each period a mean 24 hour glucose curve was constructed. Low Blood Glucose Index (LBGI) and High Blood Glucose Index (HBGI), were calculated for fasting and non-fasting periods.

Results: There was a major difference in CGM profiles amongst patients with diabetes during and outside fasting period with characteristic rapid rise in blood glucose during iftar (breaking of fasting). There was a small, but statistically significant difference ($P < 0.005$) in mean blood glucose (139.3 $\pm$ 15.3 v 136.5 $\pm$ 6.0 mg/dL), but not HBGI or LBGI between fasting and non fasting periods.

Conclusion: In this first prospective study of Ramadan fasting using CGM we have shown a small, but significant increase in mean glucose level with a major excursion at the evening meal. In our group of patients with good glycaemic control no significant increase in hypoglycaemic episodes was seen during Ramadan fasting.

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RELATIONSHIP OF HYPOGLYCAEMIA WITH QTC PROLONGATION IN PATIENTS WITH TYPE 2 DIABETES

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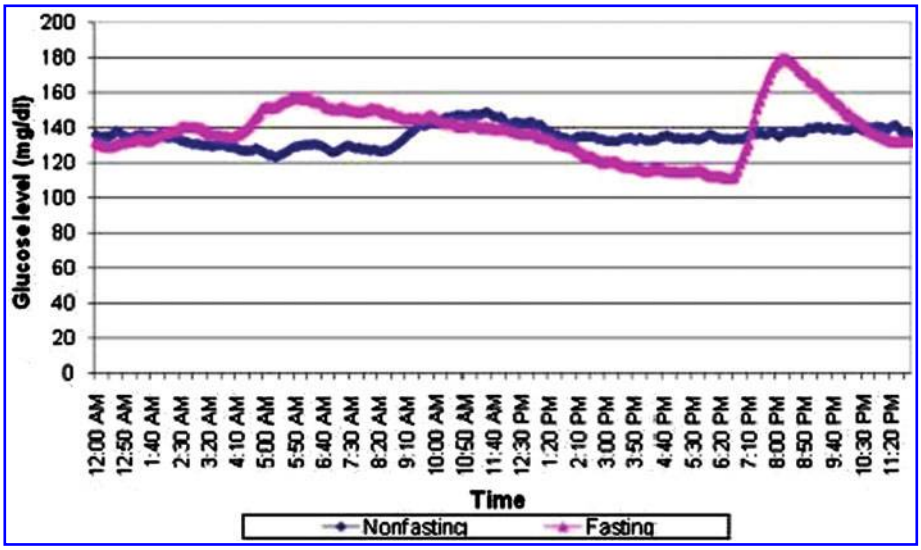
Background and aims: Recent clinical studies show that hypoglycaemia is associated with increased risk of death, mainly attributed to lethal arrhythmias and QTc prolongation. This is mostly studied in Type 1 diabetes patients. The aim of the



Average glucose level (mg/dL) before and during Ramadan fasting.

present study was to study the relationship of hypoglycaemic episodes (examined during continuous glucose monitoring subcutaneously [CGMS]) with QTc prolongation (measured by continuous ECG monitoring) in patients with type 2 diabetes (T2D).

Materials and methods: A total of 26 (14 males) patients with T2D (mean age [± SD]: 60.3 ± 10.9 years, HbA_{1c}: 6.73 ± 0.73%, diabetes duration 6.9 ± 5.0 years, 7 treated with insulin, 19 treated with insulin secretagogues) were studied with simultaneous CGMS and 24-hour ECG monitoring. Hypoglycaemia was defined as episodes of blood glucose (BG) < 70 mg/dL (3.9 mmol/L), lasting for > 5 minutes, while hyperglycaemia as episodes of BG > 200 mg/dL (11.1 mmol/L), lasting for > 5 minutes. The mean QTc of these episodes was compared with the mean QTc of normoglycaemic intervals (BG between 70–120 mg/dL [3.9–6.7 mmol/L) of similar duration.

Results: A total of 26 non-severe hypoglycaemic episodes in 13 patients (mean BG [± SD] 59.2 ± 6.9 mg/dL) and 22 hyperglycaemic episodes in 12 patients (227.8 ± 43.1 mg/dL) were identified. Mean QTc during hypoglycaemic episodes was higher than QTc during normoglycaemia (431.4 ± 36.6 vs. 416.6 ± 47.0 msec, $P = 0.015$). On the contrary, no difference was found between mean QTc during hyperglycaemic episodes and normoglycaemia.

Conclusion: Mild/moderate hypoglycaemia in T2D is associated with prolongation of the QTc interval and may contribute to the increased adverse outcomes seen with intensive treatment of the disease.

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TIMING OF INSULIN BOLUS IN PATIENTS WITH TYPE 1 DIABETES MELLITUS—EFFECT ON GLUCOSE CONTROL AND VARIABILITY USING CGMS

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Background: This pilot study was aimed to examine the effect of timing of injection, before or immediately after the meal, on overall daily glucose control and glucose variability using the continuous glucose measurements system (CGMS).

Methods: 12 patients with type 1 diabetes treated with either multiple daily insulin injections (MDI) or continuous subcutaneous insulin infusion (CSII), were connected twice to CGMS for 72 hours. During period 1 the patients injected the insulin bolus before the meal, and during period 2 after the meal.

The variability of Blood Glucose (BG) was assessed by Low BG Indices (LBGI) and High BG Indices (HBGI)- The measure of the variability of low and high BG readings. Their sum (LBGI + þHBGI) equals the BG Risk Index (BGRI)- a measure of overall variability and deviations towards hypo- and hyperglycemia.

Results: 6 patients were on CSII and 6 on MDI. The number of meals, number of insulin injections and average BG were not different between the groups. LBGI and the number of hypoglycemic events were not affected by the method of injection. BGRI were significantly higher for after the meal injection, especially on account of increased hyperglycemia($P = 0.003$). The increased HBGI and BGRI was higher for CSII ($P = 0.05$).

Satisfactory questionnaires indicated that patients would adhere to the timing of injection that they were taught initially.

Conclusion: Injecting insulin prior the meal can reduce the overall glucose variability, and remains the preferred method of injection. Larger studies are needed in order to reinforce these results.

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COMPLICATIONS WITH INSULIN PUMP CATHETERS AMONG CHILDREN AND ADOLESCENTS

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Motivation: In insulin pump therapy for children and adolescents the catheters seem to be the weak spot. In order to confirm or disprove this observation, an empirical study was initiated.

Methods: A questionnaire was developed in order to collect data regarding the frequency and kind of complications with catheters. It was handed out to the patients through the participating medical centres and out-patient clinics. Within the diabetes documentation software DPV, an input mask was developed for data retrieval. The data was processed through the University of Ulm using SAS 9.2 software.

Results: 432 paediatric patients participated in this survey. 37% reported no problems with their insulin-pump catheter. The other patients reported a total of 739 incidents: 33.2% catheter occlusions, 15.2% blood in catheter, 11.6% bent cannulas.

6% of the participants used Teflon catheters and 44% steel catheters. The complication rate shows no difference. A total of 83% of the complications occurred up to the end of the 2nd day. The complication rate does not rise with time.

84% of the participants indicated that they disinfect their skin prior to inserting the cannula. 8% do not disinfect. Complications among patients who disinfect are significantly higher than with those who do not disinfect (*P*-value 0.019; complication rate with disinfection 69.4%, without disinfection 46.9%).

Conclusion: Data about complications with insulin pump catheters was collected among 432 paediatric patients. 739 incidents were examined. The main problem seems to be catheter occlusions.

Outlook: More data will be gathered throughout this year.

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ANALYSIS OF THE EVOLUTION OF PARAMETERS FOLLOWING INSTIGATION OF INSULIN ANALOGUE TREATMENT ON DIABETIC PATIENTS IN PRIMARY HEALTHCARE, 2010

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Objectives: To analyse the changes in the regular clinical and analytical control parameters for diabetes following instigation of treatment with basal insulin analogues.

Material and methods: A retrospective observational study on a cohort of patients belonging to two contingents, in the field of basic community and teaching healthcare, recommended for treatment with basal insulin analogues by their doctors. The variables studied before and after instigation of the treatment were: age, sex, previous treatments, insulin prescribed, basal glycaemia, blood pressure (systolic SBP/diastolic DBP), body mass index (BMI), HbA1c and record of hypoglycaemias, collected in a database for two periods (basal and at 6 months).

Results: A total of 89 patients were studied, 55.1% women, with an average age of 66, treated previously with other types of insulin (39.8%), with oral anti-diabetic drugs (32.3%) and with Metformin (23.7%). The A1c values decreased from an average of $8.8 \pm 1.86\%$ to $7.9 \pm 1.21\%$ ($P < 0.01$); basal glycaemia dropped from an average of 188.9 ± 78.8 mg/dL to 153.3 ± 61.8 mg/dL ($P < 0.001$); SBP decreased from 137.0 ± 17 mmHg to 131.0 ± 17 mmHg ($P = 0.013$); DBP dropped from 76.6 ± 10 mmHg to 74.0 ± 12 mmHg ($P = 0.012$); and BMI went from 30.1 ± 4.9 to 30.2 ± 4.7 ($P < 0.001$). Finally, there were no significant differences between hypoglycaemias pre- or post-study.

Conclusions: The instigation of treatment with insulin analogues in diabetic patients entails a significant improvement in analytic parameters and better control of blood pressure without any change in hypoglycaemic events. Overall, weight increase was minimal.

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GLUCOSE VARIABILITY AS A PREDICTOR OF POOR CLINICAL OUTCOMES AMONG HOSPITAL IN-PATIENTS WITH DIABETES MELLITUS

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Objective: Hyperglycemia in hospital has been associated with poor clinical outcomes, encouraging tighter glucose control, but benefits may be offset by risk of hypoglycemia. This study aimed to assess whether blood glucose within range 4.0–11.0 mmol/L (normoglycemia) in diabetic patients hospitalized in the general medicine wards is associated with better outcomes.

Method: Retrospective cohort study of patients at Sunnybrook Health Sciences Centre between 1 November 2009 and 30 April 2010 with Type 1 or Type 2 diabetes as a co-morbidity.

Result: 300 patients had point-of-care glucose readings. 106 (35.3%) achieved normoglycemia during their entire stay, while 112 (37.3%) had hyperglycemic, 23 (7.7%) hypoglycemic episodes, and 59 (19.7%) experienced both. Hyperglycemia in the first 24 hours significantly increased the odds of having a variable glucose stay by 7.64 (95% CI: 4.08–14.30, $P < 0.001$), and having both hyper/hypoglycemia in the first 24 hours increased the odds of having a variable glucose stay by 12.25 (95% CI: 1.53–97.94, $P = 0.02$). In multivariate analysis, the average length of stay increased by 1.1 days for every day with a hypoglycemic or hyperglycemic episode and by 1.2 days for patients experiencing both ($P < 0.001$). On average, patients with > 9 days with either a hyperglycemic or hypoglycemic episode were in hospital 11.62 days longer than their expected LOS, compared with 4.24 days longer for those with normal glucose.

Conclusions: Patients with normal blood glucose levels in the first 24hours in hospital may need less frequent blood glucose testing. Variable glucose control is associated with increased length of hospital stay.

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INSULIN PUMP THERAPY IN 103 CHILDREN DURING THE FIRST YEAR OF LIFE: ANALYSIS OF THE CUMULATIVE MULTICENTER DPV REGISTER

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Introduction: The use of insulin pumps in children has increased considerably during recent years. Despite a trend to start pumps early in the course of diabetes and in younger subjects, experience on pump therapy during the first year of life is still limited.

Material and methods: The DPV initiative uses standardized longitudinal documentation based on a computer software. Every 6 months, anonymous data are exported by the 337 participating centers, integrated into a cumulative database and used for both external comparisons and patient-centered multicenter analyses.

Results: By September 2010, 11,784 pediatric patients on insulin pumps are recorded in the database: 103 patients started pump therapy during the first year of life (1604 patients between the 2nd and the 5th year, and 10,077 patients between age 5 and 20). 64% of the infants were classified as type-1-diabetes, 21.4% as connatal diabetes and 8% as diabetes due to pancreatic aplasia/hypoplasia. Mean age at start of pump therapy was 6.8 months, mean insulin dose was 10.3 U or 0.7 U/kg, with 6.6 U of prandial and 3.7 U as basal insulin. 78% of patients used insulin analogs. As indication for pump therapy, prevention of hypos (30%), increased flexibility (22%) and reduction of BG excursions (16%) were reported most frequently.

Conclusion: In conclusion, diabetes in infants during the first year of life is rare and clearly differs from older children. Experience from large multicenter registers is helpful to describe the specifics of pump therapy in this age-group.

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IMPROVED BLOOD GLUCOSE LEVELS ACHIEVED IN ICU PATIENTS USING HEMATOCRIT CORRECTED GLUCOSE METER AND BLOOD GAS ANALYZER RESULTS

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Purpose: To determine the effect of hematocrit correction of glucose meter and blood gas analyzer glucose results on the percentage of ICU patients classified in the desired 80–110 mg/dL range.

Methods: Adult ICU patient (N = 375) blood glucose levels were measured using six different glucose meters (designated Meter A through Meter F) from different manufacturers, one blood gas analyzer and the clinical laboratory plasma hexokinase method. Patient hematocrit levels were retrieved from patient medical records. Linear regression of the model (see below) was performed for each glucose meter and the blood gas analyzer to estimate hematocrit-bias and if hematocrit-bias was dependent on glucose concentration.

$$\text{Glucose}_{\text{meter}} = \beta_0 + \beta_1 \text{Glucose}_{\text{plasma, molar}} + \beta_2 \text{Hct} + \beta_3\ \text{Hct Glucose}_{\text{plasma, molar}}$$

Hematocrit corrected glucose values were determined using the β coefficients obtained from the regression analysis. The percent change of patient glucose values falling within the desired 80–110 mg/dL range after the determination of hematocrit corrected values was calculated.

Results: The changes in the percentage of patients in the 80–110 mg/dL category after hematocrit correction of the glucose data were + 2.94% (Blood Gas Analyzer), + 5.00% (Meter A), + 1.08% (Meter B), + 3.37% (Meter C), −1.43% (Meter D), + 0.74% (Meter E) and + 0.45% (Meter F).

Conclusions: The percentage of ICU patients in the desired glucose range (80–110 mg/dL) increased (exception Meter D) with the use of hematocrit corrected glucose results.

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INDIVIDUALIZATION OF MODEL PREDICTIVE CONTROL FOR THE ARTIFICIAL PANCREAS FROM STANDARD CGM TRACES

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Recent technological advancements in s.c. glucose monitoring and insulin delivery systems have stimulated the development of closed-loop systems for maintaining normoglycaemia in Type I diabetic patients. The most promising control technique is Model Predictive Control (MPC), which relies on the solution of an optimization problem based on future prediction obtained with a mathematical model of the patient. Extensive in-silico trials have demonstrated that, in order to guarantee satisfactory and reliable closed-loop control, either the cost function or the model need to be individualized to account for inter-individual variability. The MPC algorithm proposed in [1], where only the cost function was individualized, has been successfully tested in a clinical study covering night and breakfast on 20 patients [2]. Further improvements can be expected from the individualization of the model. In the literature, some authors have proposed black-box models (e.g. ARX or ARMAX) identified from ad-hoc experiments designed so as to ensure sufficiently exciting meal and insulin signals. The necessity of non-conventional experiments is a significant limitation for a wide adoption of the artificial pancreas and a major breakthrough would be the ability to identify individual patient models from standard CGM traces. In this work, we investigate the use of parametric individual models identified from data collected in closed-loop using the MPC proposed in [2]. Extensive in-silico experiments demonstrate the great potential of this approach.

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CONSISTENT TIGHT GLYCEMIC CONTROL: A ROAD TO COGNITIVE IMPAIRMENT

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Although consistent tight glycemic control helps in avoiding microvascular complications, but exploring perfection through intensive lowering of blood glucose and setting new standards of lowering HbA1c, may compel patients to sweat for irresistible desire to eat. On one hand it may increase weight and insulin resistance posing cardiovascular risks and on another hand regular occurrence develops unawareness to hypoglycemia passing through the cognitive impairment. Targeting new lows of blood glucose may induce hepatic glucose output causing undesirable glycemic variability that makes it difficult to keep round the clock optimum control and ultimately patient sweats off clinicians efforts. 200 diabetics keeping their HbA1c below 6, using technologies like insulin pump, long acting analogues, premixed analogues, premixed insulin with or without oral drugs were studied for incipient dementia, amnesia, cognitive impairment, weight gain and lack of sweating when hypoglycemia could be documented. Hypoglycemia unawareness due to blunt endocrine defenses including lack of sweating was found to be increasing with duration while cognitive impairment was noticed to be exponentially rising with tight and consistent glycemic control. On downloading glucometer it could be explored that twice a day popularly used premixed analogues caused greater diurnal glycemic variability despite achieving good A1c. High pre-dinner values forced clinicians to increase dose prior to evening meal, which led to overnight hypoglycemia promoting mid night snacking or high hepatic glucose output worsening fasting glycemic control though it was reduced using it in three divided doses.

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METHOXY POLYETHYLENE GLYCOL-EPOETIN BETA DELAYS RENAL FAILURE IN CLINICAL DIABETIC NEPHROPATHY

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Effective anemia management with Methoxy polyethylene glycol-epoetin beta, a new continuous erythropoietin receptor activator (C.E.R.A.), provides continuous stimulation of erythropoesis. With once-monthly dosing in clinical diabetic nephropathy delays renal failure maintaining stable and sustained Hb levels within the target range. Hypothesis tested in 24 anemic Type 2 DM patients with mean hemoglobin level 8.4 ± 1.5 gm% and mean A1c 7.34 ± 1.69 with clinical diabetic nephropathy with GFR < 50 mL/min/1.73 m^2. all of them randomized to receive monthly dose for 9 months while continuing anti diabetic and anti hypertensive treatment uninterrupted and unchanged. Antidiabetic and antihypertensive drugs titrated to keep plasma glucose and blood pressure in recommended range, if required. After 9 months they were re-evaluated and shown significant improvement in GFR i.e. 9.6 ± 3.1 mL/min/1.73 m^2 and hemoglobin level with mean Hb11.5 ± 0.69 gm% but no statistically significant change in A1c with mean value 7.21 ± 1.71. Thus effective and consistent stimulation of erythropoesis with maintained glycemia and blood pressure can delay renal failure in patients with clinical diabetic nephropathy improving quality of life as well as longevity. Hypothesis is true.

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RISK ESTIMATION OF HYPOGLYCEMIC REACTIONS IN WOMEN WITH DIABETES MELLITUS TYPE 1 TAKING IN ACCOUNT REGULARITY OF MENSTRUAL CYCLE

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Materials and methods: Total 155 female patients with DMT1 were examined and divided in 2 comparable by the average age (28.59 ± 7.10 years), duration of DMT1 (10.46 ± 7.28 years), HbA1c,% (9.34 ± 2.09) groups:

1 - with regular menstrual cycle (n = 117) and
2 - irregular menstrual cycle (n = 38). The study of 24-hour dynamics of glucose was performed using the system of long-term glucose monitoring (CGMS).

Results: The risk factors of hypoglycemic reactions are the period duration of normoglycemia less than 50% of day time (1st group OR = 1.06;95%CI 1.01–1,10; $P = 0017$, 2nd group OR = 1.13;95%CI 1.02–1.26; $P = 0.021$) and duration of hyperglycemia period less than 50% of day time (1st group OR = 0.93;95% CI 0.89–0.98; $P = 0.006$, 2nd group OR = 0.76;95% CI 0.59–0.98; $P = 0.037$). In the 1st group the risk factor of latent hypoglycemic reactions is the level of HbA1c less than 7% (OR = 0.63;95% CI 0.46–0.85; $P = 0.003$), in 2d group the risk factor is the level of HbA1c less than 6% (OR = 0.65;95% CI 0.44–0.96; $P = 0.029$).

Conclusions:

1. The risk factors of hypoglycemic reactions independent of regularity of menstrual cycle are the duration of period of normoglycemia and hyperglycemia less than 50% of day time.
2. The level of HbA1c less than 7% at the regular menstrual cycle and less that 6% at the disorder of menstrual cycle is the risk factor of latent hypoglycemic reactions.

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DGLUCOSE (POSTPRANDIAL - FASTING GLUCOSE) BEST PREDICTS RENAL DISEASE IN DIABETES

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This study examined if dglucose, two-hour postprandial (2hPP) minus fasting (F), predicts glycemic control and renal damage better than F, 2hPPglucose or Hemoglobin A_{1C} (HbA_{1C}). Glucose, F and 2hPP, and renal function; BUN, serum creatinine (Scr), and estimated GFR (eGFR), were obtained from 56 insulin-treated diabetic adults. 2hPP-F(d) calculated for glucose and renal function when 2hPP glucose <200 (n = 23) or >200 mg/dL(n = 33). The HbA_{1C} obtained from 42 patients. Correlation coefficients were calculated for F, 2hPP or 2hPP-F(d), renal function versus those for glucose. HbA_{1C} was correlated with F and 2hPP renal function. Variables differed significantly between F and 2hPP (t-test, $P < 0.05$) for all patients and when 2hPPglucose was < or >200 mg/dL, except dBUN at <200 mg/dL (0.6112). When F, 2hPP or 2hPP-F(d) renal function between 2hPPglucose < and >200 mg/dL were compared, dScr was significant ($P = 0.0327$). Correlation coefficients between dglucose and dScr or deGFR, were significant for all patients (r = 0.420, $P = 0.0013$, and r = -0.434, $P = 0.0008$, respectively) and for 2hPPglucose >200 mg/dL (r = 0.523, $P = 0.0018$ and r = -0.513, $P = 0.0023$, respectively) but not when 2hPPglucose <200 mg/dL. When dglucose increased by 100 mg/dL, dScr increased by 0.08 and 0.11 mg/dL, and deGFR decreased by 2.73 and 3.73 mL/min for all patients and 2hPPglucose >200 mg/dL, respectively. HbA_{1C} was poorly correlated with F BUN, Scr and eGFR (r = 0.137, $P = 0.3987$; r = 0.233, $P = 0.1485$; r = -0.127, $P = 0.4360$, respectively). We conclude that dGlucose better predicts renal function changes than F or 2hPPglucose or HbA_{1c}. Postprandial hyperglycemia (<200 mg/dL) control is crucial for renal protection in diabetes.

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NON-INVASIVE GLUCOSE MONITORING: INCREASING ACCURACY BY OVERCOMING ENVIRONMENTAL/ EXTERNAL INFLUENCES BY USING MULTI SENSORS

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Previous publications suggest improvement of glucose measurement validity through combination of three Non-invasive technologies: ultrasonic, electromagnetic and thermal. However, the measured signal in each technology is influenced by both tissue and ambient temperatures. Therefore, in order to correctly measure ambient and tissue temperatures and receive more reliable glucose readings, an external temperature sensor was added. A new temperature sensor was added in a more inert location, less accessible to the user and less influenced by the device heat dissipation. Each channel's reading was corrected and compensated by using the ambient temperature from the new sensor. A weighted combination of the 3 technologies outputs produced a final glucose reading. The new performances of

GlucoTrack® were evaluated by 8 subjects: one type 1 and seven type 2. Each subject performed the measurement procedure by him/herself in home/office environment. The device readings were compared with the participants' own (invasive) glucose monitoring device. Clarke Error Grid analysis shows 96% of the points in the clinically accepted A and B zones, of which 51% in zone A. $MARD_{mean}$ and $MARD_{median}$ are 26.6% and 19.7%, accordingly. The study demonstrates that users are able to easily operate and use the device in home alike environment and conditions. Initial outcomes of real home use conditions suggest that GlucoTrack gives good results. The additional (ambient) temperature sensor and its location is a key solution for overcoming temperature influences upon a measurement. Further efforts should be conducted in order to improve the results by reducing impacts of other external causes.

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STUDY OF THE METABOLIC EFFECT AND HISTOPATHOLOGICAL NASAL MUCOSAL CHANGES AFTER PROLONGED INTRANASAL INSULIN ADMINISTRATION

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Objective: To evaluate the histopathological and metabolic effects of administration of intranasal insulin (INI) in type 2 diabetic (T2D) individuals.

Methods: A total of 51 adult male T2D patients were divided into 4 groups: group I received INI and azone, group II received INI, azone and polyacrylic acid, group III received INI and polyacrylic acid, and in group IV, each 2 patients received INI, azone or polyacrylic acid separately. Basal nasal mucosal biopsy and oral glucose tolerance test (OGTT) were performed for all patients. On the second day, fasting blood samples were collected, and then intranasal spray was administered. The same intranasal administrations were given before each meal for 4 weeks. A second nasal biopsy was taken at the end of the 4 weeks treatment and a third biopsy one month after stopping INI.

Results: Significant drop of blood glucose and significant rise of plasma insulin were observed in groups I, II and III. However, both of these findings were more in group II, than group I and group III. The observed histological changes were in the form of local mucosal irritation of variable degrees. The severity of these changes was more marked in group II than group III and I, and least in group IV. However, the third nasal biopsy showed reversal of these changes after stopping INI administration.

Conclusions: Intranasal insulin can help in preventing rapid increments of postprandial hyperglycemia in T2D patients. The associated histopathological nasal mucosal changes are generally mild &completely reversible after stopping INI administration.

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HOW DIABETES TYPE 2 EVOLVES

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Background: This study demonstrated dominant inheritance of T2DM How T2DM evolves by comparing with pathologist's findings studying cadavers from peopleb at different stages of the disease: it begins with less beta cells and normal alpha cells, then hypertrophy of beta cells, hydropic, hyaline degeneration and fibrosis of the beta cells it correlates well with the levels of insulin, glucose and Glucagon it begins with Hyperinsulinemia-normoglycemia, then hyperinsulinemia-postprandial impaired glucose tolerance, and then full diabetes.

Methods: The method used the standard oral glucose tolerance test. Serum was sampled at 0 (fasting), 30 and 60 minutes after glucose load. Measurements of insulin Mu/mL Glucose mg/dL and Glucagon pg/mL. Hemoglobin A1C was measured.

Results:

- 29 subjects have normal levels of insulin, glucose and Glucagon.
- 39 have high values of insulin and normal values of glucose.
- 99 have the highest insulin values and glucose intolerance.
- 88 were found to have diabetes, some with insulin high, other insulin levels within the normal values and some with levels of insulin below the normal value.

Hemoglobin A1C was normal in people with postprandial glucose intolerance and diabetics.

Conclusions:

- Diabetes type 2 is dominantly transmitted.
- The only useful test to follow how T2D evolves is the standard oral glucose Tolerance test.
- Fasting and HbA1C tests are not useful tests because they can be normal in people with diabetes.

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EVALUATION OF THE EFFECTIVENESS OF PUMP THERAPY IN CHILDREN OF SAMARA REGION

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Economic problems of diabetes are based on general provisions: the definition of direct costs associated with the disease (in medicine, means for monitoring and administration tools, equipment, laboratory research, care in hospital or at home), and the definition of minor costs associated with the disease (disability, premature death).

Objective: To evaluate the clinical efficacy and pharmacoeconomic of Pump therapy in children with diabetes in the Samara region.

Material and methods: We analyzed the statistics of child endocrinological service of the Samara region for the period 2007 to 2009. System of subcutaneous injection of insulin used in the region since 2007, 130 people received insulin through the pump during the last 3 years.

Results: We analyzed data on the most common acute complications of diabetes, requiring emergency hospitalization, such as diabetic ketoacidosis. Were also analyzed data of late complications of diabetes and the costs of treating these complications. Our research work includes in comparative data of necessity of medical treatment: the total daily insulin dose, the cost of the insulin therapy with using njection pens and insulin pumps. We examined the level of hemoglobin A1c in patients who are receiving insulin by injection pens (first group) and in-

sulin pump (second group). It was lower in second group ($7.5 \pm 0.9\%$) than in the first one ($9.3 \pm 1.3\%$). We proved a general reduction in hospitalizations in patients with diabetes ketoacidosis by 47%.

Conclusions: Despite that the cost of therapy by insulin pens is lower but total cost is comparable to the cost of therapy with insulin pumps.

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IT'S TIME TO PUT THE "AGE" AND THE "FACE" ON HBA1C IN ELDERLY PATIENTS WITH DIABETES MELLITUS

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Introduction: Older patients with diabetes mellitus often have concomitant co morbidities and physical disabilities which may lead to poor quality of life. The present diabetic guidelines available to clinicians in their practice suggest aggressive and meticulous diabetic control for target HbA1c (<7%), this may lead to recurrent hypoglycaemia, and poor quality of life.

Methods: This is a cross-sectional survey of 46 diabetic patients aged between 65–90 years old who were seen in diabetic clinic. They were given questionnaire(SF36) to assess their quality of life (Mental and Physical component) in relation to to blood glucose levels and other co morbidities.

Results: Older patients and especially those with other co morbidities such as chronic kidney disease, chronic obstructive airways disease, cognitive impairment, rheumatoid arthritis and peripheral vascular disease reported one or more episodes of hypoglycaemia per month due to tight glycaemic control. We found increased poor quality of life in such patients. Older patients preferred their blood glucose to be range 7–10 mmol/L, than below 5 mmol/L, this helped to allay their anxiety.

Conclusion: We suggest that clinician's practice should aim at supporting the well-being, good quality of life and healthy lifestyle of the older people with diabetes. Glycaemic control in this age group should be individualised to reflect patient's clinical and personal needs and according to patient's informed choice. By putting the "age" and the "face" on HbA1c both patients and clinicians will be able to achieve a better balance between good quality of life and acceptable glycaemic control for this age group.

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SOUND MIND, BUT SWEET BLOOD—OLANZAPINE-INDUCED HYPERGLYCAEMIA ("OLANZAPINE ET AL" (OTHER ANTIPYSCHOTICS)—"PARTNERS IN CRIME"?)

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Background: Atypical antipyschotics agents are useful in treating patients with schizophrenia and other pyschosis, but may cause hyperglycaemia. Hyperglycaemia is not dose dependent and is reversible on stopping the treatment with atypical antipyschotics and there is relapse on restarting the treatment.. The effect and ability of various atypical antipyschotics drugs to cause diabetes is debatable. The current available evidence seems to indicate that olanzapine and clonazapine are at top of the ladder and may have higher propensity to induce diabetes compared to other atypical antipyschotics.

Methods: We present a case of a 37 year old lady with Huntington's chorea who was admitted after general deterioration over 3 days, with loss of appetite, high temperature, worsening of choreiform movements, urinary frequency and reduced level of consiousness. On admission she was found to have profound metabolic acidosis (pH 7.22), hyperglycaemic (glucose 73.3 mmol/L), renal failure and hypernatraemic(Na 170), and her urine was positive for blood, protein, glucose, and ketones. She was on olanzapine started 2 years prior to admission as well as sulpiride. There was no family history of diabetes.

Results: She was treated for urinary sepsis with IV antibiotics, IV fluid and insulin sliding scale. She was discharged home on insulin Mixtard 30: 34 units morning, and 16 units evening, and antipyschotics.

Conclusion: Health care professionals should play leading role to identify patients at risk, and monitoring those receiving antipsychotic therapy. They must be aware of the growing association between atypical antipsychotic agents and diabetes mellitus in order to detect and treat patients early.

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PIOGLITAZONE-INDUCED "ELEPHANT LEGS" (OEDEMA) AND DERMOPATHY (THICK SKIN) IN PATIENT WITH TYPE 2 DIABETES MELLITUS

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Introduction: Oedema is an adverse event associated with glitazones. Mild to moderate peripheral oedema is well known especially in patients with heart failure or those on insulin. Thickening of skin (dermopathy) may develop after initiation of pioglitazone.

Methods: We present a 60 years old lady type 2 diabetes mellitus who developed severe lower limbs oedema and thickening of skin after being started on pioglitazone. She was on metformin and gliclazide. Her glycaemic control remained poor and she was started on pioglitazone (December 2008) as an add on therapy to gliclazide and metformin. Few months after starting the pioglitazone she noticed increased bilateral leg swelling and thickening of the skin, and this resulted in blister formation and recurrent celullitis. She had no history of heart failure.

Results: Pioglitazone was stopped and patient was treated with IV antibiotics for celullitis. Few months after stopping the pioglitazone the oedema improved and thickening of the skin became less pronounced.

Conclusion: Glitazone are effective insulin sensitizers, but may cause oedema. Oedema occurs within the first few months of therapy and often is unresponsive to diuretics therapy. Although dermopathy might be a skin manifestation associated with type 2 diabetes in this case the patient states that it coincided

with initiation of pioglitazone. The mechanism of glitazone-induced-peripheral oedema is unknown but appears to be multifactorial.

(1) Expansion of plasma volume (renal sodium retention by activation of renin angiotensin system).
(2) Change in endothelial permeability.
(3) Glitazones induced cardiac hypertrophy (animal studies). Patients on glitazones should monitored for oedema, and glitazones should be stopped if oedema persist.

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HYPERGLYCEMIA DURING CHEMOTHERAPY AND ITS IMPLICATION

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Objective: Though some instances of hyperglycemia during chemotherapy are known, most of the time clear incidents and implications and literature on this subject are not clearly known in India.

Materials and methods: 585 cases undergoing chemotherapy were selected randomly. Their pre-chemo, during & after chemo & 1st &2nd week after chemo, blood sugar were recorded. Their age sex diagnosis and the chemo agents were also noted. The cases studied included diabetics and not yet known diabetes. A detailed history and examination was carried out, and following baseline investigations (F, PP, HBA1C, serum creatinine, microalbuminuria, lipids, retinal examination, chest X ray and ECG) were done. Majority of the chemo agents are continued with dexamethasone (8–16 mg) & max upto 40 mg.

Results: Among the 585 patients studied 350 cases were normal glycemic throughout chemotherapy (59.8%) and 170 (29.05%) were known diabetic which developed severe derangement of blood sugar level. Out of these known cases 5 cases developed diabetic ketoacidosis and 3 cases went into hyperosmolar nonketotic hyperglycemia. 65 cases (11.11%) developed diabetes post chemotherapy, on regular follow-up 26 cases developed frank diabetic (4.4%) and 39 cases (6.6%) glycemic status became normal after 1–2 weeks of chemotherapy.

Conclusion: This study is very significant for the necessity of awareness and precautions to be observed since asignificant number of patients without DM are also likely to go hyperglycaemic as well as diabetic ketoacidosis. These patients are managed with insulin. Goal is strict glycemic control control and prevent later control and prevent later complication.

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A TRIANGULAR APPROACH FOR CONTROL OF HYPERGLYCEMIA IN LOW SOCIOECONOMIC PERSON WITH LOW COST

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We have done a study in Type 2 DM with Low Socio Economic status people, where affordability to pay for Consultation, Purchase of Medicine was poor.

We made a triangular approach of

1) our centre
2) local rural doctor
3) Diabetic Educator

The patients were from outskirts Of Hyderabad. These patients were screened for CBP, CUE, BLOOD SUGAR LEVEL, HBA1C, SR.CREATINE LEVEL, ECG, CXR, and LIPID PROFILE. These patients were examined from head to toe for any signs of diabetes complications. The patients who were enrolled had the RBS between 200–300 mg/dL and the HBA1C ranging from 8–10.

The patients excluded from this study were Acute or Chronic complications, Metabolic syndrome, GDM, Type 1 DM, RBS $>$ 350 mg/dL, HBA1C $>$ 10.

The southern part of India is more habituated for carbohydrate rich diet. These patients were counselled about the proper diet and life style modifications. As these patients cannot afford the newer drugs, we had used only two molecules like Metformin and Sulphonylurea, some patients were on insulin. These patients were explained about the glucometer and how to use it. These patients were explained about the complication of the medicine like hypoglycaemia. We were in contact with these patients weekly twice.

After 6 months the aim was to have good glycemic control, with this systemic approach, the sugar level of the patients were under controlled and HBA1C was less than or up to 7 as per the guidelines of ADA. Controlling sugar is a team work rather than an individual approach.

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INTERRELATIONSHIP BETWEEN HIGHER BMI AND PREDIABETIC PATIENTS: DEVELOPING HYPERGLYCEMIA IN CHEMOTHERAPY PATIENTS

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Objective: During the study we have seen that prediabetic and patients with high BMI are prone to develop diabetes which could have precipitated due to steroids.

Materials and methods: 300 cases were selected randomly and studied for 6 months. Their sugar level pre, during, after chemo, day 2, day 3,1 week and regularly for 6 months were monitored. Age, Sex, Diagnosis, BMI, and the chemo agents were also noted. These patients were having the FBS(100–120 mg/dL) & PLBS (140–180 mg/dL). We have excluded patients having Diabetes, Hypertension, Coronary artery disease, CVD, acute and chronic complication of diabetes. A detailed examination was carried out and baseline investigations (F, PP, HBA1C, serum creatinine, microalbuminuria, lipids, retinal examination, chest X ray and ECG) were done.

Results: Among 300 patients studied 244 cases were normal glycemic throughout chemo (81.33%) and 56 patients (18.66%) were having BMI $>$ set normal limit. Out of 56 patients, 40 cases (13.33%) were having normal glycemic status & remaining 16 patients (5.33%), 15 cases (5%) developed diabetes and 1 case developed acute complication like DKA. These patients had BMI $> 32 \pm 5$.

32 patients with average BMI of 27.45 ± 2
18 patients with average BMI of 34.95 ± 3
6 patients with average BMI of 42.50 ± 2

Conclusion: A BMI between 25 and 30 should be viewed as medically significant and worthy of therapeutic intervention, es-

pecially in the presence of risk factors that are influenced by adiposity, such as hypertension, hyperglycemia, insulin resistance, dyslipemia, cancer. The present study was done to highlight strict glycemic control (FBS < 110 and PLBS < 140–160 mg/dL).

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PARADIGM REAL TIME 722® RESULTS IN A TYPE 1 DIABETES MELLITUS PATIENTS

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Introduction: Paradigm Real Time 722® (PRT-722) is a dual electronic device, which allows both continuous subcutaneous insulin infusion and continuous glucose monitoring (CGM). There are not available information out of controlled trial about their benefits during regular clinical practice activity.

Aim: To determine metabolic and quality of life improvement at three and twelve months in PRT-722 treated type 1 diabetes mellitus (T1DM) patients.

Subjects and methods: A close one-year duration clinical and telematic follow-up of new treated PRT-722 patients was carried out. We gathered physical exploration, insulin treatment, diabetes quality of life (DQOL), severe hypoglycemic events, capillary glucose, HbA1C and continuous glucose information of twenty-four patients at time 0, 3 and 12 months.

Results: T1DM medium duration, 15.5 ± 9.5 years. Severe hypoglycaemic events reported during 1 year before PRT-722 beginning, 1.54 ± 4.0 events. DQOL pre-PRT 722 score, 92.79 ± 18.42. CGM system frequency use: 3-months, $20.0 \pm 10.7\%$; 12-months, $20.2 \pm 13.1\%$. We detected a significant 0.61% and 0.49% HbA1C levels reduction at 3 and 12 months, respectively; rest of metabolic changes are showed in Table 1. In addition, patients referred 0.46 ± 1.6 ($P < 0.05$) severe hypoglycaemia events during first 12 months of PRT-722 treatment. We also observed a significant improvement in DQOL score at 3 (83.80 ± 21.60, $P < 0.05$) and 12 months (79.41 ± 13.81, $P < 0.05$).

Conclusions: CGM use close to 20 per cent with PRT-722 dual system was associated with a reduction of HbA1C levels and improvement of DQOL in T1DM patients at 3 and 12 months.

Table 1. Metabolic Changes

Glycemic control	*Pre-PRT 722*	*3 months*	*12 months*
MCG (mg/dL)	173.1 ± 31.2	164.6 ± 37.0	154.2 ± 23.7
MIG (mg/dL)	169.9 ± 30.6	157.6 ± 24.7	142.9 ± 19.2
AUC hypo (mg/dL)	25.8 ± 14.3	15.0 ± 8.2	10.9 ± 9.5
AUC hyper (mg/dL)	0.7 ± 1.0	0.5 ± 0.4	0.5 ± 0.6*
HbA1C (%)	7.65 ± 1.13	7.04 ± 0.68**	7.16 ± 0.76**

MCG, medium capillary glucose; MIG, medium intersticial glucose; AUC hypo, hypoglycemia area under the curve. AUC hyper, hyperglycemia area under the curve.

*$P < 0.01$

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THE RELATION BETWEEN TRADITIONAL CARDIOMETABOLIC RISK FACTORS AND GLUCOSE PARAMETERS FROM CONTINUOUS MONITORING IN PERSONS WITH TYPE 2 DIABETES

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Background: Glucose variability (GV) augments cardiovascular risk and longterm evolution in diabetes. The relation between GV and cardiometabolic risk factors in type 2 diabetes (T2D) needs further investigation for better understanding of the underlying mechanisms.

Aim: Assessment of the relation between cardiometabolic risk factors(CMRF)and continuous glucose monitoring parameters, glycated haemoglobin A1c(A1C), in persons with T2D.

Materials and methods: 30 persons with T2D(8 women,22 men, insulin-treated-14, oral-treatment-16)were assessed by continuous glucose monitoring (CGMS, Medtronic, MiniMed). Assessed CMRF:body weight, BMI, waist circumference, lipid profile (total cholesterol, HDLc, triglycerides (TG), calculated LDLc), systolic and diastolic blood pressure (SBP, DBP), personal and family history of cardiovascular disease (CVD), family history of diabetes. Assessed glucose parameters: A1C, mean amplitude of glucose excursions-MAGE (Monnier et al, 2006), glucose variability-GV-standard deviation of glucose values, glucose values number (time spent), area under the curve (AUC, glucose exposure), mean glucose values (glucose amplitude) on hypoglycemic (<70 mg/dL), intermediate (70–180 mg/dL), hyperglycemic (>180 mg/dL), optimal (90–130 mg/dL) domains.

Results: GV increased concomitently with A1C category. MAGE was higher in women. Persons with SPB >130 mmHg had lower percent of hypoglycemic values and hypoglycemic exposure, higher total glucose exposure, higher diurnal and nocturnal glucose exposure and higher glucose amplitude (mean). TG values were directly correlated with diurnal glucose exposure and inversely related to nocturnal AUC.HDLc was directly correlated with the magnitude of intermediate glucose exposure (70–180 mg/dL). Persons with family history of diabetes had higher time spent and total glucose exposure to hypoglycemia. Persons with family history of CVD had lower A1C values ($P < 0.05$). The direct correlation between worse metabolic control and hyperglycemic exposure was close to statistical significance.

Conclusion: SBP was directly correlated with total glucose status and inversely related with hypoglycemia. TG and HDLc were directly correlated with glucose status. Awareness of certain familial CMRF motivated diabetic persons to have a better glucose control with a higher exposure to hypoglycemia.

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GLUCOSE VARIABILITY IN TYPE 2 DIABETIC PERSONS EVALUATED PROSPECTIVELY BY CONTINUOUS GLUCOSE MONITORING

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Background: Actual and long-term status of blood glucose levels remanins the one of the most important determinants for diabetes evolution and appearance of chronic complications. General or postprandial glucose fluctuations are related to oxidative stress in a higher extent than sustained hyperglycemia.

Aims: To evaluate the relation between glucose variability(GV), mean amplitude of glucose excursions (MAGE) and glycated haemoglobin (A1c), anthropometric parameters, sex,

diabetes treatment, currently and prospectively in persons with type 2 diabetes.

Method and study group: 30 persons with type 2 diabetes, 8 women, 22 men, median age 64 (39–69) years, mean diabetes duration 14 (0–17) years,14 persons insulin-treated, 16 subjects on oral therapy performed continuous glucose monitoring (CGM) for 3 days. 10 of the insulin treated subjects performed a second CGM after 3 months. Assessed parameters:A1c, weight, body mass index-BMI, waist circumference; based on CGM: glucose variability(GV) and MAGE.

Results: GV and MAGE were significantly higher in insulin treated persons and women at the first visit. A1c was higher in women and in insulin treated persons. GV and MAGE were directly correlated with A1c initially and after 3 months. GV and MAGE were inversely correlated with weight initially and after 3 months ($P < 0.05$). A1c, GV and MAGE decreased after 3 months ($P > 0.05$). Weight ($P < 0.05$), BMI ($P < 0.05$) and waist circumference ($P > 0.05$) increased at second visit.

Conclusion: Glucose fluctuations are directly related to long-term glucose status (A1c), and to hyperglycemic exposure, and inversely related to parameters of normoglycemia. A1c, GV and MAGE decreased after three months, possibly also due to specific treatment adjustments based on continuous glucose monitoring. These findings need to be explored in further studies.

P-157

MASS-SPECTROMETRY AS AN ANALYTICAL TOOL FOR PANCREAS SECRETION STUDIES

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Clinical studies for artificial pancreas (AP) treatments for type 1 diabetes have shown an increase in hypoglycaemic episodes. This has driven the development for bi-hormonal pumps infusing insulin and glucagon.

Current biological assay detection techniques, Radioimmunoassay (RIA) and Enzyme-linked immunosorbent assay (ELISA), which quantify insulin and glucagon concentration require a minimum sample volume of 25–100 microlitres per individual analyte for detection. The experimental sampling frequency is inversely proportional to the number of measured analytes, limiting multiple simultaneous measurements.

Here we investigate the use of nano-High Pressure Liquid Chromatography Mass Spectrometry (nano-LCMS) as an alternative tool for analyte measurement. nano-LCMS enables the detection of multiple analytes within the same sampling volume. The potential sensitivity of nano-LCMS suggests that lower initial sample volumes would be required for analyte detection, thus significantly increasing available time-resolution for in-vitro cell secretion experiments. We demonstrate the relative merits of nano-LCMS in comparison to RIA and ELISA for glucagon and insulin measurements in the analysis of analyte secretions from in-vitro mouse pancreatic tissue studies, specifically investigating the inhibitory effects of insulin on glucagon secretion.

The development of control algorithms for AP pancreas treatments that use data from in-vivo studies is complicated by the physiological and logistical difficulties of regulating variables in pancreas secretion experiments. In-vitro pancreas tissue studies offer the ability to characterise and model cell secretion with a greater level of control, and the potential for using nano-LCMS derived data in assisting model development is presented.

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EFFICIENCY OF THE SHORT ACTING INSULIN ANALOGUES ON GLYCEMIC CONTOLE AND GLOBAL CARDIOVASCULAR RISK IN INSULIN-DEPENDENT DIABETES MELLITUS PATIENTS

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The objective of this study was the evaluation of short acting Insuline Analogues efficiency compared with short acting Human Insuline on postprandial glycemic peaks, glycemic controle and on the global cardiovascular risk in patients with Type 2 Insulin-Dependent Diabetes Mellitus (IDMD).

Material and method: We have selected a lot of 60 patients, males, with age between 55 and 65 yers, previously diagnosed with IDMD, hospitalised in an Internal Medicine Department from Western Romania. Half of the patients were treated with short acting Human Insuline: Actrapid/HumulinR/Insuman Rapid (L1) and the other half (L2) received short acting Insuline analogues (Humalog/Apidra/Novorapid). Clinical parametres evaluated were weight, height, abdominal circumference, body mass index and arterial hypertension. Biochemical parametres measured were fasting and postprandial glycemia, HbA1c, total plasma cholesterol, LDL and HDL-cholesterol, plasma tryglicerides. Global Cardiovascular risk was calculated using Euro98 Diagram.

Results: The evaluation of glycemic parametres releaved very similar average of glycemic levels (109 mg/dL/L1 versus 108 mg/dL/L2), but postprandial gycemic peaks were significantely lower in L2 group (at 1 and 2-hours: $P = 0.005$; 179 mg/dL and 135 mg/dL, versus 218 mg/dL and 179 mg/dL). Patients from L1 had higher HbA1c than those from L2 and a higher cardiovascular risk (odds ratio 1.37; $P = 0.007$).

Conclusions: Short Acting Insuline Analogues are more efficient in patients with Tip 2 Insulin-Dependent Diabetes Mellitus in glycemic controle. Lowering postprandial glycemic peaks and better glycemic control have a positive effect on the lobal cardiovascular risk.

P-159

IMPACT OF A NURSE SHORT MESSAGE SERVICE AND TELEPHONE FOLLOW-UP INTERVENTION FOR PATIENTS WITH DIABETES

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Aim: To compare the effectiveness of two methods of follow up; Short Message Service and telephone follow-up on type 2 diabetes adherence.

Background: Using telemedicine approaches may preserve the appropriate blood glucose levels and may improve adherence to diabetes control recommendations in diabetic patients.

Design: A quasi- experimental, 2-group, pretest and posttest design was used in this study to evaluate the effectiveness of nurse's follow-up via cellular phone and telephone.

Methods: The sample consisted of 77 patients with type 2 diabetes that randomly assigned to 2 groups: telephone follow-up (N = 39) and short message service (N = 38). Telephone inter-

ventions was applied for 3 months, twice a week for first month and weekly for second and third month and SMS group that received message about adherence to therapeutic regimen daily for 3 months. Data gathering instrument include data sheet to record glycosylated hemoglobin and questionnaire related to adherence therapeutic regimen. Data gathering was performed at initial the study and after 3 and 6 months. Data analyzed using statistics methods with SPSS version 11.5.

Results: showed that both interventions had significant mean changes in glycosylated hemoglobin; for the telephone group ($P = 0.000$), with a mean change of -0.93 and for the SMS group ($P = 0.000$), with a mean change of -1.01. There was no significant difference in diet adherence ($P = 0.000$), physical exercise ($P = 0.000$) and medication taking (($P = 0.000$) adherence in two groups.

Conclusion: Intervention using SMS of cellular phone and nurse-telephone follow up improved HbA1c levels and adherence to diabetes therapeutic regimen in type-2 diabetic patients.

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SUCCESSFUL IMPLEMENTATION OF BLINDED CONTINUOUS GLUCOSE MONITORING DURING A RANDOMIZED CLINCAL TRIAL

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Blinded Continuous Glucose Monitoring (CGM) enables collection of detailed retrospective data. This study was designed to investigate the use of novel procedures to enhance data capture from blinded CGM.

After a 4 week run-in, 46 patients with type 1 diabetes were randomized to 1 of 2 therapies for a 12-week treatment period, and then crossed over to the alternate treatment for 12 weeks. Blinded CGM was implemented at the end of run-in (practice only) and during the last 2 weeks of each treatment period. CGM data were deemed adequate if 80% of 288 possible daily values were captured for 3 consecutive days. CGM was continued for another week if these criteria were not met. If sensor failure occurred then patients were allowed to insert new sensors at home.

Higher than expected sensor failure rate was ~25%. During run-in practice, 12 of 45 profiles failed adequacy criteria. During treatment, only 1 of 82 attempted profiles was inadequate (6 cases required an second week of CGM). Figure 1 shows Clarke Error Grid analysis of 777 paired CGM-SMBG values.

With appropriate training, practice and opportunity to repeat blinded CGM as needed, nearly 100% of attempted profiles can be successfully obtained.

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TREATMENT OF OBESITY BY CONTROLLED FASTING IN PATIENTS WITH TYPE 1 DIABETES MELLITUS EFFECT ON INSULIN SENSITIVITY AND GLUCOSE METABOLISM

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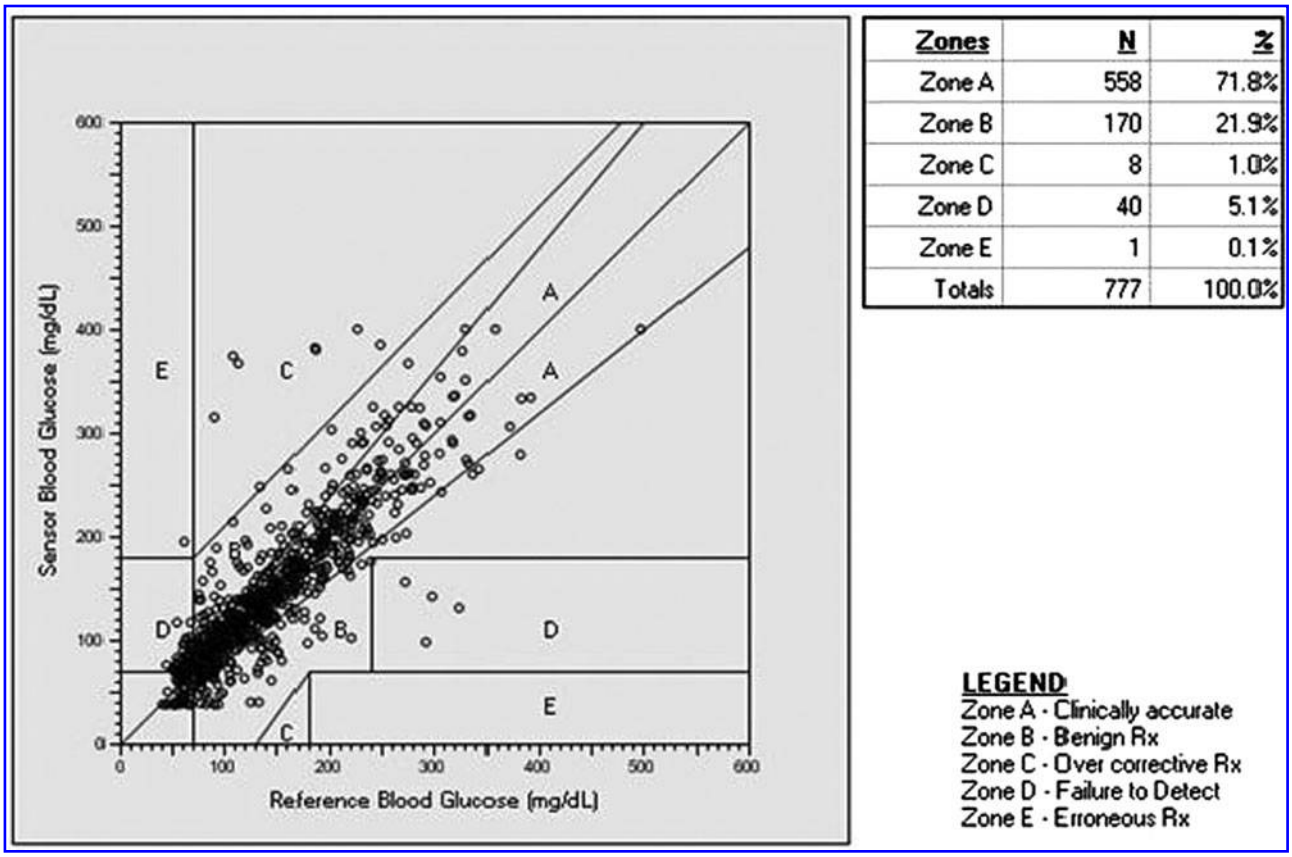

FIG. 1.

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The aim was to study the influence of 7 days of total fasting on insulin sensitivity and glucose metabolism in patients with type 1 diabetes mellitus (T1DM).

Methods: We studied 14 obese patients with T1DM (42.6 ± 9.4 years, BMI 32.4 ± 2.1 kg m^{-2}) and 13 nonobese control patients with T1DM (36.9 ± 13.9 years, BMI 22.6 ± 2.1 kg m^{-2}). Insulin sensitivity was measured in obese T1DM patients before fasting, immediately after 7 days of fasting, as well as 21 days thereafter. The control group was studied only after overnight fasting. Insulin sensitivity was measured using a two-step hyperinsulinemic-euglycemic clamp lasting 6 hours; period 1: 0 to 120 minutes, 1 mU min^{-1} kg^{-1} of insulin; period 2: 120 to 360 minutes, 10 mU min^{-1} kg^{-1} of insulin. Glucose oxidation and nonoxidative glucose disposal were measured before and during the clamp by indirect calorimetry.

Results: Fasting obese T1DM patients lost 6.1 ± 1.1 kg after the 7 days. Glycemia during fasting was maintained at 5 mmol/L by adjusting basal insulin doses. Fasting reduced insulin-mediated glucose disposal in both phases of the clamp (phase 1: from 5.18 ± 1.43 to 2.96 ± 0.49; phase 2: from 9.69 ± 1.48 to 6.78 ± 1.21 mg min^{-1} kg^{-1}, $P < 0.001$). This was caused by reduced glucose oxidation after the fasting period (phase 1: from 1.55 ± 0.64 to -0.01 ± 0.56; phase 2: from 2.81 ± 0.52 to 0.88 ± 0.98 mg min^{-1} kg^{-1}, $P < 0.001$).

Conclusion: One week of fasting transiently decreased insulin-mediated glucose disposal in T1DM patients. This was caused by reduced glucose oxidation. To our knowledge, this is the first report describing this effect.

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EFFECT OF CONFECTIONS CONTAINING EXTRACTIVE FROM *MORUS ALBA* ON GLYCEMIC AND INSLINEMIC RESPONSE IN HEALTHY SUBJECTS

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To decline the incidence and prevalence of life-style related diseases, the control of glycemic and inslinemic responses are very important. Usually sweet confections and desserts are strictly prohibited, while the onset of depression is relevant to diet therapy. The confections supplemented with suppressive effect on the postprandial elevation of blood glucose and insulin could help these problems. We have clarified that the extractive from leaves of *Morus alba* (ELM) competitively inhibited the small intestinal disaccharidases and decreased the elevation of blood glucose by the ingestion of sucrose solution in human. In this study we prepared confections (mizuyokan and daifukumochi which are Japanese traditional sweets; chiffon-cake) containing several dose of ELM and evaluated the effect on the postprandial blood glucose and insulin in ten healthy subjects using within-subjects repeated measures design. Subjects were collected blood and end-respiratory at the indicated time after the over-night fasting. The increments of blood glucose and insulin for three hours after the ingestions of mizuyokan and daifukumochi containing 30 g of sucrose and 1.5 g of ELM were significantly suppressed in comparison with placebo confections which were not added ELM, and those added 3.0 g of ELM effectively suppressed postprandial blood glucose and insulin. In the ingestion of chiffon-cake containing ELM, suppressions for the elevation of blood glucose and insulin, and excretions of breath hydrogen were dose-dependent of ELM. These results demonstrate that ELM could contribute to develop the functional foods with health claim leading the control of postprandial blood glucose and insulin for pre- and diabetic patients.

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MEMS TECHNOLOGY: A NEW BENCHMARK FOR INSULIN INFUSION PUMPS?

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MEMS (Micro-Electro-Mechanical Systems) are widely used today in many of our day life applications (Automotive, GPS navigation, cellular phones, aircrafts, …) where precision, reliability and cost are essential requirements. Using MEMS for insulin delivery requires highly sophisticated engineering and design, good understanding for safety and efficacy as well as specific needs regarding insulin stability. As a result, a pump made out of MEMS can reach new levels of accuracy, reliability, miniaturization and safety measures that would hardly be envisioned using conventional technologies.

The JewelPUMP, which is based on a MEMS chip weighting only 2 g, incorporating the entire pumping engine and multiple sensors, enables a new approach to insulin therapy.

Between many characteristic, the pump can deliver single strokes of 200 nanolitres (or 0.02 Units of insulin) with an accuracy better than 5%, ensuring a closer to physiological basal delivery rate. It can further deliver 3.5 Units/minute keeping the same accuracy level for a bolus, while very single delivery stroke is monitored by a sensor capable of detecting an occlusion within a single 0.02 Unit stroke. The pump is also capable of continuously monitoring its performance, which feature is also used to ensure a 100% quality inspection during production. Last, but not least, the MEMS pump can be produced in million units, making it a very cost effective solution for a disposable pump.

The JewelPUMP will be the smallest pump ever conceived for insulin therapy, with up to 4.5 ml insulin on board for a 6 day's use, remotely controlled by its patient's own PDA phone.

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ROSES: ROLE OF SELF-MONITORING OF BLOOD GLUCOSE AND INTENSIVE EDUCATION IN PATIENTS WITH TYPE 2 DIABETES NOT RECEIVING INSULIN: A PILOT RANDOMIZED CLINICAL TRIAL

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Introduction: Evidence supporting self-monitoring of blood glucose (SMBG) for T2DM patients not requiring insulin is still unclear.

Aims: To estimate the efficacy of a SMBG-based disease management strategy in T2DM patients treated with oral agent monotherapy.

Methods: In a 6-months randomized controlled trial we evaluated an intervention, based on quarterly face to face encounters and montlhy telephone contacts, aimed at lifestyle modifications in response to SMBG readings and timely changes in therapy. The control group received standard counselling on diet and lifestyle and quarterly follow-up. Pharmacotherapy was aimed to target HbA1c $< 7.0\%$ in both groups, using the same algorithm. Primary efficacy analysis was mean change in HbA1c levels, estimated with an ANOVA for repeated measures.

Results: Three centres recruited 62 patients (46 intervention, 16 control), of whom 5 were lost to follow-up. At baseline, both groups had a mean HbA1c value of 7.9 ± 0.6. After 6 months, the mean HbA1c reduction was 1.19 ± 0.12 in the SMBG group and 0.71 ± 0.17 in the control group, with an absolute mean difference between groups of -0.48 (95%C.I.: -0.94, -0.02;$P = 0.04$). The percentage of patients reaching the target HbA1c $< 7.0\%$ was 61.9% in the SMBG group and 20.0% in the control group ($P = 0.005$). Sixteen patients in the SMBG group (35%) and 9 patients in the control group (56%) required therapy adjustments ($P = 0.13$).

Conclusion: An SMBG disease management strategy allowing an efficient use of SMBG readings is able to improve metabolic control while reducing the need for treatment intensification among patients with TDM2 not requiring insulin.

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THE MEDTRONIC ENLITE™ SENSOR: THE NEXT STEP TOWARDS FINGERSTICK REPLACEMENT

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Objective: Continuous glucose monitoring systems (CGMS) enable users to monitor their glucose levels in real-time. However, current devices require a confirmatory fingerstick reading prior to treatment action. The next step in CGMS technology is to eliminate this confirmatory reading requirement. While Medtronic's upcoming Enlite™ sensor system is designed to work with legacy products, the system represents a significant step towards fingerstick replacement when the sensor is used in conjunction with a new glucose algorithm.

Method: Using data from a feasibility trial using the Enlite sensor ($n = 52$), the sensors selected for processing utilized the legacy glucose algorithm, a new algorithm, and the new algorithm with a retrospective single point calibration frequency.

Results: When processed using the legacy algorithm, the Enlite sensor had hypo- and hyperglycemic sensitivities of 57% and 91%, respectively. The data also showed high clinical accuracy, with coverage in the Clarke A and B zones at 96%.

Using the new algorithm, the hypoglycemic sensitivity of the system increased to 86%, while maintaining similar hyperglycemic sensitivity (89%). Additionally, the clinical accuracy (Clark A and B zones) increased to 97%. When the data was re-processed using a single calibration per day, the hypo- and hyperglycemic sensitivities were 82% and 81%, respectively. The clinical accuracy was 95%.

Conclusion: These preliminary results suggest that Medtronic's upcoming Enlite™ glucose sensor system is accurate from a clinical standpoint, and excels in detecting glycemic excursions. This level of clinical accuracy demonstrates significant progress towards fingerstick replacement.

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FETAL STEM CELLS (FSC) IN DIABETIC NEPHROPATHY

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The study included 23 patients - 15 men and 8 women aged 23–64 with 6–18-year history of types 1 and 2 DM and different stages of chronic kidney disease (CKD): stageI - 6(GFR-94,78 ± 3,95 mL/min/1,73m^2, creatininemia-0,105 ± 0,08 mmol/L, microalbuminuria-276,39 ± 18,4 mg/day), II-9(GFR-63,74 ± 2,57 mL/min/1,73 m^2, creatininemia-0,142 ± 0,09 mmol/L), III-8(GFR-43,51 ± 3,26 mL/min/1,73m^2, creatininemia-0,199 ± 0,16 mmol/L). 78% of patients presented hypertension, 100% - lipid count distortion, 33%-slight anemia, 39%-edema. All patients were taking ACE inhibitors (ARBs) and, if indicated - statins, calcium antagonists, diuretics, antiaggregants, desintoxicants. The main inclusion criterion was absence of marked carbohydrate metabolism decompensation. Patients were followed-up for 10–12 months.

We infused 1–3 ml of suspension containing fetal hematopoietic and non-hematopoietic mesenchymal and endodermal stem cells harvested from germ layers of 4–8 weeks old cadaverous embryos' internal organs and sorted thereafter (cell count-6–120×10^6/mL).

Gradual improvement of kidney function was also reported: 1,5–2-fold daily proteinuria reduction within 2–3 months in 73% of CKDI and II patients and $46.8 \pm 0.9\%$ decrease in 62% of CKDIII patients maintained for 5–12 months. In CKDII patients, GFR decrease peaked after 4–5 months to $23.7 \pm 4.1\%$, while in 75% of CKDIII patients reported unchanged GFR and creatinine, which meant no progression for 10–12 months. Blood pressure drop was reported by 72.2% of patients 2–3 months, thus the doses of hypertension drugs were lowered. Blood restoration effect of FSC was manifested by higher Hb and RBC in 1–2 months. Lipid profile improvement was reported in 82.6% of patients in 4–6 months. All the patients reported blood sugar maintenance improvement over the whole follow-up period.

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CONSIDERATION ON VALIDITY OF GLYCEMIC INDEX USING BLOOD GLUCOSE AND INSULIN LEVELS AND BREATH HYDROGEN EXCRETION IN HEALTHY SUBJECTS

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Although glycemic index (GI) is very important in choosing appropriate foods for patients with diabetes mellitus, GI itself does not provide sufficient information for choosing adequate foods. To consider the validity of GI by measurement of blood glucose and insulin levels, and breath hydrogen excretion, testing several cultivars in same type food. Twelve females, 23.8y participated in this within-subject, repeated-measures

study. To clarify variations of GI in inter-cultivars of various foods, we examined 4 white rice and 3 potato cultivars and 3 noodle brands. Starchy-foods with a glucose equivalent of 50 g were repeatedly and randomly given to each subject. Blood and end-expiration were collected at selected periods. The significant difference of GI and insulinemic index (II) was not observed among 4 white rice cultivars, though II of one cultivar were smaller than those of other 3 white rice cultivars. GI of 3 potato cultivars was relatively small, but the range of II was very big among 3 cultivars. Also, GI did not correspond to II among 3 noodle brands. AUC-3h-glucose and AUC-3h-insulin scores of white rice and noodle were significantly larger than those for 2h. The amount of breath hydrogen excretion showed a negative correlation to GI of tested foods. We should be recognized that rare foods which GI doesn't correspond to II existed in the cultivar of foods used for diet therapy of diabetes mellitus, and would like to propose the addition of other information such as II and breath hydrogen excretion of selecting foods.

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MELATONIN IMPROVES ANTIOXIDANT STATUS AND FOETAL OUTCOME IN PREGNANT DIABETIC RATS

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Diabetic pregnancy is associated with increased for congenital malformations. However, studies by Eriksson and Borg have shown that an increased generation of reactive oxygen species (ROS) or impaired ROS scavenging function may play a central role in the teratogenic process. Melatonin has been identified as a powerful direct free radical scavenger and as an indirect antioxidant.

Four groups of eight rats per group were used for the experiment. Group I consisted of normal non-diabetic pregnant rats, while group II consisted of diabetic pregnant rats. Groups III and IV consisted of diabetic pregnant rats that received 1 mg/kg and 5 mg/kg of oral melatonin respectively. Diabetes was induced by 120 mg/kg of intraperitoneal alloxan.

Results showed a significant decrease in maternal weight, foetal weight, litter size and superoxide dismutase activity, while the level of plasma malondialdehyde (MDA) and placental weight were increased in group II compared to group I. However, there was a significant increase in the maternal weight, foetal weight, litter size and superoxide dismutase activity, while the level of plasma malondialdehyde (MDA) and placental weight were reduced following treatment with melatonin in diabetic groups.

Our study showed that oral melatonin improves foetal outcome and superoxide dismutase activity in diabetic rats, while it reduced the plasma MDA level.

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USE OF THE REAL-TIME CONTINUOUS GLUCOSE MONITOR AT INITIATION OF INSULIN PUMP THERAPY IN CHILDREN AND ADOLESCENTS

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Background: REAL-Time Continuous Glucose Monitor (RT-CGM) has previously been shown to decrease hemoglobin A1c (A1c) in adults with type 1 diabetes but not in children/youth, except at pump initiation when compared to use of multiple daily injections.

Objective: This pilot study was conducted to determine if the use of RT-CGM at initiation of insulin pump therapy in children/youth can improve adherence to overall diabetes treatment compared to standard pump therapy.

Methods: 13 children have completed the study (6 M/7F; aged 11.5 ± 4.1 years; diabetes duration 2.4 ± 2.6 years; mean A1c in the previous year $8.0 \pm 0.8\%$). Participants were randomized to RT-CGM initiated 1–2 weeks before the pump start or standard glucose blood monitoring for 4 months. Objective measures of adherence (A1c, blood glucose testing and pump parameters) were collected at baseline, 1 and 4 months after pump initiation. The Self Care Inventory (SCI) score, a validated questionnaire to assess diabetes-specific adherence behaviors, was completed by parents/children.

Preliminary results: There was an improvement in A1c from baseline to 4 months in both groups, but it was significant only in the control group (-0.6% [95%CI -1.0 to -0.2]). However, the mean difference of change in A1c (baseline-4 months) between the groups was not significant (0.1% [95%CI -0.2 to 0.5]). Furthermore, there was no significant change in SCI scores from baseline to 4 months in either group.

Conclusion: We were not able to demonstrate that the use of RT-CGM at pump initiation increases adherence to overall diabetes treatment in children.

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PATIENTS WITH TYPE 2 DIABETES MELLITUS AND OXIDATIVE STRESS

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Objectives: The present study is supposed to monitor the oxidative stress parameters at obese diabetic patients vs. the non-diabetics and the correlation between the intensity of the oxidative stress and the degree of obesity, metabolic control and the presence of insulin resistance.

Material and method: We included in the study a number of 180 patients with type 2 diabetes, out of whom 120 were obese while the other 60 were non obese patients. We studied the oxidative stress parameters: the pro-oxidant activity was evaluated by dosing the malonic dialdehyde produced by the lipid peroxide and by the carbonylated proteins which resulted from the oxidation of the proteic structures, while the antioxidant activity was evaluated through the dosing of ceruloplasmine. To each patient we looked for he followings: weight, abdominal circumference, BMI, duration of diabetes evolution, the degree of metabolic control, insulinemia, the HOMA index.

Results: The obese diabetic patients presented incresed values of malonic dialdehyde and of the carbonylated proteins, as well as decreased ranges of antioxidants agents (ceruloplasmine).

Conclusions:

1. Diabetics with a severe obesity degree present an intensified activity of the prooxidant agents.
2. The oxidative stress is more increassed, the more the metabolic disorder of diabetics is higher.
3. The presence of hyper insulinism and of insulin resistance has a positive correlation to the intensification of the oxidative stress.
4. Reducing the prooxidative status may be useful in treating diabetes mellitus type 2.

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BILATERAL LOWER EXTREMITY SEQUENTIAL COMPRESSION DEVICES (SCDS) FOR THE PREVENTION OF INTRA-DIALYTIC HYPOTENSION—A NEW THERAPY FOR AN OLD PROBLEM

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Background: Intra-dialytic hypotension (IDH) is common, affecting up to 15%–50% of patients during hemodialysis. Current available therapeutic options are often ineffective. Enhanced external counter pulsation (EECP) is increasingly being utilized in the long-term management of chronic CHF and otherwise refractory angina. EECP mechanistically improves venous return, enhances peripheral resistance and cardiac lower extremity sequential compression devices (SCDs), presently used for DVT prophylaxis, could serve as mini EECP devices and stabilize the CVS during HD and prevent IDH.

Methods: We carried out an out-patient pilot study of bilateral SCDs to prevent IDH in 3 patients.

Results: Three patients, one male and two females participated in the pilot study (SEE TABLE).

Conclusions: Bilateral lower extremity SCDs were effective, convenient, and safe and prevented IDH in all three patients. We achieved ultra-filtration goals of 1–3 kg, a feat that was not possible without the SCDs. This new modality of preventing IDH is complementary to current existing therapies. Larger multicenter studies are warranted.

Results of Pilot Study

Age/Sex	*Cause of ESRD*	*Other factors contributing to IDH*	*Result of use of bilateral SCDs*
68/M	Ischemia Cardiomyopathy Sepsis	Reduced cardiac reserve Anemia Low albumin (2.9) Persistently Low SBP	Good
42/F	End Stage Liver Dx Hepato-Renal Syndr.	Alcoholic cirrhosis Low albumin (1.8) Persistently Low SBP	Excellent
86/F	Hypertension	Low albumin (2.5) Gr 2 Diastolic dysfunction	Good

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AUTOANTIGEN-SPECIFIC REGULATORY T CELLS INDUCED IN PATIENTS WITH TYPE 1 DIABETES MELLITUS BY INSULIN B-CHAIN IMMUNOTHERAPY

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There is a growing body of evidence to suggest that the autoimmunity observed in type 1 diabetes mellitus (T1DM) is the result of an imbalance between autoaggressive and regulatory cell subsets. Therapeutics that supplement or enhance the existing regulatory subset are therefore a much sought after goal in this indication. Here, we report the results of a double blind, placebo controlled, phase I clinical trial of a novel antigen-specific therapeutic in 12 subjects with recently diagnosed T1DM. Our primary objective was to test its safety. The study drug, human insulin B-chain in incomplete Freund's adjuvant (IFA) was administered as a single intramuscular injection, with subjects followed for 2 years. All subjects completed therapy and all follow-up visits. The therapy was generally safe and well-tolerated. Mixed meal stimulated C-peptide responses, measured every 6 months, showed no statistical differences between arms. All patients vaccinated with the autoantigen, but none who received placebo, developed robust insulin-specific humoral and T cell responses. Up to two years following the single injection, in peripheral blood from subjects in the experimental arm, but not the control arm, insulin B-chain-specific CD4 + T cells could be isolated and cloned that showed phenotypic and functional characteristics of regulatory T cells. The induction of a lasting, robust immune response generating autoantigen-specific regulatory T cells provides strong justification for further testing of this therapy in type 1 diabetes. (clinicaltrials.gov identifier NCT00057499).

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ANTICIPATING ERROR: ANALYSING BLOOD GLUCOSE MONITORS FOR POTENTIAL PATIENT USE ERRORS

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Introduction: Self-monitoring of blood glucose relies on the patient to effectively manage their condition, yet the potential for procedural errors exists. It is therefore essential that monitoring devices provide meaningful support of patients to both prevent and recover from situations where errors in use may occur. We use methods from the science of human-computer interaction to evaluate the support provided by a number of meters.

Background: David Price concluded that a patient's recurrent Hyperglycaemia and impending Ketoacidosis was the result of incorrect procedure when using a glucose meter. The patient had

reduced the course of insulin in reaction to frequent displays of 'LO' on the meter, indicating Hypoglycaemia. It later became apparent that the patient often failed to supply the meter with a sufficiently large sample, causing the meter to return the 'LO' message. The meter had failed to recover from an error that the patient had made and subsequently provided false information.

Results: We present an analysis of glucose meters from a range of manufacturers, demonstrating where use errors can be anticipated given the device and human behaviour. The issues that violate the principles of good interaction design range from poor error handling, to button labelling and positioning.

Conclusion: There are several issues that can be addressed to reduce future instances of patients suffering as a result incorrect usage of glucose meters. Patients are known to make errors in the SMBG process and we argue that improved device design can assist patients detect and recover from these errors.

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HOMEOSTASIS MODEL ASSESSMENT OF INSULIN RESISTANCE (HOMA-IR) THRESHOLD FOR THE DIAGNOSIS OF METABOLIC SYNDROME

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Introduction: Insulin resistance is one of the basic factors involved in the metabolic syndrome (MetS). The homeostatic model assessment (HOMA) is a validated method to measure insulin resistance from fasting glucose and insulin. To date studies regarding the cut-off values of insulin resistance for diagnosis of MetS are limited; in addition the threshold of the HOMA-IR index may vary by ethnic group.

Aim: To investigate the cut-off values of HOMA-IR index for identifying MetS in a Greek semi-urban population.

Methods: A population of 247 adult Greek individuals was studied. The MetS was defined according to the International Diabetes Federation criteria. The cut-off value of the HOMA-IR for the diagnosis of the MetS was estimated by the areas under the receiver-operating characteristic (ROC) curve.

Results: Subjects with a positive diagnostic for MetS presented higher HOMA-IR indexes values than healthy individuals (1.86 vs. 1.49; $P < 0.001$). The waist circumference($r = 0.27$), the HDL cholesterol ($r = -0.18$), triglyceride($r = 0.27$) and fasting serum glucose levels($r = 0.39$) were significantly related to the HOMA-IR index ($P < 0.005$). The area under the ROC curve (95% CI) for the HOMA-IR index was 0.630 (0.554–0.706)($P < 0.005$) and the cut-off value for the diagnosis of MetS was 1.52 (sensitivity 66.3%, specificity 53.5%).

Conclusion: Insulin resistance and MetS were significantly associated, and HOMA-IR index values were correlated with the MetS components. We suggest that the cut-off value of the HOMA-IR index can be applied to predict metabolic syndrome. Further prospective studies are warranted to elucidate the performance of this threshold in our country.

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GLUCOSE VARIABILITY IN TYPE 1 DIABETES PATIENTS WITH NEAR-TARGET HBA_{1C} LEVEL

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In this randomized controlled study 36 type 1 diabetes (DM1) adults with near-target stable for last 12 mon HbA_{1c} level were assigned to 1 year treatment in 2 arms:MDI analogs group, $n = 18$ (1), MDI analogs Usual Care (UC) group, $n = 18$ (2) with 2 control groups-healthy volunteers (HV, $n = 20$)(3) and CSII patients ($n = 20$ with mean CSII duration 12 ± 4 mon)(4). All patients were educated in the way of standard program for DM1 (including CSII educational program). Baseline characteristics of patients in each group were comparable in terms of age, diabetes duration, male/female ratio, HbA_{1c}, IU/kg ratio. As a 1st phase of our study, retrospective CGM (using Medtronic MiniMed) was performed 3 months after randomization for all groups of patients. There were significant differences in glucose variability parameters such as SD_T, MODD, $MODD_1$, $CONGA_1$, $CONGA_{24}$ in all group without statistically significant (SS) changing of HbA_{1c} in groups 1 and 4 (HbA_{1c} level $7.1 \pm 0.6\%$ and $7.2 \pm 0.5\%$, respectively). In group 2 HbA_{1c} level was increased significantly ($7.8 \pm 0.7\%$) with SS increasing of GV parameters. As a visualizing methods for GV measurement we used histograms of the distribution of the BG, fast Fourier transform methodology (with conception that $BG_{CGM\ sensor} = f(t)$) as a mark of BG stability (GS).

Conclusion: Analysis of CGM confirms results about less glycemic variability parameters in patients using CSII, regardless of HbA_{1c} level. Still important thing for effective DM1 treatment (both in glycemic targets achievement and in GV decreasing) is a frequency of visits to diabetologist. Histograms of the distribution of the BG and fast Fourier transform methodology are attractive visualizing methods for GV and GS presentation.

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PREDICTION ERROR GRID ANALYSIS (PRED-EGA): A NEW CG-EGA FOR THE ASSESSMENT OF BG-PREDICTORS

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Introduction: Prediction of the future blood glucose (BG) evolution from continuous glucose monitoring (CGM) data is a promising direction in diabetes therapy management, and several BG-predictors have recently been proposed. This raises the problem of their assessment. There were attempts to use for such assessment the continuous glucose error-grid analysis (CG-EGA), originally developed for CGM-devices. However, in CG-EGA the BG-rate of change is estimated from past BG- readings, while predictors provide BG-estimation ahead of time. Therefore, the original CG-EGA should be modified to assess predictors. Here we propose a new version of CG-EGA, the Prediction-EGA.

Data & Methods: The analysis is based both on simulated data and on data from clinical trials, performed in the European FP7-project "DIAdvisor". The BG-predictor developed in RICAM is considered for the analysis. Simulated data are used to test the ability of analyzed CG-EGA modifications to capture erroneous predictions in controlled situation. Real data are used to show the impact of different CG-EGA versions in the evaluation of a predictor.

Results: The straight-forward application of CG-EGA does not adequately classify the prediction performance. For example, we observed that about 12% of erroneous predictions in the hypo-region were not detected. In contrast, the proposed modification of CG-EGA, where the rate of change is estimated on the predicted BG-profile, is a very rigorous metric for the assessment in the hypo-region. The situation is similar for the prediction in other regions.

Conclusion: We propose a new CG-EGA, the PRED-EGA, for the assessment of BG-predictors.

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ACCURACY EVALUATION OF AN ON-LINE NEURAL NETWORK GLUCOSE PREDICTION ALGORITHM IN A CROSS-CORRELATION STUDY

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Aims: This work presents the evaluation of an on-line glucose prediction algorithm based on a neural network model (NNM) during a cross-correlation clinical study.

Material and methods: The on-line prediction algorithm was implemented on a portable personal digital assistant (PDA) where the patient types manually the last 5 continuous glucose monitoring (CGM) measurements and obtains the glucose prediction for a 30-minutes prediction horizon (PH). The patients used the PDA once a day in an inter-prandial state. They took a correction-decision before-and-after knowing the glucose estimation.

The NNM was previously trained for each patient, using his own CGM data and a generic CGM dataset. Patients were randomly assigned to start on control or experimental phase. The evaluation used CGM records from 12 patients over 2 weeks/patient.

The accuracy of the glucose predictor can be assessed only for those days where patients do not take any insulin or carbohydrate-intake correction-action and it is calculated as the root mean square error (RMSE).

Results: A total of 139 days were registered. The patients did not take any insulin or carbohydrate-intake correction-action in 62 days. The average RMSE for those days was 15.05 ± 5.59 mg/dL, ranging from 7.63 to 22.98 mg/dL.

Conclusions: The precision of the NNM is very accurate for a 30-minutes PH, although we observed an important variability among patients. This is due mainly to inter-patient differences in glucose variability, the different CGM system used for each patient and the glucose range of the generic CGM dataset used to the training of the NNM.

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PREDEVAL: A NEW TOOL FOR THE EVALUATION OF ANY GLUCOSE PREDICTION ALGORITHM

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Aims: This work presents the *PredEval* web tool that aims to help researchers in the evaluation of glucose predictor algorithms by implementing a complete evaluation methodology for comparing any glucose predictor output versus the original continuous glucose monitoring (CGM) profile.

Methods: *PredEval* estimates the mean model behaviour by the root mean square error and the average delay between the predicted and the CGM profiles. Additionally, the relative error is calculated as the absolute error normalized by the CGM value and it is classified into four groups ($\leq$10% 10–15% 15–20% ; $\geq$ 20%).

In a second phase *PredEval* focuses the analysis on the glucose peaks and valleys by calculating the peak delay (relative delay when the slope changes) and the overshooting/undershooting (difference in glucose values when the predicted profile is over/under the CGM-maximums and under/over the CGM-minimums). The predictor capacity to anticipate hypo/hyper-hyperglycemia's events is characterized by the false positive and false negative rates.

Results: PredEval is a free-access web-tool (***http://certificados.gbt.tfo.upm.es:8082/evaluador/***) that allows users to upload their original-predicted glucose profile datasets and visualizes the algorithm accuracy both as average results and profile by profile. Numerical data is sent to the user in a csv-formated file that can be imported from any spreadsheet program.

Conclusions: *PredEval* can be a useful tool to standardize the prediction algorithms evaluation methodology and to make progress in this investigation field. Up to now, it has been successfully applied to evaluate a neural network model prediction algorithm for different prediction horizons and different CGM systems.

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INTERNET VISITS IMPROVE DIABETES CONTROL IN ADOLESCENTS ON PUMP THERAPY

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Background and aims: To evaluate results from Carelink, Skype and Facebook as tools to improve diabetes control in diabetic adolescents on Medtronic PRT (insulin pump with glucose sensor).

Materials and methods: A total of 42 adolescents with type 1 diabetes, ages 14–23, were randomized in two groups: **Regular visits** (Group 1)-as standard medical protocol with regular visits at clinic, where data was downloaded at the clinic and intervention (pump settings-basal bolus insulin, education) were given to the patient and **Internet visits** (Group 2)- as protocol using Carelink personal program (Medtronic Diabetes), where the data was downloaded by the patient at home and interventions (same as group 1) were given via Skype (sound and video) and Facebook (written reports and chats). A1C was obtained before, three and six months after the study.

Results: Regular visits were 12.2 ± 1.4 patients in group 1 and Internet visits were 13.6 ± 2.1 per patient in group 2

retrospectively. There was significantly improvement in both groups (group 1 and 2 retrospectively, $7.6 \pm 0.9\%$ and $7.7 \pm 1.1\%$ on beginning with $6.3 \pm 0.8\%$ and $6.2 \pm 1.0\%$, $P < 0.05$) at the end of the study. Internet visits were more preferable by the patients.

Conclusion: This brief trial suggests that young diabetics prefer to make contact with their health care providers via internet, where new technologies using specific software like Carelink, Skype and Facebook can improve diabetes control same as regular clinic visits.

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INTERNET VISITS USING CARELINK, SKYPE AND FACEBOOK CAN IMPROVE DIABETES CONTROL IN ADOLESCENTS ON PUMP THERAPY

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Background and aims: To evaluate results from Carelink, Skype and Facebook as tools to improve diabetes control in diabetic adolescents on Medtronic PRT (insulin pump with glucose sensor).

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Results: Regular visits were 12.2 ± 1.4 patients in group 1 and Internet visits were 13.6 ± 2.1 per patient in group 2 retrospectively. There was significantly improvement in both groups (group 1 and 2 retrospectively, $7.6 \pm 0.9\%$ and $7.7 \pm 1.1\%$ on beginning with $6.3 \pm 0.8\%$ and $6.2 \pm 1.0\%$, $P < 0.05$) at the end of the study. Internet visits were more preferable by the patients.

Conclusion: This brief trial suggests that young diabetics prefer to make contact with their health care providers via internet, where new technologies using specific software like Carelink, Skype and Facebook can improve diabetes control same as regular clinic visits.

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INSULIN PUMP THERAPY USING CONTINUOUS GLUCOSE MONITORING IMPROVE PREGNANCY OUTCOME IN TYPE 1 DIABETICS

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Aim: The aim of the study is to evaluate glucose profiles and pregnancy outcome in type 1 diabetics using insulin pump with continuous glucose monitoring (CGM).

Methods: Twelve newly diagnosed pregnant women with type 1 diabetes mellitus were treated with insulin pump therapy (Medtronic 722) for 1 year. Insulin pump and CGM (Medtronic PRT) were implemented at least 3 months before conception and continuously during the pregnancy. The following parameters were analyzed HbAlc, daily insulin requirement IU/kg, lipid levels, blood pressure and renal function before and after the study. These parameters were correlated with foetal weight, APGAR score, duration of pregnancy.

Results: Significant improvement of HbA1c 7.78 ± 1.58 vs. $6.12 \pm 0.32\%$ ($P < 0.05$) was found before and the end of the study (last HbA1c before delivery). There were significant correlations between foetal weight and HbAlc ($r = -0.65$, $P < 0.05$), triglyceride levels ($r = -0.61$, $P < 0.01$) and cholesterol levels ($r = -0.65$, $P < 0{,}01$). The mean APGAR score was 8.91 ± 0.75.

Conclusion: The significant improvement of HbA1c as well the quality pregnancy outcome can be achieved with insulin pump therapy combined with CGM. The quality of glucose profile in the moment of conception was the important factor for pregnancy outcome.

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EFFICIENCY AND SAFETY OF TWO METHODS OF INSULIN THERAPY IN CHILDREN WITH DIABETES MELLITUS TYPE ONE

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Introduction: Diabetes mellitus is now uncurable. The number of newly-diagnosed children increases, the mean diagnostic age of these children decreases. There are different methods of therapy and therefore a need in comparison.

Aims: Our aim is to compere the level of T1D compensation in children on various schemes of insulin therapy.

Materials and methods: We examined 114 children. The first group were 60 children (age 7.5 ± 2.8yrs; T1D duration 1.2 ± 0.9yrs) on basis-bolus scheme. The second group were 54 children (age 10.8 ± 1.4yrs, T1D duration 1.7 ± 1.3yrs) on pump therapy. All children were hospitalized at our department and reached metabolic compensation. Duration of stay in hospital depended on the type of therapy: (7.8 ± 1.77days for the first group and 8.2 ± 1.1days for the second).

Results and discussion: Analysed parameters: HbA1c level, insulin dose per weight, microvascular complications. The need in insulin during first 24 months of T1D was significantly lower in "pump" group (0.41 ± 0.1 against 0.74 ± 0.018IU/kg at therapy start, 0.67 ± 0.1 against 1.64 ± 0.1IU/kg after one year). HbA1c level were significantly lower in the "pump" group: $6.9 \pm 0.03\%$ against $8.01 \pm 0.09\%$. There were no microvascular complications in both groups, but the "pump" group's metabolism parameters are better what makes the complications less probable. There were no severe DKA and hypoglycaemia episodes in both groups.

Conclusions: Pump therapy in children with T1D provides T1D compensation with lower doses of insulin and significantly lower HbA1c compared to other therapy methods. Pump therapy is effective and safe in children with

newly-diagnosed T1D and doesn't increase the time of first stay in hospital.

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AN EXPERIENCE OF PUMP INSULIN THERAPY IN CHILDREN UNDER 6 YEARS IN COMPARISON TO INJECTION PENS IN A NEWLY-DIAGNOSED T1D

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Introduction: The main idea behind T1D therapy is to achieve metabolic compensation for prevention of microvascular complications. The insulin pump is the only dosage system enabling individual programming for substitution therapy.

Aim: We aim at comparison of compensation state of T1D between children who use insulin pumps and those who use injection pens in bolus-basis therapy.

Materials and methods: We supervised 15 children (10 boys) with newly-diagnosed T1D, who were on pump therapy (Medtronic 712,722) and 16 children (6 boys) with newly-diagnosed T1D on bolus-basis therapy with injection pens. All children were 2–5 yrs.old. We have analysed their HbA1c at manifestation and 3, 6 and 12 month after, calculated the rate of this value, looked for echo-signs of hepathopathy, nephropaty and neuropathy after 12 month.

Results and discussion: In the "pump" group HbA1c decrease rate was 4.7% per year, while it was 3% per year in the second group. The incidence of hepathopathy in the first group was 0.3 and neurpathy - 0.2 cases/patient/year, while it was 0.6 and 3.7 cases/patient/year respectively. These values have significant differences. There were no nephropathy cases in both groups. There were no DKA or hypoglycaemia cases as well thanks to substantial awareness in parents of children from both groups.

Conclusions: Diagnostically-significant improvement in T1D compensation is evident in the group of small children on pump therapy against the group of the same age who are on injection-pen therapy.

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DIABETES ASSESSMENT SYSTEM—USING INFORMATION TECHNOLOGY TO BRIDGE THE GAP BETWEEN PRIMARY AND SPECIALIST DIABETES CARE

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Diabetes patients are commonly treated by primary health care providers (GPs) who often have limited experience in complex health problems and need specialist advice. Diabetes is relatively simple in early stage but can frequently be more complex due to interacting co-morbidities, multiple treatment targets and conflicting management priorities. We have developed algorithms embedded in a patient information management system (PIMS) used in our Diabetes Assessment Clinic (DAC) (chart to be included later) to bridge the gap between primary and specialist diabetes care.

This present study is to highlight the process of using algorithms to create feedback for GPs. The algorithms consider key patient features such as life style, renal function, risk factors for and actual complications, current management and treatment guidelines and generate comments/recommendations. For example, an EGFR < 30 mL/min would trigger recommendations about medication dosing for that individual with reference to HbA1c level. The algorithms are based on recommendations by Australian national authorities, but could be easily customised for recommendations applicable in other settings. The information management system could also be customised for use in multiple settings to support primary care providers. Data collection could occur in a variety of local or regional hospital or community centres.

Information technology incorporated into clinical practice can play a significant role in bridging the gap between the needs for specialist advice in primary care and the limited capacity of the specialist to deliver direct care. This approach also increases consistency with evidence-based expert recommendations between many individual primary healthcare providers.

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TREATMENT OF 242 PATIENTS WITH TYPE I, II, GESTATIONAL DIABETES WITH A INSULIN PREPARATION WHICH IS AVIDLY ABSORBED FROM THE AUDITORY CHANNEL: THE HAMLET/POLONIUS EFFECT

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Introduction: With the idea that molecules in the range of 4500 Dalton are readily absorbable from mucous membranes, we develop a novel Insulin preparation that can rapidly be absorbed from the external auditory channel.

Materials and methods: Different preparations were tested in phase Zero. Both radioactive and bioassays were performed. At phase I, 242 patients with either type I (22 patients), type II (190 patients) or gestational diabetes (30 patients) were enrolled to this trial. Previous hypoglycemic medications were discontinued 24 hours before the study. According to baseline BS 20–60 IU equivalents were instilled into the external auditory channel and the patients were let rest. BS was tested every 15 and later 30 minutes till 3.5 hours and serum Insulin were evaluated before and 2.5h after instillation.

Results: No patient developed local or systemic side effects. BS decreased substantially in 185 (87%) type I and II and all gestational patients and less pronounced in another 10 (4.7%) with an overall response rate of 92.7%. Response was correlated with type I and type II diabetes of shorter duration. Type II of longer duration, history of Insulin resistance, stress during study, generalized anxiety disorder and more grayish rather than pink channel were correlated with worse response. Almost all responding patients showed an absolute or relative increase of serum Insulin matched with BS. AI erased a faster response than IV Insulin in 35% of the examinees (within less than 5–15 minutes). For two months glycemic control became easier. Scintigraphy showed substantial systemic absorption.

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ECONSULTA: VIDEOCONSULTING FOR ENDOCRINOLOGY AND PRIMARY CARE COMMUNICATION IMPROVEMENT

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The main aim was to improve communication between general practitioners (GPs) and endocrinologist optimizing the resources and the quality of care regarding high prevalence pathologies such as diabetes and other metabolic disturbances. Thus, we have developed a new application for real-time communication between endocrinologist and GP's through low-cost videoconsulting, integrated into the current EHR. The videoconsulting agenda will occupy the final part of the morning, in such a way that the doctor can address its concerns about the patient on the same day they have arose. This application is based on a free videoconference software (Openmeetings) that has been complemented with additional functionalities such as virtual waiting rooms and agenda management. During the videoconference session, users can access the patient EHR and additional tools to share documents, to draw in a board and to interchange messages through a chat.

The eCONSULTA system has been integrated within the regional primary care information system, where users have to be previously authenticated. The eCONSULTA service is available in a restricted internet-based network that communicates the involved Health Care Centers. At the time of this abstract redaction, the pilot study on feasibility and users satisfaction is ongoing. Information regarding the number, complexity of the diagnoses derived to the hospital and mean waiting list are being registered to evaluate efficacy of the system.

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CARBOHYDRATE COUNTING WITH BOLUS CALCULATOR IN T1DM CHILDREN ON MDI THERAPY

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Background: Widespread use of CHO counting in T1DM children and adolescents on MDI therapy is limited.

Aim: To evaluate clinical and metabolic parameters in T1DM adolescents on MDI therapy in relation to the use of CHO counting with a bolus calculator (BC).

Subjects: 45 T1DM children (26M/19F, age 12.2 ± 3 yrs, duration of the disease 3.6 ± 2.2 yrs), were subdivided in 3 well matched groups and followed six months : Group A and Group B were educated to CHO Counting with and without BC respectively; Group C already using CHO counting was introduced to BC. At 3 months the group B was switched to the use of BC. Clinical parameters, HbA1C and glucose variability indexes (Mean $\pm$ SD, HBGI, LBGI) calculated from downloading glucose values were analyzed before (T0), during (T3) and at the end (T6) of the study.

Results: Group A and Group B were significantly different from Group C as concern HbA1C, Mean $\pm$ SD, HBGI, LBGI at T0. At the end of the study (T6) metabolic parameters of Group A and B patients were improved and no significant differences between the 3 groups were observed. Group B patients improved the metabolic parameters after 3 months of CHO counting without BC and a greater reduction of HbA1C, mean and HBGI and LBGI indexes after 3 months of BC use (T6) was found.

Conclusion: Adjustment of insulin doses according to CHO counting allowed the reduction of HbA1c and glucose variability. The bolus calculator represent a useful and ease-to use tool to further ameliorate the metabolic control.

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CLINICAL PATTERNS OF LOW BLOOD GLUCOSE IDENTIFIED BY A PATTERN ALGORITHM MAY PREDICT INCREASED RISK OF SEVERE HYPOGLYCEMIA IN THE FOLLOWING 24-HOUR PERIOD

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Introduction: Risk for hypo and hyperglycemia are significant clinical issues. Clinicians commonly use Blood Glucose (BG) pattern management to guide therapy to minimize hyper- and hypoglycemia.

Likelihood of Severe Hypoglycemia in the 24 hours after a Clinical Pattern of Low Blood Glucose (CPLBG)

Likelihood of severe hypoglycemia	BG Meter Trial	CGM Trial
At Baseline	9.0%	17.5%
Following Clinical Patterns of Low Blood Glucose (CPLBG)	23.3%	32.3%
Increase in likelihood of experiencing a severe hypoglycemia event within 24-hours after a CPLBG (compared to baseline)	260%	180%

Risk of severe hypoglycemia following CPLBG.

Objective: We evaluated the effectiveness of a pattern algorithm on the new LifeScan VerioPro meter in identifying Clinical Patterns of Low Blood Glucose (CPLBG) that may predict the risk of severe hypoglycemia.
Method: This retrospective analysis of 2 data sets of BG meter downloads assessed the frequency of severe hypoglycemia (BG < 50 mg/dL) occurring within 24-hours following a CPLBG (defined as recurrent low glucose values of BG < 70 mg/dL occurring on 2 independent days within the same 3-hour window and within 5 consecutive days**)**. The BG data sets analyzed were the entire meter downloads obtained in a published BG-meter trial (n = 208, type-1 and type-2 DM, age 13–78y, mean A1C 8.8%, followed for 4-months), and a published CGM-trial (n = 200, type-1 DM, age 8–17y, mean A1C 8.0%, followed for 6-months).
Results: Although the majority of days (78–82%) with severe hypoglycemia were not preceded by a CPLBG, the risk of a severe hypoglycemia (BG < 50 mg/dL) in the 24-hours following a CPLBG was double (180%–260%) compared to baseline.
Conclusion: In this analysis using BG meter downloads, CPLBG identified by a pattern algorithm were associated with subsequent risk of severe hypoglycemia. These findings suggest the potential of pattern algorithms to support patient self-management to prevent severe hypoglycemia which requires further clinical study.

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EFFECT OF HIGH DOSE THIAMINE ON THE LEVELS OF URINARY PROTEIN BIOMARKERS IN DIABETES MELLITUS TYPE 2

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The proteomics is known to be a valuable field of study and has become one of the most attractive sub-disciplines in clinical proteomics for human diseases. In the present research work, the levels of urinary protein biomarkers of diabetes mellitus type 2 using proteomic technology have been identified and characterized. Effect of high dose thiamine has also been observed on the levels of these marker proteins. Above 100 type 2 diabetic patients, and 50 same age and sex-matched normal healthy controls were recruited from the Sheikh Zayed Hospital, Lahore, Pakistan and 40 diabetic and 20 control has completed the trial. The urine samples from control and diabetic groups before or after thiamine therapy were further analysed and identified by 2-D liquid chromatographic system (HPLC) and mass spectrometry MALDI TOF/TOF and microTOF analysis. All the samples belonging to the control and diabetic groups were then analyzed by ELISA and estimated the levels of some proteins which were found to vary. In the urine samples, the levels of transthyretin, AMBP, haptoglobin precursor was found to be decrease while albumin, zinc α2 glycoprotein, RBP4 and E cadherin were found to be increase in the diabetic patients as compared to the controls. The level of albumin in the urine samples of diabetic patients only decreased by 34% after thiamine therapy as compared to the controls and the placebo, while other urinary protein markers did not show a significant change after the therapy. Assessment of the levels of these biomarkers will be helpful in the diagnosis and treatment of diabetes mellitus type 2.

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NOVEL FULLY IMPLANTABLE ARTIFICIAL PANCREAS WITH INSULIN REFILLING SYSTEM BASED ON SWALLOWABLE CAPSULES

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The ambitious goal to develop a closed-loop artificial pancreas relies on the integration of three elements: insulin delivery, continuous glucose sensing, and a control system regulating the proper amount of insulin delivery at the proper time. The usability and acceptability of a long-term implantable device for insulin delivery is currently limited by the need of periodic restore of insulin reservoir and battery charge. We propose a new fully implantable artificial pancreas with an insulin refilling system based on non-invasive sensorized "carrier" capsules, that can be swallowed by the patient in order to restore the insulin level in the implanted device reservoir, and with a wireless external battery charger. Such system, provided with an implanted glucose sensor and with a display, showing in real-time to the patient (and eventually to the medical doctor) the parameters characterizing the implanted device control, can open possibilities for a complete substitution of pancreas function in diabetic subjects, allowing them to have a normal daily life, including sport activities, without the need of keeping attention on transcutaneous or external devices.

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BIOAVAILABILITY OF METFORMIN GLYCINATE IN HEALTHY MEXICAN VOLUNTEERS: AN OPEN-LABEL, SINGLE-DOSE CLINICAL TRIAL

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Objective: This study was conducted to evaluate the bioavailability, safety and tolerability of metformin glycinate in healthy Mexican volunteers.

Methods: A phase I open-label clinical trial was conducted at a single location in Monterrey, México. Healthy volunteers were assigned to receive a single dose of 1,050.6 mg metformin glycinate (equivalent to 850 mg of base metformin) after a 12-hour overnight fast. For analysis of pharmacokinetic parameters, including C_{max}, t_{max}, AUC_{0t}, and $AUC_{0\infty}$, blood samples were obtained at regular intervals over a 48-hour period after administration. Tolerability and safety profiles were assessed using physical examinations, monitoring of vital signs, laboratory tests, and adverse event (AE) reports.

Results: A total of 6 women and 6 men were included in the study. All enrolled participants were Mexicans; mean [range] age was 22.91 [19 to 26] years, mean weight was 63.45 [51 to 68] kg, mean height was 164.66 [152 to 176] cm. A total of 11 participants completed the study. Mean (standard deviation or SD) C_{max} was 3,316.91 (578.05) ng/mL and mean (SD) T_{max} was 1.73 (0.97)

hours; mean (SD) $t_{1/2}$ value was 2.60 (0.42) hours. The arithmetic mean $AUC_{0\text{-}t}$ was 16,303.37(3,072.20) ng/mL*hour; and mean $AUC_{0\text{-}\infty}$17,241.15 (3,411.82) ng/mL*hour. Seven adverse events were reported in 4 subjects after drug administration; all were mild in severity and resolved spontaneously. There were no serious drug-related AEs and no deaths.

Conclusions: In this study of healthy adult Mexican volunteers, metformin glycinate was well tolerated and exhibited rapid absorption, reaching plasma concentrations after 1.73 hours.

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AUTOMATIC INITIALIZATION METHOD FOR CLOSE LOOP ALGORITHMS IN TYPE 1 DIABETES

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Background and aims: This work proposes an automatic initialization procedure to estimate the patient-dependent gain parameter in a metabolic semiclosed-loop controller. The controller has been previously assessed in simulation when the initialization was heuristically obtained by proof and error on each patient (Rodriguez-Herrero et al. 2010).

Methods: The proposed initialization method uses the total daily insulin (DIR_{OL}) and the continuous glucose data (g_{cgm}) obtained in a CSII therapy. The g_{cgm} is entered as input to the controller in order to obtain a closed-loop daily insulin (DIR_{CL}). A recursive equation calculates the gain parameter that reduces the difference between the semiclosed-loop insulin/glucose sensibility and the open-loop one. A simulation study has been performed in order to compare the heuristic initialization versus the automatic one in terms of metabolic control. The in-silico population is composed of six-patient (Hovorka et al. 2002).

Results: Table 1 summarizes the daily control outcomes for the population: insulin requirements, glucose average and standard deviation, maximun and minimum glucose, percentage of time below 120 mg/dL and the Kovatchev's risk index (BGRI). Data is presented as Average(Standard Deviation).

Conclusions: The initialization method is able to maintain all the patients controlled into the safety ranges. The method proposes least insulin than the heuristic initialization and consequently the glucose levels are slightly increased but the BGRI shows that the metabolic control is feasible.

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INDIAN MEDICINAL PLANTS *ANDERYTHRINA INDICA*, *GYMNEMA* AND *ANDROGRAPHIS* SPECIES—THE BEST CURE FOR DIABETES

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India is the treasure of medicinal plants. Most of the rural people depend upon plants to cure different diseases which direct us to do the research in medicinal plants. Diabetic mellitus was induced in adult wistar rats using the chemical compound streptozotocin which induces a type of diabetes which is similar to diabetes mellitus with non-ketosis hyperglycemia in animal species. The changes in MDA (lipid peroxidation) and glucose (by GOD method) levels in blood of both normal and diabetic rat were analyzed. Diabetes induced rats were treated with leaf extracts of *Gymnema sylvestre, Andrographis paniculata,* and bark extract of *Erytherina indica* which are of medicinal importance and possess anti diabetic and antioxidative property in order to control the glucose and MDA levels. The blood plasma of diabetic and normal rats was analyzed for the levels of MDA (lipid peroxidation) and glucose levels. Our experimental results indicated that *Gymnema sylvestre, Andrographis paniculata* and *Erytherina indica* as a dietary supplement, possesses antidiabetic effect with hyperglycemia as the major target. This suggest that *Gymnema sylvestre, Andrographis paniculata and Erytherina indica* can be used as a potential natural antidiabetic agent for treating and postponing the appearance of complications that arise due to diabetics. Further studies are in progress to isolate, identify and characterize the active principles.

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ANTI-INFLAMMATORY CYTOKINES, DEPRESSION AND TYPE 1 DIABETES

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Table 1.

Initialization	*Insulin (IU/day)*	*Average-Glucose (mg/dL)*	*Glucose-SD (mg/dL)*	*Max-glucose (mg/dL)*	*Min-glucose (mg/dL)*	*Time glucose 70 mg/dL (%)*	*Time above 140 mg/dL (%)*	*Kovatchev's index*
Heuristic	58 (45)	88 (2)	98 (7)	98 (7)	81 (3)	0	0	2.1 (0.2)
Automatic	55 (39)	100 (15)	112 (18)	112 (18)	86 (11)	0	0	1.4 (1.2)

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Introduction: Dowlati et al[1]. in a recent meta-analysis reported high levels of IL6 and TNFα in people with depression. Diabetes is associated with increased risk of psychiatric disorder, with depression being the only one that increases its prevalence in relation to general population. The aim of this study is to test whether these cytokines are elevated in patients with diabetes and depression.

Material and methods: We evaluated 37 patients with type 1 diabetes (14 M/23W), with the following characteristics: time of evolution of DM1 19 ± 10 years; HbA1c 7.6 ± 0.7%; and, type of intensive treatment: MDI (8.1%), CSII (70.3%), PRT®(Medtronic)(21.6%). Variables analyzed were:

1) Depression considering DSM-IV criteria, scale of depression in diabetes (EDDI) and Beck's inventory of depression (DI-II);
2) Anxiety (STAIE, STAIR);
3) Quality of life (DQOL); and,
4) Cytokines II-6 and TNFα (ELISA.Sigma USA. 1–400 pg/mL).

Results: For IL-6, patients with depression have significant higher values ($P < 0.05$) and these values also correlated significantly ($P < 0.05$) with scores of scales evaluating Depression (DSM-IV, r = 0.567; BDI-II, r = 0.623; and EDDI, r = 0.623) and Anxiety (STAIE, r = 0,527; STAIR, r = 0,6). Results regarding TNFα didn't reach statistical significance.

Conclusions: In our group of DM1 patients selected for the study, we can conclude that:

1) IL-6 values are increased in those with Depression;
2) IL-6 values also correlate significatively with Depression and Anxiety Scales.

Reference

[1] Dowlati et al. A meta-analysis of cytokines in Major Depression. Biol. Psychiatry 67:446–457, 2010.

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MATERNAL MORTALITY AND MORBIDITY IN OUR HOSPITAL

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Over 90% of maternal deaths occur in developing countries. The MMR is used as a measure of the quality of a health care system. Near miss cases occur more often than maternal death and may generate more information because the woman herself can be a source of data. Once severe maternal morbidity precedes maternal death, the systematic identification and the study of near miss cases may provide further understanding of the determinants of maternal mortality. The aim of this work was to study the incidence, causes and clinical characteristics of cases with maternal mortality and morbidity in El Shatby Maternity University Hospital and Gamel Abd El Naser Health Insurance Hospital in Alexandria city, Egypt. The study was conducted through a descriptive prospective approach, all pregnant women delivered in both hospitals for 6 months from 1/1/2009 to 30/6/2009 were followed to study cases of morbidity and mortality occurred in that period. Data collected from women delivered, doctors and mortality and morbidity sheets.

Results: The number of deliveries in El Shatby Hospital during the study period was 6237, number of mortality and morbidity was 2034 represent (32.6%), maternal mortality was 13 cases represents (0.2%) and maternal mortality ratio was 212 per 100 000 living birth. While in Gamel Hospital it was 812, the number of maternal morbidity and mortality was 158 represent (19.4%), and number of maternal mortality was only one case represents (0.1%) and maternal mortality ratio was 125 per 100 000 living birth.

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IMPROVEMENT OF CARE IN GESTATIONAL DIABETES MANAGEMENT BY TELEMEDICINE: A FRENCH EXPERIENCE

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Even without consensual approach, recent papers give us strong arguments to intensify screening and management of gestational diabetes.

Our experience using telemedicine in gestational diabetes management has been evaluated during a one-year period.

We demonstrate that our therapeutic education approach enhanced by a permanent electronic assistance, telemonotoring and e-consulting, offers very good results in a cohort of 113 patients.

Adequate therapeutic decisions, mainly insulin therapy initiation can be taken within a short time, needed in 44% of our patients. Continuous coaching and monitoring of insulin doses make it possible to achieve perfected glycaemic control quickly in 95%. Babies are born in excellent conditions for themselves and their mothers: with a low rate of caesarian: 20%; exceptionnal macrosomia (4%), no hypoglycemia neither neonatal respiratory distress.

Women are invited to answer an electronic questionnaire in postpartum. They expressed their total satisfaction for this practical and convivial approach. Reassured, their good psychological wellbeing seems to be reinforced.

Telemedicine in gestational diabetes management could be also a valuable educational tool and a good example of possible improvements of quality of cares in diabetology.

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RECRUITMENT AND BASELINE CHARACTERISTICS OF J-DOIT1 (JAPAN DIABETES OUTCOME TRIAL 1)

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Objective: J-DOIT1 is a cluster randomized clinical trial in high-risk individuals with type 2 diabetes designed to reduce the

development of T2DM by telephone-delivered lifestyle support in primary healthcare settings.

Research design and methods: Using a cluster-randomized design with health checkup divisions as the unit of randomization, this study evaluated telephone-delivered support for healthy eating and exercise habits targeting subjects with impaired fasting plasma glucose (FPG). Eligibility requirements were high-risk of developing type 2 diabetes (determined by annual health checkups) in individuals aged 20–65 years and with FPG levels (5.6–6.9 mmol/L).

Results: The 2847 participants recruited from 43 clusters were randomly assigned to an intervention group (n = 1338) or control group (n = 1509) between March 2007 and February 2008. Average age at entry was 49 ± 8 years, and 84.4% were male. Overall, BMI averaged 24.3 ± 3.1 kg/m^2 at baseline with 2.0% of subjects lean, 61.2% of subjects of normal weight, and 36.8% of subjects overweight or obese. There was no difference in age, sex, BMI, fasting or FPG levels, and the prevalence of syndrome according to the criteria of NCEP-ATPIII between the groups.

Conclusions: J-DOIT1 has successfully randomized a large cohort of participants of high-risk of developing T2DM with a wide distribution of age and BMI.

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TYPE 2 DIABETES: BAYESIAN NETWORK MODELLING OF GENETIC AND PHENOTYPIC TRAITS

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Type 2 diabetes is a heterogeneous disease arising from a complex interaction between genes and the environment. In an attempt to identify associations between genetic and phenotypic biomarkers, we designed a Bayesian Network inference procedure to identify probabilistic relations between genetic and phenotypic biomarkers.

Cortisol, c-peptide, growth hormone, insulin, glucagon and glucose were collected after an oral 75 g glucose challenge in 190 Caucasian patients. Genotyping of common genetic variants included 3 associated with T2D (*TCF7L2*, *KCNJ11* and *PPARG*) as well as rs6923761 in *GLP1R*, which is expressed in pancreatic beta-cells. Insulin action, beta-cells responsivity and disposition indices were calculated.

A Bayesian network framework was designed to identify probabilistic relations between the genotyped SNPs, Age, Sex, BMI, fasting and 120 minutes blood sugar and the 3 indices, modelled as random variables and linked together in a Bayesian Network. Continuous variables were quantized in tertiles, to model probability distributions with conditional probability tables. Uniform priors were chosen for phenotypic variables, whereas frequencies from the HapMap CEU populations were exploited as priors for the genetic biomarkers.

The most probable class of Bayesian Network structures given the data, in the form of a Partially Directed Acyclic Graph (PDAG), was identified with the complete Silander and Myllymäki algorithm and Bootstrap was exploited to estimate the confidence of the identified probabilistic relations.

Results suggest a probabilistic relation between *GLP1R* and both fasting blood sugar and beta-cell responsivity.

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CONTINUOUS SUBCUTANEOUS INSULIN INFUSION VERSUS MULTIPLE DAILY INJECTION THERAPY IN PREGNANT WOMEN WITH TYPE 1 DIABETES

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Background and aim: Tight glycemic control is very important in reducing the risk of pregnancy complications in women with type 1 diabetes. The aim of the study was to compare glycemic control and pregnancy outcome in women with type 1 DM with two treatment options: multiple daily insulin injections (MDI) and continuous subcutaneous infusion insulin therapy (CSII).

Patients and methods: We studied 26 pregnant women with type 1 DM. Eleven of them were on MDI and 15 on CSII. Body Mass Index, HbA1c at baseline (1st trimester) and at 3rd trimester, rate and severity of hypoglycemia, mean duration of pregnancy, newborn birth weight, neonatal hypoglycemia, incidence of large for gestational age (LGA) and small for gestational age (SGA) was recorded.

Results: There was no significant difference on all the parameters measured between the 2 groups except for neonatal hypoglycemia (1 in CSII group vs 5 in MDI, $P = 0.02$).

Conclusions: Both treatments (MDI and CSII) are effective in achieving glycemic control in pregnant women with type 1 diabetes, however neonatal hypoglycemias are less frequent with CSII.

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FACTORS RESPONSIBLE FOR POOR CONTROL OF TYPE 2 DIABETES MELLITUS—A SYSTEMATIC REVIEW AND META ANALYSIS

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Results

	Age (years)	*Diabetes Duration (years)*	*1st Trimester HBA1c (%)*	*3rd Trimester HBA1c (%)*	*Hypoglycemia (number)*	*Duration Pregnancy (weeks)*	*Newborn Weight (grams)*	*Neonatal Hypoglycemia (number)*
CSII	32 ± 4.84	15.2 ± 7	7.08 ± 1.08	5.86 ± 0.46	2.4 ± 0.91	37.67 ± 2.09	3855.33 ± 618.84	1
MDI	35 ± 4.8	15.82 ± 6	6.96 ± 0.88	6.1 ± 0.84	2.55 ± 1.13	37.45 ± 1.29	3744 ± 672	5
P	0.09	0.773	0.917	0.567	0.64	0.243	0.938	0.02

Objective: Find out the factors responsible for poor control of diabetes.

Design: Systematic review of Case control studies Data source PubMed, Lancet, New England Journal of Medicine, Diabetes care, Diabetes, International Journal of Diabetes, Indian Journal of Medical Research, Pro Quest Medical Library, Diabetes and metabolism, Diabetes educator, Bioline International Journal of Diabetes, Public Library of Science and Priory Medical Journal.

Review methods: Two reviewers independently extracted data. The outcome was Hemoglobin (HbA1c) level. Odds ratios with 95% confidence intervals and Mean difference with 95% confidence interval were pooled with a fixed effect model.

Results: Ten studies were included for meta analysis. Control of diabetes was poor among younger adults (<60 years) comparable to elders (Mantel- Haenszel Odds Ratio is 1.61 and 95% Confidence Interval is 1.11 to 2.33). There was a difference in mean BMI between poorly controlled and control led diabetics (standardized mean difference is 0.47 with 95% C.I is 0.38 to 0.55). Disease related factors, except medication compliance (Mantel- Haenszel Odds Ratio is 1.79, 95% 95% Confidence Interval is 0.59 to 5.40) were associated with poor control.

Conclusion: Age and BMI are the patient related factors of poor control of diabetes. Disease related factors of poor control are Coronary Heart Disease, neuropathy, retinopathy, renal failure and neurological disorders are the disease related factors of poor control. Adherence to diet and exercise are the treatment related factors of poor control of diabetes.

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THE FOOD, THE PICTURE, THE ANALYSIS—HOW CLOSE CAN WE GET? A (PILOT) STUDY

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Scope of the study is to present the ability to recognize automatically the different types of foods on a plate based on advanced image analysis methods. The presented work is part of a project aimed to recognize food intake and estimate the corresponding carbohydrates. It is expected that the proposed novel approach, running on smart phones, will support diabetic patient during carbohydrates counting with a more precise, easy, flexible and enjoyable way. Initially a dataset composed by more than 12,000 images of cooked foods collected from different web sources has been developed and divided into the following categories: pasta, pizza, salad, soup, rice, fish, meat, potatoes and mixed. Those images are used to design and evaluate a series of advanced computer vision based tools (Figure 1). At this stage the system is able to recognize automatically four different kinds of foods on a plate with accuracy in the order of 85% on images not used during the system's design phase. The proposed approach shows the feasibility of automatic food recognition based on computer vision techniques. The group is working towards the extension of the system to more categories and to the estimation of carbohydrate content of meals.

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FACTORS AFFECTING ADHERENCE TO CONTINUOUS GLUCOSE MONITORING IN TYPE 1 DIABETES

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Objective: To identify factors associated with persistent use of continuous glucose monitoring (CGM) in patients with type 1 diabetes.

Research design and methods: We recruited 22 patients with type 1 diabetes (aged 41 ± 12 years, 55% female, HbA1c $7.2 \pm 0.5\%$) followed at Hvidovre University Hospital, Denmark. All were treated with insulin pumps; 16 were also using CGM and 6 were former users of CGM. We conducted focus group interviews to gain detailed understanding of factors important to type 1 diabetic patients concerning sensor use. Additionally, to identify personality traits predictive of adherence to CGM, participants completed a personality inventory (NEO-PI-R).

Results: Focus group analysis showed that sensor accuracy and sensor size and body image were important to most CGM users. Current and former users of CGM differed regarding

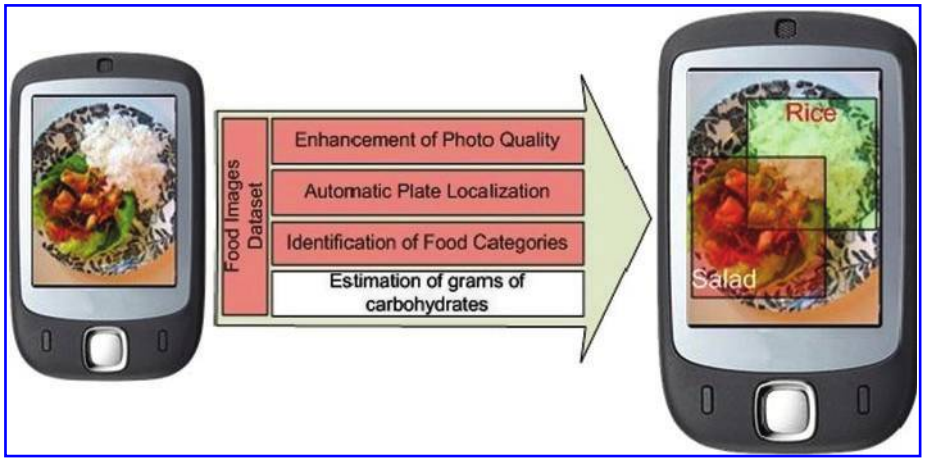

FIG. 1.

coping with alarms and ambitions of metabolic control. Logistic regression analysis of the NEO-PI-R scores identified three personality traits predictive of persistent use of CGM: Increasing scores on the scales "Angry hostility", "Achievement striving", and "Ideas" increased the probability of continuous sensor use. The sensitivity and specificity of the regression model were 81% and 83%, respectively (80% threshold).

Conclusions: This combined qualitative and quantitative study strongly suggests that personality traits influence adherence to CGM in patients with type 1 diabetes; additionally, it underscores the importance of improving sensor accuracy and reducing size in future devices.

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CLINICAL STUDY DESIGN: COLLECTION OF INFORMATION-RICH TYPE 1 DIABETES DATA

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Background: The aim of this clinical study was to obtain type 1 diabetes data to identify models for glucose prediction.

Design considerations: The study design is factorial with three daily life factors that influence blood glucose (BG) concentrations: food intake, insulin boluses and exercise. Each factor has two levels. To identify the BG response to each system input, we separated them temporally.

Each subject participated on two days, allowing testing of the performance of the model within each subject during different experimental conditions and quantification of intrasubject variability.

Methods: The study included 12 persons with type 1 diabetes using insulin pumps with optimal basal insulin and bolus guide settings. During the study, subjects also wore a continuous glucose monitor and an Actiheart to measure heart rate and activity.

We devised 24 unique sequences of events. Subjects were randomly assigned to complete two sequences on two different days. On each admission the subjects arrived fasted and received a meal with no or half meal bolus. Then they underwent exercise (mild or moderate) or received an insulin bolus (small or large). This was followed by exercise (mild or moderate), an insulin bolus (small or large) or a snack. There were no repeat events on the same day.

We took 10-min BG measurements throughout the experiment. Blood samples for insulin and glucagon analysis were drawn every 10 minutes for the first 30 minutes after an event, then every 30 minutes.

Results: Results will be presented at the meeting.

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EMPHASIZING BETTER SELF-CARE AND PATIENT ADHERENCE WITH CELL PHONE VIDEOS

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Conventionally health education is rendered through lectures/Literature, which is often ineffective because different diabetes patients need different education. We at L.K.Diabetes Centre, Indiranagar, Lucknow produce different set of films. We have classified films based on type, duration and complication of diabetes. We display them in the waiting room of our diabetes centre/during diabetes camps and distribute amongst doctors through diabetes associations. We make these films using mobile phones (Mobi-films) so that we may forward these education films as MMS (Multimedia Messaging Service) from one mobile phone to another. Doctors can transfer the education videos onto a DVD/memory stick and also upload them to the Internet. To make these films one needs a basic mobile phone/digital camera to take pictures/videos/audio. Then shift these to computer software Windows Movie Maker (available free in all computers using Windows XP). Patients should be involved in script writing and acting. Then align the pictures/videos/audio in the timeline of the software. Basic editing and background music should be added to add some spice. Cheers! A health care film is ready. I represented India in a contest called Mobile Film Makers 2006 organized by Discovery TV Channel and NOKIA and my film on diabetic foot care were selected in top 14 from Asia-Pacific region. This method of diabetes education is called Samadhan (solution) System of Diabetes Education (SSDE). SSDE has been selected for faculty lecture at ADA 2010 and IDF 2011 and shall be published in IDF Diabetes Voice October 2010.

Reference

http://indiadiabetescare.blogspot.com/ and http://www.youtube.com/watch?v = Z0k0ZIdKJoA

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EFFICACY OF ENERGY RESTRICTED DIET IN PATIENTS WITH TYPE 2 DIABETES: INFLUENCE ON CARDIOVASCULAR RISK FACTORS

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Introduction: The objective of this study was to estimate effect of energy restricted diet on risk factors of cardiovascular disease (CVD) in patients with type 2 diabetes.

Methods: One hundred twenty-six patients with type 2 diabetes (mean age 55.5 ± 1.8 years, BMI $35.0 \pm 1.5\,kg/m^2$) have included in study. Diabetes in ninety-five subjects was controlled by oral hypoglycemic agents and in thirty-one by diet alone. Mean fasting serum glucose was $10.1 \pm 1.0\,mmol\ L^{-1}$ at the beginning of the study. Eighty-two subjects had hypertension, thirty-three had coronary heart disease and seventy-eight had dyslipidemia. Patients of main group were received a moderately energy restricted diet (1500 kcal/day). Patients of control group were received a diet with caloric value of 2000 kcal. Fasting blood glucose, serum lipids, blood pressure, body weight before and after 3-week dietary management are measured.

Results: The study has shown that in main group and control after nutrition therapy the mean fasting glucose in blood reduced on 33% and 24% from initial value respectively. The systolic and diastolic blood pressure after moderately caloric restriction reduced on 26 and 18 mm Hg (on 12 and 7 mm Hg in the control) respectively. Body weight loss was been 7.1 kg in the energy restricted diet group and 4.5 kg in the control group. The effects of the both diets on serum lipids were comparable.

Conclusion: Moderate caloric restriction makes it possible to correct the risk factors of CVD in type 2 diabetes such as hyperglycemia, hypertension, and obesity.

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THE ROLE OF MEDICAL NUTRITION THERAPY IN THE COMPLEX TREATMENT OF TYPE 2 DIABETES

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Introduction: The objective was to estimate of the efficacy of a diet therapy with the specialized Glucerna SR product in the complex treatment of patients with type 2 diabetes.

Methods: A total of 102 patients (BMI $38.5 \pm 1.2\,kg/m^2$) and receiving standard glucose-lowering therapy were enrolled. Efficacy of the low-calorie Glucerna SR-including diet (1600 kcal/day) was assessed in two similar groups: main group patients ($n = 33$) received diet including Glucerna SR (230 mL as a meal substitute for second breakfast) over 3 months; control group patients ($n = 35$) were administered a standard low-calorie diet. Fasting blood glucose, serum lipids, blood pressure, body weight, and body composition parameters were evaluated in the three-week hospital stay period and after two months of ambulatory follow-up.

Results: The mean fasting glucose in the main group had reduced by three weeks from 8.3 ± 0.5 to 6.1 ± 0.3 mmol/L ($P < 0.001$) and remained within the target range by the end of the third month of observation. The mean $HbA1_C$ at 3 months of treatment decreased from 7.2 ± 0.2 to $6.1 \pm 0.2\%$ ($P < 0.0001$). In the control group, $HbA1_C$ changes were insignificant. Main group patients had more pronounced reductions in the total cholesterol, low-density lipoprotein cholesterol, and triglycerides concentrations at 3 months of treatment compared with control group. The reductions in blood pressure, BMI, and body fat content were greater in main group patients undergoing ambulatory follow-up.

Conclusion: The inclusion of the specialized product Glucerna SR in the standard diet therapy helps to improve the efficacy of complex treatment in patients with type 2 diabetes.

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EVALUATION OF ANTIDIABETIC AND ANTIOXIDANT EFFECTS OF *SEABUCKTHORN* (*HIPPOPHAE RHAMNOIDES L.)* IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE-2 DIABETIC RATS

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The present investigation was undertaken to evaluate the antidiabetic and antioxidant effect of *Seabuckthorn (Hippophae rhamnoides L.)* in streptozotocin-nicotinamide induced type-2 diabetic rats. Experimental diabetes was induced by single intraperitonial injection of streptozotocin (60 mg/kg), 15 minutes after i.p. administration of 120 mg/kg nicotinamide. *Seabuckthorn* was orally administered to normal and diabetic rats for a period of 21 days. Fasting plasma glucose, glycosylated haemoglobin, thiobarbituric acid reactive substances and tissue glutathione in pancreas were estimated following the established procedures to assess the pancreatic function. Biochemical observations were supplemented with histological examination of pancreatic tissue. Fasting plasma glucose, glycosylated haemoglobin, TBARS were significantly ($P < 0.05$) increased, whereas insulin, total haemoglobin, protein levels and glutathione were decreased significantly ($P < 0.05$) in diabetic rats. Oral administration of *Seabuckthorn* to diabetic rats significantly decreased ($P < 0.05$) fasting plasma glucose, glycosylated haemoglobin and increased insulin, haemoglobin and protein levels. Administration of *Seabuckthorn* also decreased TBARS and increased the non- enzymatic antioxidants significantly ($P < 0.05$). An oral glucose tolerance test was also performed in which we found a significant improvement in glucose tolerance in rats treated with *Seabuckthorn*. Treatment of normal rats with *Seabuckthorn* did not significantly *($P < 0.05$)* alter any of the parameters studied. Degenerative changes of pancreatic beta cells in STZ- diabetic rats were minimized to near normal morphology by administration of *Seabuckthorn* as evident by histological examination. The results of the study indicate the role of oxidative stress in the induction of diabetes and suggest a protective effect of *Seabuckthorn* in this animal model.

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REOCCURRENCE OF PERIODONTAL DISEASE IN DIABETES PATIENT

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Introduction: Periodontitis has been identified as a possible risk factor for metabolic control in subjects with diabetes. Periodontal disease involves soft and hard tissue of mouth. Sign of periodontal disease involve color changes in soft tissue; swelling and bleeding gums, bad smell, pathological pocket as well as loss of tooth function develops mobility of tooth. Uncontrolled diabetes may causes oral mucosa cheilosis, drying and cracking, burning sensation, decrease in salivary flow alteration in flora of oral cavity.

Patients and method: Total 235 patients with periodontal disease were studied for two years out of it 121 were diabetic. 154 were male and 81 were female.23 patient were not turn up for study. Patients were divided in three age groups. 25–40 Years of age group 40–55Years of age group and more than 55 Years.

Result: The results of this study showed that the sex showed no significant difference between the two groups (diabetic and non diabetic patient). There is no difference in reoccurrence of periodontal disease after treatment in male and female. In age group of 25–40 years reoccurrence of periodontal disease in diabetic patient is 35.6%in comparison to non diabetic patient where it is 18.6% whereas in age group of 40–55 it is 38.6% in diabetic patient. In third group reoccurrence of periodontal disease in diabetic patient is 41.6% with more severity.

Conclusion: In diabetic patient periodontal disease reoccurred significantly ($P \leq 0.05$) as compared to the controlled group (non diabetic patient) with high severity.

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NONINVASIVE METHOD FOR BLOOD GLUCOSE LEVEL ESTIMATION BY SALIVA

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Aims: Diagnostic devices are available in the market to measure the blood glucose level. However in all available products blood is taken as diagnostic body fluid. So necessacity arises to find some non-invasive diagnostic mean to measure body glucose level frequently without any discomfort to the patient. Hence, the present study aimed at estimation of blood and salivary glucose level in diabetic and non diabetic subjects.

Methods: Twenty diabetic and twenty non diabetic subjects were randomly selected for this study. A detailed history of each patient was obtained regarding the age, sex, duration of diabetes, associated risk factors, family history and any associated illness. The quantitative estimation of the blood and saliva glucose level were performed by glucose oxidase method, using enzymatic kits (GOD-POD).

Results: A correlation was observed between fasting saliva glucose level (SGL) and fasting blood glucose level (BGL) of diabetic as well as non diabetic subjects. The correlation coefficient of non diabetic and diabetic subjects were + 0.84 and +0.34 respectively. These values of correlation coefficient proved the correlation of fasting saliva glucose and fasting blood glucose values statistically.

Conclusion: Values observed regarding blood and saliva glucose level were found distinctly difference between normal subjects and diabetic subjects suggesting that monitoring of saliva glucose level can be used as an index of diabetes mellitus.

Keywords: Noninvasive, saliva glucose, blood glucose and diabetic mellitus.

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AN OPTIC FILTER TO PREVENT THE DEVELOPMENT OF DIABETEC RETINOPATHY AND CATARACT

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Rationale: The fact that retinas of diabetic patients are affected more than other tissues of the brain raises the possibility that light is an important factor in the pathogenesis of this process.

Aim: To evaluate the effect of filtering some "harmful" light wavelengths on the development of ocular damage in diabetic rats.

Methods: Diabetes was induced in rats by intra-peritoneal injection of streptozotocin. Ocular damage was represented by cataract development, electroretinogram (ERG) response and expression of glial fibrillary acidic protein (GFAP) in retinas. The expression of GFAP in Muller cells was used as a marker of retinal damage. Rats were kept under 12h light/12h dark conditions and were divided into 3 deferent light-conditions: unfiltered fluorescent light, filtered light −100 luks, filtered light −50 luks.

Results: The ERG responses of the rats kept under unfiltered-light condition were reduced in amplitude compared to the responses recorded from the rats kept under filtered light condition (both diabetic and control); b-wave was more affected than a-wave. ERG responses didn't differ in the filtered light groups (50 vs. 100 luks). In rats kept under filtered light conditions GFAP was demonstrated only in astrocytes, while in rats kept under unfiltered light condition it was also detected in Muller cells. Cataract was detected only in diabetic rats; rats under unfiltered light demonstrated significantly higher incidence of cataract.

Conclusion: The observations indicate that in diabetic rats, filtering "dangerous" wavelengths keep functional integrity of retinal cells and protects against cataract formation.

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DUAL ENERGY X-RAY ABSORPTIOMETRY ASSESSMENT OF FAT MASS DISTRIBUTION IN TYPE 2 DIABETES MELLITUS WOMEN

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Background and aims: The detection of the links between topographic features of adipose tissue and the various parameters of metabolic syndrome in type 2 diabetes mellitus (DM) is important for the prevention of macrovascular complications. The aim of the study was to assess the features of fat mass distribution in premenopausal and postmenopausal type 2 DM women in comparison with nondiabetic women.

Materials and methods: 205 women with type 2 DM (176 postmenopausal and 29 premenopausal women) and 70 matched controls were examined. The research involved anthropometry of patients, general clinic examination, dual energy X-ray absorptiometry (Body composition program). Fat mass distribution research was based on Total Body, Android, A/G Ratio, Trunk/Total, (Arms + Legs)/Total parameters.

Results: Fat mass distribution parameters in type 2 DM women and controls were: Total Body: $32.11 \pm 8.43\%$ vs $29.99 \pm 4.13\%$, $P < 0.01$; Android: $44.92 \pm 8.09\%$ vs $40.75 \pm 4.04\%$, $P < 0.01$; A/G Ratio: 1.14 ± 0.21 vs 1.00 ± 0.14, $P < 0.01$; Trunk/Total: 0.59 ± 0.09 vs 0.53 ± 0.04, $P < 0.01$; (Arms + Legs)/Total: 0.68 ± 0.21 vs 0.85 ± 0.18, $P < 0.01$. The android component of adipose tissue was examined in type 2 DM women depending on menopause. It was found that android component in premenopause as well as in postmenopause was statistically higher ($P < 0.05$) among women with type 2 DM (premenopausal: 45.89 ± 6.04 vs $38.83 \pm 6.57\%$; postmenopausal: 44.96 ± 6.78 vs $42.78 \pm 4.98\%$).

Conclusion: The results confirm the prevalence of central (android) distribution of body fat among women with type 2 DM. Type 2 DM women are more likely to have significant visceral fat masses at earlier age than nondiabetic women.

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METABOLIC CONTROL OF YOUTH WHO DISCONTINUED INSULIN PUMP TREATMENT

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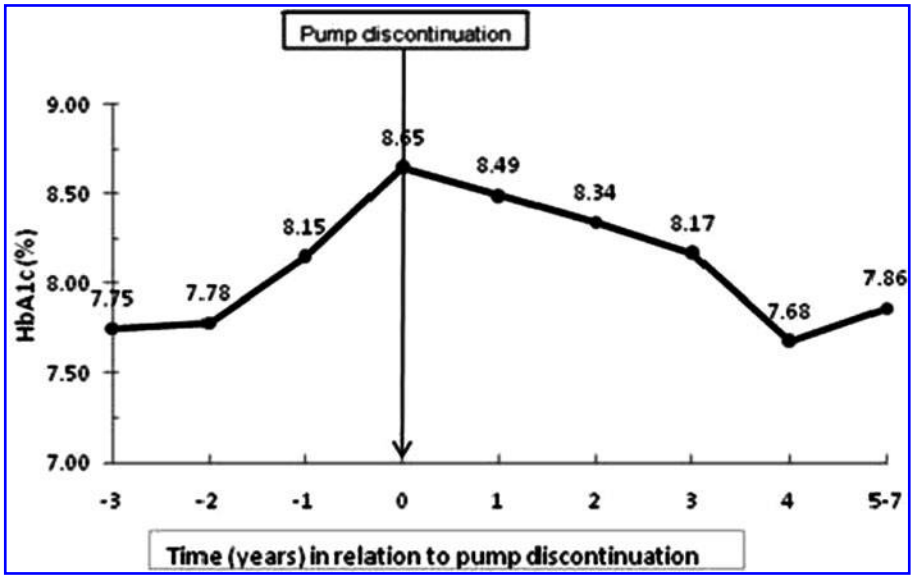
Objective: To study the metabolic control of youth with type 1 diabetes (T1DM) who discontinued insulin pump treatment and switched back to intensive treatment with multiple daily injections (MDI).



HbA1c levels in relation to pump discontinuation.

Subjects and methods: In our practice the discontinuation rate of insulin pump therapy is, in general, low (10%). In a retrospective study we collected data from 20 patients who switched from insulin pump treatment to MDI. Only patients who used the pump for at least one year (14) were included in the analysis. Their mean age at diagnosis of T1DM was 9.4 ± 4.1 years, 71% were females. The mean duration between diagnosis and insulin pump initiation was 3.5 ± 2.7 years, and mean duration on pump therapy was 3.2 ± 2.1 years. Mean age at pump discontinuation was 16.1 ± 3.2. Mean follow up after pump discontinuation was 2.9 ± 2.2 years.

Results: While on pump treatment there was a significant increase in HbA1c levels with time ($P < 0.0003$). [Figure 1]. After pump discontinuation there was a significant improvement in HbA1c level (unadjusted P for trend 0.04) over the years.

Conclusions: Girls stopped insulin pump treatment more often than boys. Pump treatment discontinuation is not necessarily bad, and some of the patients may achieve better metabolic control with MDI.

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A SMART CAST: AN ADAPTIVE, EFFICACIOUS PNEUMATIC CAST FOR HEALING OF DIABETIC PERIPHERAL NEUROPATHIC ULCERS

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An actively controlled walking cast has been designed for the specific needs of diabetic peripheral neuropathy patients. The cast is intended to aid healing of diabetic foot ulcers by relieving pressure at the location of an existing ulcer and distributing it to other areas of the lower leg. This is accomplished by a pneumatic system which maintains set pressure in multiple air bladders within the cast. The cast can respond to changes in bladder pressure due to muscle atrophy, swelling, and other external disturbances. The self-adjusting capabilities of the cast allow for treatment of ulcers using a method similar to current treatment options but with the ability to maintain the pressure distribution set by the physician with great precision. It is hypothesized that perfectly maintaining the desired off-loading from the location of existing ulcers will lead to more effective healing. Additionally, this electronically controlled adaptive cast can be used in conjunction with other technologies patented by Engineering Medix that will detect the onset of new ulcers and respond to prevent formation of additional wounds.

The pneumatic system is controlled with a PID. This controller can detect small changes in the air pressure of the bladders and bring the pressure back to the nominal value expeditiously without overshoot through use of air pump system. Bench top tests were conducted to characterize each component in the system and optimize the configuration with a mathematical model. Measurements of the behavior of the system correlate very well to predictions of the analytical model.

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IMPROVEMENT IN GLYCEMIC CONTROL FOLLOWING SWITCH OVER TO CONTINUOUS SUBCUTANEOUS INSULIN INFUSION FROM MULTIPLE DAILY INJECTIONS IN DIABETES IN PREGNANCY

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Objective: The prevalence of diabetes in pregnancy has continued to increase especially in ethnically pre-disposed populations. Management of diabetes in pregnancy requires multiple daily insulin injections with repeated monitoring of blood glucose levels. We present the data of 4 pregnant diabetic females [2 gestational diabetes (GDM), 1 type 2 diabetes (T2DM) and 1 type 1 diabetes (T1DM)] with poor glycemic control despite multiple injections of subcutaneous insulin who were initiated on continuous subcutaneous insulin infusion (CSII).

Research design and methods: All the 4 females were referral patients with duration of gestation of 12 weeks [T1DM (1st pregnancy)], 18 weeks [T2DM (2nd pregnancy)] and 18 (2nd pregnancy, 1st was also GDM) and 22 (1st pregnancy) in GDM females. After detailed counseling by the diabetes team, they were initiated on CSII. All the participants were encouraged to maintain the glucose readings in diaries by self monitoring of blood glucose and to be in touch with the study team as and when required.

Results:

Parameters	*Pre-pump*	*Post pump*	P
Premeal blood sugars (mg%)	123 ± 6	91 ± 4	<0.001
2 hr. postmeal blood sugars (mg%)	141 ± 8	116 ± 5	<0.001
Insulin dose (IU)	64 ± 12 (82 ± 16: T1DM)	44 ± 8 (68 ± 12: T1DM)	<0.001
Hypoglycemic events	No record available	2 in GDM, 3 in T2DM, 6 in T1DM	-

All 4 pregnancies were uneventful with succesful outcomes. The babies were of normal weight and there were no episodes of neonatal hypoglycemia.

Conclusions: CSII therapy seems to be safe, effective and convenient for maintaining glycemic control in pregnancies.

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LYS49-PHOSPHOLIPASE A_2 (K49-PLA_2) HOMOLOGUE IN HYPOGLYCEMIANT FRACTIONS FROM THE VENOM OF THE SNAKE *BOTHROPS JARARACA*

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The pharmacological potential of lizard's venoms was demonstrated in the case of *Heloderma suspectum*. This species contains the antidiabetic peptide exendin-4 (EX-4), a receptor agonist of GLP-1 (GLP-1R). Based on the hypothesis of molecular phylogenetic proximity of snake and lizard venoms, the search of EX-4-like peptides in *Bothrops jararaca* venom (vBj) is under advanced stage of investigation in our laboratory. This study describes a new phospholipase in vBj detected during this search. Briefly, the acidified supernatant of vBj was chromatographed in Sephadex® G-50 and the fractions eluted in the molecular weight range between 1.5 kDa-30 kDa were pooled and submitted to a gradient in RP-HPLC (ODS). The individual peaks eluted around the retention time of EX-4 were positive in anti-EX-4 EIA. The pool of these peaks lowered the glycemia in oral glucose tolerance test (OGTT) and presented the ability to bind to GLP-1R. These individual peaks rechromatographed in a shorter gradient in RP-HPLC resolved five peaks (P1-P5). The mass spectrometry peptide mass fingerprint of P1 identified the K49-phospholipase A_2 (K49-PLA_2). Since it is known that the catalytic mechanism of PLA_2 involves the binding of Ca^{2+} to Asp49, and that the substitution of Asp49Lys reduces the enzyme activity but preserves the activity on insulin release, the K49-PLA_2 of vBj could be a hypoglycemiant factor in OGTT. However, the existence of EX-4-like cannot be discarded in vBj, since antigenic and biological activities similar to EX-4 were observed in P2-P5, whose identifications are in progress.

Supported by FAPESP and CNPq.

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CLINICAL EVALUATION OF FIRST CALIBRATION TIME OF A CONTINUOUS GLUCOSE MONITOR IN DEVELOPMENT

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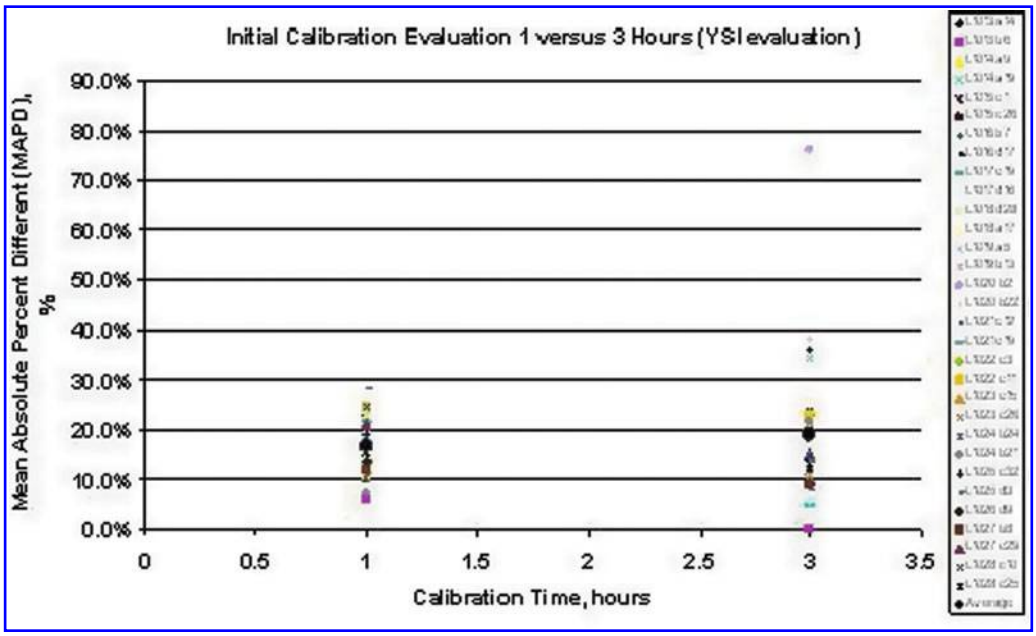

FIG. 1. Initial Calibration Evaluation.

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Objective: To determine accuracy of two initial calibration times, 1 and 3 hours, of a continuous glucose monitor (CGM) in development.

Methods: 16 subjects, at least 18 years of age, with type 1 or type 2 diabetes for at least 6 months were studied for one day in-clinic. 2 CGM sensors were inserted by the subject into the abdomen (with health care professional oversight) for evaluation of calibration at 1 hour and 3 hours. CGM Sensor glucose was compared to reference plasma (Yellow Springs Instrument, YSI, Yellow Springs, OH) obtained every 15 minutes. Sensors were removed at the end of the day. Subjects were called 2 to 3 days after sensor removal to assess any adverse events.

Results: Of the 32 sensors, 31 were able to calibrate at 1 hour and 30 at 3 hours. Mean absolute percent difference (MAPD) was computed for sensor-reference pairs (934 at 1 hour and 679 at 3 hours). MAPD was 16.7% (SE 1%) and 19.2% (SE 2.4%) at 1 and 3 hours, respectively. See Figure 1 below.

Conclusion: Mean absolute percent difference was similar at 1 hour and 3 hours in a CGM system in development. There were no device related adverse events.

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SKIN TEMPERATURE LOW AMPLITUDE OSCILLATIONS OF PATIENTS WITH DIABETES TYPE 2 DURING THE CONTRALATERAL COLD TEST

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The aim of the work is to compare the changes of the vessels' tone because of endothelial, neurogenic and miogenic mechanisms during the contralateral cold test in groups of healthy people and in type 2 diabetes patients.

Vessels' tone, which is determined by endothelial, neurogenic and miogenic mechanisms, influences on speed of a blood flow and cause the skin temperature oscillations. For all mechanisms different frequency ranges of oscillation corresponds. That's why we can use the wavelet analysis of temperature fluctuations for the research of dynamics of the vessels' tone in various ranges of frequencies.

Two groups of health people young 55 (mean $\pm$ m age 23 ± 8 years) and old 14 (56 ± 1 years) and 35 diabetic (60 ± 1) were investigated. The temperature of the right hand forefinger distal phalanx was registered 10 minutes before, 3 minute during and 10 minutes after cold test, with the following wavelet analysis of the signal.

We received the following:

- For healthy people the amplitude of skin temperature fluctuations during the indirect cold test significantly decreased and after cold test it considerably increased.
- In the group of diabetes the amplitude of skin temperature fluctuations in range of frequencies which corresponds to endothelial mechanism decreases during the cold test, but don't restore in 10 minutes after.

Finally we can conclude, that absence of similar changes in skin temperature oscillations for diabetes patients indicates the abnormalities of vascular tone regulation mechanism, especially in endothelial range.

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THERAPEUTIC POTENTIAL OF *ALOE VERA* LEAF EXTRACT ON LIPID PROFILE STATUS IN STREPTOZOTOCIN INDUCED DIABETES MALE ALBINO RATS

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Diabetes is a major Socio-economical burden with serious health consequences. The reactive oxygen species generated in this pathology alters the internal milieu of cellular system paving way to metabolic disorders. *Aloe vera* is a well known in herbal medicine which also called as a "Miracle Medicinal Plant". In the work present study the work was under taken to evaluate the potential antihyper lipidaemic effect of aloe vera leaf extract in liver and kidney tissues of Streptozotocin induced diabetes rats. The rats were divided in to five groups. Group-I (control rats), Group-II (control + *Aloe vera*), Group-III (Diabetic rats), Group-IV (Diabetic + *Aloe vera* extract) and Group-V (Diabetic + Glibenclamide). Oral administration of *Aloe vera* leaf extract (300 mg/kg BW/day) to normal and Streptozotocin induced diabetes for a period of 4 weeks has been conducted, the liver and Kidney tissues were isolated for biochemical analysis after completion of the dosage period. The levels of total lipids, triglycerides and total cholesterol in liver and Kidney tissues were increased significantly in Group-III (Diabetic rats). In case of Group-IV (Diabetic + *Aloe vera* leaf extract) to prevent the development and alteration in lipid profile status in liver and Kidney tissue maintained near normal while comparing with Group-V (Diabetic + Glibenclamide) there is a slight decrease is observed. Hence maximum prevention was observed in Group-IV than the reference drug. Therefore further studies are warranted to isolate and characterize the bioactive Antidiabetic Principles from *Aloe vera* Plant.

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ANALYSIS OF FACTORS THAT DETERMINE THE USAGE OF MODERN INSULIN PUMPS DEVICES

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To study the usage of modern insulin pump devices 114 type 1 diabetes patients treated by CSII were invited to fill an anonymous questionnaire.

Patients and methods: 65 females/49males, median of age 38 years (age range 10–76 years) Median of diabetes duration was 17 years (range 3–61 years), median of CSII treatment 6 years (range

1 month - 20 years). Questions were divided into different topics related to CSII treatment with the special focus on the use of most modern pump functions as well as to eating habits, sport and life quality evaluation. For the evaluation of life quality patients used 0 to 10 scale. The data were processed by statistical software SPSS v.17 and detailed correlation analysis was done.

Results: The median of $HbA1_C$ was 6.5% according to IFCC with the range 3.5–15%.

The real application of modern pump functions was lower than the knowledge on them (e.g. knowledge on square bolus 100%, usage 57%).

The usage of modern insulin pump functions was strongly influenced by individual characteristics as age, sex, education, diabetes duration ($P < 0.01$) as well as by the subjective evaluation of previous medical education and by the personality of the educator. Median of "life quality evaluation" was 7 but the CSII therapy was evaluated by patients as "advantageous".

The biggest impact on life quality had "the eating freedom".

Conclusion: The usage of the most modern insulin pump devices is still unsatisfactory and repeated education and motivation is the crucial to improve patient's diabetes stabilisation.

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FIRST CLINICAL EXPERIENCE USING AN ON-LINE GLUCOSE PREDICTION ALGORITHM FOR INTERPRANDIAL OPTIMISATION IN TYPE 1 DIABETES (DM 1)

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The aim of our study was to test the effectiveness and security of an on-line artificial neural network (ANN) algorithm for glucose prediction (30 min) from RT-CGM applied to correct interprandial hyperglycaemia.

Materials and methods: Twelve DM1 patients treated with insulin pumps were included in this randomized cross-over clinical study. In both phases, which lasted 6 days each one, patients tested capillary glucose in mid-afternoon and took an initial decision (no action, insulin bolus, carbohydrates intake). At that time, patients used the predictor running in a PDA, with the last 5 RT-CGM values as entries. During the experimental phase (EP), patients were allowed to modify the initial decision after knowing the prediction. A telemedicine application for the PDA was made to facilitate patients to register values and decisions and for sending data to the diabetes centre for supervision. At the end of the study, data were checked directly from pumps, meters and glucose monitors. The study period was defined as the time comprised between the initial decision and the pre-dinner glucose analysis.

Results: Patients used the prediction algorithm following the protocol in all the cases. However, only 7 patients were finally evaluable -mistakes on the introduction of CGM data-. Among them, only 2 did not obtain a higher improvement in the glucose profile during the EP. The mean blood glucose risk index reduction was also higher during the EP. No patient presented hypoglycaemia.

Conclusion: An on-line prediction algorithm based on ANN seems to help patients to better correct interprandial hyperglycaemia.

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NOCTURNAL HYPOGLYCEMIC EPISODES DETECTED WITH THE CGMS IN ADOLESCENT PATIENTS WITH TYPE 1 DIABETES MELLITUS

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It is very difficult to assess good self-control (normoglycemia) in adolescent patients and glycemic alternations in routine glycemic profiles with measurements every 3 hours could not be detected. CGMS monitoring glucose levels every 5 minutes give the chance to achieve it.

Material and methods: Our observations conducted first time in Poland from 2002 year by means of the MiniMed Continuous Glucose Monitoring System (CGMS) concerned 62 adolescent patients (48 girls and 14 boys), 13–18 years old with poor controlled type 1 diabetes (av. $HbA_1c - 10.74\%$, fructosamine - 471 mmol/L). CGMS monitor glucose levels in the range of $40 - 400$ mg/dL. CGMS was used in each patient up to 3 days. The glucose sensor was injected subcutaneously in abdomen region.

Results: A wide range in daily glucose level measurements was observed. There was a considerable increase of glycemia (>250 mg%) at the forenoon hours; most episodes occurred between 9 and 10 AM. The more frequent incidences of hypoglycemia (<40 mg%) were registered at night between 22 PM and 4 AM, in 33% of patients were at 3–4 AM. CGM could have psychoeducational importance too, because adolescents could confront data form glucose monitoring with whole day activity.

Conclusions:

1. The largest insulin requirement in adolescent with poor controlled diabetes mellitus considered forenoon hours.
2. The greater tendency to hypoglycemia episodes at the late hours at the night required nocturnal glycemic profiles to determine.
3. The CGMS is useful tool in detection of nocturnal hypoglycemia and monitoring of diabetes care.

P-222

THE FEELING OF THE QUALITY OF LIFE IN CHILDREN WITH TYPE 1 DIABETES TREATED WITH PERSONAL INSULIN PUMP

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The literature of the subject and the clinical experience show that type 1 diabetes influences the feeling of the quality of patients' life. The quality of life, in relation to health category, can be understood as ordinary or expected physical, emotional and social welfare. The initiation of the personal insulin pumps provides the possibility of improvement in quality of life, reduced by diabetes and discipline connected with it. The aim of our research is to establish to what extent and how this new therapeutical method causes the increase of the feeling of the quality of life. Material and methods. 99 children with diabetes type 1 at the age of 9–19 (53 girls and 46 boys).

The average illness lasting is about one year to 12 years (mean 4.66). The experimental group consisted of 32 children owning personal insulin pump ; the control group consisted of 67 children treated with conventional insulin therapy.

Schalock's and Keith's Quality of Life Questionnaire in Oleś modification, adapted to diabetic problems by authors was used. Results. Individual qualitative analysis of items suggests higher subjective quality of life among holders of insulin pumps in relation to children treated with conventional insulin therapy. Children owning insulin pumps have higher feeling of quality of life in dimension of capability of activity, which is expressed in independence in daily life, freedom of choice, responsibilities and capabilities to deciding in daily cases.

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LONG-TERM EFFECTS OF SMBG ON GLUCOMETABOLIC CONTROL OF PATIENTS WITH TYPE 2 DIABETES—FOLLOW-UP DATA FROM ROSSO-IN-PRAXI INTERNATIONAL

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Introduction: Self-monitoring of blood glucose (SMBG) is a simple tool to visualize lifestyle effects on blood glucose levels in insulin-naïve patients with type 2 diabetes mellitus (T2DM). Recently, we could demonstrate by the randomized controlled trial 'ROSSO-in-praxi international' that a 12-week SMBG-structured lifestyle intervention improves glucometabolic control of T2DM patients. In order to evaluate if the programme has long-term effects, patients were followed for a mean period of 1.5 years.

Methods: 125 SMBG-naïve T2DM ambulatory patients were randomly assigned to a SMBG ($n = 63$) and a control group ($n = 62$). Both groups got a manual with basic information about healthy lifestyle. Glucometabolic parameters were assessed at baseline, after 12 weeks and again after 1.5 years.

Results: During the 12 weeks of intervention participants in the SMBG group significantly improved their HbA1c (-0.51%; $P < 0.001$) and lost weight (-1.15 kg; $P < 0.05$), while HbA1c reduction (-0.23%) and weight loss (-0.64 kg) were not significant in the control group. In addition, cardiovascular risk factors such as waist circumference, systolic and diastolic blood pressure, triglyceride levels, total and LDL cholesterol significantly improved just in the SMBG group. Interestingly, after 1.5 years HbA1c level increased again in the control group, reaching baseline values. In the SMBG group improvement of HbA1c (-0.5% vs. baseline; $P = 0.0003$ for trend) as well as waist circumference, systolic and diastolic blood pressure and lipid parameters maintained stable.

Conclusions: Integration of a short-time, highly motivational and low-cost intervention into basic therapy of T2DM improves glucometabolic health and has long-term effects on glucometabolic control.

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THE DEVELOPMENT AND EVALUATION OF STRATEGIES TO ENHANCE DIABETIC OUTPATIENT CLINIC ATTENDANCE, THAILAND

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Aim: To evaluate the efficacy of an intervention to enhance attendance at diabetic outpatient clinics in Thailand.

Background: Statistics show that the number of people with type 2 diabetes is dramatically increasing each year. The greatest increase is projected to be in economically developing countries (Thailand). The Thailand Health Profile shows that diabetes has become a major health problem in the country.

Design and setting: To examine the causes of non-attendance in diabetic outpatient clinics in Thailand, a one-year retrospective analysis of anonymous patient data was undertaken. Patient data from the hospital electronic records system was analyzed. We found that 31.4% of patients failed to attend the diabetic outpatient clinic. Attendance rates were calculated with respect to demographic information. Forgetfulness was identified as one of the major factors influencing outpatient clinic appointment drop-out among these patients.

This randomized trial was combined with telephone/postal reminder campaign to evaluate the potential of an intervention to enhance attendance. The association between attendance rates and participants' illness perception was analyzed. 442 patients were chosen for participation by an independent randomization service.

Results: The overall attendance rate in patients with type 2 diabetes at the diabetic outpatient clinic, Thailand improved by almost 90% compared with the previous appointment. Despite this we did not find any significance with the intervention associated with the appointment keeping, as the attendance of both groups improved. One reason this may have occurred is the relationship formed between researcher and patients, who solved some problems before they reached their specialist.

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BARRIERS TO PUMP TREATMENT—CHARACTERISTICS OF YOUTH WHO DISCONTINUED INSULIN PUMP TREATMENT

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Objective: To assess reasons for pump treatment discontinuation among children and youth with type 1 diabetes (T1DM) and to enlighten barriers that future technology need to overcome.

Subjects and methods: Twenty patients (12 females) discontinued insulin pump treatment). Mean ± SD age at diagnosis of T1DM was 10.2 ± 3.9 years and at pump initiation 13.1 ± 5.1 years and at pump discontinuation 15.9 ± 4.8. Duration on pump therapy was 3.1 ± 2.6 years. We used a self-report scale consisting of 15 items in 3 domains: 1) diabetes control, 2) technical problems 3) body image and social acceptance. The instructions ask how much of a problem each item has been.

Results: The majority of patients (85%) thought that pump treatment enabled better flexibility with food and was not associated with pain. Nevertheless, 80% thought that their metabolic control was worse on pump than on MDI and only 45% thought that they had less hypoglycemic events on pump. Technical problems as a disadvantage of pump treatment were noted by

60% of patients. When data were assessed according to age groups we found that visibility and pump being like a foreign body were major issues among young adults (>20 years), whereas it was significantly different among the young age group (<10 years)($P < 0.05$). Body image as a reason for discontinuing pump was highly significant in females compared with males ($P < 0.04$).

Conclusions: Body image in females and visibility in youth were prominent reasons for pump discontinuation. Hopefully new techniques will help to overcome these barriers in the future.

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CONTINUOUS SUBCUTANEOUS INSULIN INFUSION (CSII), GLUCOSE MONITORING (CGM) AND GLUCOSE VARIABILITY (GV) IN DIABETES TYPE 1 (DM 1) PATIENTS DURING PREGNANCY

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Aim: To examine possibilities of CSII and CGM in euglycemia achieving, decreasing of GV and risk of pregnancy complications.

Subjects and methods: 134 pregnant women with DM1 were observed - 62 patients using CSII and 72 using multiple daily injection (MDI) regimen. The baseline characteristics in both groups were comparable (in terms of age, diabetes duration, frequency of diabetes complications). Daily insulin dose/weight (IU/kg) ratio during I, II, III trimesters and delivery were the following—CSII group: 0.56 ± 0.02; 0.72 ± 0.02; 0.93 ± 0.03; 0.16 ± 0.01 and MDI group: 0.74 ± 0.02; 0.9 ± 0.03; 1.06 ± 0.05; 0.29 ± 0.02 respectively. All patients had 72 hours CGM in all trimesters of pregnancy. Blood coagulation test, functional kidney test, blood pressure measurement were performed for all patients.

Results: In group using CSII euglycaemia level (3.3–7.8 mmol/L) were achieved in $74.7 \pm 4.0\%$ of time, in group using MDI in $58.3 \pm 3.2\%$ ($P < 0.01$) without increasing hypoglycaemia rate (in CSII group duration of hypoglycemia was $3.9 \pm 0.9\%$ of detections, in MDI group $7.6 \pm 1.8\%$ of detections). Glucose variability coefficients (SD_T, $CONGA_{24}$, MODD) were significantly lower in CSII group ($P < 0.001$) and positive correlations between GV measurements (SD_T, $CONGA_{24}$) and fibrinogen level, proteinuria level were observed.

Conclusion: GV could be detected as important negative factor in development of endothelial dysfunction. CSII and CGM are the best way for decreasing of glucose variability and euglycemia achieving.

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HUMAN EMBRYONIC STEM CELL MODEL SYSTEM AS A POTENTIAL BASIS FOR INSULIN PRODUCTION WITH B-CELLS MARKER BY IMMUNOHISTOCHEMICAL STAINING IN TYPE 2 DIABETES

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Type 2 diabetes is characterized by peripheral insulin resistance with an insulin-secretory defect that varies in severity. For type 2 diabetes mellitus to develop, both defects must exist: all overweight individuals have insulin resistance, but only those with an inability to increase β-cells production of insulin develop diabetes. In the progression from normal glucose tolerance to abnormal glucose tolerance, postprandial glucose levels first increase. Eventually, fasting hyperglycemia develops as inhibition of hepatic gluconeogenesis declines. The relative paucity of donations for pancreas or islet allograft transplantation has prompted the search for alternative sources for β-cell replacement therapy. In the current study, we used pluripotent undifferentiated human embryonic stem (hES) cells as a model system for lineage-specific differentiation. Using hES cells in both adherent and suspension culture conditions, we observed spontaneous *in vitro* differentiation that included the generation of cells with characteristics of insulin-producing β-cells.

Immunohistochemical staining for insulin was observed in a surprisingly high percentage of cells. Secretion of insulin into the medium was observed in a differentiation- dependent manner and was associated with the appearance of other β-cell markers. These findings validate the hES cell model system as a potential basis for enrichment of human β-cells or their precursors, as a possible future source for cell replacement therapy in diabetes.

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DIAGNOSTIC EXPERT SYSTEM FOR DIABETES USING SOFT COMPUTING TOOLS

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The objective of the paper is to develop a diagnostic expert system for Diabetes using neural network models consisting of Back Propagation Algorithm (BPA), Radial Basis Function (RBF) Network, Learning Vector Quantization (LVQ) and Neuro-fuzzy approaches. Data for diabetes is collected which is preprocessed and normalized and is divided into training and testing data sets. The various network models were constructed, initialized and trained by the data. The performance was measured on the testing data and we also compared the performance of various networks in terms of factors like accuracy of diagnosis, training time and number of hidden nodes to find out the best diagnostic model. The results obtained clearly indicate that the system finds better accuracy and usage of soft computing approaches than that of the previous works.

The performance of each of the diagnostic system is basically application dependent, and depends a lot on the kind of data set being used for training and testing. Also, all the neural networks have their own advantages and disadvantages. So we conducted the experiment with different neural networks to find the best system for diagnosis. The simulation model can be well applied for diagnosis of patients. The work can be generalized by including other diseases like AIDS, Lungs Disorder, Cardiac Diseases, Skin disorders and Hepatitis. Further the system can be made fault tolerant by comparison with other ANN and AI techniques.

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UP-TO-DATE POSSIBILITIES OF NON-INVASIVE SKIN MICROCIRCULATION EXAMINATION WITH DIABETES MELLITUS PATIENTS

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	Transcutaneous oxymetry	*Capillaroscopy*	*Laser Doppler flowmetry*	*iontophoresis*	*Laser Doppler perfusion imaging*	*photopletysmography*	*Near-infrared spectroscopy*	*Magnetic resonance imaging*
Financial accessibility	++	++	++	++	+	+	+	−
Area examined/ The depth of examined area	−/+	−/−	−/+	−/+	++/+	+/++	+/+	+++/++
Vessel morphology display	−	++	−	−	−	−	−	+
Blood stream evaluation	+	+	++	++	+	++	++	++
Resolving capacity	−	++	−	−	+	−	−	++
Metabolism evaluation	++	−	−	−	−	−	++	−
User intensity	++	+	++	++	−	+	+	+
Subjective influence	−	++	+	−	−	−	−	+
Time demand	+	+	+	++	−	−	+	++

The broken skin microcirculation itself probably is not a main cause of the diabetic foot syndrome nevertheless together with neuropathy and other risk factors may take part in its rise. Its role has been a matter of more and more discussions. Whereas the minimum dimensions and slow blood flow this part of the blood vessel system was inaccessible to examination for a long time. The objective of this work is presenting a complex overview of the methods that have been used to examine microcirculation recently.

Each method has its own advantages and disadvantages. It is not possible to state which is the best one. It depends on the microcirculation area to be examined (area, depth), whether we are interested in total flow or vessels morphology etc. Last but not least the personal and financial possibilities of the workplace are important.

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COMPARISON OF GLUCOSE CONTROL BY INSULIN PUMP, MDI AND PRE MIX INSULIN IN ROUTINE CLINICAL PRACTICE

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Access to insulin pump therapy (CSII) in the UK NHS is regulated by NICE (technology appraisal guidance 151). This limits CSII to type 1 diabetes with HbA1c > 8.5% or recurrent hypoglycaemia if <8.5% and after trying optimised multidose injection (MDI) where possible. Hence the most difficult patients are selected as the potential health and economic benefit is maximised for the increased costs of CSII over MDI. Patients selected for CSII under these criteria might continue after CSII to have worse glycaemic control and other risk factors as they were at a more severe stage of diabetes. The aim of this study was to see if this was the case in routine clinical practice.

Method: Audits of glycaemic control and risk factors were undertaken in various insulin treated populations attending one consultant's clinics: type 1 CSII, type 1 (non CSII), type 2 (various regimes).

Results: In CSII (97), type 1 (98 non CSII), type 2 (MDI 49, basal only 50, pre-mix 51): Mean HbA1c 7.8%, 8.3%, 8.5%, 8.5%, 8.4%; blood pressure 133/75, 136/80, 142/80, 147/80,151/78 BMI 26.9 kg/m^2, 26.4, 30.7, 34.7, 32.2; microalbuminuria 11%, 15%, 21%, 25%, 30%. Data on weight, lipids and insulin doses will be presented.

Conclusions: The HbA1c reduction of 0.5% in our practice with CSII is similar to reported RCTs. However intensive insulin therapy in type 2 patients without CSII did not achieve target levels due to limiting hypoglycaemia. Use of CSII in type 2 patients could prove cost effective if glycaemic targets could be achieved.

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DIABETES-INDUCED DECREASED CYP3A ACTIVITY INCREASED NATEGLINIDE BIOAVAILABILITY IN RATS: AN EXPERIMENTAL EVIDENCE

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Nateglinide (NT) is an antidiabetic agent that is often used for the control of postprandial hyperglycemia. The bioavailability of NT is about 60% in healthy volunteers. It is metabolized by CYP3A, CYP2C8, and CYP2C9. Incidentally, some reports suggest that in diabetes there is marked decrease in the liver microsomal enzymes. How does this, would affect the bioavailbility of CYP3A substrate such as nateglinide has been investigated in rats. Type I and type II diabetes was induced in Sprague-Dawley rats respectively by alloxan (40 mg/kg, i.v.) and streptozotocin

(50 mg/kg, i.p.), and induction of diabetes was confirmed by estimating blood glucose levels.

After 18 h of fasting, non-diabetic, type I diabetic, and type II diabetic rats received nateglinide (50 mg/kg), and its plasma levels were estimated by HPLC at 0.25, 0.5, 1, 2, and 3 h after oral administration. In another set of above groups, the prevailing activity of CYP3A was determined by erythromycin N demethylase (EMD) assay. The AUC and Cmax values for nateglinide from respective groups indicated that the bioavailability of nateglinide was significantly higher in type 1 (30%) as well as type 2 (20%) diabetic group. The EMD assay revealed that CYP3A activity was lesser in diabetic groups as compared to non-diabetic group. The later results suggest that the increased bioavailability of nateglinide was due to its decreased metabolism via CYP3A, and that further population pharmacokinetic investigations are necessary to substantiate the outcome.

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KCNJ11 ACTIVATING MUTATION IN NEONATAL DIABETES: MASHHAD EXPERIENCE

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Objective: Patients with permanent neonatal diabetes usually present within the first six month of life and require insulin treatment. Closure of ATP-sensitive K + channels (KATP channels) in response to metabolically generated ATP or binding of sulfonylurea drugs stimulates insulin release from pancreatic β-cells. Heterozygous Activating Mutation in the KCNJ11 gene encoding the Kir6.2 subunit of this channel is the most common cause of neonatal diabetes.

Methods: We sequenced the KCNJ11 gene in 20 patients with neonatal diabetes by implementing DNA amplification of blood sample by Polymerase Chain Reaction (PCR).

Results: We collected 20 patients diagnosed with diabetes before 6 months of age. We identified 2 known mutations in *KCNJ11* in 4 (20%) patients who were diagnosed with diabetes before 6 months of age. Two patients with R201C mutation and a patient with E227K mutation. Another patient with W68R novel mutation.

Conclusions: Heterozygous Activating Mutation in the gene encoding Kir6.2 cause permanent neonatal diabetes. Identification of the genetic cause of permanent neonatal diabetes may facilitate the treatment of disease with sulfonylurea. Genetic testing, which is critical for guiding appropriate management, should be considered in patients diagnosed with diabetes before 1 year of age.

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GLUCOMEN®DAY: FROM INTERSTITIAL FLUID TO VENOUS BLOOD CONTINUOUS GLUCOSE MONITORING FOR USE IN CRITICAL CARE

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An increasing number of studies is confirming the importance of a tight control of the glycemic levels in critically ill patients. Achievement of normoglycemic regimes by avoiding hypoglycemic and hyperglycemic stresses is a crucial therapeutic target in Intensive Care Units (ICUs) for reducing mortality, morbidity and in-hospital stay.

Continuous Glucose Monitoring Systems (CGMs) are tools whose importance for the management of diabetes in non-hospitalised patients is increasingly growing. These technologies are not currently used in ICU primarily for two reasons, both related to the fact that CGMS commonly operate in the interstitial fluid (ISF):

1. Glucose concentration in the interstitial compartment changes with a significant time lag as compared to the venous one;
2. Under conditions of shock, edema, or anaerobic glucose utilization, correlation between glucose in blood and subcutaneous tissue might significantly (and unpredictably) decrease.

Hence, because of these inherent limitations, current real-time CGM technologies do not allow for appropriate medical actions to be promptly taken.

GlucoMen®Day is a new CGMs from A. Menarini Diagnostics the clinical performance of which in measuring ISF glucose have been validated through several clinical studies. As compared to the needle-type CGMs, the microdialytic platform of GlucoMen®Day could offer as a major advantage the possibility to operate in both interstitial and venous compartments.

Herein, we present the promising results of a new device configuration, equipped with a microdialytic probe for measuring glucose directly in the venous stream through a standard peripheral intravenous catheter with negligible time lag high accuracy, and low calibration frequency.

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MINIMISING THE IMPACT OF TIME LAG VARIABILITY ON ACCURACY EVALUATION OF CONTINUOUS GLUCOSE MONITORING SYSTEMS

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Continuous Glucose Monitoring (CGM), crucial tool for the management of diabetes in insulin-treated patients, is expected to represent the natural evolution of Self-Monitoring of Blood Glucose (SMBG).

However, current CGM systems (CGMs) measure the glucose concentration in the intracellular interstitial fluid (ISF), where glucose concentration changes with a significant time lag with respect to the corresponding venous blood (VB) values.

Several studies have demonstrated that ISF glucose is highly correlated to VB glucose only during periods when the glucose concentration is relatively stable. In case of rapid changes, the time lag between the two compartments significantly reduces this correlation, thus resulting in an apparent deterioration of the accuracy of the CGMs (which are necessarily calibrated using either capillary or venous blood glucose reference data).

In recent years, Kovatchev's group has introduced the Poincarè Plot method for assessing the average time lag. The calculated time lag value is used to shift the reference data points with respect to the CGM profile prior to calculating the accuracy of the CGM device.

Herein, we present an adaptive time lag correction method based on an ISF-VB intercompartment diffusion model. Such a model was elaborated using the CGM/reference data pairs coming from two independent clinical trials performed using the GlucoMen®Day CGMs (A. Menarini Diagnostics). Accuracy of the CGM device was evaluated according to Kovatchev's CG-EGA criteria. Functioning and benefits of using this adaptive time lag compensation method for highlighting the "true" accuracy performance of any CGM device are discussed in details.

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ASSESSMENT OF BLOOD GLUCOSE CONTROL IN THE PEDIATRIC INTENSIVE CARE UNIT: EXTENSION OF THE GLYCEMIC PENALTY INDEX TOWARDS CHILDREN AND INFANTS

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Introduction: Blood glucose (BG) control performed by intensive care unit nurses is becoming standard practice for critically ill patients. The performance of BG control (for "adult" critically ill patients) is easily assessed by the Glycemic Penalty Index (GPI). As targetted normal BG ranges are lower in critically ill children and infants, risks of potentially detrimental hypoglycemia are higher. Therefore, the adult GPI needs to be adapted for children (1–16 years) and infants (<1 year).

Method: Different BG ranges correspond to penalty values that behave as a staircase "clinical expertise" penalty function which is transformed into an optimized smooth polynomial function by minimizing the squared differences. A penalty is assigned to each measured BG based on this smooth polynomial function. The average of all penalties of a patient represents the patient-specific GPI. This glucose trajectory evaluation method does not assume any relationship between successive measurements and is not effected by outliers.

Result: The GPI formula generates a number between 0 and 100 with an "ideal" level of 0 (indicating all measured BG values fall within the normoglycemic range). Penalization in the hypoglycemic range is more severe than in the hyperglycemic range in order to reflect the cautious clinical approach to hypoglycemia. The cut-off GPI that shows "clinically acceptable" BG behavior is (mathematically) defined as 23 for children and 14 for infants.

Conclusion: The extended GPI version allows to mathematically and easily interpret BG profiles obtained in children/infants. The determined cut-off GPI values need to be confirmed by clinical studies.

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AMBULATORY BLOOD PRESSURE MONITORING IN TYPE 2 DIABETES

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Hypertension is a major risk factor for the development and progression of chronic complications in type 2 diabetes. The office blood pressure evaluation does not allow evaluating nocturnal dipping patterns, presence of white-coat hypertension, blood pressure loads, and patients with masked hypertension. Patients in masked hypertension group otherwise have not been identifiable because their routine office blood pressure exam is normal and physicians would not be aware they belong to a high-risk group. Blood pressure evaluation over a 24-h ambulatory blood pressure monitoring (ABPM) period correlates better with outcomes than ordinary office blood pressure measurements in diabetics.

In this study 20 Type 2 Diabetic patients were enrolled and circadian pattern of these subjects were recorded by the help of ABPM monitor TM2430 made by A&D Japan for 7 days. The monitor was set to record day time BP every half hourly and night time every hourly. Blood pressure data series of each individual were analyzed by the COSINOR method to evaluate their circadian rhythm. By this we found 24 hours cosine curve. This analysis provides estimates of the parameters of circadian pattern of blood pressure like MESOR (Midline Estimating Statistics of Rhythm), Amplitude, Acrophase, Hyperbaric Index, Percentage Time Elevation. The results indicate that more than 60% Type 2 diabetic subjects are having MESOR hypertension. More than 14% of total patients apart from having MESOR do have CHAT. 5% subjects have Reverse Dipping and 10% have non dipping pattern. About 7% subjects were having masked hypertension.

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LONG-TERM EFFECTS OF METFORMIN AND ROSIGLITAZONE THERAPY IN A RAT MODEL OF METABOLIC SYNDROME WITH HIGH FRUCTOSE/FAT DIETS

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Metabolic syndrome and diabetes are close related to diet and calories intake. There are few studies about the chronic effects of metformin and rosiglitazone in animal models with high fructose or high fat diets. In this study, groups of Wistar rats were fed with 30% fructose or 30% fat diets during 4, 6 and 7 months, and oral-treated with metformin 200 mg/kg and/or 8 mg/kg rosiglitazone, daily during 2 and 3 months. Oral Glucose Tolerance Test (OGTT), systolic and diastolic blood pressure (BP), and QPCR gene expression in pancreas and liver were analyzed.

In 4 months, both diets induced an evident metabolic syndrome model, showing the increase of BP, impaired OGTT, insulin resistance, and alterations in mRNA levels of different proteins involved in glucose metabolism in pancreas and liver. With 2 months of metformin and rosiglitazone administration, BP was significantly diminished in both diets, but OGTT was less improved after 3 months of treatment. Insulin, glucagon and PDX-1 expressions were significantly altered, possibly as a process of islets compensation. Hepatic glucokinase and GLUT2 expressions indicated modification in glucose sensing and metabolism, improved with both drugs. The synergic effect was not statistical different in all groups. Morfological alterations were also observed in kidney, pancreas and adipose tissue from animals fed with fructose or fat diet, and changes lightly improved after pharmacological treatments. In conclusion, monotherapies improve many alterations in a long-term metabolic syndrome model.

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NON-INVASIVE GLUCOSE MEASUREMENT: ON THE WAY TO PRECISE AND SPECIFIC GLUCOSE VALUES

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In spite of different approaches to measure glucose non-invasively there is no such device available to the patient, possibly due to low specificity of involved techniques. Mid-infrared spectroscopy (MIR) is specific but poses other problems: low penetration depth of the energy, difficult quantification of absorption and measuring tissue glucose instead of blood glucose.

We solved these problems by combining the high energy emitted by MIR quantum cascade lasers (QCL) with a photoacoustic (PA) detection. After optimisation in phantoms and volunteers we performed a series of 12 oral glucose tolerance tests (oGTs) involving two different patients. The data are presented as the ratio of the PA induced by specific and unspecific wavelengths.

MIR has a high variation and signals induced by glucose are small *in vitro*. We found a correlation between two specific wavelengths PA and the blood glucose value ($r = 0.6648$, $P < 0.00496$ and $r = 0.6249$, $P < 0{,}00965$) when pooling the data of intervals 10 mg% wide. A multiple regression improved the r factor. In a simultaneous oGT in the two persons the slope of the correlation was identical.

MIR induced PA yields specific information about the glucose in the tissue. As *in vitro* the quality of the correlation of the PA and the blood glucose improves with an increase of the number of wavelengths applied. Currently, we improve the system towards clinically relevant performance by replacing the fixed wavelengths of individual QCL by >100 wavelengths from a single tunable QCL.

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STEVIA: A NEW WEAPON TO TACKLE DIABETES WAR

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In this paper, we presenting a very effective herbal treatment for diabetes. A South American native sweetner plant of sunflower family named **"Stevia"** has a great potential to fight with this deadly disease. This paper will cover all the aspects related to benefits of including this agent in patient's diet.

It reduces the calories from your food which make it a suitable replacement of sugar for calorie concious as well diabetic people. It is also being found by people that the regular use of stevia helps in reactivating the insulin secreting cells in pancreas. The raw stevia leaves are around 35–40 times sweeter than sugar, stevia extract are sweet upto 300 times of ordinary sugar. The stevia is safest low calorie sweetner without any side effect. Stevia is also useful in lowering blood sugar and it reduces the tooth cavity hence it can be used in tooth pastes in place of sugar to avoid tooth decay.

More recent medical research has shown promise in **treating obesity and hypertension**. Stevia has a negligible effect on blood glucose, even enhancing glucose tolerance; therefore, it is attractive as a natural sweetener to diabetics and others on carbohydrate-controlled diets.

Stevia is useful in regulating blood sugar, preventing hypertension, treatment of digestive disorders and prevention of tooth decay. Studies shows that it is natural antibacterial and antiviral agent as well. On top of that, calorie and carbohydrate free and hence a great sweetener choice for diabetics, and those watching their weight.

P-240

INDIVIDUALIZE MPC-BASED ARTIFICIAL PANCREATIC BETA-CELL USING ADVISORY MODE EVALUATION

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Objective: Model predictive control (MPC) has been implemented in artificial pancreatic beta-cell to connect insulin pump and continuous glucose monitoring system (CGMS). History data from insulin pump and CGMS can be used to individualize input variation weight for MPC using advisory mode evaluation method.

Methods: Using type 1 diabetes model proposed by University of Cambridge and log-normal distribution sampling, eight virtual subjects were built in MATLAB® (The MathWorks, Inc., Natick, MA). Each subject has an optimal open-loop therapy, where the bolus is based on perfect meal size estimation and the basal rate is optimized. Taking open-loop CGM readings as inputs, MPC can design insulin delivery rate offline. The optimal input variation weight is such that the total insulin dose under MPC is the same with that under the open-loop therapy.

Results: The individualized MPC was compared with the optimal open-loop therapy on eight virtual subjects as summarized in the table below. All subjects followed a 24-h scenario of three meals at {7:00, 12:00, 18:00} with {40 g, 60 g, 85 g} CHO, respectively.

Conclusions: An automatic scheme was presented to individualize MPC. The suggested weights are good starting points for further optimization.

Glycemic Control Performance Comparison

Subject		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
% within [70 180] mg/dL	Open-loop	97.6	83.3	100	94.4	57.3	66.3	54.5	100
% within [70 180] mg/dL	MPC	90.6	62.5	100	64.2	77.8	79.9	32.6	100
Blood glucose index	Open-loop	1.91	3.58	0.80	2.39	7.48	5.82	22.40	0.41
Blood glucose index	MPC	3.37	8.36	0.95	5.55	4.98	4.31	32.69	0.70

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PLANNING CLOSED-LOOP (CL) STUDIES IN HOME SETTING—SIMULATIONS SUPPORT FOR HAZARD ANALYSIS (HA)

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Glucose Control Measures

	Baseline	*Hazard 1*	*Hazard 2*	*Hazard 3*	*Hazard 4*	*Hazard 5*
Glucose (mM)	7.8 ± 1.4	8.1 ± 1.4	6.8 ± 1.3	9.1 ± 1.3	7.6 ± 1.4	8.2 ± 1.3
Time in target 3.9–8.0 mM (%)	60 (27–82)	51 (18–74)	71 (42–90)	50 (0–65)	62 (36–82)	52 (14–81)
Time >8.0 mM (%)	38 (16–73)	47 (26–82)	20 (0–51)	50 (36–100)	36 (16–64)	46 (19–86)
Severe hypo events ≤ 2.0 mM	0	0	2	0	0	0

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Introduction: Closed-Loop systems have been studied widely in the controlled clinic setting. Unsupervised home use of such systems raises concerns reflected in carefully compiled HA for overnight home use of Florence CL system.

Aim: To provide mitigations for HA for home use of Florence CL system using simulations.

Methods: Simulation environment comprising 18 virtual subjects with type 1 diabetes and combining experimentally-derived characteristics of FreeStyle Navigator® measurement error was used to simulate overnight CL with Florence MPC-based controller. Four levels of calibration error were applied. Simulated study started at 17:00 and continued for 15 h. CL commenced at 21:00 and continued for 11 h. Protocol included an evening meal (70 g CHO at 18:00) and a snack (20 g CHO at 21:00) accompanied by insulin boluses.

Results: Simulation studies provided mitigations for five identified hazards:

(1) failure to deactivate CL at breakfast time leading to overrunning it until midday,
(2) overestimation and
(3) underestimation of meal size by 50%,
(4) overestimation and
(5) underestimation of total daily dose of insulin by 30%.

Table shows glucose control measures obtained from these simulated studies compared to baseline; values are mean ± SD or median(IQR), N = 72.

Conclusion: Simulation studies provided valuable support in mitigating identified hazards.

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IN VITRO STABILITY OF INSULIN LISPRO, ASPART, AND GLULISINE IN SIMULATED CONTINUOUS SUBCUTANEOUS INSULIN INFUSION (CSII) USING ACCU-CHEK® PLASTIC CARTRIDGES

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Background: This study was designed to assess compatibility of plastic cartridges with rapid-acting insulin preparations by analyzing insulin stability and potency after simulated storage and use during CSII therapy.

Methods: Accu-Chek® Plastic Cartridges were filled with Insulin Lispro 100 IU/mL, Insulin Glulisine 100 IU/mL and Insulin Aspart 100 IU/mL. Filled cartridges were capped with a corresponding closure system and stored in the refrigerator for 32 days at 5 ± 3°C to simulate suggested maximum storage conditions. The cartridges were then placed on a shaker (30 oscillations/min., 2 ± 0.5 cm amplitude) for 7 days at 37 ± 2°C to simulate worst case use conditions during insulin pump therapy. Quality parameters of insulin were analyzed and compared to control samples.

Results: There was no significant reduction in insulin potency. The pH remained stable throughout the study. Impurities were not observed. The quality parameters A-21 desamido insulin and high molecular-weight proteins remained within specified limits of the Pharmacopoeia. The concentration of preservatives decreased but remained at levels to ensure preservative efficacy.

Conclusions: Study results confirm compatibility of Accu-Chek® Plastic Cartridges with insulin Lispro, Aspart and Glulisine for a storage time of 32 days in the refrigerator and an in-use time of 7 days.

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RETROSPECTIVE CONTINUOUS GLUCOSE MONITORING: A REVIEW OF USES OVER THE LAST 10 YEARS

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Aim: Retrospective continuous glucose monitoring systems (CGMS) have been used in a variety of settings ranging from diabetes treatment studies, to drug efficacy trials and behavioral modification approaches. The outcomes assessed with CGMS, the type of trial, and the disease state studied in the last 10 years are reported.

Methods: A retrospective literature review was conducted using the Biomedical Reference Collection, Medline Full Text, and ClinicalTrials.gov to identify studies that used retrospective, blinded, or professional CGMS. Studies were then separated by purpose and disease state.

Results: Since 2000, 257 studies using CGMS were reported in the literature. Among these, 26% reported that the primary purpose was to evaluate CGMS itself as a tool to assess glycemia, 39% reported using CGMS to understand glycemic patterns and/or to make therapy recommendations, 22% used CGMS to determine secondary glycemic endpoints in pharmaceutical trials, and 13% used CGMS to assess a behavioral intervention. The disease state most frequently studied was type 1 diabetes (46%). Other diagnoses included type 2 diabetes (23%), pregnancy (7%), cystic fibrosis (2%), cardiovascular disease (2%), ICU patient status (2%), and renal disease (10%). Healthy individuals were used as subjects in 7% of the studies.

Conclusion: Retrospective CGMS has been used to elucidate glycemia and glycemic patterns in a variety of settings and disease states. Clinical evidence continues to accumulate as to the value of retrospective CGMS as a tool to assess and potentially improve patient outcomes.

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SWEETENED WHEY PROTEIN BEVERAGES ATTENUATE PLASMA GLUCOSE, HUNGER AND CALORIE INTAKE IN YOUNG WOMEN

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The high prevalence of diabetes and obesity calls for food-based prevention. Whey protein has potential as a functional food component to reduce the blood glucose spike and regulate body weight by providing satiety signals. Sweetened beverages are popular and may prove an effective vehicle for delivering whey protein for consumption. However, whey protein is insulinotropic and may excessively reduce blood glucose when mixed with sugars.

Aims: The current study was designed to investigate the effect of whey-glucose mixed drinks relative to glucose or whey alone on a) glycemic response, b) appetite response and c) calorie intake.

Methods: Test beverages were given randomly once a week after an overnight fast as 50 g or 75 g glucose, 25 g whey protein or 25 g whey protein mixed with 50 g glucose. Blood glucose and appetite were measured simultaneously at 0, 15, 30, 45, 60, 90, 120, 150 and 180 min by a portable glucometer and visual analogue questionnaires. Pizza was served at 180 min. Calories consumed from the pizza were calculated.

Results: The blood glucose incremental area under the curve showed 50% reduction after the whey-glucose mixed drink compared to pure glucose drinks. Calorie intake was reduced after both whey protein drinks with or without glucose mixed. The change from baseline blood glucose was associated with reduced appetite and calorie intake.

Conclusion: Whey protein drinks prevented excessive fluctuation in blood glucose response and suppressed appetite and calorie intake at meal time over three hours in young women.

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ASSESSMENT OF LINEAR TECHNIQUES TO MODEL MULTISENSOR DATA FOR NON-INVASIVE CONTINUOUS GLUCOSE MONITORING

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Aim: Solianis Monitoring AG recently proposed a multi-sensor approach for Non-Invasive Glucose Monitoring (NIGM) [Caduff et al., Biosensors & Bioelectronics, 2009] based on a combination of dielectric and optical sensors. The aim of this work is to assess the performance of three linear model estimation techniques for the estimation of glucose profiles from sensor measurements.

Methods: Thirty-two runs (study visit of approximately eight hours) were obtained from four type-1-diabetic subjects. Reference Blood Glucose (BG) concentration was assessed by the HemoCue Glucose Analyser. Training and test set consisted both of sixteen runs. BG profiles and Multisensor measurements were associated by means of a linear model through Ordinary Least Squares (OLS), Stepwise, Partial Least Squares (PLS) and Least Absolute Shrinkage and Selection Operator (LASSO). Twelve regressors were used by the last three methods.

Results: Model estimates were compared to the reference BGs by Root Mean Square Error (RMSE).

	Stepwise	*PLS*	*LASSO*
RMSE improvement w.r.t. OLS [%]	29.6	−11.5	31.4

Stepwise and LASSO avoid the typical overfitting of OLS. PLS performs worst because of its sensitivity to the noise in the optical channels affecting the latent variables. Compared to Stepwise, variables selected with LASSO seem to generalise better in external validation.

Conclusions: A linear model allows the estimation of glucose profiles when suitable estimation techniques are used. LASSO and Stepwise outperform PLS and OLS as PLS is less robust to measurement errors and OLS is prone to overfitting.

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AN AUTO-TUNING NONLINEAR MODEL PREDICTIVE CONTROL FOR BLOOD GLUCOSE CONTROL IN TYPE 1 DIABETES

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Background: This paper presents a personalized insulin infusion advisory system (IIAS) which serves as a control algorithm towards the development of a closed-loop artificial pancreas using the subcutaneous (sc) route. The IIAS has been *in silico* tested with the 10 adults' population with Type 1 Diabetes Mellitus (T1DM), available in the training version of the UVa T1DM simulator.

Methods: The IIAS is based on Nonlinear Model Predictive Control (NMPC). The personalized model used to provide the NMPC with glucose predictions, is based on the combined use of two Compartmental Models, which simulate sc insulin kinetics and glucose absorption into the blood from the gut, respectively, and a Recurrent Neural Network, which models the patient's glucose kinetics. Moreover, a novel tuning algorithm updates online the NMPC parameters. The IIAS has been tested for its ability to handle meal disturbances, fasting conditions, inter-patient variability, and robustness against over- (OEE) and under- (UEE) estimation errors up to 25% of carbohydrates' amount in ingested meals.

Results: The mean values presented in Table I, demonstrate that most of the time, blood glucose levels are kept within the target range while the risk indices associated with extreme glucose deviations have low values.

IIAS' Performance

Simulation scenario	% within target (70–180 mg/dL)	Risk index
Accurate meal announcement	97.49 ± 2.76	1.45 ± 0.66
OEE	97.92 ± 2.78	1.31 ± 0.61
UEE	94.51 ± 6.60	2.00 ± 1.34

Conclusions: The results indicate that tight glycemic control is achieved.

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ASSESSMENT OF REPEATABILITY PRESSURE MEASUREMENT DEVICE (LORAN PLATFORM) FOR ITS APPROPRIATE USE IN BIOMECHANICAL RESEARCH AND IN CLINICAL PRACTICE

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Diabetic foot ulcers result from abnormal mechanical loading of the foot, such as repetitive moderate pressure applied to the foot plantar surface while standing or walking. Diabetic peripheral neuropathy causes changes in foot structures, increasing foot plantar pressure, which is a predictive risk factor for the development of diabetic foot ulcerations that may end up in amputation.

Previous studies have used various systems in order to analyze foot pressures. It is important that measurements provide sufficient repeatability compared to the measurement parameters being used for evaluation purposes. Differences in sensor technology, matrix spatial resolution, pressure range, sampling rate, calibration procedures, raw data, ageing, lead to significant differences in the plantar pressure measurements overall accuracy. This study was designed to assess the repeatability of the Loran Platform (2304 resistive sensors, sampling at 30 Hz) and to evaluate the variability of plantar pressure and postural balance during barefoot standing position in non diabetic and diabetic subjects.

Measurements were taken from eight non-diabetic subjects and eight diabetic patients in an early stage of the illness. Five variables were measured with the platform during barefoot standing. Ten measurements were taken using two different techniques for feet and posture positioning, during three sessions, once a week. A novel standard method was developed in order to obtain repeatability measurements from the Loran Platform; algorithms were developed in order to export the system's data automatically and to improve the resolution of the obtained images, for its appropriate use in biomechanical research and in clinical practice.

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USER CHARACTERISTIC IMPACTS ON THE PERFORMANCE OF A CONTINUOUS GLUCOSE MONITORING SYSTEM

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Introduction: This report evaluated the impact of subject characteristic on the performance of a seven day, real-time, continuous glucose monitoring (CGM) SEVEN®PLUS system.

Materials and methods: Study design was reported previously. For each subject, CGM system performance was measured as the average difference in reference to laboratory standard (YSI). Subject characteristics were evaluated with the CGM system performance measures using regression methods and correlation coefficients.

CGM User Characteristics

User Characteristics	Average (Standard Deviation)	Minimum	Maximum
Age (years)	46.9 (12.3)	23	92
Duration of Diabetes (years)	20.0 (12.0)	1	53
BMI (kg/m^2)	28.4 (7.8)	19.7	56.4
T1D/T2D (N)	39/10		
Male/Female (N)	30/19		
CSII/MDI (N)	23/25		

Results: Forty-nine subjects provided venous YSI reference measures while using CGM. User characteristics represented a convenient study population from three research centers (Table 1). According to the WHO's classification of obesity, 16 (33%), 20 (41%), 9 (18.4%), and 4 (8%) were classified as Normal, Overweight, Obese, and Morbid Obesity, respectively. None of the subject characteristics was a significant independent variable for the CGM performance in reference to YSI as the dependent variables in regression analysis. No statistical significant correlation coefficient was observed (all $P > 0.5$).

Conclusions: There is no apparent impacts from user characteristics (age, gender, BMI, duration of diabetes, type of diabetes and method of insulin delivery) on the performance of the SEVEN®PLUS system.

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GLOBAL CARDIOVASCULAR RISK ASSESSMENT IN PATIENTS WITH POSTPRANDIAL DYSMETABOLISM

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The postprandial dysmetabolism is caracterised by exaggerated supraphysiological postprandial spikes in blood glucose and lipids and is regarded as a risky state for cerebro- and cardiovascular disease. Althought hyperlipidemia, hyperglycemia and blood presure contribute to the maintenance of mental activity. We aimed to assess cardiovascular risk (CVR) in patients with metabolic syndrome (MS) or newly diagnosed type 2 diabetes mellitus (DM) and postprandial dysmetabolism.

Material and method: We have selected a group of patients with MS (group A) and a group B with newly diagnosed type 2 DM. We studied familial clustering for obesity, postprandial glycemia, HDL-cholesterol, LDL-cholesterol, total cholesterol, post-prandial triglycerides level, and the presence of atherosclerotic cardiovascular diseases due to this frequent morbid

association. Cardiovascular risk was calculated using the diagram for evaluating the coronary risk (Euro98 Diagram).

Results: We noticed the presence of hypertension in 68% of patients of group A vs. 76% in group B, post-prandial dyslipemia 90% vs. 92%, central obesity 81% vs. 92% for women and 90% vs. 94% for men. In the group B we observe the presence of high CVR in 35% of patient compared with group A, were the incidence of a high value score risk was present only in 10% of the patients.

Conclusion: The presence of postprandial dysmetabolism increase the cardiovascular risk, aspect confirmed by our measurement using Euro98 Diagram. Post-prandial dysmetabolism associated with Metabolic Syndrome and/or newly diagnosed type 2 diabetes mellitus is an independent predictor for future acute cardiovascular events.

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PRODUCTION OF BIOFILM FORMATION BY GRAM-NEGATIVE ORGANISMS ISOLATED FROM DIABETIC FOOT ULCER PATIENTS AND ITS CORRELATION WITH CLINICAL PROFILE

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Out of 342 diabetic patients, 102 patients with Texas grade 1–3 DFU were hospitalized in Centre for Diabetes and Endocrinology, J.N. Medical College, A.M.U. Aligarh, India from April 2009 to Feb 2010. Of these, 57 had gram-negative bacterial infection were included in this study. All patients were subjected to detailed clinical, biochemical, and examination of foot. Gram-negative organisms were screened for extended spectrum beta lactamase and biofilm production. Among 57 diabetic foot ulcer patients, 70% were males. 44 (77.1%) had type 2 diabetes mellitus. The duration of the ulcer was >1 month in 54.3% cases. The presence of sensory neuropathy was observed in 72.5% patients. Poor glycemic control and poor HbA1c (>8) was observed in 68.7% and 86.2% patients respectively. *Escherichia coli* was the most common (42.2%) isolate followed by *Pseudomonas aeruginosa* (23.7%), *Klebsiella oxytoca* (11.3%), *Klebsiella pneumonia* (9.2%), *Proteus vulgaris* (5.1%), *Acinetobacter sp* (5.1%), *Proteus mirabilis (2%)* and *Morganella morganii* (2.6%) were isolated. Of the 57 patients, 97 gram-negative bacilli were isolated. Out of these, 59.4% were biofilm producers. The antibiotics resistance was higher in biofilm producers when compared with non-biofilm producers. Of the 67 ESBL positive isolates, 74.6% were biofilm producers. Factors responsible for ulceration which lead to infection were duration of ulcer >1 month, ulcer size >4 cm^2, hospital stay, poor glycemic control, higher HbA1c, presence of neuropathy, higher antibiotic resistance & ESBL production by biofilm producers. Among the gram negative bacilli, higher degree of antibiotic resistance was associated with biofilm production.

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THE EFFICACY OF BOLUS CALCULATOR ON METABOLIC CONTROL IN PEDIATRIC PATIENTS USING CSII

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Aim: To assess the efficacy of a bolus calculator (BC) on glycemic control in type 1 diabetic pediatric patients using continuous subcutaneous insulin infusion (CSII) treatment.

Methods: 383 downloaded pump data of 129 patients were analyzed. 117 children used Paradigm pumps (Medtronic), 12 - Spirit pumps (Roche). Patients were divided into two groups regarding "active" BC feature during last examination (group 1 - BC active, group 2 - BC inactive. Last HbA1c, insulin requirement (IU/kg), number of boluses per day, number of hypoglycemias requiring assistance per week were considered.

Results: 39 patients qualified to group 1 (30.2%), 90 (69.8%) - to group 2. In group 1 only 19 patients were "active" BC users (group 1a) (48.7% of the group, 14.7% of whole studied population). Both groups did not differ regarding metabolic control (HbA1c: $7.4\% \pm 1.5$ vs $7.9\% \pm 1.4$, $P = 0.17$), insulin requirement (0.84 ± 0.4 UI/kg/day vs 0.87 ± 0.4 UI/kg/day, $P = 0.7$), bolus quantity (6.9 ± 2.2/day vs 7.5 ± 2.6/day, $P = 0.2$) as well as number of hypoglycemias (0.564 ± 0.8/week vs 0.565 ± 0.9/week, $P = 0.99$). Similarly, no differences were observed when comparing group 1a vs 1b as well as group 1a vs (1b + 2).

Conclusions: No differences in studied parameters between groups is probably due to small size of groups and the fact that in group 1 less that 50% of patients were really using this feature. Moreover, when using bolus calculator, patients tended to modify suggested insulin dose.

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Incidence, risk factors for amputation among patients with diabetic foot ulcer in a North Indian tertiary care hospital

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ABSTRACT

Objective: Aim of this study was to evaluate the incidence and risk factors for amputation among patients with diabetic foot ulcer (DFU).
Research design and methods: We performed a prospective study of 162 DFU in patients treated in a multidisciplinary based diabetes and endocrinology centre of Jawaharlal Nehru Medical College of Aligarh Muslim University, Aligarh, India during the period of December 2008–March 2011. Detailed history and physical examination was carried out for every subject. Risk factors for amputation were determined by univariate analysis with 95% of CI.
Results: The overall amputation rate was 28.4%. On univariate analysis, male sex [OR 2.8, RR 1.28], hypertension [OR 2.83, RR 1.31], neuropathy [OR 3.01, RR 1.35], nephropathy [OR 2.24, RR 1.26], LDL-C (>100 mg/dl) [OR 2.53, RR 1.28], total cholesterol (>150 mg/dl) [OR 3.74, RR 1.52],HDLC(<40 mg/dl) [OR 1.19, RR 1.18], triglycerides (>200 mg/dl) [OR 5.44, RR1.76], previous antibiotic use [OR 9.12, RR 1.92], osteomyelitis [OR 6.97, RR 2.43] and biofilm infection [OR 4.52, RR 1.41] were significant risk factors.
Conclusion: The risk factors for amputation were presence of PVD, leukocytosis, neuropathy, nephropathy, hypertension, dyslipidemia, over use of antibiotics, osteomyelitis, biofilm production and higher grade of ulcer.

1. Introduction

Diabetic foot ulcer (DFU) and infections are a major medical, social, economic problem and a leading cause of morbidity and mortality, especially in the developing countries like India [1]. DFUs precede 70–80% of lower extremity amputations [2]. Other risk factors for amputation in diabetic patients include peripheral neuropathy, peripheral vascular disease (PVD), excessive planter pressure, poor glycemic control, chronic renal failure and a prevalence of retinopathy [2]. The prevalence of PVD reported in diabetic subjects is 10%, whereas in non-diabetics it is 2.6% [3]. Recently in India, Vishwanathan and Kumpatla [4] in a multicentric study have reported that 35.3% DFU patients had PVD. Ischemia may be the cause of or contribute towards the progression of trophic lesions in the foot, which form favourable locations for infections to take hold. The coexistence of neuropathy, ischemia and leukocyte immune function disorders in diabetic patients favour the development of severe and extensive infections in the lower limbs and that, if not adequately treated, may lead to amputation and death. Amputation affects not only the quality of life and physiological welfare, but it is also often a premortal events. Following major leg amputation, mortality ranges from 20% to 50% within 3 years and reaches 70% in 5 years [2]. Despite of all these, factors for amputation have not been clearly established and vary significantly from hospital to hospital study.

The worst outcome of DFU is lower limb amputation. DFU continues to be a major reason for lower extremity non-traumatic amputation worldwide. Recently, *Global Lower Extremity Amputation Study Group* estimated that 25–90% of all amputations were associated with diabetes [5]. Because of the large diabetes population in the country like India, the patients with diabetic foot problems are also increasing dramatically. However, in spite of vast population, data about diabetic foot problem are sparse in India. Therefore, we plan to investigate the incidence and potential risk factors in predicting amputations in DFU patients in a tertiary care hospital. Identification of risk factors for amputations would lead to prompt and proper care of patients with DFU and also aid in preventing amputation.

2. Materials and methods

2.1. Study design

Prospective, hospital based study. Total of 469 diabetic patients admitted in the Centre for Diabetes and Endocrinology, Jawaharlal

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Nehru Medical College Hospital, Aligarh Muslim University, Aligarh, India, 162 who developed Ulcer in their foot during Dec 2008 to March 2011 were included in this study. Multiple ulcers during one hospitalization were considered as one ulcer episode and the worst outcome of various ulcers was recorded as final outcome. All the subjects gave informed consent and clearance was obtained from the Institutional Ethics Committee (IEC).

2.2. Clinical examination

A detailed history and physical examination was carried out for every subject. Age, sex, anthropometric measurements (body mass index), duration of diabetes, glycemic control prior to and during the hospital stay, lipid profile, presence of retinopathy, nephropathy (creatinine >1.5 mg% or presence of micro- or macroalbuminuria), neuropathy (absence of perception of the Semmes–Weinstein monofilament at 2 of 10 standard planter sites on either foot), peripheral vascular disease (ischaemic symptoms and intermittent claudication of rest pain, with or without absence of pedal pulses or posterior tibial pulses), hypertension, duration, site, and size of ulcer, history of smoking, history of previous amputation and clinical outcome were noted in every patient. Clinical assessment for signs of infection (swelling, exudates, surrounding cellulitis, odour, tissue necrosis, crepitation and pyrexia) was made by one researcher classifying the ulcers and determining the presence of clinical signs of infection. Ulcer size was determined by multiplying the longest and the widest diameters and expressed in centimetres square. The wound was graded and staged at the time of hospitalization according to the University of Texas Wound classification system as grade 1 (superficial wound, not involving, tendon, capsule or bone), grade 2 (wound penetrating to tendon or capsule) and grade 3 (wound penetrating bone or joint). Grade 0 patients (pre- or post-ulcerative site that has healed) were excluded from the study. Diagnosis of extension to the bone was made in majority of patients by probing with a sterile steel probe. In the absence of sinus tract or an exposed bone, a standard radiograph showing signs of osteomyelitis in the bone was considered definitive and later on MRI was done to confirm the osteomyelitis in suspected patients. Amputation was defined as the complete loss in the transverse anatomical plane of any part of the lower limb.

2.3. Microbiological methods

Culture of specimens, antimicrobial susceptibility testing, ESBL and MRSA were described elsewhere [1]. Biofilm production assay was adopted from our previous study [6].

2.4. Statistical analysis

Patients were divided into an amputation group (patients underwent major and minor amputations) and non-amputation group. Quantitative variables were expressed as mean ± sd. The normality of the distribution of each continuous variable was assessed using the Kolmogorov–Smirnov test. If normality was established, Odds ratios (strength of association) and Risk ratio (the probability of the amputation) with 95% confidence interval (CI) were reported for independent variables associated with the outcome variable: amputation and non-amputation. All analysis was performed using SPSS 19.0 software. *P*-values less that 0.05 were considered significant.

3. Results

Baseline characteristics of patients with and without amputation are shown in Table 1. Males were predominant 105(64.8%).

Table 1
Baseline characteristics of diabetic foot ulcer patients with and without amputation.

Factors	Non-amputation 116(71.6)	Amputation 46(28.4)	*P* value
Sex (M/F)	69/47	37/9	0.006
Age (years)	46.9 ± 12.8	49.8 ± 13.6	0.182
Smoking (Yes/No)	30/16	73/43	0.278
Duration of diabetes(years)	17.7 ± 5.3	13.3 ± 5.7	<0.001
Duration of ulcer (days)	42.2 ± 45.5	45.6 ± 59.8	<0.001
Size of ulcer (cm^2)	25.9 ± 57.9	16.4 ± 18.7	<0.001
HbA1c(%)	9.8 ± 1.9	9.1 ± 2.16	0.015
WBC count($10^3/\mu l$)	10.2 ± 4.7	9.59 ± 3.4	0.006
Neutrophil granulocyte ($10^3/\mu l$)	4.9 ± 1.23	5.3 ± 1.61	0.085
Hb (g/dl)	10.3 ± 2.0	10.3 ± 2.4	0.084
Serumcreatinine (mg/dl)	1.21 ± 0.52	1.45 ± 0.67	<0.001
LDL-C (mg/dl)	100.4 ± 28.5	105.1 ± 35.0	0.102
HDL-C (mg/dl)	43.8 ± 8.6	43.4 ± 5.8	0.200
Total cholesterol (mg/dl)	174.3 ± 33.4	183.6 ± 36.4	0.138
Triglycerides (mg/dl)	143.6 ± 73.1	164.6 ± 102.5	<0.001
SGOT/AST (IU/L)	18.8 ± 10.5	18.4 ± 8.4	0.002
SGPT/AST (IU/L)	17.2 ± 11.0	16.7 ± 9.1	<0.001
Urinary albumin (mg/L)	23.07 ± 16.5	26.41 ± 17.8	0.325
Hospital stay(days)	24.0 ± 17.0	27.5 ± 22.1	<0.001

Data are mean ± sd or *N*(%) unless otherwise indicated.
WBC, white blood cells, Hb, haemoglobulin; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; SGOT/AST,serum glutamic oxaloacetic transaminase/aspartate transaminase; SGPT/AST, serum glutamate–pyruvate transaminase/aspartate transaminase.

All patients had ulcers graded 1–3 in the university of Texas classification system. Majority of subjects had T2DM 134(82.7%). The mean age of the subjects was 51.1 ± 11.4 years. The mean duration of diabetes was 13.5 ± 4.6 years, and nearly 111(68.5%) had the condition for ≤10 years. Eighty two patients (50.6%) had neuropathy, 72(54.4%) nephropathy, 82(50.6%) retinopathy, 92(56.7%) were hypertensive and 4.32% had PVD. Nearly one third 60(37%) of subjects had ulcer for >1 month before presentation at the hospital. The infection in ulcer was superficial in 48(29.6%) cases, subcutaneous 94(58%) and osteomyelitic in 20(12.3%) patients. Glycemic control was poor in 123(69.7%) during the first five days of hospital stay. Glycemic control was poor in majority of the subjects 147(90.7%) at the time of admission in the hospital. Majority of the ulcers were found on interdigit (43.2%), followed by plantar surface (28.4%), heel (29.5%), margins (12.6%), malleoli (16.7%), and leg (3.6%) and on multiple areas (≥2 sites) as 31.4%. Size of ulcer ≤4 cm^2 was observed in 38(23.4%) patients and ≥4 cm^2 in 124(76.5%) in which majority of the patients were males. Grade I ulcer was found in 48(29.6%), Grade II in 94(58%) and Grade III in 20(12.3%) patients.

3.1. Microbiological observations

A total of 275 (aerobic + anaerobic) bacteria were isolated, averaging of 1.70 (1.57 aerobic, 0.10 anaerobic) species per patient. 35% patients had monomicrobial infection and polymicrobial etiology was observed in 65%.

In the direct microscopic examination of gram stained ulcer samples, 96% had corresponding results to the culture growth on next day, in 2% samples direct result differ in their culture growth and in 2% cases, direct examination could not be done. Among the aerobic bacterial isolates, gram positive cocci comprised of 34.5% and gram negative bacilli of 65.4%. Gram positive to gram negative ratio was 1:1.8.The frequency of bacterial isolates from the DFU is shown in Table 2. *Escherichia coli* was the most common isolate, accounting for 27.8%; followed by *Staphylococcus aureus* 23.5%, *Pseudomonas aeruginosa* 15.6%, *Klebsiella oxytoca* 7%, *Klebsiella pneumoniae* 5.8%, *Proteus vulgaris* and *Enterococcus faecalis* 3.5% each, *Acinetobacter* sp. 3.1%, *Coryneform* sp. 2.7%, beta haemolytic *streptococcus* sp and CONS 2.3% each, *Proteus mirabilis 1.5%* and *Morganella morganii 0.7%*. Anaerobic gram-positive cocci were found

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Table 2
Frequency of distribution of isolates from 162 DFU patients in relation to treatment.

Name of isolates	Total *N*(%)	Amputed foot *N*(%)	Non-amputed foot *N*(%)
Aerobic			
Gram positive cocci	88(34.5)	19(21.5)	69(78.4)
1. *Staphylococcus aureus*	60(23.5)	15(25)	45(75)
2. *Enterococcus faecalis*	9(3.5)	2(22.2)	7(77.8)
3. *Beta hemolytic streptococcus*	6(2.3)	2(33.3)	4(66.6)
4. *CONS*	6(2.3)	–	6(100)
5. *Coryneform sp*	7(2.7)	–	7(100)
Gram negative bacilli	167(65.4)	70(41.9)	97(58.0)
6. *Escherichia coli*	71(27.8)	36(50.7)	35(49.2)
7. *Pseudomonas aeruginosa*	40(15.6)	11(27.5)	29(72.5)
8. *Klebsiella oxytoca*	18(7.0)	10(55.5)	8(44.4)
9. *Klebsiella pneumoniae*	15(5.8)	5(33.3)	10(66.6)
10. *Proteus vulgaris*	9(3.5)	4(44.4)	5(55.5)
11. *Proteus mirabilis*	4(1.5)	1(25)	3(75)
12. *Acinetobacter sp*	8(3.13)	3(37.5)	5(62.5)
13. *Morganella morganii*	2(0.7)	–	2(100)
Total aerobic	255(93.7)	89(34.9)	166(65.0)
Anaerobic			
Gram positive cocci	10(58.8)		
14. *Peptostreptococcus sp*	6(35.2)	–	–
15. *Peptostreptococcus anaerobius*	4(23.5)	–	–
Gram positive bacilli	5(29.4)		
16. *Propionibacterium sp*	3(17.6)	–	–
17. *Clostridium perfringens*	1(5.8)	–	–
18. *Eggerthella lenta*	1(5.8)	–	–
Gram negative bacilli	2(11.7)		
19. *Bacteroides ureolyticus*	2(11.7)	–	–
Total anaerobic	17(6.25)		
Total	272(100)		

N(%): number(percentage).

in 10(6.1%) patients, 5(3.0%) patients had infection by anaerobic gram-positive bacilli and only two (1.2%) patients had infection by anaerobic gram negative bacilli. The remaining 145 patients (89.5%) were found negative for anaerobic culture. Among the anaerobic bacteria isolated, gram positive cocci comprised of 58.8%, gram positive bacilli 29.4% and gram negative bacilli for 11.7%. *Peptostreptococcus* sp. was the most common isolate, accounting for 35.2%; followed by *Peptostreptococcus anaerobius* 23.5%, *Propionibacterium* sp. 17.6%, *Bacteroides ureolyticus* 11.7%, *Clostridium perfringens* 5.8% and *Eggerthella lenta 5.8%* were isolated from DFU patients.

3.2. Antibiotic resistance profile

The result of resistance studies are summarized in Fig. 1. Higher percentage of resistance (75.2%) was shown among the Penicillin group [*Group A:81.7%*; *Group B: 68.7%*], followed by Lincosamides (71.7%):[*Group A:86.8%*; *Group B: 55.6%*], Macrolide (69.8%): [*Group A:68.2%*; *Group B: 71.4%*], Monobactam (63.5%): [*Group A:58.5%*; *Group B: 68.8%*], Aminoglycoside (61%): [*Group A:62.6%*; *Group B:59.8%*], Quinolones and Fluoroquinolones (60.5%): [*Group A:72.7%*; *Group B:48.3%*], Cephalosporin (57.2%):[*Group A: 70.9%*; *Group B:43.5%*], Chloramphenicol (50.8%): [*Group A:40.4%*; *Group B: 61.2%*], beta-lactamase inhibitor(21.7%): [*Group A:22.2%*; *Group B:21.2%*] and Carbapenems (23.1%): [*Group A:24.7%*; *Group B: 21.6%*]. All the anaerobes were susceptible to metronidazole, amoxicillin + clavulanate and Imipenem.

3.3. ESBL, MRSA and biofilm production

Bacteria isolated from patients in whom amputation was done showed high positivity in production of ESBL, in both screening and confirmatory test. These were 73.4% and 72.5% in screening and 73.6% and 56.7% by confirmatory ESBL test by bacteria isolated from amputated patients. Infection by MRSA producing *S. aureus* isolates were higher (93.3%) from amputated patents as compared to non-amputated patients (68.1%). Similarly, biofilm production in isolates of amputated foot was 65.1% and in non-amputated foot as 61.9%.

In a Kolmogorov–Smirnov normality test, there was no significant difference in age, haemoglobin level, total serum protein, total cholesterols, HDL-C and LDL-C between the two groups. When compared with DFU patients without amputation, patients with amputation had longer hospital stay [$P < 0.001$], longer duration of ulcer [$P < 0.001$], ulcer size >4 cm^2 [$P < 0.001$], duration of diabetes [$P < 0.001$], smoking [$P < 0.001$], nephropathy [$P = 0.02$], HbA1c >7% [$P < 0.015$], increased level of white blood cells (WBC) counts [$P < 0.006$], triglycerides [$P < 0.001$], SGOT/AST [$P = 0.002$], SGPT/AST [$P < 0.001$], serum albumin [$P < 0.011$] and serum globulin [$P < 0.001$] (Table 1).

In a univariate analysis (Table 3), odds ratio and risk ratio were calculated in between the two groups. Significant factors which were most likely to have an risk factor for amputation were male sex [$P = 0.01$, OR 2.8, RR 1.28], PVD [$P = 0.02$, OR 6.95, RR 2.5], hypertension [$P = 0.009$, OR 2.83, RR 1.31], chronic sensory peripheral neuropathy [$P < 0.002$, OR 3.01, RR 1.35], nephropathy [$P = 0.02$, OR 2.24, RR 1.26], SGPT [$P < 0.000$, OR 5.96, RR 1.51], LDL-C (>100 mg/dl)[$P = 0.014$, OR 2.53, RR 1.28], total cholesterol (>150 mg/dl) [$P < 0.0003$, OR 3.74, RR 1.52], triglycerides (>200 mg/dl) [$P > 0.005$, OR 5.44, RR 1.76], previous antibiotic use [$P < 0.0001$, OR 9.12, RR 1.92], infection type such as osteomyelitis [$P < 0.007$, OR 3.7, RR 1.67], subcutaneous wound [$P = 0.002$, OR 0.33, RR 0.72] and biofilm production [$P < 0.0008$, OR 4.52, RR 1.41]. While the factors having odds and risk ratio in favour of risk factors

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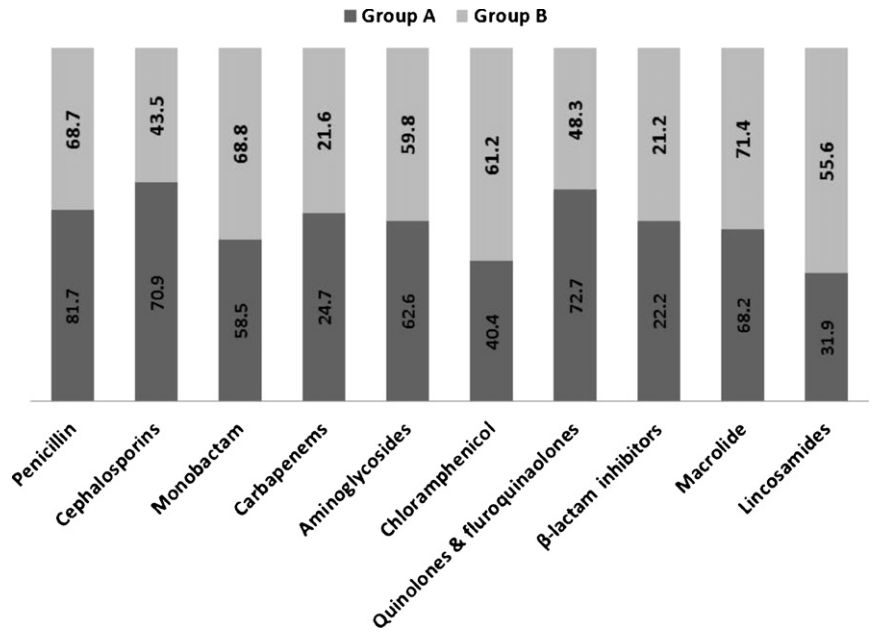

Fig. 1. Distribution of resistance percentage of bacteria isolated from DFU in relation to treatment.
[Group A = resistance % of bacteria isolated from amputated foot; group B = resistance % of bacteria isolated from non-amputated foot].

were age [$P = 0.69$, OR 1.26, RR 1.06], T2DM [$P = 0.81$, OR 1.23, RR 1.05], size of ulcer [$P = 0.51$, OR 1.45, RR 1.10], SGOT [$P = 0.365$, OR 1.46, RR 1.11], HDL-C (<40 mg/dl) [$P = 0.132$, OR 1.19, RR 1.18], smoking [$P = 0.857$, OR 1.10, RR 1.02] and superficial infection in wound[$P = 0.252$, OR 1.60, RR 1.15].

4. Discussion

Foot complications among people with diabetes are common. Most of the foot problems associated with diabetes in India are neuropathic and infective rather that vascular in origin as in devel-

Table 3
Univariate analysis of risk factors for amputation inDFU.

N = 162	Amputation	Conservative	*P* value	OR(95%CI)	RR(95%CI)
N	**46(28.4)**	**116(71.6)**	–	–	–
Sex (Male)	37(80.4)	69(59.4)	**0.01**	**2.8(1.23–6.34)**	**1.28(1.07–1.54)**
Age >41 years	35(76.0)	83(71.5)	0.69	1.26(0.57–2.78)	1.06(0.86–1.31)
T2DM	39(84.7)	95(81.8)	0.81	1.23(0.48–3.13)	1.05(0.83–1.34)
Size of ulcer (>4 cm^2)	39(84.7)	92(79.3)	0.51	1.45(0.57–3.65)	1.10(0.88–1.37)
Complications					
Hypertension	34(73.9)	58(50.0)	**0.009**	**2.83(1.33–6.01)**	**1.31(1.08–1.58)**
Retinopathy	21(45.6)	61(52.5)	0.53	0.75(0.38–1.50)	0.92(0.76–1.23)
Neuropathy	32(69.5)	50(43.1)	**0.002**	**3.01(1.45–6.24)**	**1.35(1.10–1.65)**
Nephropathy	27(58.6)	45(38.7)	**0.02**	**2.24(1.18–4.49)**	**1.26(1.02–1.55)**
PVD	5(10.6)	2(1.7)	**0.02**	**6.95(1.2–37.2)**	**2.5(0.79–8.33)**
Grade of ulcer (Texas)					
1	17(36.9)	31(26.7)	0.252	1.60(0.77–3.3)	1.15(0.91–1.41)
2	18(39.1)	76(65.5)	**0.002**	**0.33(0.16–0.68)**	**0.72(0.58–0.90)**
3	11(23.9)	9(7.7)	**0.007**	**3.7(1.43–9.7)**	**1.67(1.02–2.7)**
Previous antibiotic use	35(76.0)	30(25.8)	**<0.001**	**9.12(4.11–20.1)**	**1.92(1.46–2.52)**
Bacterial infection type					
Superficial	17(36.9)	31(26.7)	0.252	1.60(0.77–3.3)	1.15(0.91–1.41)
Subcutaneous	18(39.1)	76(65.5)	**0.002**	**0.33(0.16–0.68)**	**0.72(0.58–0.90)**
Osteomyelitis	11(23.9)	9(7.7)	**0.007**	**3.7(1.43–9.7)**	**1.67(1.02–2.7)**
HbA1c (>7%)	40(86.9)	107(92.2)	0.367	0.56(0.18–1.67)	0.82(0.53–1.26)
Smoking history	30(65.2)	73(62.9)	0.857	1.10(0.54–2.25)	1.02(0.84–1.25)
WBC	27(58.6)	93(80.1)	**<0.004**	**2.80(1.39–5.66)**	**1.35(1.08–1.69)**
Hb	24(52.1)	48(41.3)	<0.224	1.54(0.77–3.07)	1.13(0.92–1.38)
Serum creatinine(>1.5 mg/dl)	23(50.0)	26(22.4)	**<0.0004**	**3.46(1.67–7.14)**	**1.54(1.13–1.98)**
SGOT/AST(>34 IU/L)	20(43.4)	41(35.3)	0.365	1.46(0.72–2.94)	1.11(0.90–1.37)
SGPT/AST(>35 IU/L)	39(84.7)	56(48.2)	**<0.0001**	**5.96(2.46–14.4)**	**1.51(1.26–1.83)**
Lipid profile					
LDL-C (>100 mg/dl)	33(71.7)	58(50.0)	0.14	2.53(1.21–1.55)	1.28(1.05–1.55)
Total cholesterol (>150 mg/dl)	25(54.3)	28(24.1)	**0.0003**	**3.74(1.82–7.68)**	**1.52(1.16–2.0)**
HDL-C (<40 mg/dl)	10(21.7)	40(34.4)	0.133	0.52(0.23–1.17)	0.84(0.70–1.02)
Triglycerides (>200 mg/dl)	27(58.6)	24(20.6)	**<0.005**	**5.44(2.6–11.4)**	**1.76(1.30–2.3)**
Biofilm infection	39(84.7)	64(55.1)	**<0.0008**	**4.52(1.87–10.9)**	**1.41(1.18–1.69)**

OR: Odds ratio; RR: Risk Ratio; PVD: peripheral vascular disease; WBC, white blood cells, Hb, haemoglobulin; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; SGOT/AST, serum glutamic oxaloacetic transaminase/aspartate transaminase; SGPT/AST, serum glutamate–pyruvate transaminase/aspartate transaminase. Data are in *N*(%) otherwise indicated.
The bold values indicate that those are the main factors which are associated with the risk of amputation in the Diabetic foot ulcer patients.

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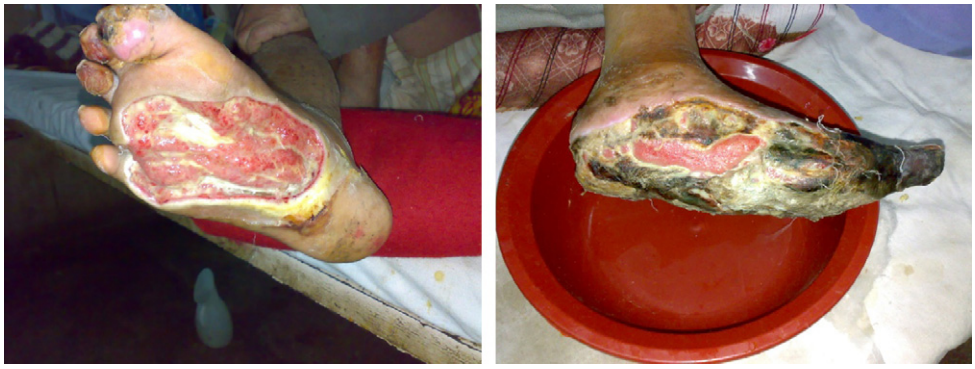

Fig. 2. Images of diabetic foot ulcer patients.

oped countries [7]. The rate of amputation varies significantly in different regions of the world. In USA, the Strong Heart Study has reported only 4.4% amputation in their 8 years follow-up of 1974 DFU patients [8]. In India, Vishwanathan and Kumpatla in their multicentric study have reported high prevalence of amputation rate of 65.2%, which include 3, 4, 3 and 21 study centres from North, East, West and South India, respectively [4]. Recently, we have reported 22.5% amputation in our previous study [1]. In the present study, the overall amputation rate was 28.4%, which was higher from our previous report [1]. The reduction of foot amputations as compared to report of Vishwanathan and Kumpatla from South India [4] was probably achieved by well-organized multidisciplinary foot care teams, good glycemic control, offloading, debridement and proper selection of antibiotics and by educating patients on foot care in the present study (Fig. 2).

Several risk factors for amputation among the patients with diabetes have been cited in the literature including age [8], male sex [9,10], size of ulcer [11], hypertension [9,10], neuropathy [10], nephropathy [8,10,12], poor glycemic control [8], white blood cells [13,14] and lipid abnormalities [15,16]. Although there are inconsistencies among studies regarding all the various risk factors for amputation, PVD was identified by different studies as an independent risk factor. In our analysis presence of PVD also led to a significant higher rate of amputations [$P = 0.02$, OR 6.95; RR 2.5], which was compatible with previous studies. The development of a foot ulcer was considered to be the result of multiple factors including peripheral neuropathy, foot deformity, external trauma, PVD and peripheral oedema [12]. Most important risk factor for amputation reported from India was infection, glycemic control, duration of diabetes, vascular complications [4,16]. Adequate blood supply was vital for ulcer healing and combating the ulcer infections. However, patients with diabetes had a higher prevalence of PVD than patients without diabetes [3]. Thus, it was not surprising that PVD was associated to be one of the important risk factors with amputation.

In the present study, the risk of amputation was associated with male sex, a consistent finding in at least two previous prospective studies in American Indians. In the Oklahoma Indian Diabetes Study [9] ($N = 875$), risk of incidence in men was twice then in women, and in a study of 4399 Pima Indians, the rate of amputation in men was 2.6 times higher than in women, adjusted for age and diabetes duration [10]. However, the observation that men are at higher risk is not a universal finding. In a national study of 20-year risk of amputation, sex did not predict amputation [8]. One reason for the absence of a sex effect in the latter study may be due to the fact that it included a mix of individuals with and without diabetes, potentially masking an important sex effect in studies exclusively of individuals with diabetes. Several factors might explain the persistent observation that men with diabetes experience more amputation(s) than women with diabetes. It is possible that men experience more minor trauma to the foot that ultimately results in amputation in view of their outdoor activities, walking barefoot especially among rural subjects in India.

Presence of chronic sensory peripheral neuropathy was found to be a predictive risk factor for amputation in our study, similar to findings by Nelson et al. [10]. A threefold sex differences in amputation risk in the current report were similar to the studies conducted by others [9,10]. Nephropathy [$P = 0.02$, OR 2.24, RR 1.26] was found to be significant predictive factors for limb loss in our cohort. Studies conducted by Resnick et al. [8] and Nelson et al. [10] also found nephropathy to be a significant prognostic factor. However, Gurlek et al. [17] did not find nephropathy to be a significant predictive factor. In contrast, retinopathy was not found to be a significant factor in our study, similar to findings by Gurlek et al. [17]. The size of ulcer >4 cm^2 is an important predictor of amputation in the present study. The greater the area and the longer the time taken by the ulcer for healing and more the possibility that amputation would be undertaken [11]. In accordance with our study results, Winkley et al. [18] also found patients who underwent amputation had increased ulcer size.

Leukocyte Count (WBC) count was also found to be independent risk factors for overall amputation in our study with the odds and risk ratios of 2.80 and 1.35, respectively. In accordance with present observation, Yesil et al., also found that patients who underwent amputation had increased WBC counts at baseline [13]. Recently WBC count >11,000 cells/μl in a severe diabetic foot ulcer patients was reported to be single most important predictive marker for poor clinical response [14]. Diabetes typically exhibited mixed dyslipidemia characterized by elevated triglycerides and low level of HDL-C. These lipid abnormalities and high serum cholesterol level have proven to be a major risk factor for cardiovascular risk and have commonly been linked with worst outcome [15]. In the present study, the levels of triglycerides (>150 mg/dl), cholesterol (>150 mg/dl), LDL-C (>100 mmol/l), HDL-C (<40 mg/gl) were associated with the risk of amputation as also reported by Chaturvedi et al. [16].

Poor glycemic control was significant risk factors for poor outcome in the present study as reported by other workers [8]. Duration of diabetes was not found to be a predictive factor for amputation in our study, similar to findings by Gurlek et al. [17]. Our finding of no association between smoking and amputation risk is consistent with findings from a study of Pima Indians [10] and Gurlek et al. [17]. A likely explanation for our finding is that smokers in this part of India (North), smoke fewer cigarettes per day than the national average.

Other risk factors that are significant were type of infection in foot. In the present study, subcutaneous and osteomyelitis infection were significantly associated the risk of amputations. However osteomyelitis was found to be a significant prognostic factor [17]. The Biofilm infection in their ulcer was also found to be

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an independent and the most important risk factor for amputation with odds and risk ratio of 4.52 and 1.41, respectively. According to our literature search, this was the first report of biofilm production as risk factor for amputation. The resistance in the biofilm producing bacteria was high as compared to non-biofilm producers, reported in our previous studies [6]. The biofilm producing bacteria have close cell–cell contact that permits easy transfer of plasmids to one another than in the planktonic state. These plasmids can encode for resistance to several different antimicrobial agents. The biofilm also provides a physical protection to bacteria because antimicrobial agents are also ineffective in penetrating the biofilm, decreasing the concentration acting on the bacterial cells within the biofilm and as a consequence their efficacy [19]. In addition, biofilm also appear to have an antiphagocytic property within the biofilm, which renders leukocytes present within the matrix ineffective [20]. Additionally, there appears to be a component within the polysaccharide that inactivates and traps both complement and host antibodies. These factors lead to an accumulation of host immune factors that can lead to host tissue damage and eventually chronic inflammation.

Diabetic foot infections are usually polymicrobial in nature and this has been well documented in the literature. In the present study polymicrobial etiology was found in 65% and monomicrobial in 35% patients with the rate of isolation of about 1.70 bacteria per patient which is lower than the previous studies [21] that showed rate of isolation between 2.3 and 5.8. The major infective organisms in diabetic foot ulcers in our patients appear to be different. We found gram negative aerobic bacteria were most frequently isolated which is in accordance with our previous reports [1,6] and in tune with a similar study from reported from different regions of India [21]. The gram positive to gram negative ratio was 1:1.8 which is in similar to the findings reported earlier [1]. MRSA were found in 73.3% of *S. aureus* isolates which differs from the older studies that shows predominance of gram positive ones [22]. Gram-negative bacteria that are regarded as normal flora of the skin may cause severe tissue damage in diabetics. We suggest that they should be regarded as significant in diabetic foot ulcers. In our anaerobic study, *Peptostreptococcus* sp was the most predominant one which is, in accordance to the previous study [23]. Other anaerobes isolated in their study were *Bacteroides fragilis*, *Clostridium* sp, *Eggerthella lenta* and *Propionibacterium*. We recovered fewer anaerobic species compared with earlier anaerobic culture reports [21]. In the present study, we have isolated few anaerobic bacteria from DFU patients because most of our patients did not have chronic draining wounds, and only 12% had gangrene associated with their infections. This may be an indication of fewer anaerobic species among nonthreatening lower-extremity infections, which is also reported earlier by Lipsky and Berendt [24].

The present study confirms the prevalence of both MRSA isolates and ESBL producing gram negative bacteria (73.3% & 68.67.8%, respectively) as compared with previous studies [25]. None of the gram positive isolates were resistant to vancomycin (VRSA). Gram negative bacilli were isolated as ESBL producers in 67.8% similar to our previous findings [1].

The present study has some important limitations. First, information on neuropathic assessment, degree of blood pressure control, lipid controls and assessment of chronic glycemia in the past was not available. To what extent estimates of these risk factors assessment have influenced the risk factors is difficult to estimate. Further, the high rate of antibiotic resistance may be due to the fact that being a tertiary care hospital, referring hospital/physicians have already tried and failed to control infection using combination of different antimicrobials. The facilities for microbiological studies at the first contact physician/surgeon are usually not available at district hospitals/smaller cities in India. Further, the indiscriminate use of antimicrobials therapy without establishing the etiology is a common practice. All these factors are disconcerting because infection with these organisms limits the choice of antibiotic treatments and may lead to a worse outcome. These observations are important for patient's management and underscore the need for institutional infections control committee to develop antibiotic treatment policies.

In summary, this study showed the profile of amputation in a north Indian tertiary care hospital. Infection was found to be the major cause of amputation. The risk factors for the amputation in these patients with a diagnosis of DFU include presence of peripheral neuropathy, co-morbid conditions like nephropathy and dyslipidemia, elevated WBC, biofilm production, high grade of ulcer and bacterial infection type (subcutaneous and osteomyelitis). Following a diagnosis of DFU, more intensive surveillance and aggressive care by a multidisciplinary team involved in diabetic foot care may improve patient's outcome and reduce the amputation. The result of this study, therefore, alerts us the need for proper management of the patients and in deciding the antibiotic treatment policies.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Clinico-microbiological study and antimicrobial drug resistance profile of diabetic foot infections in North India

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ABSTRACT

The study was carried out in diabetic patients with foot ulcer to determine the microbiological profile of infected ulcer, antibiotic resistance of the isolates and to find out the potential risk factors for infection with multidrug resistance and the outcome of these infections. A detailed clinical history and physical examination was carried out in each patient. Pus samples for bacterial culture were collected from 102 patients admitted with diabetic foot infections. All patients had ulcer with Texas grades 1–3. Seventeen patients (16.6%) had coexisting osteomyelitis. Aerobic gram negative bacilli were tested for extended spectrum β lactamase (ESBL) production by phenotypic and genotypic methods. Staphylococcus isolates were tested for susceptibility to oxacillin and cefoxitin by disk method. Potential risk factors for MDRO positive samples were explored. Gram negative aerobes were most frequently isolated (63.8%), followed by gram positive aerobes (36.1%) and anaerobes (31.4%). Forty five percent of patients were positive for MDROs. ESBL production and methicillin resistant was noted in 68.5% and 43.2% of bacterial isolates respectively. 34.5% gram negative strains were positive for $bla_{CTX\text{-}M}$ gene followed by bla_{SHV} (23%) and bla_{TEM} (7.4%). Among the anaerobic organism 17(31.4%) from 54 patients, most commonly isolated were *Peptostreptococcus* sp. (35.2%). MDRO positive status was associated with the presence of neuropathy ($P < 0.001$), osteomyelitis ($P < 0.001$), and ulcer size >4 cm^2 ($P < 0.001$) but not with patients characteristic, ulcer type and type of diabetes, or duration of hospital stay. Poor glycemic control in 68.6% patients, duration of infection > 1 month (36.2%) and ulcer size > 4 cm^2 (75.4%) were independently associated with risk of MDR organisms infection.

1. Introduction

Diabetic foot ulceration and infections are a major medical, social, economic problem and a leading cause of morbidity and mortality, especially in the developing countries like India [1–3]. Fifteen percent of all diabetic patients develop a foot ulcer at some point in their lives which is highly susceptible to infections that spreads rapidly, leading to overwhelming tissue destruction and subsequent amputation [4,5]. In addition to foot ulcers, diabetic patients have also functional changes in microcirculation and changes in cellular activity and growth factor activation processes, which increasingly disrupts wound healing [6]. Antimicrobial resistance is considered to be a major public health threat [7]. The most important cause of antimicrobial resistance is overusing an inappropriate use of antibiotics [8,9]. Diabetic patients with foot ulcers have several factors that may be associated with a high risk of multidrug resistant microorganisms (MDRMs) carriage, such as inappropriate antibiotic treatment, chronic course of the wound, and frequent hospital admissions. Furthermore, peripheral arterial diseases are often present in patients with diabetic foot ulcers (DFUs) and may lead to poor penetration of antibiotics into the lower limb tissues, thereby promoting selection of resistant bacterial strains. Although in DFU, different microorganisms or mixed bacteria are usually responsible for the infection depending on the status of the ulcer, *Staphylococcus aureus* and coagulase negative Staphylococci are the most frequently isolated microorganisms [10,11]. In recent years, there has been an increase in the incidence and prevalence of ESBLs. Currently there is paucity of data on extended spectrum beta lactamases (ESBLs)-producing organisms from diabetic foot infections especially in this part of World. Infection with MDROs may increase the duration of hospital stay, cost of management and may cause additional morbidity and mortality [7]. Early diagnosis of microbial infections is aimed to institute the appropriate antibacterial therapy to avoid further complications. Therefore, this study was planned with the objective to determine the bacterial profile and antimicrobial resistance profile of organisms isolated from patients with DFUs. The potential risk factors for infection of ulcers with

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MDRMs and the outcome of these infections were also studied.

2. Materials and methods

2.1. Study design

Out of 342 diabetic patients admitted in the Centre for Diabetes and Endocrinology, J.N.M.C, A.M.U. Aligarh, India, 102 who developed ulcer in their foot during December 2008 to February 2010 were included in this study. All the subjects gave informed consent and clearance was obtained from the Institutional Ethics Committee (IEC).

2.2. Clinical examination

A detailed history and physical examination was carried out for every subject. Age, sex, anthropometric measurements (body mass index), duration of diabetes, glycemic control during the hospital stay, lipid profile, presence of retinopathy, nephropathy (creatinine > 1.5 mg% or presence of micro or macroalbuminuria), neuropathy (absence of perception of the Semmes–Weinstein monofilament at 2 of 10 standard planter sites on either foot), peripheral vascular disease (ischaemic symptoms and intermittent claudication of rest pain, with or without absence of pedal pulses or posterior tibial pulses), hypertension, duration, site, and size of ulcer, history of smoking, history of previous amputation and clinical outcome were noted in every patient. Clinical assessment for signs of infection (swelling, exudates, surrounding cellulitis, odor, tissue necrosis, crepitation and pyrexia) was made by one researcher classifying the ulcers and determining the presence of clinical signs of infection. Ulcer size was determined by multiplying the longest and the widest diameters and expressed in centimeters squared [11,12]. The wound was graded and staged at the time of hospitalization according to the University of Texas wound classification system [13] as grade 1 (superficial wound, not involving, tendon, capsule or bone), grade 2 (wound penetrating to tendon or capsule) and grade 3 (wound penetrating bone or joint). Grade 0 patients (pre- or post-ulcerative site that has healed) were excluded from the study. Diagnosis of extension to the bone was made in majority of patients by probing with a sterile steel probe. In the absence of a sinus tract or an exposed bone, a standard radiograph showing signs of osteomyelitis in the bone was considered definitive and later on MRI was done to confirm the osteomyelitis in suspected patients.

2.3. Microbiological methods

Culture specimens were obtained at the time of admission; after the surface of the wound had been washed vigorously by saline, and followed by debridement of superficial exudates. Specimens were then obtained by scrapping the base of ulcer or the deep portion of the wound edge with a sterile curette after cleaning the base of ulcer with a sterile swab stick [3,13,14]. The soft tissue specimens and pus aspirated from syringe were promptly sent to the Microbiology Department and processed for aerobic and anaerobic bacteria. Standard methods for isolation and identification of aerobic [15,16] and anaerobic bacteria were used [17,18].

2.4. Susceptibility testing

Antimicrobial susceptibility testing of aerobic isolates was performed using the disk diffusion method as described by the CLSI [19]. Antimicrobial disks used were imepenem (10 μg), aztreonam (30 μg), amoxyclav (30 μg), cefpodoxime (10 μg), metronidazole (5 μg), cefepime (30 μg), cefoperazone (75 μg), cefoperazone/sulbactum (75/10 μg), cefixime (5 μg), piperacillin (100 μg), piperacillin/tazobactum (100/10 μg), ceftazidime (30 μg), ceftazidime/clavulanic acid (30/10 μg), amikacin (30 μg), amoxycillin (20 μg), cephotaxime (30 μg), ofloxacin (5 μg), cephotaxime/clavulanic acid (30/10 μg), ceftriaxone (30 μg), cefoxitin (30 μg), oxacillin (1 μg), chloramphenicol (30 μg), gentamicin (10 μg), gatifloxacin (5 μg), levofloxacin (5 μg), sparfloxacin (5 μg), streptomycin (10 μg), vancomycin (30 μg), clindamycin (2 μg), tobramycin (10 μg), azithromycin (15 μg), erythromycin (15 μg), and bacitracin (μg). All disks were obtained from Hi-Media Labs, Mumbai, India. Interpretative criteria for each antimicrobial tested were those recommended by manufacturer's guideline (Hi-Media Labs, Mumbai, India).

2.5. Phenotypic methods for MRSA and ESBL detection

Staphylococcus species were tested for methicillin resistance by using 1-μg oxacillin disk [20] and 30-μg cefoxitin disk [21]. Gram-negative bacilli were first screened for the production of ESBL by disk diffusion method using cephotaxime, ceftriaxone, aztreonam, cefepime, cefoxitin and ceftazidime and later on confirmed by cephalosporin/clavulanate combination disk test (disk potential test) using ceftazidime, ceftazidime + clavulanic acid, cephotaxime, cephotaxime + clavulanic acid, piperacillin, cefoparazone + sulbactam, cefoparazone and piperacillin + tazobactum [22]. *E. coli ATCC* 25922 (non ESBL-producer), *Klebsiella pneumoniae 700603* (ESBL-producer) and *S. aureus* ATCC 25923 were used as control strains respectively. A microorganism was classified as MDRO if it was found to be resistant to two or more classes of antimicrobials and included MRSA, ESBL producing organisms [7].

2.6. Molecular methods for ESBL detection

2.6.1. Preparation of DNA template

Template DNA was prepared from freshly cultured bacterial isolates by suspending 3–5 colonies in 50 μl of molecular grade water, and then heating at 95 °C for 5 min and immediately chilling at 4 °C. Positive controls harboring $bla_{CTX\text{-}M}$, bla_{TEM} and bla_{SHV} and negative control (*E. coli* ATCC 25922) were processed in the same way for DNA extraction.

2.6.2. Detection of bla genes by PCR

Molecular detection of $bla_{CTX\text{-}M}$, bla_{TEM} and bla_{SHV} was performed in ceftriaxone resistant gram negative isolates by using polymerase chain reaction (PCR) according to the methods described previously with minor modifications [23,24]. The primers and cycling conditions for the detection of *bla* genes were same as described by Shahid et al. [25].

2.7. Antibiotic treatment

Antibiotics were selected according to published recommendation [7]. In mild infections amoxicillin clavulanic acid was given empirically by the oral route. However in moderate infections intravenous route was preferred taking into consideration the likelihood of osteomyelitis. Considering that the causative agent was polymicrobial, we initiated ampicillin-sulbactam plus an aminoglycoside/quinolone or piperacillin-tazobactam or ceftriaxone plus metronidazole/clindamycin. In the presence of severe infections, surgical debridement and amputation were performed immediately after admission. Metronidazole (500 mg I.V. every 8 h) was added to the drug regimen if cellulitis or gangrene was also present. Combinations of extended spectrum antibiotics were initiated and the treatment was later modified in accordance with the culture results. The duration of the treatment was at least 4–6 weeks and

Table 1
Association of study characteristics in two groups of diabetic patients according to the infection of foot ulcer with MDR and non-MDRs (data are mean ± SD or *n* (%) unless otherwise indicated).

N = 102	Total	MDR	Non-MDR	*P* value	O.R. (95% CI)
Gender distribution		*n* = 46	*n* = 56		
Male	67(65.6)	30(65.2)	37(66)	0.72	0.50(0.13–1.8)
Female	37(34.3)	16(34.7)	19(33.9)	0.67	0.49(0.17–1.82)
Age distribution (years)	**49.11 ± 12.46**				
<40	16(15.6)	10(21.7)	8(14.2)	–	–
41–60	65(63.72)	29(63)	36(64.2)	**0.000**	**0.72(0.19–2.68)**
61–80	19(18.62)	7(15.2)	12(21.4)	–	–
Type of diabetes					
Type 1	14(13.72)	6(13)	8(14.2)	–	1.00
Type 2	88(86.27)	40(86.9)	48(85.7)	0.067	3.94(0.97–16.4)
Duration of diabetes (years)	**15.5 ± 6.40**				
≤10	72(70.5)	23(50)	49(87.5)	–	1.00
11–20	26(25.4)	20(43.4)	6(10.7)	0.04	0.74(0.09–2.03)
≥21	4(3.9)	3(6.5)	1(1.7)	–	0.86(0.72–1.05)
Duration of ulcer	**41.52 ± 47.58**				
<1 month	63(61.76)	32(69.5)	31(55.3)	0.032	1.00
>1 month	37(36.27)	14(30.4)	23(41)	0.001	0.54(0.12–1.4)
Size of ulcer	**20.14 ± 44.85**				
≤4 cm^2	25(24.5)	6(13)	20(35.7)	0.47	1.00
>4 cm^2	**77(75.49)**	40(86.9)	37(66)	**<0.001**	**12.6(3.9–33.5)**
Complications					
Hypertension	69(67.6)	44(95.6)	25(44.6)	**0.004**	**3.48(1.26–9.67)**
Retinopathy	54(52.9)	39(84.70)	15(26.7)	**0.000**	**0.84(0.34–2.34)**
Neuropathy	47(46)	37(80.4)	27(48.2)	**<0.001**	**1.32(0.46–3.91)**
Nephropathy	64(62.7)	32(69.5)	15(26.7)	**0.01**	**4.37(1.06–14.6)**
Grade of ulcer (Texas)					
1	16(15.6)	9(19.50)	7(12.5)	0.56	0.43(0.16–1.13)
2	59(57.8)	36(78.2)	23(41)	**0.001**	**0.62(0.13–2.63)**
3	27(26.4)	21(45.60)	6(10.7)	**<0.001**	**2.34(0.74–7.39)**
Nature of ulcer					
Non-necrotic	79(77.4)				
Necrotic	23(22.5)				
Hospital stay (days)	**22.9 ± 15.54**	**26.9 ± 7.2**	**17.9 ± 2.9**	**0.005**	**10.76(7.3–23.8)**
Management					
Amputation	23(22.5)	19(41.3)	4(7.1)	**<0.001**	**5.13(1.77–13.3)**
Conservative	79(77.4)	27(58.6)	52(92.8)	–	–
Previous antibiotic use					
Present	48(47)	36(78.2)	12(21.4)	**0.002**	**0.25(0.09–3.25)**
Absent	54(52.9)	10(21.7)	44(78.5)	–	–
Discharge status					
Alive	97(95)	42(91.3)	55(98.2)	0.06	1.00
Dead	5(4.9)	4(8.6)	1(1.7)	**0.002**	**3.2(1.2–8.2)**
Bacterial infection type					
Superficial	16(15.6)	9(19.5)	7(12.5)	0.56	0.43(0.16–1.13)
Subcutaneous	59(57.8)	36(78.2)	23(41)	**0.001**	**0.62(0.13–2.63)**
Osteomyelitis	27(26.4)	21(45.6)	6(10.7)	**<0.001**	**2.34(0.74–7.39)**

The bold values indicate that those are the main factors which are associated with the MDR infections in the Diabetic foot ulcer patients.

prolonged in cases of osteomyelitis. All patients also received an intensive insulin treatment.

2.8. Statistical analysis

The data were analyzed using SPSS version 13.0 for descriptive statistics. Quantitative variables were expressed as mean ± SD while qualitative variables were expressed as percentage (%). Continuous variables were compared using 2 sample *t* tests for independent samples. Odds ratios and 95% confidence interval (CI) were reported for independent variables associated with the outcome variable: presence of MDR infection.

3. Results

Males were predominant 67(65.6%) in the study subjects. All patients had ulcers graded 1–3 in the University of Texas classification system. Majority of subjects had T2DM 88(86.2%). The mean age of the subjects was 49.1 ± 12.4 years. The mean duration of diabetes was 15.5 ± 6.6 years, and nearly 72(70.5%) had the condition for ≤10 years. Forty-seven patients (46%) had neuropathy, 64(62.7%) nephropathy, 54(52.9%) retinopathy, and 69(67.6%) were hypertensive. Osteomyelitis was present in 27(26.4%) subjects. Nearly one third 37(36.2%) had lesions for >1 month before presentation at the hospital. The ulcer was necrotic in 23(22.5%) cases. Glycemic control was poor in 67(65.6%). HbA1c was <7% in 12 patients (11.7%), 7–8% in 2(1.9%) and >8% in 88(86.2%) subjects. More than 46(45%) received surgical treatment, mainly in the form of debridement. 23(22.5%) patients were subject to amputation and 5(4.9%) died during the hospital stay (mean hospital stay 22.9 ± 15.5) [Table 1]. Glycemic control at the time of discharge was good in 67(55.8%, HbA1c <7%), poor in 40(39.2%, HbA1c >8%) and satisfactory in 5(4.9%, HbA1c 7–8%) subjects (Figs. 1 and 2). Majority of the ulcer was found on interdigits (42.15%), followed by plantar surface (26.4%), heel (23.5%), margins (15.6%), malleoli (13.7%), and leg (4.9%) and on multiple areas (≥2 sites) was 26.4% which include 22 males and only 7 females. Size of ulcer ≤4 cm^2 was observed in 24.5% patients [11 males; 14 females], ≥4 cm^2 in 75.4% in which majority of the patients were males.

Grade I ulcer was found in 15.6%, grade II in 57.8% and grade III in 26.4% patients. The number of DFU patients and their type of isolates (MDR+ and MDR−) within grade is summarized in Table 2.

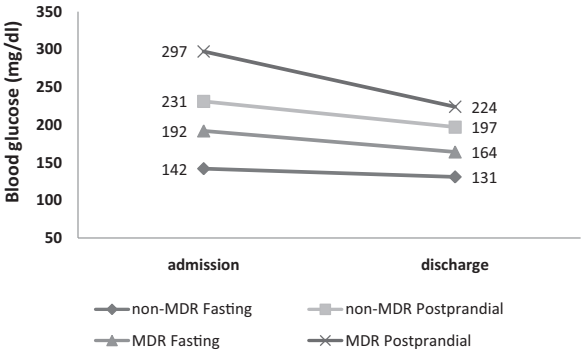

Fig. 1. Fasting and postprandial blood glucose level among diabetic foot ulcer infected with and without MDROs at the time of admission and discharge from the hospital.

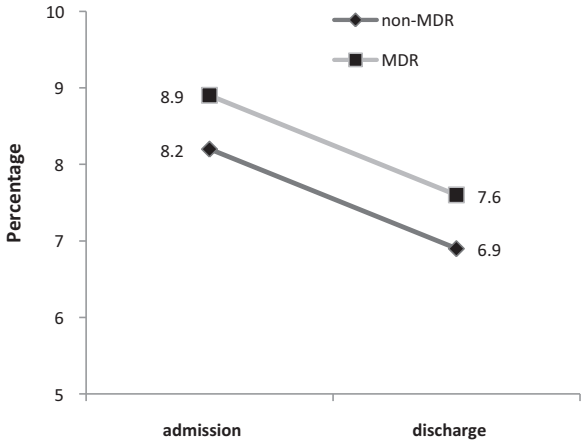

Fig. 2. HbA1c values among the diabetic foot ulcer infected with and without MDROs at the time of admission and discharge from the hospital.

In grade 1, MDR positive and MDR− infection was seen in 5.8% ($P = 0.005$) and 9.8% ($P = O.17$) respectively. In grade 2, MDR positive and MDR− infection was seen in 22.5% ($P = 0.001$) and 35.2% ($P = O.147$) and in grade 3, MDR positive and MDR− infection was seen in 16.6% ($P = 0.006$) and 9.8% ($P = O.035$) patients respectively. In addition, there was a significant relation between the depths of DFU (subcutaneous wound and presence of osteomyelitis) with MDRM infection. In superficial wound, growth of bacteria was significantly lower when compared with subcutaneous and bone involvement infection ($P = 0.001$, $P = 0.000$ respectively). When MDR growth was compared, there was significant correlation with the subcutaneous and osteomyelitis ($P = 0.001$ and $P = 0.006$ respectively). No significant relation was present in MDR-negative with superficial, subcutaneous and presence of osteomyelitis ($P = 0.17$, $P = 0.147$ and $P = 0.035$ respectively).

3.1. Microbiological observations

A total of 152 aerobic bacteria were isolated, averaging of 1.49 species per patient. 38% patients had monomicrobial infection and polymicrobial etiology was observed in 62%.

In the direct microscopic examination of ulcer samples, 94% had corresponding results to the culture growth on next day, in 2% samples direct result differ in their culture growth and in 4%

Table 2
The microorganism growth rate according to the grade of diabetic foot (superficial, subcutaneous or presence of osteomyelitis) and frequency of aerobic and anaerobic bacteria isolated from 102 and 54 diabetic foot ulcer patients respectively. *n* (%).

	MDR		Non-MDR		Total	
	n (%)	*P*	*n* (%)	*P*	*n* (%)	*P*
Superficial	6(5.8)	0.005	10(9.8)	0.17	16(15.6)	0.103
Subcutaneous	23(22.5)	0.001	36(35.2)	0.147	59(57.8)	0.001
Osteomyelitis	17(16.6)	0.006	10(9.8)	0.035	27(26.4)	0.000

	Name of isolates	Total
Aerobic		
	Gram positive cocci	55(36.1)
1	*Staphylococcus aureus*	37(24.3)
2	*Enterococcus faecalis*	5(3.2)
3	*Beta hemolytic streptococcus*	5(3.2)
4	*CONS*	4(2.6)
5	*Coryneform* sp.	4(2.6)
	Gram negative bacilli	97(63.8)
6	*Escherichia coli*	41(42.2)
7	*Pseudomonas aeruginosa*	23(23.7)
8	*Klebsiella oxytoca*	11(11.3)
9	*Klebsiella pneumoniae*	9(9.2)
10	*Proteus vulgaris*	5(5.1)
11	*Proteus mirabilis*	2(2.0)
12	*Acinetobacter* sp.	5(5.1)
13	*Morganella morganii*	1(1.0)
	Total aerobic	152
Anaerobic		
	Gram positive cocci	10(58.8)
14	*Peptostreptococcus* sp.	6(35.2)
15	*Peptostreptococcus anaerobius*	4(23.5)
	Gram positive bacilli	5(29.4)
16	*Propionibacterium* sp.	3(17.6)
17	*Clostridium perfringens*	1(5.8)
18	*Eggerthella lenta*	1(5.8)
	Gram negative bacilli	2(11.7)
19	*Bacteroides ureolyticus*	2(11.7)
	Total anaerobic	17

Table 3
Antimicrobial resistance pattern of bacteria isolated from diabetic foot ulcers in diabetic patients ($N = 152$; n = total number of bacteria isolates of a given type).

	Antimicrobial	*Ps* 23(15.1)	*Ec* 41(26.7)	*Pv* 5(3.2)	*Pm* 2(1.3)	*Mm* 1(0.6)	*Ko* 5(7.2)	*Kp* 7(4.6)	*Ac* 5(3.2)	*Sa* 37(24.3)	Bhs 5(3.2)	*CONS* 4(2.6)	*Cr* 4(2.6)	*En* 5(3.2)	Total
Penicillin	Amoxycillin	16(69.6)	31(75.6)	0(0)	2(100)	1(100)	10(90.9)	8(88.9)	5(100)	18(48.6)	4(80)	3(75)	3(75)	1(20)	95(62.5)
	Amoxyclav	17(73.9)	35(85.4)	5(100)	2(100)	1(100)	3(27.3)	8(88.9)	5(100)	¤	¤	¤	¤	¤	76(50.0)
	Piperacillin	18(78.3)	38(92.7)	3(60)	2(100)	1(100)	11(100)	7(77.8)	4(80)	¤	¤	¤	¤	¤	84(55.3)
	Oxacillin	£	£	£	£	£	£	£	£	16(43.2)	3(60)	4(100)	1(25)	4(80)	39(25.7)
Cephalosporins	Cefoxitin	20(87.0)	12(29.3)	3(60)	1(50)	1(100)	11(100)	2(22.2)	3(60)	18(48.6)	3(60)	3(75)	4(100)	0(0)	78(51.3)
	Ceftriaxone	23(100)	18(43.9)	2(40)	2(100)	1(100)	3(27.3)	7(77.8)	4(80)	26(70.3)	1(20)	3(75)	1(25)	2(40)	93(61.2)
	Cefpodoxime	21(91.3)	21(51.2)	3(60)	2(100)	0(0)	3(27.3)	7(77.8)	4(80)	¤	¤	¤	¤	¤	61(40.1)
	Ceftazidime	11(47.8)	16(39.0)	1(20)	1(50)	0(0)	11(100)	8(88.9)	5(100)	¤	¤	¤	¤	¤	53(34.9)
	Cefotaxime	16(69.6)	33(80.5)	5(100)	2(100)	1(100)	11(100)	7(77.8)	4(80)	14(37.8)	4(80)	3(75)	4(100)	2(40)	106(69.7)
	Cefoparazone	20(87.0)	40(97.6)	5(100)	2(100)	1(100)	11(100)	7(77.8)	5(100)	¤	¤	¤	¤	¤	91(59.9)
	Cefixime	15(65.2)	34(82.9)	3(60)	2(100)	0(0)	11(100)	8(88.9)	1(20)	27(73.0)	3(60)	1(25)	4(100)	2(40)	111(73.0)
	Cefepime	15(65.2)	31(75.6)	3(60)	2(100)	0(0)	11(100)	1(11.1)	1(20)	¤	¤	¤	¤	¤	64(42.1)
Monobactum	Aztreonam	17(73.9)	17(41.5)	2(40)	2(100)	1(100)	3(27.3)	8(88.9)	3(60)	¤	¤	¤	¤	¤	53(34.9)
Carbapenems	Imepenem	12(52.2)	15(36.6)	0(0)	0(0)	0(0)	0(0)	1(11.1)	0(0)	¤	¤	¤	¤	¤	28(18.4)
Aminoglycosides	Amikacin	16(69.6)	14(34.1)	3(60)	1(50)	1(100)	9(81.8)	1(11.1)	1(20)	12(32.4)	1(20)	3(75)	4(100)	5(100)	71(46.7)
	Gentamycin	15(65.2)		3(60)	2(100)	0(0)	10(90.9)	7(77.8)	4(80)	14(37.8)	3(60)	3(75)	0(0)	4(80)	74(48.7)
	Streptomycin	£	£	£	£	£	£	£	£	32(86.5)	3(60)	4(100)	0(0)	5(100)	44(28.9)
	Tobramycin	£	£	£	£	£	£	£	£	21(56.8)	3(60)	4(100)	4(100)	4(80)	36(23.7)
Chloremphenecol	Chloramphenicol	14(60.9)	16(39.0)	3(60)	1(50)	1(100)	1(9.1)	2(22.2)	2(40)	4(10.8)	4(80)	3(75)	0(0)	4(80)	55(36.2)
Quinalones and fluro-quinalones	Ofloxacin	14(60.9)	29(70.7)	4(80)	2(100)	1(100)	11(100)	7(77.7)	4(80)	20(54.1)	3(60)	4(100)	1(25)	0(0)	100(65.8)
	Sparfloxacin	£	£	£	£	£	£	£	£	31(83.8)	4(80)	4(100)	0(0)	5(100)	44(28.9)
	Gatifloxacin	20(87.0)	26(63.4)	4(80)	1(50)	0(0)	9(81.8)	1(11.1)	2(40)	16(43.2)	3(60)	1(25)	0(0)	1(20)	84(55.3)
	Levofloxacin	16(69.6)	27(65.9)	3(60)	1(50)	1(100)	9(9.1)	1(11.1)	2(40)	25(67.6)	3(60)	1(25)	1(25)	3(60)	93(61.2)
β-Lactum inhibitors	Ceftazidime + clavulanic acid	4(17.4)	2(4.9)	0(0)	0(0)	0(0)	11(100)	2(22.2)	4(80)	¤	¤	¤	¤	¤	23(15.1)
	Cephotaxime + clavulanic acid	0(0.0)	13(31.7)	0(0)	0(0)	0(0)	0(0)	2(22.2)	2(40)	20(54.1)	0(0)	2(50)	0(0)	1(20)	40(26.3)
	Piperacillin + tazobactum	15(65.2)	4(9.8)	3(60)	1(50)	0(0)	11(100)	2(22.2)	4(80)	¤	¤	¤	¤	¤	40(26.3)
	Cefoparazone + sulbactum	13(56.5)	5(12.2)	3(60)	1(50)	0(0)	11(100)	1(11.1)	4(80)	¤	¤	¤	¤	¤	38(25.0)
Macrolides	Erythromycin	£	£	£	£	£	£	£	£	17(45.9)	2(40)	4(100)	1(25)	4(80)	28(18.4)
	Azithromycin	£	£	£	£	£	£	£	£	30(81.1)	3(60)	4(100)	1(25)	5(100)	43(28.3)
Lincsamides	Clindamycin	£	£	£	£	£	£	£	£	16(43.2)	3(60)	4(100)	4(100)	4(80)	31(20.4)
Glycopeptides	Vancomycin	£	£	£	£	£	£	£	£	0(0.0)	0(0)	00	0(0)	0(0)	0(0)
Bacitracin		£	£	£	£	£	£	£	£		3(60)				3(100)

£, not tested; ¤, all Staphylococcus resistant to oxacillin have been considered resistant to all β-lactum; *Ps*: *Pseudomonas aeruginosa*, *Ec*: *Escherichia coli*, *Pv*: *Proteus vulgaris*, *Pm*: *Proteus mirabilis*, *Mm*: *Morganella morganii*, *Ko*: *Klebsiella oxytoca*, *Kp*: *Klebsiella pneumoniae*, *Ac*: *Acinetobacter* sp., *Sa*: *Staphylococcus aureus*, Bhs: beta hemolytic *streptococcus*, *CONS*: coagulase negative *staphylococcus* sp., *Cr*: *Coryneform* sp., *En*: *Enterococcus faecalis*.

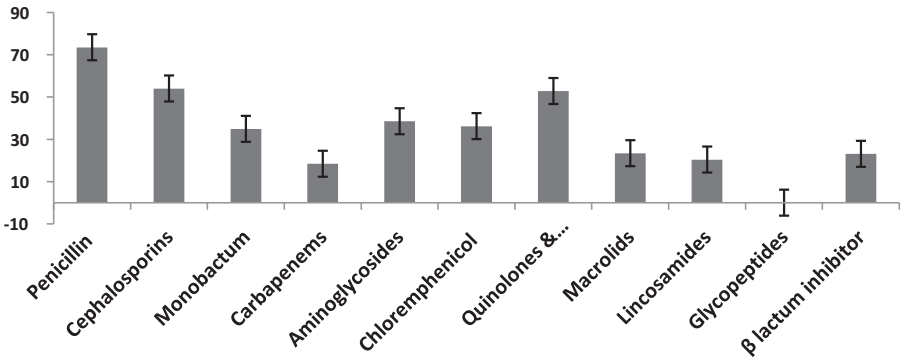

Fig. 3. Resistance percentage of antibiotic groups.

cases, direct examination could not been done. Among the bacterial isolates, gram positive cocci comprised of 36.1% and gram negative bacilli for 63.8%. Gram positive to gram negative ratio was 1:1.7. The frequency of bacterial isolates from the DFU is shown in Table 3. *Escherichia coli* was the most common isolate, accounting for 42.2%; followed by *S. aureus* 24.3%, *Pseudomonas aeruginosa* 23.7%, *Klebsiella oxytoca* 11.3%, *K. pneumoniae* 9.2%, *Proteus vulgaris* and *Acinetobacter* 5.1% each, beta hemolytic *streptococcus* spp. 3.2%, *Enterococcus faecalis* 3.2%, *CONS* 2.6%, *Coryneform* sp. 2.6%, *Proteus mirabilis* 2% and *Morganella morganii* 1%. Anaerobic culture was done in fifty four DFU patients in whom the pus sample was aspirated by the sterile syringe. Air from the syringe was removed immediately. Anaerobes alone were observed in two patients (3.7%). Both aerobic and anaerobic organisms were isolated in the remaining patients (96.2%). Anaerobic gram-positive cocci were found in 10 patients, 5 patients had infection by gram-positive bacilli and only two patients had infection by gram negative bacilli. The remaining 37 patients (57.5%) were found negative for anaerobic culture. Among the anaerobic bacteria isolated, gram positive comprised of 88.2% and gram negative for 11.7%. The frequency of bacterial isolates from the DFU was shown in Table 2. *Peptostreptococcus* sp. was the most common isolate, accounting for 35.2%; followed by *Peptostreptococcus anaerobius* 23.5%, *Propionibacterium* sp. 17.6%, *Bacteroides ureolyticus* 11.7%, *Clostridium perfringens* 5.8% and *Eggerthella lenta* 5.8% were isolated from 54 DFU patients.

3.2. Antibiotic resistance profile

The results of resistance studies are summarized in Table 3 and Fig. 3. High degree of antibiotic resistance was exhibited by *P. mirabilis* (69.6%), followed by *K. oxytoca* (67.6%), *P. aeruginosa* (65.8%), *Acinetobacter* sp. (63.5%), *P. vulgaris* (53%), *M. morganii* (52.2%), *E. coli* (51.5%) and *K. pneumoniae* (50.7%). Higher percentage of resistance (73.5%) was shown among the Penicillin group [*P. aeruginosa* (73.9%), *E. coli* (84.5%), *P. vulgaris* (53.3%), *K. oxytoca* (72.7%), *K. pneumoniae* (85.2%), *Acinetobacter* sp. (93.3%) and *S. aureus* (35.8%)], followed by cephalosporin group (54%) [*P. aeruginosa* (76%), *E. coli* (62.5%), *P. vulgaris* (62.5%), *P. vulgaris* (62.5%), *P. mirabilis* (87.5%), *M. morganii* (50%), *K. oxytoca* (81.8%), *K. pneumoniae* (65.2%), *Acinetobacter* sp. (67.5%) and *S. aureus* (61.5%)], quinolones and fluroquinolones (52.8%): [*P. aeruginosa* (72.5%), *E. coli* (66.6%), *P. vulgaris* (73.3%), *P. mirabilis* (66.6%), *M. morganii* (66.6%), *K. oxytoca* (63.6%), *K. pneumoniae* (33.3%), *Acinetobacter* sp. (53.3%), *S. aureus* (61.5%), *CONS* (45%) and *E. faecalis* (45%)], aminoglycosides group (38.5%) [*P. aeruginosa* (72.5%), *E. coli* (66.6%), *P. vulgaris* (73.3%), *P. mirabilis* (66.6%), *M. morganii* (66.6%), *K. oxytoca* (63.6%), *K. pneumoniae* (33.3%), *Acinetobacter* sp. (53.3%) and *S. aureus* (52.7%)], with beta lactam inhibitors (32.2%) [*P. aeruginosa* (23.9%), *E. coli* (14.6%), *P. vulgaris* (30%), *P. mirabilis* (25%), *K. oxytoca* (75%), *K. pneumonia* (19.4%) and *Acinetobacter* sp. (70%)] and carbapenems (18.4%) [*P. aeruginosa* (52.2%), *E. coli* (36.6%), *P. vulgaris* (0%), *P. mirabilis* (0%), *M. morganii* (0%), *K. oxytoca* (0%), *K. pneumoniae* (11.1%) and *Acinetobacter* sp. (0%)]. All the anaerobes were susceptible to metronidazole, amoxicillin + clavulanate and imepenem.

3.3. Phenotypic ESBL and MRSA detection

Eighty (83.3%) gram negative DFU isolates were ESBL positive (Table 4a and b) by disk diffusion method using cephotaxime, followed by 71(73.2%) for cefpodoxime, 68(70.1%) for aztreonam, 65(67%) for ceftriaxone and 63(64.9%) was shown for ceftazidime. About 81(83.5%) gram negative DFU isolates were ESBL positive by disk potential method using cefoparazone/cefoparazone + sulbactum followed by 77(79.4%) by piperacillin/piperacillin + tazobactum, 60(61.9%) by ceftazidime/ceftazidime + clavulanic acid and the cephotaxime/cephotaxime + clavulanic acid shows only 50(51.5%) ESBL production. *Staphylococcal* isolates identified as MRSA were 16(43.2%) and 18(48.6%) by using 1 μg oxacillin disk and 30 μg cefoxitin disk respectively. None of the *S. aureus* were vancomycin resistant (VRSA) including those which are resistant to oxacillin and cefoxitin antibiotics.

3.4. Occurrence of bla genes

The frequency of the occurrence of various *bla* genes in DFU isolates is shown in Fig. 4, CTX-M was found to be the most prevalent ESBL noticed in 34.5%, followed by TEM in 23% isolates and SHV beta-lactamases were noticed in 7.4% isolates respectively.

Table 1 also shows the result of univariate analysis of factors to be associated with the presence of MDR infections. The age distribution [O.R. 0.72, $P = 0.000$], duration of ulcer > 1 month [O.R. 0.54, $P = 0.001$] was observed in 78.5% patients having MDR infection.

Table 4
(a) Screening test result of ESBL producing gram negative bacilli, and (b) confirmatory test result of ESBL producing gram negative bacilli from 102 DFU patients.

(a) Screening result	ESBL
Aztreonam	68(70.1)
Cefpodoxime	71(73.2)
Ceftazidime	63(64.9)
Cephotaxime	80(83.5)
Ceftriaxone	65(67)
(b) Confirmatory	ESBL
Ceftazidime + clavulanic acid	60(61.9)
Cephotaxime + clavulanic acid	50(51.5)
Piperacillin + tazobactum	77(79.4)
Cefoparazone + sulbactum	81(83.5)

(a) Screening result	ESBL
AZTREONAM	68(70.1)
CEFPODOXIME	71(73.2)
CEFTAZIDIME	63(64.9)
CEPHOTAXIME	80(83.5)
CEFTRIAXONE	65(67)
(b) Confirmatory	**ESBL**
CEFTAZIDIME+CLAVULANIC ACID	60(61.9)
CEPHOTAXIME+CLAVULANIC ACID	50(51.5)
PIPERACILLIN+TAZOBACTUM	77(79.4)
CEFOPARAZONE+SULBACTUM	81(83.5)

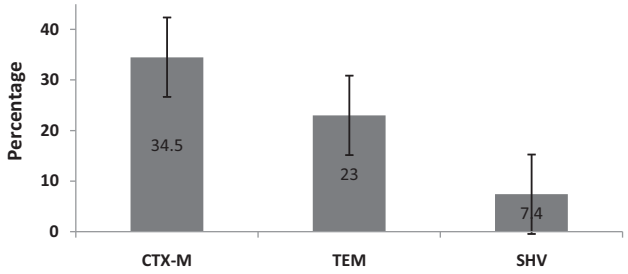

Fig. 4. PCR assay results for bla_{CTX-M}, bla_{TEM} and bla_{SHV}.

The size of ulcer more than 4 cm^2 [O.R. 12.6, $P < 0.001$] was found in 85.7% patients with MDR infection and in 14.2% patients having ulcer size less than 4 cm^2 in MDR infections. The hypertension [O.R. 3.48, $P = 0.004$], retinopathy [O.R. 0.84, $P = 0.000$], neuropathy [O.R. 1.32, $P < 0.001$], previous antibiotic use [O.R. 0.25, $P = 0.002$] were significantly associated with MDR positive infection. There was a significant relation between the bacterial growth with MDR when compared with the depth of diabetic foot ulcer (superficial wound, subcutaneous wound and presence of osteomyelitis). The presence of osteomyelitis [O.R. 2.34, $P < 0.001$] and subcutaneous wound [O.R. 0.62, $P = 0.001$] was significantly associated with the presence of MDR organisms infection (Fig. 5).

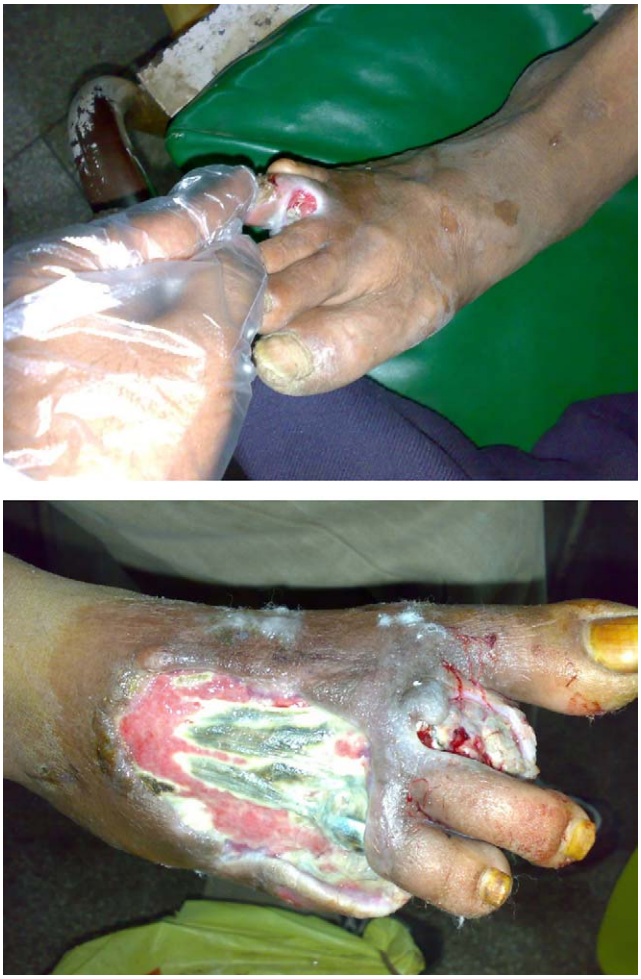

Fig. 5. Images of diabetic foot ulcer patients.

4. Discussion

This study presents a comprehensive clinical and microbiological profile of infected diabetic foot ulcers in hospitalized patients.

With the rise in the prevalence of diabetes mellitus there is increasing problem of infections among diabetic patients especially the diabetic foot infection which according to some studies accounts for 20% of hospital admissions [2]. As multidrug resistance is a growing problem, effort was made to study the association of different study characteristics with the presence of MDR organisms. The soft tissue samples were also used for bacteriological cultures, including osteomyelitis, although bone biopsy may be a better sample but it is not routinely performed in our hospital.

Diabetic foot infections are usually polymicrobial in nature and this has been well documented in the literature. In the present study polymicrobial etiology was found in 62% and monomicrobial in 38% patients with the rate of isolation of about 1.49 bacteria per patient which is lower than the previous studies [3,26] which showed rate of isolation between 2.3 and 5.8. The major infective organisms in diabetic foot ulcers in our patients appear to be different. We found gram negative aerobic bacteria were most frequently isolated which is in accordance with the previous reports [3] and in tune with a similar study from the Southern parts of India [2]. The ratio of gram positive to gram negative ratio was 1:1.7 which is similar to the findings reported earlier [27]. Gram positive organisms which include MRSA were found in 48.6% of isolates which differs from the older studies which shows predominance of gram positive ones [4,28,29]. Gram-negative bacteria that are regarded as normal flora of the skin may cause severe tissue damage in diabetics. We suggest that they should be regarded as significant in diabetic foot ulcers. In our anaerobic study, *Peptostreptococcus* sp. was the most predominant one which is, in accordance to the previous studies [30,31]. Other anaerobes isolated in their study were *Bacteroides fragilis*, *Clostridium* sp. and *Propionibacterium*. *Clostridium* sp. was the most commonly isolated anaerobe, followed by *Bacteroides* [32]. Compared with earlier anaerobic culture reports, we recovered fewer anaerobic species [33,34]. Most of our patients did not have chronic draining wounds, and only 6% had gangrene associated with their infections. This may be an indication of fewer anaerobic species among nonthreatening lower-extremity infections, which is also reported earlier [35].

The present study confirms that MDRO infection is extremely common in hospitalized patients with DFUs similar in tune with the report of Hartemann-Heurtier et al. [7]. Almost 46(46%) of our patients were infected with MDROs. The prevalence of both MRSA isolates and ESBL producing gram negative bacteria was higher (48.6% and 68.5% respectively) in our population as compared with previous studies [7].

A high degree of antibiotic resistance was observed in the present study, which may be due to the fact that ours is a tertiary care hospital with widespread usage of broad spectrum antibiotics leading to selective survival advantage of pathogen. Moreover most of our patients had received some antimicrobial treatment before

presenting at our centre. The antimicrobial resistance pattern was similar to the recent studies done in India and outside [2,36]. In our study, 43.2% of isolated *S. aureus* was methicillin resistant by using 1 μg oxacillin disk and 48.6% of isolated *S. aureus* was resistant to 30 μg cefoxitin disk. Cefoxitin disk shows high percentage of sensitivity over oxacillin disk. None of the gram positive isolates were resistant to vancomycin (VRSA). These observations are important, especially for patients' management and deciding the antibiotic treatment policies.

In the present study, gram negative bacilli were isolated as ESBL producers in 68.5% similar to the observation by Mathur et al. from India [37]. Babypadmini and Appalaraju have shown 40% of *K. pneumoniae* isolates and 41% of *E. coli* isolates to be ESBL producers in their study cohort [38]. A study conducted in Brazil [41] says, the prevalence was only 6% among *E. coli* isolates. Gadepalli et al. have reported 54.5% *E. coli* isolates to be ESBL producers in diabetic foot infections [3]. There is paucity of data on the prevalence of ESBLs in diabetic foot infection in gram negative bacteria other than *E coli* and *Klebsiella* sp. On screening for ESBL, 72.8% of *Acinetobacter* spp. were ESBL producers followed by *P. aeruginosa* (71.2%), *K. oxytoca* (63.3%), *K. pneumoniae* (58.8%), *E. coli* (56.2%) and *P. vulgaris* (45.9%). On confirmation of ESBL production by disk potentiation test, highest prevalence of ESBL was observed in *Acinetobacter* spp. (61%) followed by *P. aeruginosa* (60%), *P. vulgaris* (47%), *K. pneumoniae* (47%), *K. oxytoca* (39%), and *E. coli* (36.5%). In a recent study Shobha et al. have reported 27.3% *K. pneumonia*, 25.2% *E. coli*, 21.42% *Pseudomonas* spp., 25% *Enterobacter* spp. and 17% *Acinetobacter* spp. to be ESBL producer [39].

The duration of infection > 1 month, prior antibiotic use and ulcer size >4 cm^2 were independent predictors of infection with MDR organism. Thus patients with a large ulcer, with a history of prior antibiotic use and duration of infection > 1 month were more likely to harbor MDROs. In the present study mean duration of ulcer was found to be 41.5 ± 7.5 days with 36.2% having ulcer for more than 1 month. About 75.4% presented with a large ulcer of approximate size of >4 cm^2 thereby accounting for approximately 84.3% of the patients with bacterial growth from subcutaneous region and presence of osteomyelitis in relation to MDR. 47% of patients had used antibiotics prior to reporting to the hospital. The reasons for presentation with advanced grade and stage of ulceration could be because of lack of structured health care delivery in the country, attempted self-medication and trust in traditional healers [40]. Moreover, inadequate antibiotic treatment and the use of non sterile instruments for dressing results in the growth of multi resistant organisms necessitating hospital admission and surgical intervention [41]. Moreover prolonged or broad-spectrum antibiotic therapy predisposes patients to infections with antibiotic-resistant organisms like MRSA [7]. This could explain the high level of MDR infection in our study. We could not get the previous hospitalized details for the same wound in our patients that could have helped us explaining the reasons for the high prevalence of MDROs in our patients.

Furthermore, the occurrence of ESBL in the bacterial isolates was substantiated by $bla_{CTX\text{-}M}$, bla_{TEM} and bla_{SHV} in DFU using PCR to find out true resistance to antibiotics. This genomic study helps us to find out the nature of resistance whether plasmid mediated or some environmental effect. We found 34.5% $bla_{CTX\text{-}M}$ as the most prevalent ESBL gene followed by 23% bla_{TEM} and 7.4% bla_{SHV} in the DFU isolates tested. From the study of *bla* genes(CTX-M, SHV AND TEM), we interpolate that the nature of resistance developed by organism was mainly influence environmental (45.6% out of 68.5% phenotypic ESBL positive isolates). To the best of our knowledge, no such type of report have been reported from isolates of DFUs, however, the $bla_{CTX\text{-}M}$ as the most prevalent and widely disseminated genes in the clinical bacterial population had been reported from India [23,24,42].

A significant relation between the longer hospitalization period and the infection with MDR was found in the present study, which is in accordance with existing literature [43]. It is known that MDR infections are resistant to several antibiotics and therefore patient should be treated with extended spectrum antibiotics for longer durations. As a result, duration of hospital stay in infections with MDR is usually longer and concomitantly treatment more costly. Furthermore, in our study, mortality from infections with MDR is twice as high as mortality from infections with microorganisms sensitive to antibiotics (8.6%) [$P = 0.002$, O.R. = 3.2]. For these reasons, risk factors for acquisition of MDR should be determined and decreased as soon as possible.

Resistance to antibiotics is seen when they are used for a prolonged period of time. This resistance is an acquired form rather than an intrinsic one. The former develops following a mutation in the DNA of a microorganism or by acquisition of a new DNA. Acquisition of new DNA is accomplished by genetic elements such as plasmids or transposons. Resistance plasmids may have approximately 10 resistance genes for various antibiotics. Bacteria can transmit these characteristics to other bacteria [44,45]. Frequent or unnecessary use of antibiotics result in a selection favouring resistant bacteria. In this study history and discharge summaries showed that overwhelming majority (26.4%) of the diabetic patients with osteomyelitis who were referred to our centre had received an antibiotic treatment before. However, which antibiotics and in what doses they were given was not clear, only the duration of the therapy was evident. Although the number of patients who had no antibiotic therapy was low in our series MDR presence was higher (41.3%) in those who had previous antibiotic therapy. Also infection with MDR was significantly higher in this population when compared to other bacteria (MDR, negative).

Presence of vascular disease characterized by disrupted micro- and macro-circulations cause a delay in wound healing in diabetic patients [6,46]. Disruption of wound healing results from a decreased blood flow into the ulceration and an aberrant expression of growth factors and cytokines as well. These factors, which delay wound healing, cause foot ulcers. Infections of these foot ulcers require a longer duration of treatment with antibiotics and the use of an appropriate antibiotic in an appropriate dosage [47]. Insufficient blood flow which causes ischemia will hinder penetration of antibiotics into the wound and therefore facilitate infections with MDR [48]. In fact, we found that MDR was more frequently isolated in neuroischemic cases (38%). Although this increase was statistically insignificant, we believe that an increase in the number of cases could positively affect significance. To our knowledge, there has been no study confirming or negating this finding in the literature. Manual minimum inhibitory concentration (MIC) was not carried out as it was time consuming and tedious for all the ESBL-producing clinical isolates obtained in the present study.

Our results indicate adequate control of blood glucose level is more common in patients with non-MDR infected ulcers as compared with MDR infected ulcers, and further, higher mortality rates were reported in patients with DFU whose blood glucose levels were poorly controlled. Thus, MDRs might lead to higher mortality among DFU which need to be investigated. We found duration of hospital stay and cost was more in MDRs as compared with non-MDRs. The duration of hospital stay may also depend on the management policy of the hospital. In our hospital, patients are discharged once the healing begins and are advice to come to follow-up at out-patients clinic every week.

5. Conclusion

A detailed knowledge of the susceptibility to antimicrobial agents is necessary to facilitate the development of effective strate-

gies to combat the growing problem of resistance especially the MRSA and ESBL strains. The prevalence of MDR organisms is alarmingly high in the diabetic foot patients in India because of indiscriminate use of antibiotics. The findings of the present study suggest that prospective multicentre studies are required to assess the appropriate empirical antibiotic regimen in diabetic foot ulcer infections. The study also directs us that proper management of diabetic foot ulcers with appropriate antibiotics groups such as aminoglycoside, macrolides and chloramphenicol for *S. aureus* isolates and third and fourth generation cephalosporin's, β-lactum inhibiters and amino-glycoside for gram negative bacilli along with good glycemic control must be implemented to decrease the incidence of MDR organisms for better clinical outcome. Empirical treatment with amino-glycoside may begun initially as they cover both gram positive and gram negative bacteria. However chloramphenicol can be used as a reserve drug in infection refractory to DFU with conventional drugs.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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A study of biofilm production by gram-negative organisms isolated from diabetic foot ulcer patients

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Abstract
The present study was undertaken to study the difference in antibiotic resistance profile and minimum antibiotic concentration (MIC) of biofilm producing and non-biofilm producing gram-negative bacilli isolated from diabetic foot ulcer (DFU) patients in a tertiary care hospital in North India. Among the diabetic foot patients, 73.6% were males and 15% were females. 77.1% had T2DM whereas only 24.4% patients had T1DM. Poor glycemic control and poor HbA1c (>8) was observed in 68.7% and 70.1% patients respectively. Among the 57 patients, 97 gram-negative bacilli were isolated in which mixed bacterial infection was found in 67.8% and monomicrobial in 32.2% only. *Escherichia coli* was the most common (42.2%) isolate followed by *Pseudomonas aeruginosa* (23.7%)*, Klebsiella oxytoca* (11.3%)*, Klebsiella pneumonia* (9.2%)*, Proteus vulgaris* (5.1%)*, Acinetobacter* sp (5.1%), *Proteus mirabilis* (2%) and *Morganella morganii* (1.0%). 77.1% DFU patients had infection by biofilm producing organisms. BFP positive status was associated with the presence of neuropathy (O.R. 7.65), osteomyelitis (O.R. 3.14), duration of ulcer (O.R. 25.7), grade of ulcer (O.R. 9.12), necrotising ulcer (O.R. 14.4) and ulcer size >4cm^2 (O.R. 3.30) but not with patients characteristic, type of diabetes and type of diabetes, or duration of hospital stay. Poor glycemic control in 56.1% patients, amputation (24.5%), hospital stay (38.5%) and age distribution were independently associated with risk of biofilm producing infection in diabetic foot patients.

Keywords: Diabetic foot ulcer; bacterial profile; antibiotic resistance; biofilm production.

Introduction

Toole *et al.* (2005) who observed that, the bacteria are not free floating but grow upon submerged surfaces. The basic architecture of biofilms shows that the microcolony is actually the basic structural unit of the biofilm. The exhaustive structural analysis of hundreds of monospecies *in vitro* biofilms, and of dozens of multispecies natural biofilms, has shown that microcolonies are discrete matrix-enclosed communities of bacterial cells that may include cells of one or of many species. Depending on the species involved, the microcolony may be composed of 10–25% cells and 75–90% extracellular exopolysaccharide matrix (EEM). The matrix material often appears to be most dense in the area closest to the core of the microcolony, which is characterized by their lack of Brownian movement. Costerton *et al.* (1999) showed the arrangement of micro-colonies are in horizontal array in thin biofilms, but also form a vertical arrays in very thick sessile communities. Biofilm EEM, which is also referred to as *slime* (although not everything described as slime is a biofilm), is a polymeric conglomeration generally composed of extracellular DNA, proteins, polysaccharides, adhesins (PS/A) and autolysin (encoded by *atlE* gene) are involved in regulation of biofilm production present in various configurations. The *ica* gene codes for intracellular adhesion (ICA) and may also code for PS/A and, is required for biofilm production (Toole *et al*., 2005; Donlan *et al.,* 2002; Carol *et al.,* 2005).

Biofilm which forms on living or non-living surfaces establishes a protective environment of microbial life in natural, industrial and hospital settings (Stoodley *et al.,* 2004), which are, physiologically distinct from planktonic cells of the same organism, which, by contrast, are single-cells that may float or swim in a liquid medium (Karatan *et al*., 2009; Hoffman *et al*., 2005). When a cell switches to the biofilm mode of growth, it undergoes a phenotypic shift in behavior in which large suites of genes are differentially regulated (An *et al*., 2007). Biofilms are also often the site for quorum sensing influence the availability of key nutrients for biofilm formation, chemotaxis towards surface, motility of bacteria, surface adhesion and presence of surfactants are certain factors which influence biofilm formation (Carol *et al*., 2005; Thomas *et al*., 2007). According to a recent public announcement from National Institute of Health (NIH), more than 60% of all infections are caused by biofilm (Kim *et al*., 2001). Moreover, these ulcers adversely influence the patients' quality of life, leading to decrease in

social, physical and physiological functions (Raiber *et al.*, 1998). Various factors including defects in host defense mechanisms (impaired leukocyte functions) are responsible for this increase in infection rates. Wound infection is known to impair wound healing in both acute and chronic DFUs (Robson *et al.,* 1997). That most of the infections in DFU are polymicrobial in nature have recently been documented in our studies also (Zubair *et al.,* 2010a,b). Although the numbers and type of bacteria in a wound are critical for infection to occur, recently a new concept of bacterial biofilms has emerged as a potential way to better understand how bacteria deter healing. Therefore, a better understanding of bacterial biofilms is needed, and this may ultimately result in development of novel therapeutics for the prevention and treatment of DFU infections. The biofilm producing organisms have an inherent resistance to antibiotics and in the long run they may be very damaging because of the development of immune complex diseases (Donlan *et al.,* 2002; Raad *et al.,* 1995; Souli *et al.,* 1998).

There are only scarce reports on biofilm formation by clinical isolates from DFU especially in North India. Keeping this in mind, the present study was undertaken to study the difference in their antibiotic resistance profile and minimum antibiotic concentration of biofilm producing and non-biofilm producing gram-negative bacilli isolated from diabetic foot ulcer in a tertiary care hospital in North India.

Materials and Methods

Study Design

The study was carried out prospectively at the Diabetic and Endocrinology ward, J.N. Medical College, Aligarh Muslim University. Aligarh, India, from June 2009 to February 2010. Subjects studied were all in-patients of the male and female ward who had ulcer/infection in their foot with gram-negative bacterial infection.

Clinical Examination

A detailed clinical history and physical examination was carried out for every subject, which include a record of age, sex, anthropometric measurements, duration of ulcer, duration of diabetes and glycemic control. Foot ulcers were categorized into six grades (0-5) based on Meggit Wagner Classification System (Wagner *et al*, 1981). Neuropathy was quantified in each patient assessing vibration sensation using a 128 Hz tuning fork and a 10g monofilament (absence of perception of the Semmes Weinstein monofilament at 2 of 10 standardized plantar sites on either foot).

Ulcers were assessed for signs of infection (swelling, exudates, surrounding, cellulitis, odor, tissue necrosis and crepitation) and size was determined by multiplying the longest and widest diameters expressed in cm^2. Each patient was included only once in the study. All cases were monitored until discharged from the hospital. All the subjects gave informed consent and clearance was obtained from the hospital ethics committee.

Microbiological Methods

The microbiological methods described by Gadepalli *et al.* (2006) as adopted in our previous studies (Zubair *et al.,* 2010b, c) were used. Total transfer time to the laboratory was not more than 30 minutes. Direct microscopic examination of ulcer sample was performed and all the bacterial isolates were identified to the species level using standard identification techniques (Collee *et al.*, 1996).

Susceptibility Testing

Antimicrobial susceptibility testing was performed as described by the CLSI and adopted by us elsewhere (Zubair *et al.,* 2010b,c). Antimicrobial discs used were Aztreonam (30µg), Imipenem (10µg), Amoxyclav (30µg), Cefpodoxime (10µg), Cefepime (30µg), Cefoperazone (75µg), Cefoperazone/sulbactam (75/10µg), Cefixime (5µg), Piperacillin (100µg), Ceftazidime (30µg), Piperacillin/tazobactam (100/10µg), Ceftazidime/clavulanic acid (30/10µg), Amoxycillin (20µg), Cephotaxime (30µg), Cephotaxime/clavulanic acid (30/10µg), Ceftriaxone (30µg), Cephoxitin (30µg), Amikacin (30µg), Chloramphenicol (30µg), Gentamicin (10µg), Gatifloxacin (5µg), Ofloxacin (5µg), Levofloxacin (5µg). All discs were obtained from Hi-Media Laboratory, Mumbai, India. Interpretative criteria for each antimicrobial tested were those recommended by manufacturer's guidelines (Hi-Media Labs, Mumbai, India).

Biofilm Assay - Tissue Culture Plate (TCP) method

The biofilm assay described by Mathur *et al*. (2006) was adopted. Stated briefly, 10 ml of trypticase soy broth (TSB) with 1% glucose was inoculated with a loopful of test organism from overnight culture on nutrient agar. The TSB broth was incubated at 37^oC for 24 hours. The culture was further diluted 1:100 with fresh medium and flat bottom tissue culture plates (96 wells) were filled with 200µl of diluted cultures individually. Uninoculated

sterile broth served as blank. Similarly, control organisms were also diluted and incubated. The culture plates were incubated at 37^{o}C for 24 hours. After incubation, gentle tapping of the plates was done. The wells were washed with 200 µl of phosphate buffer saline (pH 7.2) four times to remove free-floating bacteria. Biofilms which remained adherent to the walls and the bottoms of the wells were fixed with 2% sodium acetate and stained with 0.1% crystal violet. Excess stain was washed with deionized water and plates were dried properly. Optical densities (OD) of stained adherent biofilm were obtained with a micro ELISA auto-reader at wavelength of 570 nm. Experiments were performed in duplicate and the average of OD values of sterile medium were calculated and subtracted from all test values.

Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined in doubling dilutions from 512 µg/ml to 0.05 µg/ml (CLSI). Antibiotic powders were obtained from Hi-Media Labs, Mumbai, India, except potassium clavulanate (clavulanic acid) which was procured from the Center for Diabetes and Endocrinology, A.M.U., Aligarh.

Antibiotic Treatment

Antibiotics were selected according to published recommendation (Hartemann-Heurtier *et al*., 2009). In mild infections amoxicillin clavulanic acid was given empirically by the oral route. However, in moderate infections intravenous route was preferred taking into consideration the likelihood of osteomyelitis. Considering that the causative agent was polymicrobial, we initiated ampicillin-sulbactam plus an aminoglycoside/quinolone or piperacillin-tazobactam or ceftriaxone plus metronidazole/clindamycin. In the presence of severe infections, surgical debridement and amputation were performed immediately after admission. Metronidazole (500 mg I.V. every 8 hours) was added to the drug regimen if cellulitis or gangrene was also present. The treatment was later modified in accordance with the culture results. The duration of the treatment was at least 4-6 weeks and prolonged in cases of osteomyelitis. All patients also received an intensive insulin treatment.

Statistical Analysis

The data was analyzed using SPSS version 17.0 for descriptive statistics. Quantitative variables were expressed as mean±sd while qualitative variables were expressed as percentage (%). Continuous variables were compared using 2 sample *t tests* for independent samples. Odds ratios and 95% confidence interval (CI) were reported for independent variables associated with the outcome variable: presence of anaerobic infection.

Results

Clinical

Males were predominant 42(73.6%) in the study subjects. Majority 44(77.1%) of subjects had T2DM. The mean age of the subjects was 49.1±12.4 years. The mean duration of diabetes was 12.6±6.4 years. Thirty-four patients (59.4%) had neuropathy, 35(61.4%) nephropathy, 32(56.1%) retinopathy, and 33(57.8%) were hypertensive. Osteomyelitis was present in 18(31.5%) subjects. Majority (77.0%) of the DFU patients were from Meggit Wagner grade II to grade IV. Grade I ulcer was found in 8.7%, Grade II in 14%, Grade III in 28%, Grade IV in 35%, and Grade V in 8.7% of patients. Majority of the subjects 31(54.3%) had lesions for >1 month before presentation at the hospital. The ulcer was necrotic in 25(43.8%) cases. Glycemic control was poor in 67(65.6%). HbA1c was <7% in 12 patients (21%), 7%-8% in 5(8.7%) and >8% in 40(70.1%) subjects. More than 38(66.6%) received surgical treatment, mainly in the form of debridement. 19(33.3%) patients were subject to amputation and 3(5.3%) died during the hospital stay (mean hospital stay 19.6±12.5) (Table 1). Majority of the ulcers were found on interdigits and the plantar surface (47.3% each), followed by heels (42.1%), margins (28%), malleoli (24.5%), and legs (8.7%) and on multiple (≥2 sites) 47.3%. Size of ulcer ≤4cm^2 was observed in 21% patients and ≥4cm^2 in 64.9% patients.

Microbiological Observations

A total of 97 gram-negative bacteria were isolated from 57 DFU patients, averaging 1.7 species per patient. Monomicrobial infection was observed in 32.2% patients whereas polymicrobial etiology was observed in 67.8% patients. In the direct microscopic examination of ulcer samples, 96% findings correspond with the culture growth on next day and in 4% patients, direct smear result differed with their culture growth. The frequency of bacterial isolates from DFU is shown in Table 2. *Escherichia coli* was the most common isolate, accounting for 41(42.2%), followed by *Pseudomonas aeruginosa* 23(23.7%), *Klebsiella oxytoca* 11(11.3%), *Klebsiella*

pneumoniae 9(9.2%), *Proteus vulgaris* 5(5.1%), *Acinetobacter* sp. 5(5.1%), *Proteus mirabilis* 2(2%) *and Morganella morganii* 1(1%).

Biofilm Assay

Among the 97 gram-negative bacterial isolates, 60(59.4%) were biofilm producers. A total of 80% *P. vulgaris* isolates were biofilm producers, followed by *K. pneumoniae* (77.7%), *E. coli* (63.4%), *K. oxytoca* (63.4%), *Acinetobacter* sp. (60%) and *P. aeruginosa* (52.1%). The lone isolate of *M. morganii* was a biofilm producer (Table 2).

Antibiotic Resistance Profile of BFP and BFN Isolates

The result of resistance studies are summarized in Fig. 1. High degree of antibiotic resistance was exhibited by all the BFP isolates compared with NBP. High degree of resistance by BFP isolates was observed against cefoparazone (79.6%) followed by piperacillin (68.4%), cephotaxime (67.3%), amoxyclav (64.3%), cefixime (64.3%), amoxycillin (63.3%), ofloxacin (63.3%), cefepime (59.2%), gatifloxacin (57.1%), levofloxacin (51.0%), cefpodoxime (49.0%), ceftriaxone (44.9%), ceftazidime (42.9%), amikacin and gentamicin (40.8% each), astreonam (39.8%), cephoxitin (36.7%), chloramphenicol (31.6%), imepenem (24.5%), piperacillin+tazobactum (21.4%), cefotaxime+clavulanic acid (12.2%), and Ceftazidime+clavulanic acid (9.2%).

Minimum Inhibitory Concentration (MIC)

The MIC values of the piperacillin (with/without tazobactam), cefoparazone (with/without sulbactam), ceftazidime (with/without clavulanic acid) and levofloxacin between the BFP and NBP were given in Table 3. Percentage of BFP isolates that had an MIC of ≥2µg/ml was 93.3% for cefoparazone followed 90% for piperacillin, 81.6% for ceftazidime, and 75% for levofloxacin. The isolates that had an MIC ≥2µg/ml antibiotics with inhibitor were 80% for piperacillin+tazobactum, followed by 73.3 % for cefoparazone+sulbactum and 48.3% for ceftazidime + clavulanic acid.

Correlation of Biofilm Assay and Clinical Characteristics of DFU Patients

Table 1 also shows the result of univariate analysis of factors to be associated with the presence of biofilm producing organism infections. The age distribution [O.R. 1.23, P = 0.489], Type 2 diabetes [O.R. 2.16, P<0.207], duration of ulcer >1 month [O.R. 25.7, P < 0.001] was observed in 52.6% patients having biofilm producing infection. The size of ulcer more than 4 cm^2 [O.R. 3.30, P < 0.89] was found in 64.9% patients with biofilm positive infection and in 14.0% patients having ulcer size less than 4 cm^2. The neuropathy [O.R. 7.65, P < 0.003], osteomyelitis [O.R. 3.14, P <0.136], necrotising ulcer [O.R. 14.4, P< 0.002] and poor glycemic control (HbA1c : >8%)[O.R. 1.66, P<0.32] were significantly associated with biofilm producing bacterial infection. There was a significant relation between the biofilm producing bacterial growth with Wagner's grading. Majority of the biofilm positive patients were from grade 4 [O.R. 9.12, P<0.001] followed by grade 3 [O.R. 2.56, P< 0.23], grade 2 [O.R. 2.27, P< 0.40] and grade 5 [O.R. 1.5, P< 0.68]. (Fig. 5).

Discussion

This study presents a comprehensive clinical and microbiological profile of infected diabetic foot ulcers in hospitalized patients with special reference to the study of biofilm production in the gram-negative bacterial isolates.

With the rise in the prevalence of diabetes mellitus there is increasing problem of infections, especially foot infections. According to some studies, patients with diabetic foot infections account for 20% of hospital admissions (Shankar *et al.,* 2005). India is the home for the largest number of diabetic individuals. As higher resistance is a growing problem, effort was made to study the association of different study characteristics with the presence of resistant organisms. The prevalence of diabetic foot ulcers among male subjects was found to be 73.6% against 26.3% in female i.e. a ratio of 2.3:1 which may be due to higher level of outdoor activity among males compared to females (Zubair *et al.,* 2010b,c). With increasing duration of diabetes, there is increased risk of diabetes related complications especially chronic complications like sensory neuropathy. This study also reports a high prevalence of neuropathy (59.4%). There was a marked variation of sensory neuropathy from our earlier studies (Zubair *et al.,* 2010b,c), which showed a slightly higher percentage (66.6% & 78.5%) of neuropathy in North India. Ako *et al.* (2006) in a Nigerian study, showed the increase in neuropathy to 77.8% and 56.8% in a South Indian study (Shankar *et al.,* 2005). This marked variation in the prevalence may be due to difference in the methods used for the diagnosis of these conditions (10g monofilament or biothesiometer).

In Table 1, duration of infection >1month, prior antibiotic use and ulcer size >4cm^2 were independent predictors of

infection. Thus patients with a large ulcer, with a history of prior antibiotic use and duration of infection >1month were more likely to harbor BFP organisms. In the present study, mean duration of ulcer was found to be 41.5±47.6 days with 54.3% having ulcer for more than 1 month. About 78.9% presented with a large ulcer of approximate size of >4cm^2, thereby accounting for approximately 77.1% of the patients presenting with Wagner's grade II and IV. The reasons for presentation with advanced grade and stage of ulceration could be because of lack of structured health care delivery in the country, attempted self-medication and trust in traditional healers (Boulton *et al*., 2001; Zubair *et al*., 2010b,c). Diabetic foot infections are usually polymicrobial in nature and this has been well documented in the literature. In our study also, we found polymicrobial etiology in 67.8% and monomicrobial in 32.2% patients with the rate of isolation of about 1.7 bacteria per patient which is higher than the previous reports (Zubair *et al.,* 2010a,b,c) whereas Gerding *et al*., (1995) and Gadepalli *et al*. (2006) have reported higher isolation rate of 2.0%-5.8%. The present study also confirms the high resistance among the DFU isolates which was extremely common in hospitalized patients with diabetic foot ulcers. This is in accordance with the reports of Hartemann-Heurtier *et al*. (2009) and Zubair *et al*. (2010a,b,c).

This high degree of antibiotic resistance may be due to the fact that ours is a tertiary care hospital with widespread usage of broad spectrum antibiotics leading to selective survival advantage of pathogen. Our results of antimicrobial resistance pattern were similar to the recent studies done in India and outside (Shankar *et al,* 2005; Raja *et al.,* 2007). Gram-negative bacteria that are regarded as normal flora of the skin, like *P. aeruginosa*, may cause severe tissue damage in diabetics and should never be automatically disregarded as insignificant in diabetic foot ulcers (Zubair *et al.,* 2010b).

Another reason for this high antimicrobial resistance among the BFP appears to be due to the close cell-cell contact that permits bacteria to more effectively transfer plasmids to one another than in the planktonic state. These plasmids can encode for resistance to several different antimicrobial agents (Mah and Toole, 2001). Another factor contributing to resistance is quorum sensing, which through the processes described above can force bacteria into a slow-growing state when placed in an environment with adverse growth conditions; when in this state of intermission, bacteria are less susceptible to antimicrobial attack (Mertz, 2003). The biofilm also provides a physical protection to bacteria because antimicrobial agents are also ineffective at penetrating the biofilm, decreasing the concentration acting on the bacterial cells within the biofilm and as a consequence their efficacy (Mah and Toole, 2001). In addition to the resistance to antimicrobials, biofilms also appear to have an antiphagocytic property within the biofilm, which renders leukocytes present within the matrix ineffective (Leid, 2002). Additionally, there appears to be a component within the polysaccharide that inactivates and traps both complement and host antibodies. These factors lead to an accumulation of host immune factors that can lead to host tissue damage and eventually chronic inflammation (Percival and Bowler, 2004).

The idea of disrupting a biofilm that is already formed is attractive. This could be accomplished in a number of ways, including physical methods and/or application of topical substances. Among potential physical methods, debridement, electrical stimulation, or ultrasound could be used. Debridement may not only remove the bacteria and biofilm but also may aid in the removal of necrotic tissue for which the bacteria would thrive on. Electrical stimulation has been used over the years to assist penetration of various topical agents but have a limited application (e.g., electroporation and electrophoresis have been shown to enhance the penetration of a photosensitizer) (Johnson and Oseroff, 2002).

Changing the perspective about chronic infectious disease to include biofilm enables two important insights. First, it opens new methods for detection and treatment. Second, it provides a global reconceptualization of many chronic infectious diseases as resulting from a biofilm, allowing biofilm principles to be shared across disciplines. Recent studies have investigated new methods for detecting the components of a biofilm. Several investigations have used modern molecular methods, such as denaturing gradient gel electrophoresis and denaturing high performance liquid chromatography, along with imaging techniques including fluorescent in situ hybridization. Also, molecular methods such as polymerase chain reaction (PCR) and pyrosequencing in conjunction with conventional culture methods have been used to determine the bacterial species composition of chronic infections (Dowd *et al.*, 2008). Performing molecular tests as part of routine bacterial analysis is becoming a real option for clinical laboratories. These tests could include

methods such as PCR, reverse transcriptase–PCR, microarrays, antigen testing, and rapid sequencing. Only a few of these methods are being used to test for certain pathogens, but culture-free identification of all pathogens and their corresponding resistance markers may soon become routine (Espy *et al.*, 2006). A biofilm focus also provides new strategies for treatment of chronic infections. Biofilm-based treatments might block initial bacterial attachment to a surface, block or destroy EPS formation, interfere with cell-cell signalling pathways, and use bacteriostatic or bactericidal agents at the same time. Concomitant therapies that not only attempt to eradicate bacteria but also affect the biofilm's community structure and communications may prove more effective than a single or sequential strategy such as antibiotic therapy (Ehrlich *et al.*, 2005). This multimodality approach to therapy is commonly used in other areas of medicine, such as the treatment of human immunodeficiency virus for which combination antiretroviral therapy is used to achieve the best clinical outcome.

Conclusion

Diabetic foot infections are a significant burden on patients as well as a burden on the health care delivery system. It is important to quickly and effectively identify and treat these ulcers and prevent complications. Biofilm formation on these wounds may be responsible for the chronicity of these wounds and for their common infectious complications. The presence of biofilm also represents an important barrier to effective treatment. Although in vitro study of novel approaches to control or eradicate biofilm formation are being performed, *in vivo* testing is necessary because various factors (e.g., wound fluid, proteases, growth factors, and so forth) need to be taken into consideration to determine the true efficacy of these agents. Treating the DFU by shifting from the planktonic model of microbiology to the biofilm model makes available new methods for detection and treatment. Because of molecular methods, science now has the ability to detect biofilms and understand the implications of interspecies chaos that contribute to infections. With these new scientific approaches along with coordination of clinical and laboratory efforts, education, and research, it is possible to imagine overcoming much of biofilm disease.

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Table 1: Demographic presentation of DFU patients in response to biofilm assay positive and negative bacterial infections (mean±sd and n(%) of otherwise indicated).

N=57	Total	Biofilm + (n=44)	Biofilm – (n=13)	P-value	OR(95%CI)
Sex					
Male	42(73.6)	32	10	0.532	0.8(0.18-3.4)
Female	15(26.3)	12	3		
Age distribution (years)	49.1±12.4	44.6±7.3	54.3±10.2		
<40	10(17.54	7(12.2)	3(5.2)		
41-60	33(57.8)	26(45.6)	7(12.2)	0.489	**1.23(0.35-4.3)**
>61	14(24.5)	11(19.2)	3(5.2)		
Type of Diabetes					
Type 1	14(24.4)	9(15.7)	5(8.7)		
Type 2	44 (77.1)	35(61.4)	9(15.7)	0.207	**2.16(0.57-8.0)**
Duration of Ulcer	41.5 ± 47.5	39.6±2.6	22.7±1.0		
< 1month	26 (45.6)	14(24.5)	12(21.0)		
>1 month	31(54.3)	30(52.6)	1(1.7)	**0.0001**	**25.7(3.0-217.7)**
Hospital stay(days)	19.6 ± 12.5	20.6±12.3	9.2±10.2		
≤20	19(33.3)	10(17.5)	9(15.70		
20-40	24(42.1)	22(38.5)	2(3.5)	0.46	**1.22(0.42-3.5)**
>40	14(24.5)	12(21.0)	2(3.5)		
Ulcer Grade (Wagner)					
grade 0	3(5.2)	0(0)	3(5.2)	-	-
grade 1	5(8.7)	0(0)	5(8.7)	-	-
grade 2	8(14)	7(12.2)	1(1.7)	0.40	**2.27(0.25-20.3)**
grade 3	16(28)	14(24.5)	2(3.5)	0.23	**2.56(0.5-13.1)**
grade 4	20(35)	19(33.3)	1(1.7)	**0.001**	**9.12(1.08-76.3)**
grade 5	5(8.7)	4(7.0)	1(1.7)	0.68	**1.2(0.12-11.7)**
Status					
discharge	54 (94.7)	41(71.9)	12(22.2)		
Dead	3 (5.3)	2(3.5)	1(1.7)	0.656	**0.878(0.08-9.2)**
Treatment					
conservative	38(66.6)	30(52.6)	8(14.0)		
amputation	19 (33.3)	14(24.5)	5(8.7)	0.447	**0.74(0.28-2.6)**
Diabetes duration(years)	12.6 ± 6.40	14.9±2.6	7.6±2.7		
Size of ulcer	20.14 ± 44.85	19.2±3.7	9.8±2.6		
≤4 cm^2	12 (21)	7(12.2)	5(8.7)		
>4 cm^2	45 (78.9)	37(64.9)	8(14.0)	0.89	**3.30(0.83-13.1)**
Complications					
neuropathy	38(66.6)	34(89.4)	4(10.5)	**0.003**	**7.65(1.9-30.1)**
nephropathy	35(61.4)	27(77.1)	8(22.8)	0.627	0.49(0.27-3.54)
retinopathy	32(56.1)	22(68.7)	10(31.2)	0.078	0.30(0.07-1.24)
hypertension	33(57.8)	24(72.7)	9(27.2)	0.269	0.53(0.14-1.99)
osteomyelitis	18(31.5)	16(88.8)	2(11.1)	0.136	**3.14(0.61-15.9)**
Nature of Ulcer					
necrotising	25(43.8)	24(96)	1(4)	**0.002**	**14.4(1.72-120)**
non-necrotising	32(56.1)	20(62.5)	12(37.5)		
Body Mass Index	20.59±4.41	20.3±2.1	18.6±1.8		
Plasma Glucose					
fasting	174.28±85.33	184.7±24.7	142.4±2.8		
postprandial	222.72±92.18	238.4±32.7	187.4±12.7		
HbA1c %	10.11±2.50	10.7±1.7	7.1±2.5		
<7 %(good control)	12(21.0)	9(15.7)	3(5.2)		
7-8 % (fair control)	5(8.7)	3(5.2)	2(3.5)		
>8 % (poor control)	40(70.1)	32(56.1)	8(14.0)	0.32	**1.66(0.45-6.11)**

Table 2: Gram-negative bacilli isolated from 57 diabetic foot ulcers (N=97).

	Name of DFU isolates	Biofilm assay		Total
		Positive	***Negative***	
1	*Escherichia coli*	26(63.4)	15(36.5)	**41(42.2)**
2	*Pseudomonas aeruginosa*	12(52.1)	13(47.9)	**23(23.7)**
3	*Klebsiella oxytoca*	7(63.6)	4(36.4)	**11(11.3)**
4	*Klebsiella pneumoniae*	7(77.7)	2(22.3)	**9(9.2)**
5	*Proteus vulgaris*	4(80)	1(20)	**5(5.1)**
6	*Proteus mirabilis*	-	2(100)	**2(2.0)**
7	*Acinetobacter sp*	3(60)	2(40)	**5(5.1)**
8	*Morganella morganii*	1(100)	-	**1(1.0)**
	Total	**60(59.4)**	**37(38.1)**	**97**

Fig. 1: Average resistance percentage of biofilm positive and negative gram-negative DFU isolates tested against various antibiotics.

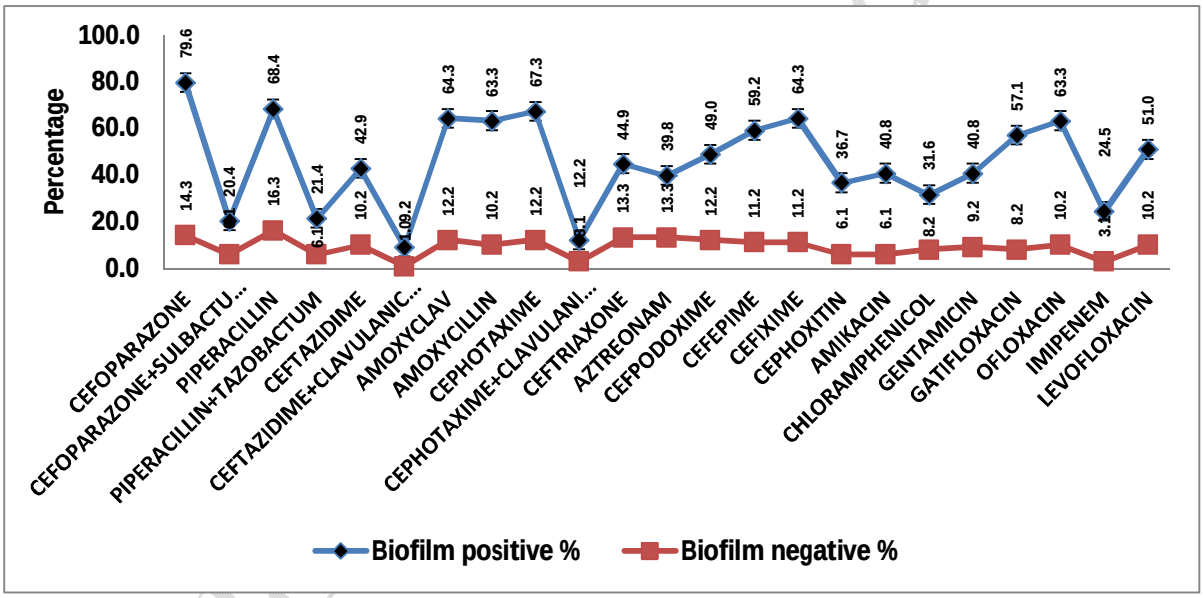


Table 3: MIC of gram-negative bacilli (GNB) isolated from 57 DFU patients (N=97).

MIC	Biofilm producers	Non-biofilm producers
	≥2µg/ml	**≥2µg/ml**
Piperacillin	54(90)	6(10)
Piperacillin+Tazobactum	48(80)	12(20)
Cefoparazone	56(93.3)	1(6.7)
Cefoparazone+Sulbactum	44(73.3)	16(27)
Ceftazidime	49(81.6)	11(18.4)
Ceftazidime+Clavulanic acid	29(48.3)	31(51.6)
Levofloxacin	45(75)	15(25)

Fig. 2: Fasting and postprandial blood glucose level among DFU patients having infection with the biofilm producing and non-producing gram-negative bacterial infections at the time of admission and discharge from the hospital.

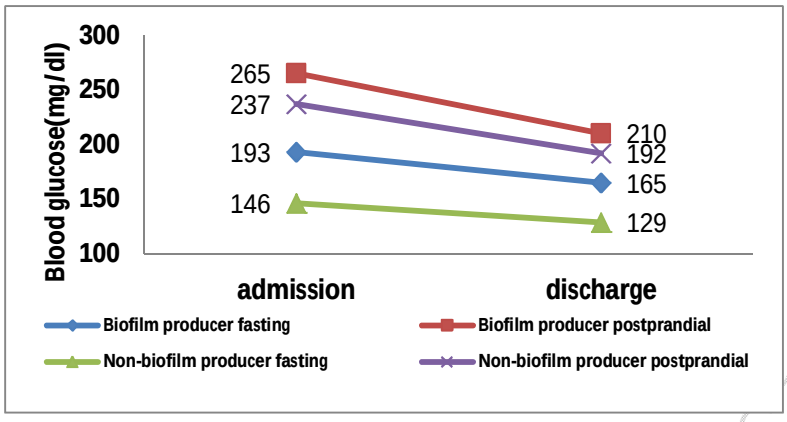


Fig. 3: HbA1c values among the DFU patients having infection with the biofilm producing and non-producing gram-negative bacterial infections at the time of admission and discharge from the hospital.

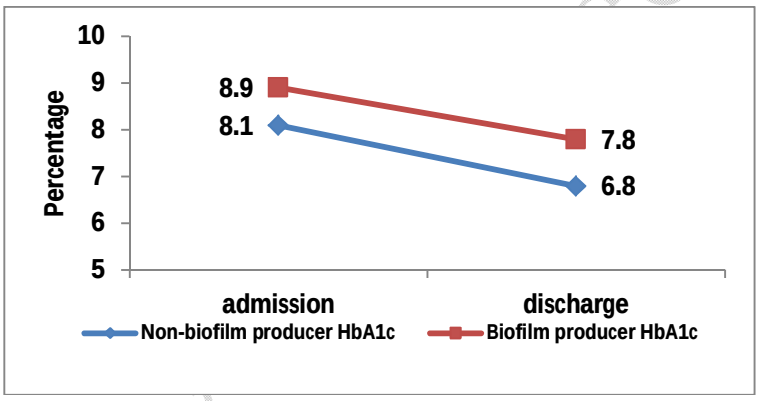


Fig. 4: Tissue culture plate showing the result of biofilm assay, A1 and B1 were blank.

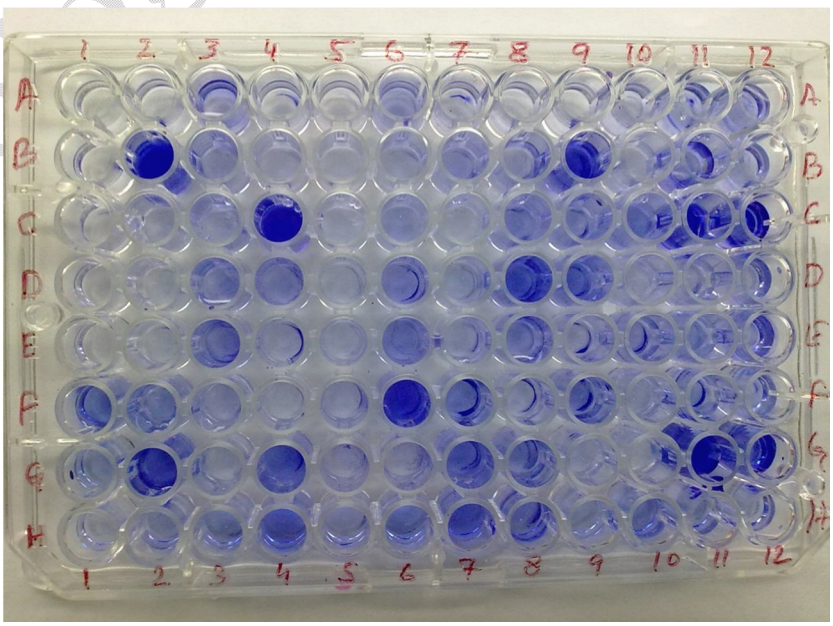
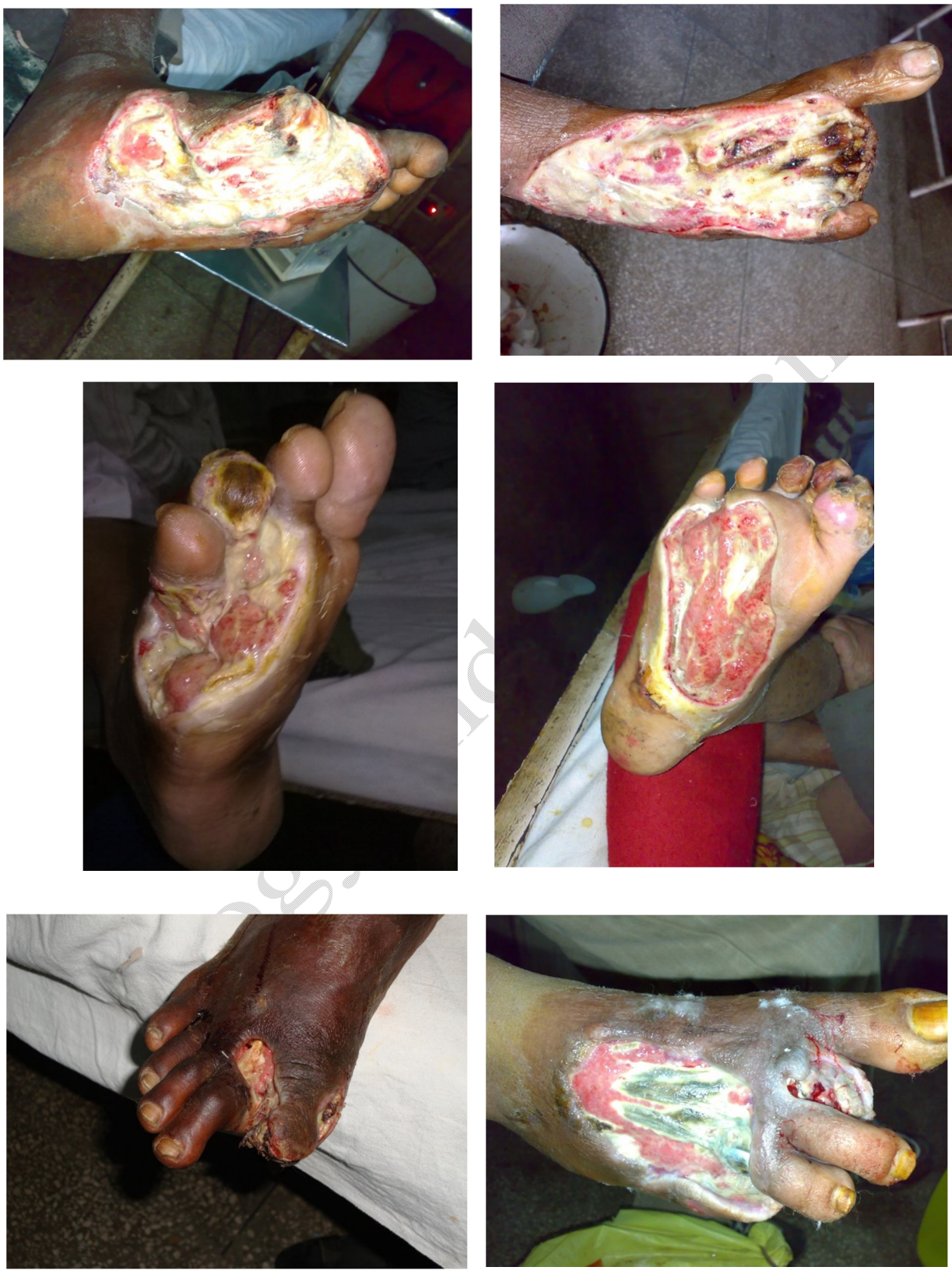


Fig. 5: Images of Diabetic Foot Ulcer.



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Prevalence of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* isolated from diabetic foot ulcer patients

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ABSTRACT

Metallo-beta-lactamase (MBL)-producing *Pseudomonas aeruginosa* strains have been reported to be an important cause of nosocomial infections. There is not enough information from India regarding their prevalence in diabetic foot ulcer (DFU) patients. The present study was undertaken over a period of two year from December 2008 to March 2011 to study the incidence of MBL producing *P. aeruginosa* isolated from 162 DFU patients with various grades of ulcer (Texas classification). Forty isolates of *P. aeruginosa* were obtained from patients. These isolates were subjected to susceptibility testing to anti-*pseudomonal* drugs as per Clinical Laboratory and Standards Institute (CLSI) guidelines, and were further screened for the production of MBL by disc potentiation testing using ethylenediaminetetraacetic acid (EDTA)-impregnated imipenem and meropenem discs. Of the 40 isolates of *P. aeruginosa*, 22 (55%) isolates were found resistant to carbapenems (imipenem) and 18 (81.1%) were found to be MBL producers using imipenem + (EDTA) and 15(68.1%) by meropenem + EDTA. This rapid dissemination of MBL producers is worrisome and necessitates the implementation of not just surveillance studies but also proper and judicious selection of antibiotics, especially carbapenems.

1. Introduction

The introduction of carbapenems into clinical practice represented a great advance for the treatment of serious bacterial infections caused by beta-lactam-resistant bacteria. Due to their broad spectrum activities and stability to hydrolysis by most beta-lactamase, the carbapenems have been the drug of choice for the treatment of infections caused by penicillin or cephalosporin-resistant Gram-negative bacilli, especially ESBL positive Gram-negative infections [1]. The carbapenems available for use in India are imipenem and meropenem [2]. However, carbapenem resistance has been observed frequently in non-fermenting bacilli *Pseudomonas aeruginosa* and *Acinetobacer* spp. Resistance to carbapenem is due to decreased outer membrane permeability, increased efflux systems, alteration of penicillin-binding proteins and carbapenem hydrolyzing enzymes-carbapenemase. These carbapenemase are class B metallo-β-lactamases (MBLs; IMP, VIM) or class D oxacillinases (OXA 23–27) or class A clavulanic acid inhibitory enzymes (SME, NMC, IMI, and KPC) [3]. The IMP and VIM genes responsible for MBL production are horizontally transferable via plasmids and can rapidly spread to other bacteria [4]. They have potent hydrolyzing activity not only against carbapenem but also against other β-lactam antibiotics [5].

Thus, MBL-producing *P. aeruginosa* strains have been reported to be important causes of nosocomial infections associated with clonal spread [6]. There is not enough information from the Indian subcontinent regarding the prevalence of MBL-producing *P. aeruginosa* isolated from DFU patients.

2. Materials and methods

2.1. Study design

The present study was undertaken over a period of two year from December 2008 to March 2011 to study the incidence of MBL *P. aeruginosa* isolated from DFU patients admitted to our hospital. Ethical clearance was obtained from Institutional Ethics Committee (IEC).

2.2. Clinical examination

The clinical examination of DFU patients was adopted from our previous studies [7]. The wound was graded and staged at the time of hospitalization according to the University of Texas Wound classification system as grade 1 (superficial wound, not involving, tendon, capsule or bone), grade 2 (wound penetrating to tendon or capsule) and grade 3 (wound penetrating bone or joint). Grade 0

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patients (pre- or post-ulcerative site that has healed and have no infection) were excluded from the study.

2.3. Microbiological methods

Method of culture of specimens, identification was described elsewhere [7,8] and antimicrobial susceptibility testing was performed using the disk diffusion method as described by the CLSI [9]. Antimicrobial disk used were imipenem (10 μg), aztreonam (30 μg), amoxyclav (30 μg), cefpodoxime (10 μg), ofloxacin (5 μg), cefotaxime (30 μg), cefepime (30 μg), cefixime (5 μg), cefoperazone (75 μg), cefoperazone/sulbactam (75/10 μg), amikacin (30 μg), piperacillin (100 μg), piperacillin/tazobactam (100/10 μg), ceftriaxone (30 μg), ceftazidime (30 μg), ceftazidime/clavulanic acid (30/10 μg), gentamicin (10 μg), amoxycillin (20 μg), cefotaxime/clavulanic acid (30/10 μg), levofloxacin (5 μg), cefoxitin (30 μg), chloramphenicol (30 μg), and gatifloxacin (5 μg). All discs were obtained from Hi-Media labs, Mumbai, India. Inter-pretative criteria for each antimicrobial tested were those recommended by manufacturer's guideline (Hi-Media labs, Mumbai, India).

2.4. Metallo-beta-lactamase detection

Screening and confirmation for the detection of MBL was done by disc potentiation test with EDTA-impregnated imipenem discs and EDTA-impregnated meropenem discs [10].

2.4.1. Disc potentiation test methods

1. Test organism was inoculated onto plates of Mueller–Hinton agar plate (opacity adjusted to 0.5 McFarland opacity standards).
2. A 0.5-m EDTA solution was prepared by dissolving 186.1 g of disodium EDTA $2H_2O$ in 1000 ml of distilled water and adjusting it to pH 8.0 by using NaOH. The mixture was sterilized by autoclaving.
3. Two 10-μg imipenem discs and meropenem discs were placed on the plate; 5 μl of EDTA solution was added to one of the disc each.
4. The inhibition zones of the imipenem and imipenem-EDTA discs and meropenem and meropenem-EDTA discs were compared after 16–18 h of incubation at 35 °C.
5. An increase in the zone size of at least 7 mm around the imipenem-EDTA disc and meropenem-EDTA discs was recorded as an MBL-positive strain.

3. Results

Of the 40 isolates of *P. aeruginosa*, 22 (55%) were found resistant to carbapenems (imipenem) and 15 (68.1%) were found to be MBL producers confirmed by the disc potentiation method (Figs. 1 and 2). The ATCC 27853 *P. aeruginosa* did not exhibit any zone size enhancement with EDTA-impregnated imipenem discs.

Of the 15 MBL-producing isolates, 4 (26.6%) were grade I DFU patients, 9 (60%) grade II and 2 (13.3%) had grade III University of Texas foot grade. Of the 40 patients, 28 (70%) were males and 12 (30%) were females; median age of these patients was 51.7 years (35–87 years).

Overall, the antibiotic sensitivity pattern of *P aeruginosa* is reflected in Fig. 3. Among diabetic patients, ceftazidime with clavulanic acid showed maximum sensitivity (82.5%) followed by ceftazidime (55%), cefoparazone + sulbactam (40%), chloramphenicol (37.5%), ofloxacin (35%), cefepime (32.5%), cefixime (32.5%), gentamycin (32.5%), piperacillin + tazobactam (30%), amoxicillin

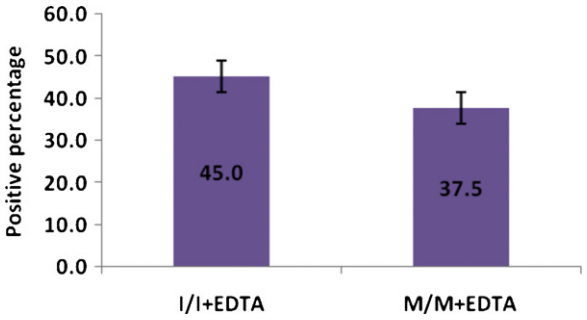

Fig. 1. MBL positive percentage of *P. aeruginosa* strains isolated from DFU infection.

(27.5%), amikacin (27.5%), levofloxacin (27.5%), amoxiclav (25%), aztreonam (22.5%), piperacillin (20%), cefoxitin (10%), gatifloxacin (10%) and cefoparazone (10%). While all strains of *P. aeruginosa* shows 100% resistant to ceftriaxone.

Among the 15 patients with MBL-positive isolates, two patients expired; mortality rate being 13.3%. Among the 15 patients with MBL-producing *P. aeruginosa*, 3 (20%) needed amputation of non-healing DFU because of deterioration in clinical condition due to progressive disease. The mean hospital stay of patients in whom MBL producers were isolated was 35 days (range 16–60 days).

4. Discussion

P. aeruginosa producing MBLs was first reported from Japan in 1991. Since then, they have been described from various parts of the world including Asia, Europe, Australia, South America and North America [11].

In various studies across the world, varying resistance (4–60%) has been seen towards imipenem and meropenem [12,13]. In 2002 from India, Navneeth et al. first reported MBL production by *P. aeruginosa* to be 12% [14]. Since then, the incidence of MBL production by *P. aeruginosa* has been reported to be 10–30% from various clinical specimens across the country [15]. A study conducted by Jesudason et al. reported 42% MBL production by *Pseudomonas* [16]. Another study conducted by Sarkar et al. reported 54.54% MBL production by *Pseudomonas* [17]. We report 68.1% MBL production by *P. aeruginosa* of the 22carbapenem-resistant isolates.

Various methods have been recommended for screening MBL. These include the modified Hodge test, double-disc synergy test using imipenem and EDTA discs or ceftazidime and EDTA discs, EDTA-impregnated imipenem discs [10] and EDTA-impregnated meropenem discs [18].

For MIC detection of imipenem, the *E*-test strip is recommended where one-half of the strips are impregnated with an imipenem gradient across seven dilutions and the other half with another imipenem gradient overlaid with a constant concentration of EDTA [18].

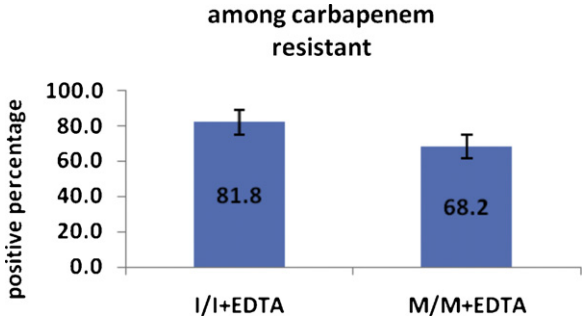

Fig. 2. MBL positivity among the carbapenems resistant *P. aeruginosa* isolated from DFU infection.

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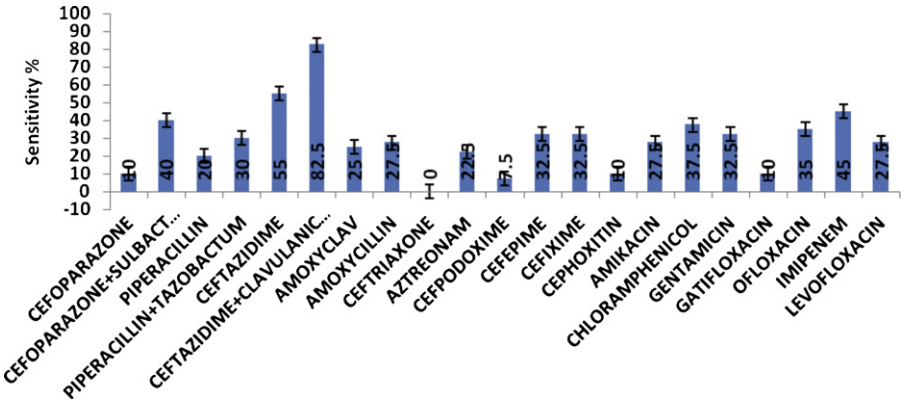

Fig. 3. Overall antibiotic sensitivity pattern of *P. aeruginosa* isolated from infections of diabetic foot ulcer patients.

We used disc potentiation test with EDTA-impregnated imipenem and meropenem discs. *E*-test strips were not used in this study as they are very expensive. Data extrapolated from in vitro studies suggest that polymyxin B or colistin represent the best treatment options for MBL-producing *P. aeruginosa* treatment options [19]. In the present study, ceftazidime with clavulanic acid showed maximum sensitivity (82.5%) followed by ceftazidime (55%), cefoparazone + sulbactam (40%), chloramphenicol (37.5%), ofloxacin (35%), cefepime (32.5%), cefixime (32.5%), gentamycin (32.5%), piperacillin + tazobactam (30%), amoxicillin (27.5%), amikacin (27.5%), levofloxacin (27.5%), amoxiclav (25%), aztreonam (22.5%), piperacillin (20%), cefoxitin (10%), gatifloxacin (10%) and cefoparazone (10%). While all strains of *P. aeruginosa* shows 100% resistant to ceftriaxone.

Among the diabetic patients, foot infections being polymicrobial, there is a high incidence of multidrug-resistant *P. aeruginosa*. Gadepalli et al. have reported *P. aeruginosa* strains showing 100% sensitivity to carbapenems, followed by 88.8% sensitivity to cefoperazone–sulbactam and ticarcillin–clavum combinations [8]. In our earlier reports of antimicrobial study of diabetic foot ulcer, 23 *P. aeruginosa* was isolated from 102 DFU patients' shows, carbapenems were positive in 32.4% isolates and resistance against penicillin group was 73.9%, cephosporins (76%), aminoglycosides (72.5%). While for beta lactam the resistance reported was 32.2% [7].

P. aeruginosa are responsible for 3–7% infections and high mortality rates (27–48%) in critically ill patients [20].

In conclusion, this rapid dissemination of MBL producers necessitates the implementation of not just surveillance studies but also proper and judicious selection of antibiotics, especially carbapenems.

Conflict of interest

The authors declare that there are no conflicts of interest.

Source of funding

None.

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Clinico-microbiological study and antimicrobial drug resistance profile of diabetic foot infections in North India

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ABSTRACT

The study was carried out in diabetic patients with foot ulcer to determine the microbiological profile of infected ulcer, antibiotic resistance of the isolates and to find out the potential risk factors for infection with multidrug resistance and the outcome of these infections. A detailed clinical history and physical examination was carried out in each patient. Pus samples for bacterial culture were collected from 102 patients admitted with diabetic foot infections. All patients had ulcer with Texas grades 1–3. Seventeen patients (16.6%) had coexisting osteomyelitis. Aerobic gram negative bacilli were tested for extended spectrum β lactamase (ESBL) production by phenotypic and genotypic methods. Staphylococcus isolates were tested for susceptibility to oxacillin and cefoxitin by disk method. Potential risk factors for MDRO positive samples were explored. Gram negative aerobes were most frequently isolated (63.8%), followed by gram positive aerobes (36.1%) and anaerobes (31.4%). Forty five percent of patients were positive for MDROs. ESBL production and methicillin resistant was noted in 68.5% and 43.2% of bacterial isolates respectively. 34.5% gram negative strains were positive for $bla_{CTX\text{-}M}$ gene followed by bla_{SHV} (23%) and bla_{TEM} (7.4%). Among the anaerobic organism 17(31.4%) from 54 patients, most commonly isolated were *Peptostreptococcus* sp. (35.2%). MDRO positive status was associated with the presence of neuropathy ($P < 0.001$), osteomyelitis ($P < 0.001$), and ulcer size >4 cm^2 ($P < 0.001$) but not with patients characteristic, ulcer type and type of diabetes, or duration of hospital stay. Poor glycemic control in 68.6% patients, duration of infection > 1 month (36.2%) and ulcer size > 4 cm^2 (75.4%) were independently associated with risk of MDR organisms infection.

1. Introduction

Diabetic foot ulceration and infections are a major medical, social, economic problem and a leading cause of morbidity and mortality, especially in the developing countries like India [1–3]. Fifteen percent of all diabetic patients develop a foot ulcer at some point in their lives which is highly susceptible to infections that spreads rapidly, leading to overwhelming tissue destruction and subsequent amputation [4,5]. In addition to foot ulcers, diabetic patients have also functional changes in microcirculation and changes in cellular activity and growth factor activation processes, which increasingly disrupts wound healing [6]. Antimicrobial resistance is considered to be a major public health threat [7]. The most important cause of antimicrobial resistance is overusing an inappropriate use of antibiotics [8,9]. Diabetic patients with foot ulcers have several factors that may be associated with a high risk of multidrug resistant microorganisms (MDRMs) carriage, such as inappropriate antibiotic treatment, chronic course of the wound, and frequent hospital admissions. Furthermore, peripheral arterial diseases are often present in patients with diabetic foot ulcers (DFUs) and may lead to poor penetration of antibiotics into the lower limb tissues, thereby promoting selection of resistant bacterial strains. Although in DFU, different microorganisms or mixed bacteria are usually responsible for the infection depending on the status of the ulcer, *Staphylococcus aureus* and coagulase negative Staphylococci are the most frequently isolated microorganisms [10,11]. In recent years, there has been an increase in the incidence and prevalence of ESBLs. Currently there is paucity of data on extended spectrum beta lactamases (ESBLs)-producing organisms from diabetic foot infections especially in this part of World. Infection with MDROs may increase the duration of hospital stay, cost of management and may cause additional morbidity and mortality [7]. Early diagnosis of microbial infections is aimed to institute the appropriate antibacterial therapy to avoid further complications. Therefore, this study was planned with the objective to determine the bacterial profile and antimicrobial resistance profile of organisms isolated from patients with DFUs. The potential risk factors for infection of ulcers with

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MDRMs and the outcome of these infections were also studied.

2. Materials and methods

2.1. Study design

Out of 342 diabetic patients admitted in the Centre for Diabetes and Endocrinology, J.N.M.C, A.M.U. Aligarh, India, 102 who developed ulcer in their foot during December 2008 to February 2010 were included in this study. All the subjects gave informed consent and clearance was obtained from the Institutional Ethics Committee (IEC).

2.2. Clinical examination

A detailed history and physical examination was carried out for every subject. Age, sex, anthropometric measurements (body mass index), duration of diabetes, glycemic control during the hospital stay, lipid profile, presence of retinopathy, nephropathy (creatinine > 1.5 mg% or presence of micro or macroalbuminuria), neuropathy (absence of perception of the Semmes–Weinstein monofilament at 2 of 10 standard planter sites on either foot), peripheral vascular disease (ischaemic symptoms and intermittent claudication of rest pain, with or without absence of pedal pulses or posterior tibial pulses), hypertension, duration, site, and size of ulcer, history of smoking, history of previous amputation and clinical outcome were noted in every patient. Clinical assessment for signs of infection (swelling, exudates, surrounding cellulitis, odor, tissue necrosis, crepitation and pyrexia) was made by one researcher classifying the ulcers and determining the presence of clinical signs of infection. Ulcer size was determined by multiplying the longest and the widest diameters and expressed in centimeters squared [11,12]. The wound was graded and staged at the time of hospitalization according to the University of Texas wound classification system [13] as grade 1 (superficial wound, not involving, tendon, capsule or bone), grade 2 (wound penetrating to tendon or capsule) and grade 3 (wound penetrating bone or joint). Grade 0 patients (pre- or post-ulcerative site that has healed) were excluded from the study. Diagnosis of extension to the bone was made in majority of patients by probing with a sterile steel probe. In the absence of a sinus tract or an exposed bone, a standard radiograph showing signs of osteomyelitis in the bone was considered definitive and later on MRI was done to confirm the osteomyelitis in suspected patients.

2.3. Microbiological methods

Culture specimens were obtained at the time of admission; after the surface of the wound had been washed vigorously by saline, and followed by debridement of superficial exudates. Specimens were then obtained by scrapping the base of ulcer or the deep portion of the wound edge with a sterile curette after cleaning the base of ulcer with a sterile swab stick [3,13,14]. The soft tissue specimens and pus aspirated from syringe were promptly sent to the Microbiology Department and processed for aerobic and anaerobic bacteria. Standard methods for isolation and identification of aerobic [15,16] and anaerobic bacteria were used [17,18].

2.4. Susceptibility testing

Antimicrobial susceptibility testing of aerobic isolates was performed using the disk diffusion method as described by the CLSI [19]. Antimicrobial disks used were imepenem (10 μg), aztreonam (30 μg), amoxyclav (30 μg), cefpodoxime (10 μg), metronidazole (5 μg), cefepime (30 μg), cefoperazone (75 μg), cefoperazone/sulbactum (75/10 μg), cefixime (5 μg), piperacillin (100 μg), piperacillin/tazobactum (100/10 μg), ceftazidime (30 μg), ceftazidime/clavulanic acid (30/10 μg), amikacin (30 μg), amoxycillin (20 μg), cephotaxime (30 μg), ofloxacin (5 μg), cephotaxime/clavulanic acid (30/10 μg), ceftriaxone (30 μg), cefoxitin (30 μg), oxacillin (1 μg), chloramphenicol (30 μg), gentamicin (10 μg), gatifloxacin (5 μg), levofloxacin (5 μg), sparfloxacin (5 μg), streptomycin (10 μg), vancomycin (30 μg), clindamycin (2 μg), tobramycin (10 μg), azithromycin (15 μg), erythromycin (15 μg), and bacitracin (μg). All disks were obtained from Hi-Media Labs, Mumbai, India. Interpretative criteria for each antimicrobial tested were those recommended by manufacturer's guideline (Hi-Media Labs, Mumbai, India).

2.5. Phenotypic methods for MRSA and ESBL detection

Staphylococcus species were tested for methicillin resistance by using 1-μg oxacillin disk [20] and 30-μg cefoxitin disk [21]. Gram-negative bacilli were first screened for the production of ESBL by disk diffusion method using cephotaxime, ceftriaxone, aztreonam, cefepime, cefoxitin and ceftazidime and later on confirmed by cephalosporin/clavulanate combination disk test (disk potential test) using ceftazidime, ceftazidime + clavulanic acid, cephotaxime, cephotaxime + clavulanic acid, piperacillin, cefoparazone + sulbactam, cefoparazone and piperacillin + tazobactum [22]. *E. coli ATCC* 25922 (non ESBL-producer), *Klebsiella pneumoniae 700603* (ESBL-producer) and *S. aureus* ATCC 25923 were used as control strains respectively. A microorganism was classified as MDRO if it was found to be resistant to two or more classes of antimicrobials and included MRSA, ESBL producing organisms [7].

2.6. Molecular methods for ESBL detection

2.6.1. Preparation of DNA template

Template DNA was prepared from freshly cultured bacterial isolates by suspending 3–5 colonies in 50 μl of molecular grade water, and then heating at 95 °C for 5 min and immediately chilling at 4 °C. Positive controls harboring $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} and negative control (*E. coli* ATCC 25922) were processed in the same way for DNA extraction.

2.6.2. Detection of bla genes by PCR

Molecular detection of $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} was performed in ceftriaxone resistant gram negative isolates by using polymerase chain reaction (PCR) according to the methods described previously with minor modifications [23,24]. The primers and cycling conditions for the detection of *bla* genes were same as described by Shahid et al. [25].

2.7. Antibiotic treatment

Antibiotics were selected according to published recommendation [7]. In mild infections amoxicillin clavulanic acid was given empirically by the oral route. However in moderate infections intravenous route was preferred taking into consideration the likelihood of osteomyelitis. Considering that the causative agent was polymicrobial, we initiated ampicillin-sulbactam plus an aminoglycoside/quinolone or piperacillin-tazobactam or ceftriaxone plus metronidazole/clindamycin. In the presence of severe infections, surgical debridement and amputation were performed immediately after admission. Metronidazole (500 mg I.V. every 8 h) was added to the drug regimen if cellulitis or gangrene was also present. Combinations of extended spectrum antibiotics were initiated and the treatment was later modified in accordance with the culture results. The duration of the treatment was at least 4–6 weeks and

Table 1
Association of study characteristics in two groups of diabetic patients according to the infection of foot ulcer with MDR and non-MDRs (data are mean ± SD or *n* (%) unless otherwise indicated).

N = 102	Total	MDR	Non-MDR	*P* value	O.R. (95% CI)
Gender distribution		*n* = 46	*n* = 56		
Male	67(65.6)	30(65.2)	37(66)	0.72	0.50(0.13–1.8)
Female	37(34.3)	16(34.7)	19(33.9)	0.67	0.49(0.17–1.82)
Age distribution (years)	**49.11 ± 12.46**				
<40	16(15.6)	10(21.7)	8(14.2)	–	–
41–60	65(63.72)	29(63)	36(64.2)	**0.000**	**0.72(0.19–2.68)**
61–80	19(18.62)	7(15.2)	12(21.4)	–	–
Type of diabetes					
Type 1	14(13.72)	6(13)	8(14.2)	–	1.00
Type 2	88(86.27)	40(86.9)	48(85.7)	0.067	3.94(0.97–16.4)
Duration of diabetes (years)	**15.5 ± 6.40**				
≤10	72(70.5)	23(50)	49(87.5)	–	1.00
11–20	26(25.4)	20(43.4)	6(10.7)	0.04	0.74(0.09–2.03)
≥21	4(3.9)	3(6.5)	1(1.7)	–	0.86(0.72–1.05)
Duration of ulcer	**41.52 ± 47.58**				
<1 month	63(61.76)	32(69.5)	31(55.3)	0.032	1.00
>1 month	37(36.27)	14(30.4)	23(41)	0.001	0.54(0.12–1.4)
Size of ulcer	**20.14 ± 44.85**				
≤4 cm^2	25(24.5)	6(13)	20(35.7)	0.47	1.00
>4 cm^2	**77(75.49)**	40(86.9)	37(66)	**<0.001**	**12.6(3.9–33.5)**
Complications					
Hypertension	69(67.6)	44(95.6)	25(44.6)	**0.004**	**3.48(1.26–9.67)**
Retinopathy	54(52.9)	39(84.70)	15(26.7)	**0.000**	**0.84(0.34–2.34)**
Neuropathy	47(46)	37(80.4)	27(48.2)	**<0.001**	**1.32(0.46–3.91)**
Nephropathy	64(62.7)	32(69.5)	15(26.7)	**0.01**	**4.37(1.06–14.6)**
Grade of ulcer (Texas)					
1	16(15.6)	9(19.50)	7(12.5)	0.56	0.43(0.16–1.13)
2	59(57.8)	36(78.2)	23(41)	**0.001**	**0.62(0.13–2.63)**
3	27(26.4)	21(45.60)	6(10.7)	**<0.001**	**2.34(0.74–7.39)**
Nature of ulcer					
Non-necrotic	79(77.4)				
Necrotic	23(22.5)				
Hospital stay (days)	**22.9 ± 15.54**	**26.9 ± 7.2**	**17.9 ± 2.9**	**0.005**	**10.76(7.3–23.8)**
Management					
Amputation	23(22.5)	19(41.3)	4(7.1)	**<0.001**	**5.13(1.77–13.3)**
Conservative	79(77.4)	27(58.6)	52(92.8)	–	–
Previous antibiotic use					
Present	48(47)	36(78.2)	12(21.4)	**0.002**	**0.25(0.09–3.25)**
Absent	54(52.9)	10(21.7)	44(78.5)	–	–
Discharge status					
Alive	97(95)	42(91.3)	55(98.2)	0.06	1.00
Dead	5(4.9)	4(8.6)	1(1.7)	**0.002**	**3.2(1.2–8.2)**
Bacterial infection type					
Superficial	16(15.6)	9(19.5)	7(12.5)	0.56	0.43(0.16–1.13)
Subcutaneous	59(57.8)	36(78.2)	23(41)	**0.001**	**0.62(0.13–2.63)**
Osteomyelitis	27(26.4)	21(45.6)	6(10.7)	**<0.001**	**2.34(0.74–7.39)**

The bold values indicate that those are the main factors which are associated with the MDR infections in the Diabetic foot ulcer patients.

prolonged in cases of osteomyelitis. All patients also received an intensive insulin treatment.

2.8. Statistical analysis

The data were analyzed using SPSS version 13.0 for descriptive statistics. Quantitative variables were expressed as mean ± SD while qualitative variables were expressed as percentage (%). Continuous variables were compared using 2 sample *t* tests for independent samples. Odds ratios and 95% confidence interval (CI) were reported for independent variables associated with the outcome variable: presence of MDR infection.

3. Results

Males were predominant 67(65.6%) in the study subjects. All patients had ulcers graded 1–3 in the University of Texas classification system. Majority of subjects had T2DM 88(86.2%). The mean age of the subjects was 49.1 ± 12.4 years. The mean duration of diabetes was 15.5 ± 6.6 years, and nearly 72(70.5%) had the condition for ≤10 years. Forty-seven patients (46%) had neuropathy, 64(62.7%) nephropathy, 54(52.9%) retinopathy, and 69(67.6%) were hypertensive. Osteomyelitis was present in 27(26.4%) subjects. Nearly one third 37(36.2%) had lesions for >1 month before presentation at the hospital. The ulcer was necrotic in 23(22.5%) cases. Glycemic control was poor in 67(65.6%). HbA1c was <7% in 12 patients (11.7%), 7–8% in 2(1.9%) and >8% in 88(86.2%) subjects. More than 46(45%) received surgical treatment, mainly in the form of debridement. 23(22.5%) patients were subject to amputation and 5(4.9%) died during the hospital stay (mean hospital stay 22.9 ± 15.5) [Table 1]. Glycemic control at the time of discharge was good in 67(55.8%, HbA1c <7%), poor in 40(39.2%, HbA1c >8%) and satisfactory in 5(4.9%, HbA1c 7–8%) subjects (Figs. 1 and 2). Majority of the ulcer was found on interdigits (42.15%), followed by plantar surface (26.4%), heel (23.5%), margins (15.6%), malleoli (13.7%), and leg (4.9%) and on multiple areas (≥2 sites) was 26.4% which include 22 males and only 7 females. Size of ulcer ≤4 cm^2 was observed in 24.5% patients [11 males; 14 females], ≥4 cm^2 in 75.4% in which majority of the patients were males.

Grade I ulcer was found in 15.6%, grade II in 57.8% and grade III in 26.4% patients. The number of DFU patients and their type of isolates (MDR+ and MDR−) within grade is summarized in Table 2.

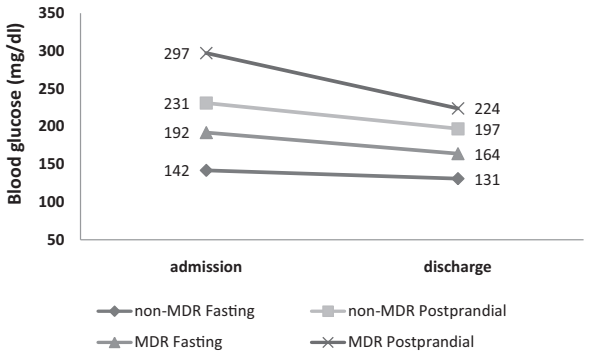

Fig. 1. Fasting and postprandial blood glucose level among diabetic foot ulcer infected with and without MDROs at the time of admission and discharge from the hospital.

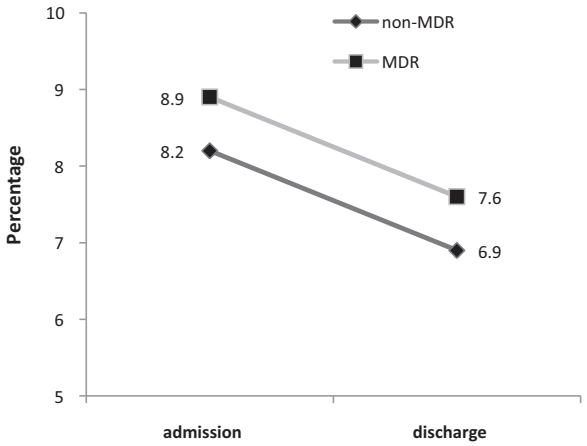

Fig. 2. HbA1c values among the diabetic foot ulcer infected with and without MDROs at the time of admission and discharge from the hospital.

In grade 1, MDR positive and MDR− infection was seen in 5.8% ($P = 0.005$) and 9.8% ($P = O.17$) respectively. In grade 2, MDR positive and MDR− infection was seen in 22.5% ($P = 0.001$) and 35.2% ($P = O.147$) and in grade 3, MDR positive and MDR− infection was seen in 16.6% ($P = 0.006$) and 9.8% ($P = O.035$) patients respectively. In addition, there was a significant relation between the depths of DFU (subcutaneous wound and presence of osteomyelitis) with MDRM infection. In superficial wound, growth of bacteria was significantly lower when compared with subcutaneous and bone involvement infection ($P = 0.001$, $P = 0.000$ respectively). When MDR growth was compared, there was significant correlation with the subcutaneous and osteomyelitis ($P = 0.001$ and P = 0.006 respectively). No significant relation was present in MDR-negative with superficial, subcutaneous and presence of osteomyelitis ($P = 0.17$, $P = 0.147$ and $P = 0.035$ respectively).

3.1. Microbiological observations

A total of 152 aerobic bacteria were isolated, averaging of 1.49 species per patient. 38% patients had monomicrobial infection and polymicrobial etiology was observed in 62%.

In the direct microscopic examination of ulcer samples, 94% had corresponding results to the culture growth on next day, in 2% samples direct result differ in their culture growth and in 4%

Table 2
The microorganism growth rate according to the grade of diabetic foot (superficial, subcutaneous or presence of osteomyelitis) and frequency of aerobic and anaerobic bacteria isolated from 102 and 54 diabetic foot ulcer patients respectively. *n* (%).

	MDR		Non-MDR		Total	
	n (%)	*P*	*n* (%)	*P*	*n* (%)	*P*
Superficial	6(5.8)	0.005	10(9.8)	0.17	16(15.6)	0.103
Subcutaneous	23(22.5)	0.001	36(35.2)	0.147	59(57.8)	0.001
Osteomyelitis	17(16.6)	0.006	10(9.8)	0.035	27(26.4)	0.000

	Name of isolates	Total
Aerobic		
	Gram positive cocci	55(36.1)
1	*Staphylococcus aureus*	37(24.3)
2	*Enterococcus faecalis*	5(3.2)
3	*Beta hemolytic streptococcus*	5(3.2)
4	*CONS*	4(2.6)
5	*Coryneform* sp.	4(2.6)
	Gram negative bacilli	97(63.8)
6	*Escherichia coli*	41(42.2)
7	*Pseudomonas aeruginosa*	23(23.7)
8	*Klebsiella oxytoca*	11(11.3)
9	*Klebsiella pneumoniae*	9(9.2)
10	*Proteus vulgaris*	5(5.1)
11	*Proteus mirabilis*	2(2.0)
12	*Acinetobacter* sp.	5(5.1)
13	*Morganella morganii*	1(1.0)
	Total aerobic	152
Anaerobic		
	Gram positive cocci	10(58.8)
14	*Peptostreptococcus* sp.	6(35.2)
15	*Peptostreptococcus anaerobius*	4(23.5)
	Gram positive bacilli	5(29.4)
16	*Propionibacterium* sp.	3(17.6)
17	*Clostridium perfringens*	1(5.8)
18	*Eggerthella lenta*	1(5.8)
	Gram negative bacilli	2(11.7)
19	*Bacteroides ureolyticus*	2(11.7)
	Total anaerobic	17

Table 3
Antimicrobial resistance pattern of bacteria isolated from diabetic foot ulcers in diabetic patients ($N = 152$; n = total number of bacteria isolates of a given type).

	Antimicrobial	*Ps* 23(15.1)	*Ec* 41(26.7)	*Pv* 5(3.2)	*Pm* 2(1.3)	*Mm* 1(0.6)	*Ko* 5(7.2)	*Kp* 7(4.6)	*Ac* 5(3.2)	*Sa* 37(24.3)	Bhs 5(3.2)	*CONS* 4(2.6)	*Cr* 4(2.6)	*En* 5(3.2)	Total
Penicillin	Amoxycillin	16(69.6)	31(75.6)	0(0)	2(100)	1(100)	10(90.9)	8(88.9)	5(100)	18(48.6)	4(80)	3(75)	3(75)	1(20)	95(62.5)
	Amoxyclav	17(73.9)	35(85.4)	5(100)	2(100)	1(100)	3(27.3)	8(88.9)	5(100)	¤	¤	¤	¤	¤	76(50.0)
	Piperacillin	18(78.3)	38(92.7)	3(60)	2(100)	1(100)	11(100)	7(77.8)	4(80)	¤	¤	¤	¤	¤	84(55.3)
	Oxacillin	£	£	£	£	£	£	£	£	16(43.2)	3(60)	4(100)	1(25)	4(80)	39(25.7)
Cephalosporins	Cefoxitin	20(87.0)	12(29.3)	3(60)	1(50)	1(100)	11(100)	2(22.2)	3(60)	18(48.6)	3(60)	3(75)	4(100)	0(0)	78(51.3)
	Ceftriaxone	23(100)	18(43.9)	2(40)	2(100)	1(100)	3(27.3)	7(77.8)	4(80)	26(70.3)	1(20)	3(75)	1(25)	2(40)	93(61.2)
	Cefpodoxime	21(91.3)	21(51.2)	3(60)	2(100)	0(0)	3(27.3)	7(77.8)	4(80)	¤	¤	¤	¤	¤	61(40.1)
	Ceftazidime	11(47.8)	16(39.0)	1(20)	1(50)	0(0)	11(100)	8(88.9)	5(100)	¤	¤	¤	¤	¤	53(34.9)
	Cefotaxime	16(69.6)	33(80.5)	5(100)	2(100)	1(100)	11(100)	7(77.8)	4(80)	14(37.8)	4(80)	3(75)	4(100)	2(40)	106(69.7)
	Cefoparazone	20(87.0)	40(97.6)	5(100)	2(100)	1(100)	11(100)	7(77.8)	5(100)	¤	¤	¤	¤	¤	91(59.9)
	Cefixime	15(65.2)	34(82.9)	3(60)	2(100)	0(0)	11(100)	8(88.9)	1(20)	27(73.0)	3(60)	1(25)	4(100)	2(40)	111(73.0)
	Cefepime	15(65.2)	31(75.6)	3(60)	2(100)	0(0)	11(100)	1(11.1)	1(20)	¤	¤	¤	¤	¤	64(42.1)
Monobactum	Aztreonam	17(73.9)	17(41.5)	2(40)	2(100)	1(100)	3(27.3)	8(88.9)	3(60)	¤	¤	¤	¤	¤	53(34.9)
Carbapenems	Imepenem	12(52.2)	15(36.6)	0(0)	0(0)	0(0)	0(0)	1(11.1)	0(0)	¤	¤	¤	¤	¤	28(18.4)
Aminoglycosides	Amikacin	16(69.6)	14(34.1)	3(60)	1(50)	1(100)	9(81.8)	1(11.1)	1(20)	12(32.4)	1(20)	3(75)	4(100)	5(100)	71(46.7)
	Gentamycin	15(65.2)		3(60)	2(100)	0(0)	10(90.9)	7(77.8)	4(80)	14(37.8)	3(60)	3(75)	0(0)	4(80)	74(48.7)
	Streptomycin	£	£	£	£	£	£	£	£	32(86.5)	3(60)	4(100)	0(0)	5(100)	44(28.9)
	Tobramycin	£	£	£	£	£	£	£	£	21(56.8)	3(60)	4(100)	4(100)	4(80)	36(23.7)
Chloremphenecol	Chloramphenicol	14(60.9)	16(39.0)	3(60)	1(50)	1(100)	1(9.1)	2(22.2)	2(40)	4(10.8)	4(80)	3(75)	0(0)	4(80)	55(36.2)
Quinalones and fluro-quinalones	Ofloxacin	14(60.9)	29(70.7)	4(80)	2(100)	1(100)	11(100)	7(77.7)	4(80)	20(54.1)	3(60)	4(100)	1(25)	0(0)	100(65.8)
	Sparfloxacin	£	£	£	£	£	£	£	£	31(83.8)	4(80)	4(100)	0(0)	5(100)	44(28.9)
	Gatifloxacin	20(87.0)	26(63.4)	4(80)	1(50)	0(0)	9(81.8)	1(11.1)	2(40)	16(43.2)	3(60)	1(25)	0(0)	1(20)	84(55.3)
	Levofloxacin	16(69.6)	27(65.9)	3(60)	1(50)	1(100)	9(9.1)	1(11.1)	2(40)	25(67.6)	3(60)	1(25)	1(25)	3(60)	93(61.2)
β-Lactum inhibitors	Ceftazidime + clavulanic acid	4(17.4)	2(4.9)	0(0)	0(0)	0(0)	11(100)	2(22.2)	4(80)	¤	¤	¤	¤	¤	23(15.1)
	Cephotaxime + clavulanic acid	0(0.0)	13(31.7)	0(0)	0(0)	0(0)	0(0)	2(22.2)	2(40)	20(54.1)	0(0)	2(50)	0(0)	1(20)	40(26.3)
	Piperacillin + tazobactum	15(65.2)	4(9.8)	3(60)	1(50)	0(0)	11(100)	2(22.2)	4(80)	¤	¤	¤	¤	¤	40(26.3)
	Cefoparazone + sulbactum	13(56.5)	5(12.2)	3(60)	1(50)	0(0)	11(100)	1(11.1)	4(80)	¤	¤	¤	¤	¤	38(25.0)
Macrolides	Erythromycin	£	£	£	£	£	£	£	£	17(45.9)	2(40)	4(100)	1(25)	4(80)	28(18.4)
	Azithromycin	£	£	£	£	£	£	£	£	30(81.1)	3(60)	4(100)	1(25)	5(100)	43(28.3)
Lincsamides	Clindamycin	£	£	£	£	£	£	£	£	16(43.2)	3(60)	4(100)	4(100)	4(80)	31(20.4)
Glycopeptides	Vancomycin	£	£	£	£	£	£	£	£	0(0.0)	0(0)	00	0(0)	0(0)	0(0)
Bacitracin		£	£	£	£	£	£	£	£		3(60)				3(100)

£, not tested; ¤, all Staphylococcus resistant to oxacillin have been considered resistant to all β-lactum; *Ps*: *Pseudomonas aeruginosa*, *Ec*: *Escherichia coli*, *Pv*: *Proteus vulgaris*, *Pm*: *Proteus mirabilis*, *Mm*: *Morganella morganii*, *Ko*: *Klebsiella oxytoca*, *Kp*: *Klebsiella pneumoniae*, *Ac*: *Acinetobacter* sp., *Sa*: *Staphylococcus aureus*, Bhs: beta hemolytic *streptococcus*, *CONS*: coagulase negative *staphylococcus* sp., *Cr*: *Coryneform* sp., *En*: *Enterococcus faecalis*.

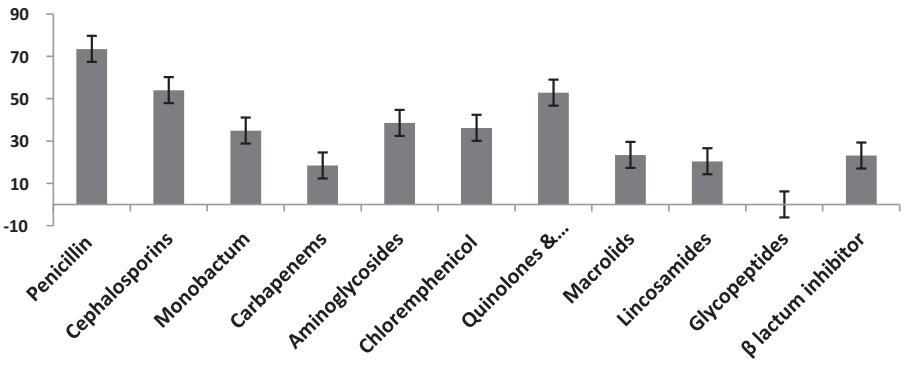

Fig. 3. Resistance percentage of antibiotic groups.

cases, direct examination could not been done. Among the bacterial isolates, gram positive cocci comprised of 36.1% and gram negative bacilli for 63.8%. Gram positive to gram negative ratio was 1:1.7. The frequency of bacterial isolates from the DFU is shown in Table 3. *Escherichia coli* was the most common isolate, accounting for 42.2%; followed by *S. aureus* 24.3%, *Pseudomonas aeruginosa* 23.7%, *Klebsiella oxytoca* 11.3%, *K. pneumoniae* 9.2%, *Proteus vulgaris* and *Acinetobacter* 5.1% each, beta hemolytic *streptococcus* spp. 3.2%, *Enterococcus faecalis* 3.2%, *CONS* 2.6%, *Coryneform* sp. 2.6%, *Proteus mirabilis* 2% and *Morganella morganii* 1%. Anaerobic culture was done in fifty four DFU patients in whom the pus sample was aspirated by the sterile syringe. Air from the syringe was removed immediately. Anaerobes alone were observed in two patients (3.7%). Both aerobic and anaerobic organisms were isolated in the remaining patients (96.2%). Anaerobic gram-positive cocci were found in 10 patients, 5 patients had infection by gram-positive bacilli and only two patients had infection by gram negative bacilli. The remaining 37 patients (57.5%) were found negative for anaerobic culture. Among the anaerobic bacteria isolated, gram positive comprised of 88.2% and gram negative for 11.7%. The frequency of bacterial isolates from the DFU was shown in Table 2. *Peptostreptococcus* sp. was the most common isolate, accounting for 35.2%; followed by *Peptostreptococcus anaerobius* 23.5%, *Propionibacterium* sp. 17.6%, *Bacteroides ureolyticus* 11.7%, *Clostridium perfringens* 5.8% and *Eggerthella lenta* 5.8% were isolated from 54 DFU patients.

3.2. Antibiotic resistance profile

The results of resistance studies are summarized in Table 3 and Fig. 3. High degree of antibiotic resistance was exhibited by *P. mirabilis* (69.6%), followed by *K. oxytoca* (67.6%), *P. aeruginosa* (65.8%), *Acinetobacter* sp. (63.5%), *P. vulgaris* (53%), *M. morganii* (52.2%), *E. coli* (51.5%) and *K. pneumoniae* (50.7%). Higher percentage of resistance (73.5%) was shown among the Penicillin group [*P. aeruginosa* (73.9%), *E. coli* (84.5%), *P. vulgaris* (53.3%), *K. oxytoca* (72.7%), *K. pneumoniae* (85.2%), *Acinetobacter* sp. (93.3%) and *S. aureus* (35.8%)], followed by cephalosporin group (54%) [*P. aeruginosa* (76%), *E. coli* (62.5%), *P. vulgaris* (62.5%), *P. vulgaris* (62.5%), *P. mirabilis* (87.5%), *M. morganii* (50%), *K. oxytoca* (81.8%), *K. pneumoniae* (65.2%), *Acinetobacter* sp. (67.5%) and *S. aureus* (61.5%)], quinolones and fluroquinolones (52.8%): [*P. aeruginosa* (72.5%), *E. coli* (66.6%), *P. vulgaris* (73.3%), *P. mirabilis* (66.6%), *M. morganii* (66.6%), *K. oxytoca* (63.6%), *K. pneumoniae* (33.3%), *Acinetobacter* sp. (53.3%), *S. aureus* (61.5%), *CONS* (45%) and *E. faecalis* (45%)], aminoglycosides group (38.5%) [*P. aeruginosa* (72.5%), *E. coli* (66.6%), *P. vulgaris* (73.3%), *P. mirabilis* (66.6%), *M. morganii* (66.6%), *K. oxytoca* (63.6%), *K. pneumoniae* (33.3%), *Acinetobacter* sp. (53.3%) and *S. aureus* (52.7%)], with beta lactam inhibitors (32.2%) [*P. aeruginosa* (23.9%), *E. coli* (14.6%), *P. vulgaris* (30%), *P. mirabilis* (25%), *K. oxytoca* (75%), *K. pneumonia* (19.4%) and *Acinetobacter* sp. (70%)] and carbapenems (18.4%) [*P. aeruginosa* (52.2%), *E. coli* (36.6%), *P. vulgaris* (0%), *P. mirabilis* (0%), *M. morganii* (0%), *K. oxytoca* (0%), *K. pneumoniae* (11.1%) and *Acinetobacter* sp. (0%)]. All the anaerobes were susceptible to metronidazole, amoxicillin + clavulanate and imepenem.

3.3. Phenotypic ESBL and MRSA detection

Eighty (83.3%) gram negative DFU isolates were ESBL positive (Table 4a and b) by disk diffusion method using cephotaxime, followed by 71(73.2%) for cefpodoxime, 68(70.1%) for aztreonam, 65(67%) for ceftriaxone and 63(64.9%) was shown for ceftazidime. About 81(83.5%) gram negative DFU isolates were ESBL positive by disk potential method using cefoparazone/cefoparazone + sulbactum followed by 77(79.4%) by piperacillin/piperacillin + tazobactum, 60(61.9%) by ceftazidime/ceftazidime + clavulanic acid and the cephotaxime/cephotaxime + clavulanic acid shows only 50(51.5%) ESBL production. *Staphylococcal* isolates identified as MRSA were 16(43.2%) and 18(48.6%) by using 1 μg oxacillin disk and 30 μg cefoxitin disk respectively. None of the *S. aureus* were vancomycin resistant (VRSA) including those which are resistant to oxacillin and cefoxitin antibiotics.

3.4. Occurrence of bla genes

The frequency of the occurrence of various *bla* genes in DFU isolates is shown in Fig. 4, CTX-M was found to be the most prevalent ESBL noticed in 34.5%, followed by TEM in 23% isolates and SHV beta-lactamases were noticed in 7.4% isolates respectively.

Table 1 also shows the result of univariate analysis of factors to be associated with the presence of MDR infections. The age distribution [O.R. 0.72, $P = 0.000$], duration of ulcer > 1 month [O.R. 0.54, $P = 0.001$] was observed in 78.5% patients having MDR infection.

Table 4
(a) Screening test result of ESBL producing gram negative bacilli, and (b) confirmatory test result of ESBL producing gram negative bacilli from 102 DFU patients.

(a) Screening result	ESBL
Aztreonam	68(70.1)
Cefpodoxime	71(73.2)
Ceftazidime	63(64.9)
Cephotaxime	80(83.5)
Ceftriaxone	65(67)
(b) Confirmatory	ESBL
Ceftazidime + clavulanic acid	60(61.9)
Cephotaxime + clavulanic acid	50(51.5)
Piperacillin + tazobactum	77(79.4)
Cefoparazone + sulbactum	81(83.5)

(a) Screening result	ESBL
AZTREONAM	68(70.1)
CEFPODOXIME	71(73.2)
CEFTAZIDIME	63(64.9)
CEPHOTAXIME	80(83.5)
CEFTRIAXONE	65(67)
(b) Confirmatory	**ESBL**
CEFTAZIDIME+CLAVULANIC ACID	60(61.9)
CEPHOTAXIME+CLAVULANIC ACID	50(51.5)
PIPERACILLIN+TAZOBACTUM	77(79.4)
CEFOPARAZONE+SULBACTUM	81(83.5)

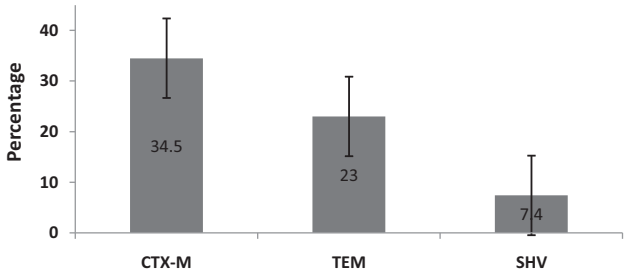

Fig. 4. PCR assay results for bla_{CTX-M}, bla_{TEM} and bla_{SHV}.

The size of ulcer more than 4 cm^2 [O.R. 12.6, $P < 0.001$] was found in 85.7% patients with MDR infection and in 14.2% patients having ulcer size less than 4 cm^2 in MDR infections. The hypertension [O.R. 3.48, $P = 0.004$], retinopathy [O.R. 0.84, $P = 0.000$], neuropathy [O.R. 1.32, $P < 0.001$], previous antibiotic use [O.R. 0.25, $P = 0.002$] were significantly associated with MDR positive infection. There was a significant relation between the bacterial growth with MDR when compared with the depth of diabetic foot ulcer (superficial wound, subcutaneous wound and presence of osteomyelitis). The presence of osteomyelitis [O.R. 2.34, $P < 0.001$] and subcutaneous wound [O.R. 0.62, $P = 0.001$] was significantly associated with the presence of MDR organisms infection (Fig. 5).

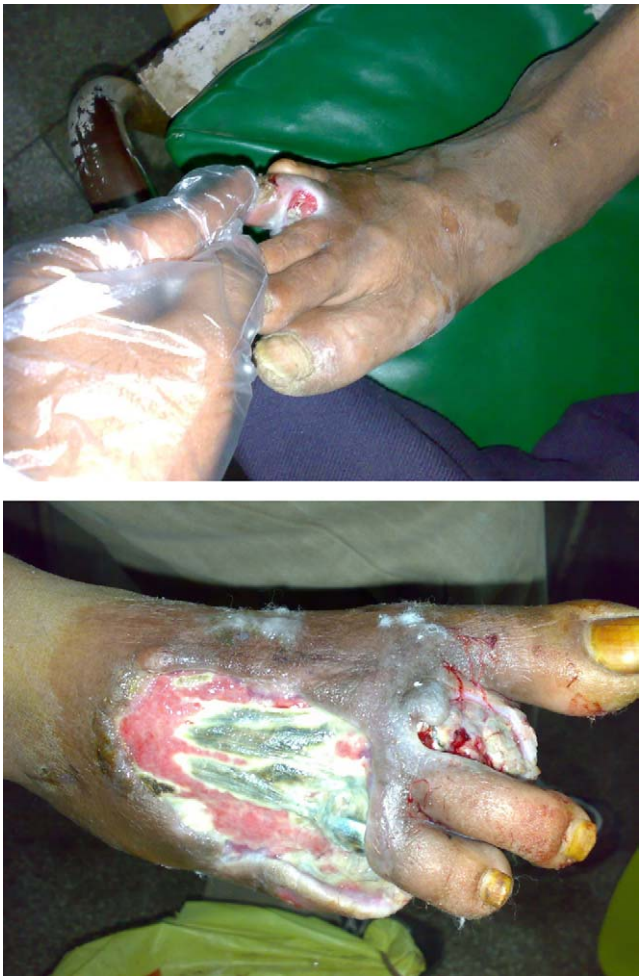

Fig. 5. Images of diabetic foot ulcer patients.

4. Discussion

This study presents a comprehensive clinical and microbiological profile of infected diabetic foot ulcers in hospitalized patients.

With the rise in the prevalence of diabetes mellitus there is increasing problem of infections among diabetic patients especially the diabetic foot infection which according to some studies accounts for 20% of hospital admissions [2]. As multidrug resistance is a growing problem, effort was made to study the association of different study characteristics with the presence of MDR organisms. The soft tissue samples were also used for bacteriological cultures, including osteomyelitis, although bone biopsy may be a better sample but it is not routinely performed in our hospital.

Diabetic foot infections are usually polymicrobial in nature and this has been well documented in the literature. In the present study polymicrobial etiology was found in 62% and monomicrobial in 38% patients with the rate of isolation of about 1.49 bacteria per patient which is lower than the previous studies [3,26] which showed rate of isolation between 2.3 and 5.8. The major infective organisms in diabetic foot ulcers in our patients appear to be different. We found gram negative aerobic bacteria were most frequently isolated which is in accordance with the previous reports [3] and in tune with a similar study from the Southern parts of India [2]. The ratio of gram positive to gram negative ratio was 1:1.7 which is similar to the findings reported earlier [27]. Gram positive organisms which include MRSA were found in 48.6% of isolates which differs from the older studies which shows predominance of gram positive ones [4,28,29]. Gram-negative bacteria that are regarded as normal flora of the skin may cause severe tissue damage in diabetics. We suggest that they should be regarded as significant in diabetic foot ulcers. In our anaerobic study, *Peptostreptococcus* sp. was the most predominant one which is, in accordance to the previous studies [30,31]. Other anaerobes isolated in their study were *Bacteroides fragilis*, *Clostridium* sp. and *Propionibacterium*. *Clostridium* sp. was the most commonly isolated anaerobe, followed by *Bacteroides* [32]. Compared with earlier anaerobic culture reports, we recovered fewer anaerobic species [33,34]. Most of our patients did not have chronic draining wounds, and only 6% had gangrene associated with their infections. This may be an indication of fewer anaerobic species among nonthreatening lower-extremity infections, which is also reported earlier [35].

The present study confirms that MDRO infection is extremely common in hospitalized patients with DFUs similar in tune with the report of Hartemann-Heurtier et al. [7]. Almost 46(46%) of our patients were infected with MDROs. The prevalence of both MRSA isolates and ESBL producing gram negative bacteria was higher (48.6% and 68.5% respectively) in our population as compared with previous studies [7].

A high degree of antibiotic resistance was observed in the present study, which may be due to the fact that ours is a tertiary care hospital with widespread usage of broad spectrum antibiotics leading to selective survival advantage of pathogen. Moreover most of our patients had received some antimicrobial treatment before

presenting at our centre. The antimicrobial resistance pattern was similar to the recent studies done in India and outside [2,36]. In our study, 43.2% of isolated *S. aureus* was methicillin resistant by using 1 μg oxacillin disk and 48.6% of isolated *S. aureus* was resistant to 30 μg cefoxitin disk. Cefoxitin disk shows high percentage of sensitivity over oxacillin disk. None of the gram positive isolates were resistant to vancomycin (VRSA). These observations are important, especially for patients' management and deciding the antibiotic treatment policies.

In the present study, gram negative bacilli were isolated as ESBL producers in 68.5% similar to the observation by Mathur et al. from India [37]. Babypadmini and Appalaraju have shown 40% of *K. pneumoniae* isolates and 41% of *E. coli* isolates to be ESBL producers in their study cohort [38]. A study conducted in Brazil [41] says, the prevalence was only 6% among *E. coli* isolates. Gadepalli et al. have reported 54.5% *E. coli* isolates to be ESBL producers in diabetic foot infections [3]. There is paucity of data on the prevalence of ESBLs in diabetic foot infection in gram negative bacteria other than *E coli* and *Klebsiella* sp. On screening for ESBL, 72.8% of *Acinetobacter* spp. were ESBL producers followed by *P. aeruginosa* (71.2%), *K. oxytoca* (63.3%), *K. pneumoniae* (58.8%), *E. coli* (56.2%) and *P. vulgaris* (45.9%). On confirmation of ESBL production by disk potentiation test, highest prevalence of ESBL was observed in *Acinetobacter* spp. (61%) followed by *P. aeruginosa* (60%), *P. vulgaris* (47%), *K. pneumoniae* (47%), *K. oxytoca* (39%), and *E. coli* (36.5%). In a recent study Shobha et al. have reported 27.3% *K. pneumonia*, 25.2% *E. coli*, 21.42% *Pseudomonas* spp., 25% *Enterobacter* spp. and 17% *Acinetobacter* spp. to be ESBL producer [39].

The duration of infection > 1 month, prior antibiotic use and ulcer size >4 cm^2 were independent predictors of infection with MDR organism. Thus patients with a large ulcer, with a history of prior antibiotic use and duration of infection > 1 month were more likely to harbor MDROs. In the present study mean duration of ulcer was found to be 41.5 ± 7.5 days with 36.2% having ulcer for more than 1 month. About 75.4% presented with a large ulcer of approximate size of >4 cm^2 thereby accounting for approximately 84.3% of the patients with bacterial growth from subcutaneous region and presence of osteomyelitis in relation to MDR. 47% of patients had used antibiotics prior to reporting to the hospital. The reasons for presentation with advanced grade and stage of ulceration could be because of lack of structured health care delivery in the country, attempted self-medication and trust in traditional healers [40]. Moreover, inadequate antibiotic treatment and the use of non sterile instruments for dressing results in the growth of multi resistant organisms necessitating hospital admission and surgical intervention [41]. Moreover prolonged or broad-spectrum antibiotic therapy predisposes patients to infections with antibiotic-resistant organisms like MRSA [7]. This could explain the high level of MDR infection in our study. We could not get the previous hospitalized details for the same wound in our patients that could have helped us explaining the reasons for the high prevalence of MDROs in our patients.

Furthermore, the occurrence of ESBL in the bacterial isolates was substantiated by $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} in DFU using PCR to find out true resistance to antibiotics. This genomic study helps us to find out the nature of resistance whether plasmid mediated or some environmental effect. We found 34.5% $bla_{\text{CTX-M}}$ as the most prevalent ESBL gene followed by 23% bla_{TEM} and 7.4% bla_{SHV} in the DFU isolates tested. From the study of *bla* genes(CTX-M, SHV AND TEM), we interpolate that the nature of resistance developed by organism was mainly influence environmental (45.6% out of 68.5% phenotypic ESBL positive isolates). To the best of our knowledge, no such type of report have been reported from isolates of DFUs, however, the $bla_{\text{CTX-M}}$ as the most prevalent and widely disseminated genes in the clinical bacterial population had been reported from India [23,24,42].

A significant relation between the longer hospitalization period and the infection with MDR was found in the present study, which is in accordance with existing literature [43]. It is known that MDR infections are resistant to several antibiotics and therefore patient should be treated with extended spectrum antibiotics for longer durations. As a result, duration of hospital stay in infections with MDR is usually longer and concomitantly treatment more costly. Furthermore, in our study, mortality from infections with MDR is twice as high as mortality from infections with microorganisms sensitive to antibiotics (8.6%) [$P = 0.002$, O.R. = 3.2]. For these reasons, risk factors for acquisition of MDR should be determined and decreased as soon as possible.

Resistance to antibiotics is seen when they are used for a prolonged period of time. This resistance is an acquired form rather than an intrinsic one. The former develops following a mutation in the DNA of a microorganism or by acquisition of a new DNA. Acquisition of new DNA is accomplished by genetic elements such as plasmids or transposons. Resistance plasmids may have approximately 10 resistance genes for various antibiotics. Bacteria can transmit these characteristics to other bacteria [44,45]. Frequent or unnecessary use of antibiotics result in a selection favouring resistant bacteria. In this study history and discharge summaries showed that overwhelming majority (26.4%) of the diabetic patients with osteomyelitis who were referred to our centre had received an antibiotic treatment before. However, which antibiotics and in what doses they were given was not clear, only the duration of the therapy was evident. Although the number of patients who had no antibiotic therapy was low in our series MDR presence was higher (41.3%) in those who had previous antibiotic therapy. Also infection with MDR was significantly higher in this population when compared to other bacteria (MDR, negative).

Presence of vascular disease characterized by disrupted micro- and macro-circulations cause a delay in wound healing in diabetic patients [6,46]. Disruption of wound healing results from a decreased blood flow into the ulceration and an aberrant expression of growth factors and cytokines as well. These factors, which delay wound healing, cause foot ulcers. Infections of these foot ulcers require a longer duration of treatment with antibiotics and the use of an appropriate antibiotic in an appropriate dosage [47]. Insufficient blood flow which causes ischemia will hinder penetration of antibiotics into the wound and therefore facilitate infections with MDR [48]. In fact, we found that MDR was more frequently isolated in neuroischemic cases (38%). Although this increase was statistically insignificant, we believe that an increase in the number of cases could positively affect significance. To our knowledge, there has been no study confirming or negating this finding in the literature. Manual minimum inhibitory concentration (MIC) was not carried out as it was time consuming and tedious for all the ESBL-producing clinical isolates obtained in the present study.

Our results indicate adequate control of blood glucose level is more common in patients with non-MDR infected ulcers as compared with MDR infected ulcers, and further, higher mortality rates were reported in patients with DFU whose blood glucose levels were poorly controlled. Thus, MDRs might lead to higher mortality among DFU which need to be investigated. We found duration of hospital stay and cost was more in MDRs as compared with non-MDRs. The duration of hospital stay may also depend on the management policy of the hospital. In our hospital, patients are discharged once the healing begins and are advice to come to follow-up at out-patients clinic every week.

5. Conclusion

A detailed knowledge of the susceptibility to antimicrobial agents is necessary to facilitate the development of effective strate-

gies to combat the growing problem of resistance especially the MRSA and ESBL strains. The prevalence of MDR organisms is alarmingly high in the diabetic foot patients in India because of indiscriminate use of antibiotics. The findings of the present study suggest that prospective multicentre studies are required to assess the appropriate empirical antibiotic regimen in diabetic foot ulcer infections. The study also directs us that proper management of diabetic foot ulcers with appropriate antibiotics groups such as aminoglycoside, macrolides and chloramphenicol for *S. aureus* isolates and third and fourth generation cephalosporin's, β-lactum inhibiters and amino-glycoside for gram negative bacilli along with good glycemic control must be implemented to decrease the incidence of MDR organisms for better clinical outcome. Empirical treatment with amino-glycoside may begun initially as they cover both gram positive and gram negative bacteria. However chloramphenicol can be used as a reserve drug in infection refractory to DFU with conventional drugs.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

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ABSTRACT BOOK

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differential parameters found including history of diabetes, physical status and laboratory data other than renal parameters between the two groups.

We showed the association between the delayed HRR and SMI in type 2 diabetic patients. The delayed HRR may be a predictor of SMI in diabetic patients. Diabetic autonomic neuropathy might also play a role in SMI.

2397-PO

Training Modifies the Effect of Lipid Infusion on Insulin Resistance

LISA S. CHOW, DOUGLAS G. MASHEK, ELIZABETH R. SEAQUIST, *Minneapolis, MN, Saint Paul, MN*

Insulin resistance is a precursor to type 2 diabetes (DM2). Insulin resistance correlates with intramyocellular lipid (IMCL) content in untrained people. Yet endurance athletes have normal insulin sensitivity despite IMCL levels comparable to people with DM2. As lipid infusion is an established method of inducing insulin resistance, we performed this pilot study with the hypothesis that training modifies the extent to which lipid infusion produces insulin resistance.

Trained and sedentary subjects were recruited. Activity was self-described (trained:>45 min.running 5d/wk; sedentary: <30 min exercise/wk). Fitness was measured by VO_2 max and fat free mass (FFM) by DEXA. Insulin sensitivity was measured by a hyperinsulinemic (1.5 mU/kg FFM/min), euglycemic clamp at baseline (3hr) and at a separate visit in conjunction with a lipid infusion (6 h Intralipid:90ml/h, no heparin).

The trained and sedentary subjects were similar in terms of age, BMI, FFM and baseline free fatty acid (FFA) levels. The trained subjects were more fit (higher VO_2max) and more insulin sensitive (higher glucose infusion rate (GIR)) than sedentary subjects. Following the lipid infusion which achieved similar FFA levels in both groups, the trained and sedentary subjects had similar GIR.

mean±SE	Trained (n=6)	Sedentary (n=4)	P value
Age (yrs)	26.5±3.5	20.3±0.3	p=0.14
BMI (kg/m^2)	23.5±0.5	22.3±0.9	p=0.31
FFM (kg)	54.6±5.5	49.1±3.3	p=0.42
VO_2max (ml/kg/min)	47±1.8	39.3±1.4	p=0.01
Baseline FFA (umol/l)	485±78	651±55	p=0.11
Baseline GIR (mg/kgFFM/min from 3 hr clamp)	14.6 ±1.6	7.3±1.0	p=0.004
Post Lipid GIR (mg/kgFFM/min from 6 hr clamp)	9.13±0.9	7.51±1.3	P=0.34
Post Lipid FFA (umol/l)	780 ± 53	885±180	p=0.61

In the setting of lipid infusion, trained subjects had higher decline in the GIR compared with sedentary subjects. The lack of decline in the GIR of sedentary subjects may be related to their lower baseline insulin sensitivity and our study's mild lipid exposure. Trained subjects appear to be more responsive to the adverse effects of lipid infusion on insulin sensitivity than sedentary subjects, with the benefit of training on skeletal muscle insulin sensitivity readily abrogated by lipid infusion.

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FOOT CARE—LOWER EXTREMITIES

2398-PO

Clinico-Bacterial Study and Drug Resistance Profile of Diabetic Foot Infections in North India

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Aims: A prospective study was carried out with the objective to determine the clinical characteristics, bacterial profile and antibiotic resistance especially methicillin resistance, ESBL production and their correlation with the clinical findings. Methods: - Sixty patients with Wagner grade 0-5 Diabetic Foot Ulcers (DFU) were hospitalized in Centre for Diabetes and Endocrinology, J.N. Medical College, A.M.U. Aligarh, India during Dec 2008 to Nov 2009. All patients were subjected to detailed assessment, biochemical profile along with examination of foot. Ulcer samples were collected for bacterial culture and sensisitivity testing at Department of Microbiology. *Staphylococcal* isolates were tested for methicillin resistance by using 1-μg oxacillin disc and Gram's negative organisms were screened for extended spectrum β lactamase (ESBL) production by double disc diffusion method. Data was analyzed using SPSS version 13.0. Results: Bacterial infection was found in 86.6% cases (Polymicrobial (40%), Monomicrobial (48.5%). 41.3% isolates were Gram positive and 58.6% isolates gram negative. *Staphylococcus aureus* isolates were common (28%), followed by *Escherichia coli* (26.6%), *Pseudomonas aeruginosa* (10.6%), β *hemolytic streptococcus* (9.3%) *Klebsiella pneumonia* (6.6%), *Klebsiella oxytoca* (5.3%), *Enterococcus faecalis* (4%), *Acinetobacter sp* (4%), *Proteus vulgaris* (2.6%), *Coryneform sp* (2.6%) and CONS (2.6%). 23.3% DFU patients had infection by MDR organisms. Methicillin resistance was found in 23.8% *Staphylococcus aureus* isolates while 57.9% gram negative organisms were ESBL producers. Majority of the patients (63.3%) having foot ulcers were in the age group 40-60 years (Wagner grade 2-5). Duration of infection > 1month and ulcer size > 4cm^2 was independently associated with risk of MDR organism's infection. Conclusions: - Infection with MDR organism was common DFU. The presence of infection, ulcer size, Wagner grading, duration of infection and hospital stay were the factors more likely to be associated with adverse clinical outcome.

2399-PO

Dynamic Analysis of Foot Plantar in Chinese Normal Population and Diabetes Mellitus

CHUAN YANG, HUISHEN XIAO, LI YAN, LIHONG CHEN, GUOJUAN LAO, *Guangzhou, China*

Diabetic foot is a special serious complication in Chinese diabetes mellitus. The abnormal foot plantar pressure maybe one of most important factors on diabetes mellitus' foot ulcers.

Enrolled were 1003 normal subjects, with average (45.17±14.61) years old and 1123 diabetics, with average(58.04±11.05) . There were not serious foot diseases, for example, foot ulcers, intermittent claudication, clubfoot, Charcot joint et al. All of them were registrated in our department.

Every subject was evaluated with the EMED-AT system. Each trail included at least five acceptable separate barefoot for each foot . The mean Maximum force (N), Peak pressure (kPa), Contact time (ms) and Force-time integral (N*s) was calculated.

Compared to normal group(617.23±108.66 N), maximum force was higher significantly in diabetic group(633.24±113.00N) (P<0.05). But the peak pressure(431.73±106.19kPa) in normal was higher than that(423.07±111.22kPa) in diabetic group. (P<0.05) . Further analysis, we found the peak pressure in various fields of foot plantar was difference between normal and diabetic group. The maximum peak pressure was local in M5, which was the second metatarsal head in normal group(316.08±108.38kPa) , but in diabetic group, the maximum peak pressure was in M9(299.21±115.56kPa), which was the first phalanx. Special, the maximum peak pressure in M8, which was the fifth metatarsal head, the diabetic group(201.00±104.14kPa) was higher than normal group(169.57±84.22) significantly.

For the Contact time, in the diabetic group was substantial higher than normal group in every field of foot plantar. The force-time integral(impulse), impulse, can show the force continued effect on foot plantar and it was the most important factor to induce the foot ulcer. In the diabetic group, the force-time integral(559.39±165.09 N*s) was significantly. In normal group, the force time integral was 386.73 ±88.37 N*s.

There were a lot of changes in Chinese diabetes mellitus comparing with normal population. (1)The maximum peak pressure were different distribution between diabetes group and normal group; (2)The impulse was specifically increased in diabetic group.

2400-PO

Effects of a Loofa Scrub Soap Prepared from Dead Sea for Care of Diabetic Foot

DARIA MAGGI, EMANUELA CAMERA, ELVA ABRIL, MAURO PICARDO, ANNA RITA MAURIZI, SILVIA MANFRINI, PAOLO POZZILLI, *Roma, Italy*

Functional and mechanical properties of skin in diabetic patients are impaired because of hyperglycaemia and decreased insulin signal. In diabetes a reduced hydration state of statum corneum (SC) with decreased sebaceous gland activity but no impairment of the SC barrier function have been recently reported.

The aim of this study was to evaluate SC hydration and transepidermal water loss (TEWL) in diabetic subjects following the use of a natural loofa scrub soap (nutrimed), a mineral product prepared from the Dead Sea which is ideal for removing dead skin cells.

For author disclosure information, see page A781.

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Clinico-bacteriology and risk factors for the diabetic foot infection with multidrug resistant microorganisms in north India

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Abstract
This study was carried out in diabetic patients with foot ulcer, to determine the bacterial profile of infected ulcer, antibiotic resistance of the isolates and to find out the potential risk factors for infection with multidrug resistance. Gram-negative bacilli were screened for extended spectrum β lactamase (ESBL) production and *Staphylococcus aureus* were screened for methicillin resistance. In the 60 diabetic foot patients, 37(61.6%) were males and 23(38%) were females. 49(81.6%) had T2DM, whereas only 11(18.3%) patients had T1DM. The presence of sensory neuropathy was observed in 66.6% patients. Bacterial infection was found in 86.6% DFU cases, 40% cases had mixed bacterial infection while 48.5% cases had monomicrobial infections. 23.3% DFU patients had infection by multidrug resistant (MDR) organisms. ESBL producer was found in 45.3% gram-negative isolates. 33 % gram-negative strains were positive for $bla_{CTX\text{-}M}$ gene followed by bla_{SHV} (20%) and bla_{TEM} (6.6%) Poor glycemic control in 63.3% patients, duration of infection > 1month (43.3%) and ulcer size > 4cm2 (78.1%) was independently associated with risk of MDR organism infection.

Keywords: Diabetic foot ulcers; multidrug-resistant organisms; risk factors; outcome.

Introduction

Diabetic foot ulceration and infections are a major medical, social, economic problem and a leading cause of morbidity and mortality, especially in the developing countries like India (Ako et al., 2006; Shankar et al., 2005; Gadepalle et al., 2006). Fifteen percent of all diabetics develop a foot ulcer at some point in their lives which is highly susceptible to infections and that spreads rapidly, leading to overwhelming tissue destruction and subsequent amputation (Lipsky et al., 2004). The major predisposing factor to foot ulceration leading to infection is usually related to peripheral neuropathy (Joshi et al., 1999). Mostly the diabetic foot infections are mixed bacterial infections (Viswanathan et al., 2002; Chincholikar and Pal, 2002) and the proper management of these infections requires appropriate antibiotic selection based on culture and antimicrobial susceptibility testing. Sometimes, initial management comprises empirical antimicrobial treatment based on susceptibility data (Goldstein et al., 1996). Knowledge of the agent(s) that cause infected DFU is helpful in selecting definitive antibiotic therapy. In recent years, there has been an increase in the incidence and prevalence of ESBLs. Currently, there was paucity of data on ESBL-producing organisms from diabetic foot infections especially in this part of world. Infection with multidrug resistant organisms (MDR organisms) may increase the duration of hospital stay, cost of management and may cause additional morbidity and mortality (Hartemann-Heurtier et al., 2004). Early diagnosis of microbial infections is aimed to institute the appropriate antibacterial therapy to avoid further complications. Therefore, this study is planned with the objective to determine the bacterial profile and antibiotic resistance to find out the potential risk factors for infection with multidrug resistance.

Materials and Methods

A total of 230 diabetic patients were admitted in the Centre for Diabetes and Endocrinology, J.N.M.C, A.M.U., Aligarh, India, 60 of them who developed ulcer in their foot during Dec 2008 to Nov 2009 were included in this study.

Clinical examination

A detailed clinical history and physical examination was carried out for every subject. Age, Sex, anthropometric measurements, duration of ulcer, duration of diabetes, glycemic control, lipid profile, presence of retinopathy, serum creatinine level or presence of micro/macro-albuminuria, hypertension, history of smoking, history of previous amputation, duration of hospital stay and clinical outcome were noted for every patient. Foot ulcers were categorized into six grades (grade 0 - grade 5) based on Meggit Wagner Classification System (Wagner, 1981). Neuropathy was quantified in each patient, assessing vibration sensation using a

128 HTZ tuning fork and a 10g monofilament (absence of perception of the Semmes Weinstein monofilament at 2 of 10 standardized plantar sites on either foot).

Ulcers were assessed for signs of infection (swelling, exudates, surrounding, cellulitis, odor, tissue necrosis and crepitation) and size was determined by multiplying the longest and widest diameters expressed in centimeters squared (cm^2), and the diagnosis of extension to the bone was made by probing with a sterile probe by the resident posted in the ward. Plain radiograph was performed to all the subjects and MRI was done in Osteomyelitis suspects. The lesions were then categorized into 3 main clinical groups: (I) skin ulcer (Wagner 1 and 2); (II) deep tissue ulcer with suspected osteomyelitis (Wagner 3); and (III) gangrenous lesion (Wagner 4 and 5). All cases were monitored until discharged from the hospital. All the subjects gave informed consent and clearance was obtained from the hospital ethics committee.

Microbiological methods

Pus sample were obtained by scrapping the base of ulcer or the deep portion of the wound edge with a sterile curette (Gadepalle et al., 2006; Motta et al., 2003), which was transported to the Microbiology Department and processed for aerobic bacteria. Total transfer time to the laboratory was not more that 30 mins. Direct microscopic examination of ulcer sample was performed. Standard methods for isolation and identification of aerobic bacteria were used (Collee et al., 1996; Collee and Marr, 1996).

Susceptibility testing

Antimicrobial susceptibility testing was performed using the disk diffusion method as described by the CLSI (Clinical and Laboratory Standards Institute, 2007), Antimicrobial disk used were Imepenem (10µg), Aztreonam (30µg), Amoxyclav (30µg), Cefpodoxime (10µg), Cefepime (30µg), Cefoperazone (75µg), Cefoperazone/sulbactam (75/10µg), Cefixime (5µg), Piperacillin (100µg), Piperacillin/tazobactam(100/10µg), Ceftazidime (30µg), Ceftazidime/clavulanic acid (30/10µg), Amoxycillin (20µg), Cephotaxime (30µg), Cephotaxime/clavulanic acid (30/10µg), Ceftriaxone (30µg), Cefoxitin (30µg), Amikacin (30µg), Chloramphenicol (30µg), Gentamicin (10µg), Gatifloxacin (5µg), Ofloxacin (5µg), Levofloxacin (5µg), Sparfloxacin (5µg), Streptomycin (10µg), Erythromycin (15µg), Tobramycin (10µg), Clindamycin (2µg), Azithromycin (15µg), Oxacillin (1µg), Vancomycin (30µg) and Bacitracin (µg). All discs were obtained from Hi-Media labs, Mumbai, India. Inter-pretative criteria for each antimicrobial tested were those recommended by manufacturer's guideline (Hi-Media labs, Mumbai, India).

Phenotypic methods for MRSA and ESBL detection

Staphylococcus species were tested for methicillin resistance by using 1-µg oxacillin disc (National Committee for Clinical Laboratory Standards, 2004) and 30 µg cefoxitin disk (Anand et al., 2009). Gram-negative bacilli were first screened for the production of ESBL by disc diffusion method using Cephotaxime, Ceftriaxone, Aztreonam, Cefepime, Cefoxitin and Ceftazidime and later on confirmed by Cephalosporin/Clavulanate combination disk test (disk potential test) using Ceftazidime, ceftazidime+clavulanic acid, cephotaxime, cephotaxime+clavulanic acid, piperacillin, piperacillin+tazobactam, cefoparazone and cefoparazone+sulbactam (David and Robert, 2005). *E. coli ATCC* 25922 (non ESBL-producer), *K. pneumoniae 700603* (ESBL-producer) and *Staphylococcus aureus* ATCC 25923 were used as control strains respectively. A microorganism was classified as MDRO if it was found to be resistant to two or more classes of antimicrobials and included MRSA, ESBL producing organisms (Hartemann-Heurtier et al., 2004).

Molecular methods for ESBL detection

Preparation of DNA template: Template DNA was prepared from freshly cultured bacterial isolates by suspending 3-5 colonies in 50 µl of molecular grade water, and then heating at 95°C for 5 minutes and immediately chilling at 4°C. Positive controls harboring bla_{CTX-M}, bla_{TEM} and bla_{SHV} and negative control (*E. coli* ATCC 25922) were processed in the same way for DNA extraction.

Detection of bla genes by PCR

Molecular detection of bla_{CTX-M}, bla_{TEM} and bla_{SHV} was performed in gram-negative isolates by using polymerase chain reaction (PCR) according to the methods described previously with minor modifications (Ensor et al., 2006; Shahid et al., 2009). The primers and cycling conditions for detection of *bla genes* were same as described by Shahid et al (Shahid et al., 2009).

Antibiotic treatment

Antibiotics were selected according to published recommendation (Hartemann-Heurtier et al., 2004). In mild infections amoxicillin clavulanic acid was given

empirically by the oral route. However in moderate infections intravenous route was preferred taking into consideration the likelihood of osteomyelitis. Considering that the causative agent was polymicrobial, we initiated ampicillin-sulbactam plus an aminoglycoside/quinolone or piperacillin-tazobactam or ceftriaxone plus metronidazole/clindamycin. In the presence of severe infections, surgical debridement and amputation were performed immediately after admission. Metronidazole (500mg I.V. every 8 hours) was added to the drug regimen if cellulitis or gangrene was also present. Combinations of extended spectrum antibiotics were initiated and the treatment was later modified in accordance with the culture results. The duration of the treatment was at least 4-6 weeks and prolonged in cases of osteomyelitis. All patients also received an intensive insulin treatment.

Statistical analysis

The data was analyzed using SPSS version 13.0 for descriptive statistics. Quantitative variables were expressed as means ±SD while qualitative variables were expressed as percentage (%).

Results

In the 60 diabetic foot patients studied (TABLE 1a & 1b), the male to female ratio was 1.6:1. The average age of patients was 48.7±11.4 years (average ± s.d.). Majority of patients 38 (63.3%) were in the age group 41-60 years which include 21 males and 17 females, followed by 13 (21.6%) in age group 21-40 years (10 Males and 3 females).

Table 1a: Demographic details of DFU patients (data expressed as mean±sd or n% unless otherwise indicated).

	Overall Mean±sd or n(%)	**Male Mean±sd or n(%)**	**Female Mean±sd or n(%)**
Age Distribution (years)	**48.70±11.41**	**46.97±12.36**	**49.41±9.6**
0-20	1 (1.6)	1(1.6)	**-**
21-40	13(21.60)	10(16)	3(5)
41-60	38(63.33)	21(35)	17(28.33)
61-80	8(13.33)	5(8.3)	3(5.00)
Type of Diabetes			
Type 1	11 (18.33)	8 (13.3)	3 (5.0)
Type 2	**49 (81.66)**	29 (48.3)	20 (33.33)
Duration of Ulcer (days)	**41.72 ± 5.61**	**44.65 ± 6.07**	**35.33 ± 3.86**
< month	30(51)	18(30)	12(20)
> month	**23(38.3)**	12(20)	11(18.3)
Hospital Stay (days)	**22.13 ± 16.50**	**21.73 ± 11.41**	**21.83 ± 22.74**
Ulcer Grade (Wagner)		**37(61.6)**	**23(38.3)**
grade 0	3(5)	2(3.3)	1(1.6)
grade 1	20(33.3)	10(16.6)	10(16.6)
grade 2	21(35)	14(5.4)	7(11.6)
grade 3	12(20)	9(15)	3(5)
grade 4	1(1.6)	1(1.6)	-
grade 5	3(5)	1(1.6)	2(3.3)
Discharge Status			
alive	57(95)	34(56.66)	23(38.3)
dead	3(5.0)	3(5.0)	-
Habit			
Non-smoker	16(26.6)	7(11.6)	9(15)
Smoker	21(35.0)	20(33.33)	1(1.6)
Alcoholic	5(8.3)	5(8.3)	-
Fundus Examination	**55**	**34(61.18)**	**21(38.18)**
Normal	25(45.45)	17(30.90)	8(14.54)
Diabetic Retinopathy	29(52.72)	17(30.90)	12(21.81)
Cataract	1(1.81)	**-**	1(1.81)
History of Amputation			

Present	12(20)	8(13.3)	4(6.6)
Absent	48(80)	33(55)	15(25)
Duration of Diabetes (years)	**7.93 ± 5.95**	**8.78 ± 5.67**	**6.13 ± 5.07**
0-10	29(69)	19(45.2)	10(23.8)
11-20	12(28.5)	8(19)	4(9.5)
21-30	1(2.3)	1(2.3)	-
Size of Ulcer	**23.58±56.26**	**29.48±69.89**	**13.81±17.94**
≤4 cm^2	12(21.81)	6(10.90)	6(10.90)
>4 cm^2	43(78.1)	28(50.9)	15(27.2)
Site of Ulcer			
Planter	11(18.3)	7(11.6)	4(6.6)
Margin	10(16.6)	6(10.0)	4(6.6)
Heel	13(21.6)	9(15.0)	4(6.6)
Interdigital	20(33.3)	12(20.0)	8(13.3)
Malleoli	5(8.3)	2(3.3)	3(5.0)
Leg	3(5.0)	1(1.6)	2(3.3)
Multiple areas	7(11.6)	4(6.6)	3(5.0)

In presenting complaints, 65% patients had polyuria, polydipsia (32%), polyphagia (24%), weight loss (38%), weakness (39%), swelling in feet (42%), burning during micturition (27%), and pain in leg (35%). Among the DFU patients, 49 (81.6%) had type 2 diabetes mellitus, whereas only 11 (18.3%) patients had type 1 diabetes mellitus. The duration of diabetes for more than 10 years was observed in 28.5 % (11-20 yrs duration) and 2.3 % (>21 yrs) patients whereas 69% had diabetes for less than 10 years.

Table 1b: Clinical characteristics of DFU patients (data expressed as mean±sd or n% unless otherwise indicated).

Routine Investigations (at the time of admission)			
Blood Picture	58	35(60)	28(48.2)
WBC	10.57 ± 4.48	10.80 ± 5.32	9.80 ± 2.80
Hb	11.24±2.16	11.03±2.26	11.09±2.04
RBC	4.9±1.750	5.0±2.20	4.6±0.60
Renal Function Test	58	35(60.3)	23(39.6)
Blood sugar	202.0 ± 105	187.33 ± 114.7	215.58 ± 86.8
Blood urea	39.49 ± 15.2	39.40 ± 14.28	37.97 ± 16.97
Serum Creatinine	2.08 ± 0.4	2.06 ± 0.3	2.03 ± 0.5
Liver Function Test	55	33(60)	22(40)
SGOT/AST	19.75 ± 11.1	20.91 ± 11.5	17.17 ± 10.3
SGPT/AST	18.33 ± 12.2	19.17 ± 13.0	16.04 ± 10.8
alkaline phosphate	13.98 ± 8.7	13.23 ± 9.7	14.47 ± 7.3
bilirubin	1.73 ± 0.1	1.71 ± 0.1	1.69 ± 0.0
Serum Protein	56	33(58.9)	23(41)
Total serum protein	7.56 ± 0.8	7.54 ± 0.8	7.28 ± 0.8
Serum albumin	4.35±0.5	4.32±0.6	4.21±0.4
Serum globulin	4.28±1.1	4.22±0.7	4.20±1.5
Plasma Glucose	58	35	23
Fasting	164.50±82.96	163.86±89.23	158.62±74.31
Normal ≤125	22(37.9)	14(24.1)	8(13.7)
Postprandial	215.30±94.23	213.86±102.35	208.86±82.53
Normal ≤195	28(48.2)	16(27.5)	12(20.6)
HbA1c	11.01±2.52	10.99±2.54	10.51±2.53
6-7 %(good control)	3(6)	2(4)	1(2)
7-8 % (fair control)	2(40	1(2)	1(2)
>8 % (poor control)	41(82)	25(50)	16(32)

The mean HbA1c 11±2.52 was observed in a total of 50 patients with only 14% patients achieving an HbA1c of < 7.0 %. The mean duration of foot infection was 41.7±5.6 days of which 56.6% patients had foot infection of less than 1 month and 43.3% patients reported history of infection for more than 1 month. The mean duration of hospital stay was 22.1±16.5 days. Ulcer was found on plantar surface in 18.3% patients, on interdigits (33.3%), on margins (16.6%), on heel (21.6%), on malleoli (8.3%), and on multiple areas (≥2) was 11.6%. Size of ulcer <4 cm^2 was observed in 21.8% patients, between 4-8 cm^2 in 30.9%, 8 – 12 cm^2 in 21.8% and > 12 cm^2 in 25.4% patients. Patients were graded according to *Meggit Wagner Classification*. Grade I ulcer was found in 33.3%, Grade II in 35%, Grade III in 20%, Grade IV in 1.6%, and Grade V in 5% of patients. The number of DFU patients and their type of isolates within the Wagner grade was summarized in Table 2.

Table 2: Type of flora isolated from different grades of DFU patients.

Wagner Grade and Type of Isolate				
Wagner's Grade	**Patients**	**Sterile**	**Mono**	**Poly**
Grade-0	3	3	-	-
Grade-1	20	5	11	4
Grade-2	21	-	14	7
Grade-3	12	-	7	5
Grade-4	4	-	1	-
Grade-5	3	-	1	2
Total	**60**	**8**	**34**	**18**

In Grade 1**,** monomicrobial infection and polymicrobial infection was seen in 55% and 20% patients respectively whereas 25% showed no growth in their culture report. In Grade 2, monomicrobial infection (66.6%) and polymicrobial infection (33.3%), In Grade 3, monomicrobial infection (58.3%) and polymicrobial infection (41.7%), In Grade 5, monomicrobial infection (33.3%) and polymicrobial infection (66.7%) whereas all the patients showing monomicrobial infection were from Grade 4. 80% of patients were managed conservatively with medical therapy and/or debridement and in 20% of patient's amputation was done. 52.7% reported diabetic retinopathy in which 17 were males and 12 were females. The presence of sensory neuropathy was observed in 66.6% patients whereas 15% had no sensory neuropathy. Nephropathy and hypertension was present in 39% and 55.8% of patients respectively.

Microbiological observations

A total of 75 bacterial isolates were isolated, averaging 1.2 species per patient. 56.6% patients had monomicrobial infection and polymicrobial etiology was observed in 33% while 13.3% showed no growth in their culture report. In the direct microscopic examination of ulcer samples, 93% showed corresponding result to the culture growth on next day, 2% direct results differ in their culture growth and in 5% cases, direct examination could not been done. Among the bacterial isolates, gram-positive cocci comprised of 44% and gram-negative bacilli accounted for 56%. Gram-positive to gram-negative ratio was 1:1.3. *Staphylococcus aureus* was the most common isolate, accounting for 28%; followed by *Escherichia coli* 26.6%, *Pseudomonas aeruginosa* 10.6%, beta hemolytic *Streptococcus spp* 6.6%, *Klebsiella oxytoca 5.3%, Enterococcus faecalis 4%, Acinetobacterspp 4%, Coryneformspp 2%, CONS 2%* and *Proteus vulgaris 2%.* Prevalence of various bacterial isolates in different Wagner grades of foot ulcer was shown in Table 3.

Table 3: Frequency of isolates within Wagner grades.

Frequency of Isolates Within Wagner's Grades							
S. No.	**Name of Isolate**	**I**	**II**	**III**	**IV**	**V**	**Total**
		n	**n**	**n**	**n**	**n**	**n (%)**
1	*Staphylococcus aureus*	7	8	4	1	1	21(28)
2	*Escherichia coli*	4	8	4	2	2	20(26.6)
3	*Pseudomonas aerugenosa*	3	1	2	1	1	8(10.6)
4	*Klebsiella pneumoniae*	1	2	1	1	-	5(6.6)
5	*β hemo_streptococcus*	2	1	2	-	-	5(6.6)
6	*Klebsiella oxytoca*	-	-	2	1	1	4(5.3)
7	*Enterococcus faecalis*	-	3	-	-	-	3(4)
8	*Acinetobacter*	-	1	2	-	-	3(4)
9	*CONS*	-	2	-	-	-	2(2.6)
10	*Coryneformspp*	1	-	1	-	-	2(2.6)
11	*Proteus vulgaris*	-	1	-	-	1	2(2.6)
	Total	**18**	**27**	**18**	**6**	**6**	

In grade 1, prevalence of *S. aureus* was predominant compared to others whereas in grade 2 and 3, *E coli* and *S. aureus* showed equal number of prevalence. In grade 5, the prevalence of *E. coli* was doubled when compared with *S. aureus.* The maximum number of isolates (28) was from grade 2 infected patients followed by 21 in grade 1, 9 in grade 3, 6 in grade 5 and only 1 in grade 4.

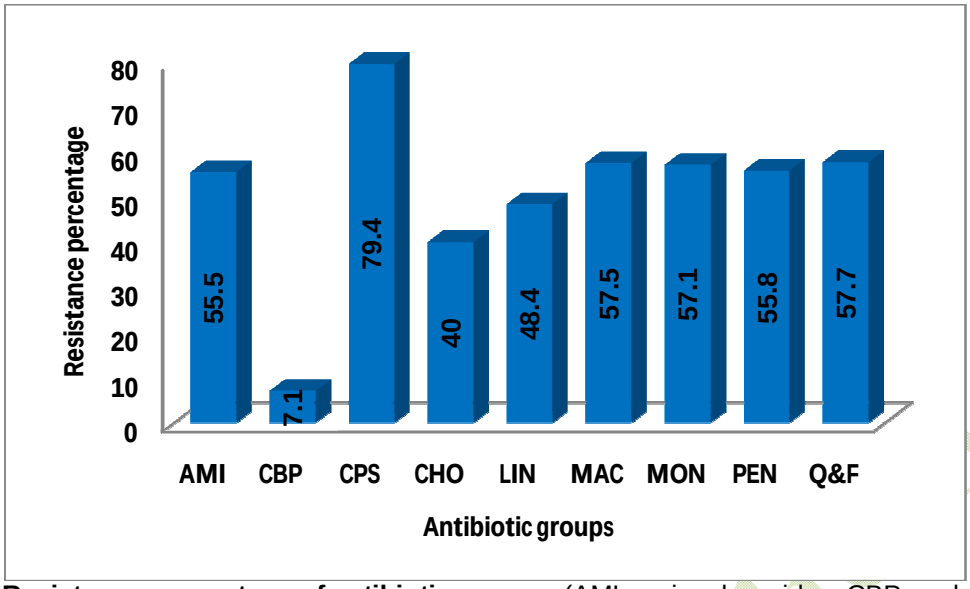
The result of resistance studies are summarized in Table 4. High degree of antibiotic resistance was observed in gram-negative bacilli (55.9%) compared to 48.3% by gram-positive cocci. In gram-positive bacteria, CONS exhibited a higher frequency (73.8%) of resistance to the antibiotics tested, followed by beta hemolytic *streptococcus* (52.7%), *E. faecalis* (52.7%), *S. aureus* (43%) and 42.8% by *Coryneform spp.* All the gram-positive isolates were uniformly susceptible to vancomycin. Methicillin resistance was found in 57.1% *S. aureus* isolates by using 1 µg oxacillin disk and 71.4% by 30 µg Cefoxitin disk. Among gram-negative bacilli, *Acinetobacter spp* showed 75.3% of resistance to the antibiotics tested, followed by *K. oxytoca* (59.7%), *P. aeruginosa* (55.9%), *E. coli* (53%), *K. pneumonia* (48.6%), and *P. vulgaris* (47%). On an average, 23.3% patients having infections in there foot by MDR Organisms.

The resistance percentage of total bacterial isolates in different groups of antibiotics tested is shown in Graph 1. Higher percentage of resistance (79.4%) was shows among the cephalosporin group followed by quinalones and fluoroquinalones (57.7%), macrolides (57.5%), monobactam (57.1%), penicillin (55.8%), aminoglycosides (55.5%), lincosamides (48.4%), chloremphenecol (40%) and carbapenems (7.1%).

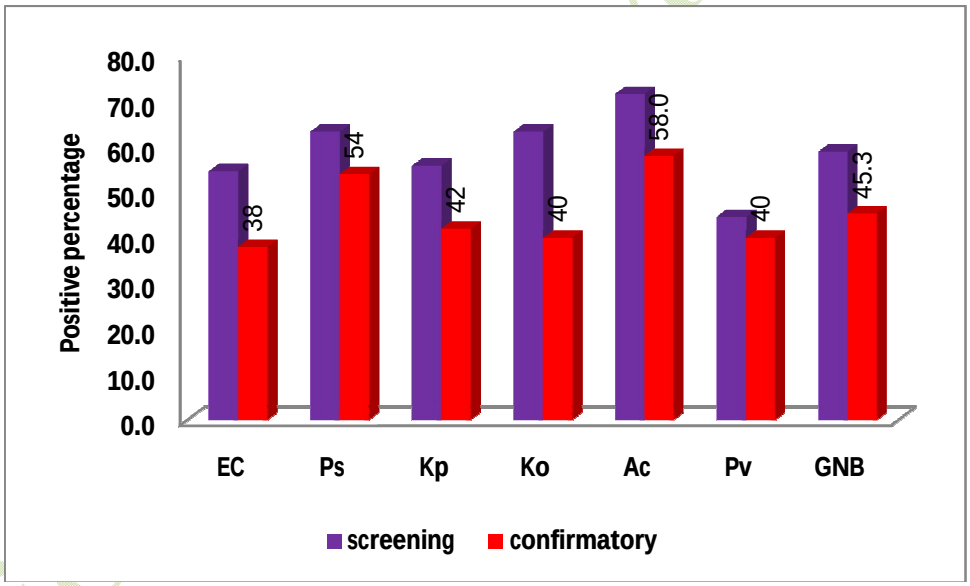
Phenotypic ESBL detection in DFU isolates

The result of phenotypic test was shown in Graph 2. Based on Kirby-Bauer disc diffusion method average production of ESBL was inferred in 71.6% of *Acinetobacter spp* followed by *P. aeroginosa* (63.3%), *K. oxytoca* (63.3%), *K. pneumoniae* (55.8%), *E. coli* (54.6%) and *P. vulgaris* (44.6%). About 57.9% gram-negative DFU isolates were ESBL positive by disc diffusion method.

In Disk potentiation method, average production of ESBL was 58% in *Acinetobacterspp* followed by *P. aeruginosa* (54%), *K. pneumoniae* (42%), *K. oxytoca* (40%), *P. vulgaris* (40%) and *E. coli* (38%). 45.3% of gram-negative DFU isolates were ESBL positive by disk potentiation test.



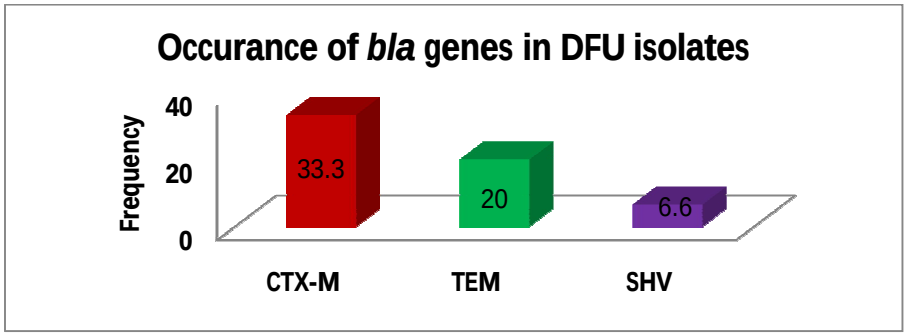
Graph 1: Resistance percentage of antibiotic groups. (AMI: aminoglycosides, CBP: carbapenems, CPS: cephalosporins, CHO: choramphenecol, LIN: lincosamides, Mac: macrolides, MON: monobactam, PEN: penicillin and Q&F: quinalones & fluoroquinalones)



Graph 2: Screening and confirmatory test result for ESBL detection in DFU isolates. (EC:*Escherichia coli*, Ps: *Pseudomonas aerugenosa*, Kp: *Klebsiella pneumoniae*, Ko: *Klebsiella oxytoca*, Ac: *Acinetobacter*, Pv: *Proteus vulgaris*, GNB: total *gram-negative*)

Occurrence of bla genes

The frequency of the occurrence of various *bla* genes in DFU isolates is shown in Graph 3, CTX-M was found to be the most prevalent ESBL noticed in 33.3%, followed by TEM in 20% isolates and SHV beta-lactamases were noticed in 6.6% isolates, respectively.



Graph 3: PCR assay results for bla_{SHV}, bla_{TEM} and bla_{CTX-M} genes.

Table 5 shows the result of factors to be associated with the presence of MDR infections. The duration of ulcer > 1 month was observed in 78.5% patients having MDR infection. The size of ulcer more than 4cm^2 was found in 85.7% patients with MDR infection and in 14.2 % patients having ulcer size less than 4 cm^2 in MDR infections. Fasting blood sugar 176±85.3 mg, HbA1c >8 (85.7%), presence of sensory neuropathy in 78.5% MDR patients and previous antibiotic use in 71.4% were significantly associated with MDR organisms infection in DFU patients.

Table 5: Association of clinical data of DFU patients with or without MDR organism infections (data expressed as mean±sd or n% unless otherwise indicated).

N 60	Non-MDR n 47	MDR n 14
Gender Distribution		
Male	29(61.7)	8(61.5)
Female	18(38.2)	6(42.8)
Age Distribution (years)		
0-20	1(2.1)	-
21-40	11(23.4)	3(23)
41-60	28(59.5)	10(76.9)
61-80	7(14.8)	1(7.6)
Type of Diabetes		
Type 1	10(21.2)	1(7.6)
Type 2	37(78.7)	13(92.8)
Duration of Diabetes (years)		
0-10	33(70.2)	8(61.5)
11-20	9(19.1)	2(15.3)
21-30	4(8.5)	3(23)
Duration of Ulcer		
< month	31(65.9)	3(23)
>month	16(34)	11(78.5)
Hospital Stay (days)	**21.7±17.5**	**20.6±15.2**
Size of Ulcer		
≤4 cm^2	11(23.4)	2(14.2)
>4 cm^2	31(65.9)	12(85.7)
Ulcer Grade (Wagner)		
grade 0	3(6.3)	0(0)
grade 1	17(36.1)	3(23)
grade 2	16(34)	5(38.4)
grade 3	9(19.1)	4(28.5)
grade 4	0(0)	1(7.6)
grade 5	2(4.2)	1(7.6)
Fundus Examination		

Diabetic Retinopathy	22(46.8)	9(69.2)
Discharge Status		
Alive	46(97.8)	12(85.7)
Dead	1(2.1)	2(15.3)
Management		
Amputation	3(6.3)	10(71.4)
Conservative	44(93.6)	4(30.7)
Blood Picture		
WBC	9.5±4.6	10.6±3.6
Hb	9.5±14.1	6.2±6.2
RBC	10.5±2.1	10.1±2.2
Previous Antibiotic Use		
Present	16(34)	9(64.2)
Absent	31(65.9)	4(28.5)
RFT		
Blood Sugar	218±110	150.8±48.7
Blood Urea	38.4±14.8	40.8±16.5
Serum Creatinine	1.0±0.3	1.3±0.7
LFT		
SGOT/AST	18.9±10.9	19.5±11.6
SGPT/AST	17.7±12.1	17.1±12.4
Alkaline Phosphate	12.3±8.6	16±8.6
Bilirubin	0.7±0.1	0.8±0.1
Serum Protein		
TSP	6.7±0.8	6.7±0.9
SA	3.4±0.6	3.2±0.5
SG	3.1±0.7	4.0±1.8
Plasma Glucose		
Fasting	176.6±85.3	127±55.4
Postprandial	231.3±97.6	167.1±50.3
HbA1c		
6-7 %(good control)	2(4.2)	1(7.1)
7-8 % (fair control)	5(10.6)	1(7.1)
>8 % (poor control)	30(63.8)	12(85.7)
Lipid Profile (mg/dl)		
Total lipid	555.3±187.9	543±165.3
Cholesterol	170.6±38.6	155.3±47.2
Triglycerides	176.6±124.8	160.3±100.4
HDL	42±7.3	39.3±10.5
LDL	90.2±28.8	84±14
VLDL	35.2±24.7	32±19.9
Phospholipids	215.4±27	202.8±34.5

Discussion

This study presents a comprehensive clinical and microbiological profile of infected diabetic foot ulcers in hospitalized patients. With the rise in the prevalence of diabetes mellitus there is increasing problem of infections among diabetic patients especially the diabetic foot infection which according to some studies accounts for 20% of hospital admissions (Shankar et al., 2005). India is the home of the largest number of diabetic individuals and their socio-economic conditions are poor. As multidrug resistance is a growing problem, effort was made to study the association of different characteristics with the presence of MDR Organisms. The prevalence of diabetic foot ulcers among male subjects was found to be 56.6% against 30% in female i.e. a ratio of 3.5:1, which may be due to higher level of outdoor activity among males compared to females. Diabetic retinopathy was observed in 52.7% patients studied. There may be one possible of the 13.3% DFU patients which shows no growth in their culture report, that,

in India, most of the patients went to local practitioner, who have little knowledge on DFU treatment. With increasing duration of diabetes, there is increased risk of diabetes related complications especially chronic complications like sensory neuropathy. This study also reports a high prevalence of neuropathy (66.6%). The prevalence of sensory neuropathy in earlier studies shows marked variation. It was 77.8% in a Nigerian study (Ako et al., 2006) and 56.8% in a south Indian study (Shankar et al., 2005). This marked variation in the prevalence may be due to difference in the methods used for the diagnosis of these conditions (10g monofilament or biothesiometer).

In the statistical analysis, duration of infection >1month, prior antibiotic use and ulcer size >4cm^2 were independent predictors of infection with MDR Organism. Thus patients with a large ulcer, with a history of prior antibiotic use and duration of infection >1month were more likely to harbor MDRO's. In the present study, mean duration of ulcer was found to be 41.7±5.6 days with 38.3% having ulcer for more than 1 month. About 78.1% presented with a large ulcer of approximate size of >4cm^2 thereby accounting for approximately 61.6% of the patients presenting with Wagner's grade II and above. 41.6% of patients had used antibiotics prior to reporting to the hospital. The reasons for presentation with advanced grade and stage of ulceration could be because of lack of structured health care delivery in the country, attempted self-medication and trust in traditional healers (Boulton and Vileikte, 2001). Moreover inadequate antibiotic treatment and the use of non sterile instruments for dressing results in the growth of multi resistant organisms necessitating hospital admission and surgical intervention (Armstrong and Lipsky, 2004). Prolonged or broad-spectrum antibiotic therapy predisposes patients to infections with antibiotic-resistant organisms like MRSA (Hartemann-Heurtier et al., 2004). This could explain the high level of MDRO infection in our study.

A bacteriological evaluation of diabetic foot ulcer infections showed that the prevalence of gram-negative organisms were found to be more than gram-positive organisms which is in accordance with the previous findings (Gadepalli et al., 2006). The gram-positive to gram-negative ratio was 1:1.3 which is similar to the findings reported earlier (Tentolouris et al., 1999).

Diabetic foot infections are usually polymicrobial in nature and this has been well documented in the literature. In our study also, we found polymicrobial etiology in 13.3% and monomicrobial in 30% patients with the rate of isolation of about 1.25 bacteria per patient which is lower than the previous studies (Gadepalli et al., 2006; Gerding, 1995) which shows rate of isolation between 2.3 -5.8. Gram-positive organisms which include MRSA were found in 23.3% of patients in reversal to the older studies which show predominance of gram-positive ones (Lipsky et al., 2004a; Lipsky et al., 1990; Lipsky et al., 2004b). The present study confirms that MDR organisms are extremely common in hospitalized patients with diabetic foot ulcers. This is in accordance with the reports of Heartemann-Heurtier (Hartemann-Heurtier et al., 2004).

There is high degree of antibiotic resistance found in our isolates, may be due to the fact that ours is a tertiary care hospital with widespread usage of broad spectrum antibiotics leading to selective survival advantage of pathogen. The antimicrobial resistance pattern was similar to the recent studies done in India and outside (Shankar et al., 2005; Raja, 2007). Gram-negative bacteria that are regarded as normal flora of the skin, like *P. aeruginosa*, may cause severe tissue damage in diabetics and should never be automatically disregarded as insignificant in diabetic foot ulcers (Mike and Ali, 2004). In our study, 57.1% of isolated *S aureus* were methicillin resistant by using 1µg oxacillin disk and 71.4 % of isolated *S. aureus* was resistant to 30µg cefoxitin disk. None of the gram-positive isolates were resistant to vancomycin (VRSA). Clinical isolates of vancomycin resistant *Enterococcus* (VRE) and MRSA resistant have also been reported from treated patients (Herrero et al., 2002; Tsiodras et al., 2001).

Mathur et al. have reported 68% prevalence of ESBL producers from India (Mathur et al., 2002). Babypadmini et al. have shown 40% of *K. pneumoniae* isolates and 41% of *E. coli* isolates to be ESBL producers in their study cohort (Babypadmini and Appalaraju, 2004). Currently there is paucity of data on the prevalence of ESBLs in diabetic foot infection. In a study conducted in Brazil, Motta et al. (2003) say that the prevalence was only 6% among *E. coli* isolates. Gadepalli et al. have reported 54.5% *E. coli* isolates to be ESBL producers (Gadepalli et al., 2006) , which have caused diabetic foot infections. In a recent study, Shobha et al. have reported 27.3% *K. pneumonia, 25.2% E. coli*, 21.42% *Pseudomonas* spp, 25% *Enterobacter* spp and 17% *Acinetobacter* spp to be ESBL producer (Shobha et al., 2009). In this study, 71.6% of *Acinetobacterspp* were positive for ESBL

screening, followed by *P. aeroginosa* (63.3%), *K. oxytoca* (63.3%), *K. pneumoniae* (55.8%), *Escherichia coli* (54.6%) and *P. vulgaris* (44.6%). The high percentage of ESBL production by disk potentiation test was observed in *Acinetobacter* spp (58%) followed by *P. aeruginosa* (54%), *K. pneumoniae* (42%), *K. oxytoca* (40%), *P. vulgaris* (40%) and *E. coli* (38%). The bla_{CTX-M} is among the most prevalent and widely disseminated genes in the clinical bacterial population in India (Ensor et al., 2006; Walsh et al., 2007). In the present study, we found 33.3% bla_{CTX-M} as the most prevalent ESBL gene followed by 20% bla_{TEM} and 6.6% bla_{SHV} in the DFU isolates tested.

It is known that MDR infections are resistant to several antibiotics, and therefore, they can be treated with extended spectrum antibiotics for longer durations. As a result, duration of hospital stay for infections with MDRMs can be longer and their treatment can be more costly. Furthermore, mortality from infections with MDRMs is twice as high as mortality from infections with microorganisms sensitive to antibiotics (Eckman et al., 1995).

In our study, the prevalence of multi-resistant bacterial strains also portends the possibility of longer period of hospitalization for patients as healing may be compromised when bacterial are highly resistant to antimicrobials. The prevalence of both MRSA isolates and ESBL producing gram-negative isolates was in accordance with the reports of Hartemann-Heurtier et al. (Hartemann-Heurtier et al., 2004). Manual minimum inhibitory concentration (MIC) was not carried out as it was time consuming and tedious for all the ESBL-producing clinical isolates obtained in the present study.

Table 4: Antimicrobial resistance pattern of bacteria isolated from diabetic foot ulcers in diabetic patients (N=75).

Antibiotic groups	Antimicrobial agents	EC	Ps	Kp	Ko	Ac	Pv	Sa	Ef	bHS	CONS	TOTAL
		20	8	5	4	3	2	21	3	5	2	
		n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	N(%)
Penicillin	Amoxycillin	17(85)	6(75)	4(80)	3(75)	3(100)	2(100)	¤	‡	‡	‡	35(83.3)
	Amoxyclav	13(65)	5(62.5)	4(80)	3(75)	3(100)	0(0)	6(28.6)	1(33.3)	5(100)	1(50)	42(56)
	Piperacillin	17(85)	6(75)	3(60)	4(100)	3(100)	1(50)	¤	‡	‡	‡	34(80.9)
	Oxacillin	‡	‡	‡	‡	‡	‡	12(57.1)	2(66.7)	3(60)	2(100)	20(60)
Cephalosporins	Cefoxitin	10(50)	6(75)	2(40)	1(25)	2(66.6)	1(50)	15(71.4)	0(0)	3(60)	1(50)	41(54.6)
	Ceftriaxone	11(55)	8(100)	3(60)	2(50)	3(100)	0(0)	12(57.1)	2(66.7)	1(20)	2(100)	44(58.6)
	Cefpodoxime	14(70)	7(87.5)	3(60)	3(75)	3(100)	1(50)	¤	‡	‡	‡	31(73.8)
	Ceftazidime	10(50)	6(75)	4(80)	4(100)	3(100)	0(0)	¤	‡	‡	‡	27(64.2)
	Cefotaxime	15(75)	5(62.5)	3(60)	4(100)	3(100)	2(100)	4(19.0)	2(66.7)	5(100)	2(100)	46(61.3)
	Cefoparazone	20(100)	6(75)	5(100)	4(100)	3(100)	2(100)	¤	‡	‡	‡	40(95.2)
	Cefixime	16(80)	4(50)	4(80)	4(100)	1(33.3)	1(50)	11(52.4)	2(66.7)	3(60)	1(50)	48(64)
	Cefepime	13(65)	4(50)	2(40)	4(100)	1(33.3)	1(50)	¤	‡	‡	‡	25(59.1)
Monobactum	Aztreonam	10(50)	5(62.5)	4(80)	2(50)	2(66.66)	1(50)	¤	‡	‡	‡	24(57.1)
Carbapenems	Imepenem	1(5)	2(25)	0(0)	0(0)	0(0)	0(0)	¤	£	‡	‡	3(7.1)
Aminoglycosides	Amikacin	11(55)	5(62.5)	1(20)	4(100)	1(33.3)	1(50)	3(14.3)	3(100)	1(20)	2(100)	34(45.3)
	Gentamycin	8(40)	4(50)	3(60)	4(100)	3(100)	1(50)	4(19.0)	2(66.7)	3(60)	2(100)	35(46.6)
	Streptomycin	‡	‡	‡	‡	‡	‡	16(76.2)	3(100)	3(60)	2(100)	25(75.7)
	Tobramycin	‡	‡	‡	‡	‡	‡	11(52.4)	2(66.7)	3(60)	2(100)	18(54.5)
Chloremphenecol	Chloramphenicol	10(50)	3(37.5)	2(40)	1(25)	1(33.3)	1(50)	3(14.3)	2(66.7)	4(80)	1(50)	30(40)
Quinalones & fluroquinalones	Ofloxacin	14(70)	3(37.5)	3(60)	4(100)	3(100)	2(100)	10(47.6)	0(0)	3(60)	2(100)	44((58.6)
	Sparfloxacin	‡	‡	‡	‡	‡	‡	16(76.2)	3(100)	3(60)	2(100)	25(75.7)
	Gatifloxacin	11(55)	6(75)	1(20)	2(50)	2(66.6)	2(100)	6(28.6)	1(33.3)	3(60)	1(50)	36(48)
	Levofloxacin	9(45)	5(62.5)	1(20)	2(50)	2(66.6)	1(50)	11(52.4)	2(66.7)	3(60)	1(50)	37(49.3)
β lactum inhibitors	Ceftazidime+Clavulanic acid	2(20)	1(12.5)	1(20)	0(0)	3(100)	0(0)	¤	‡	‡	‡	7(16.6)
	Cephotaxime+Clavulanic acid	6(30)	(0)	1(20)	0(0)	1(33.3)	0(0)	14(66.7)	2(66.7)	0(0)	1(50)	25(33.3)
	Piperacillin+Tazobactum	3(15)	4(50)	1(20)	4(0)	3(100)	1(50)	¤	‡	‡	‡	16(38.0)
	Cefoparazone+Sulbactum	4(20)	2(25)	1(20)	0(0)	3(100)	1(50)	¤	‡	‡	‡	11(26.1)
Macrolids	Erythromycin	‡	‡	‡	‡	‡	‡	7(33.3)	2(66.7)	2(40)	2(100)	14(24.4)
	Azithromycin	‡	‡	‡	‡	‡	‡	15(71.4)	3(100)	3(60)	2(100)	24(72.7)
Lincsamides	Clindamycin	‡	‡	‡	‡	‡	‡	9(42.9)	2(66.7)	3(60)	2(100)	16(48.4)
Glycopeptides	Vancomycin	‡	‡	‡	‡	‡	‡	0(0)	0(0)	0(0)	0(0)	0(0)
Bacitracin		‡	‡	‡	‡	‡	‡	‡	‡	3(60)	‡	3(60)

‡ Not tested, ¤ All Staphylococcus resistant to oxacillin have been considered resistant to all β-lactum
Ps: *Pseudomonas aeruginosa*, Ec: *Escherichia coli*, Pv: *Proteus vulgaris*, Ko: *Klebsiella oxytoca*, Kp: *Klebsiella pneumoniae*, Ac: *Acinetobacter sp*, Sa: *Staphylococcus aureus*, BHS: Beta hemolytic *streptococcus*, CONS: Coagulase negative *staphylococcus sp*, Ef: *Enterococcus faecalis*

In conclusion, a detailed knowledge of the susceptibility to antimicrobial agents is necessary to facilitate the development of effective strategies to combat the growing problem of resistance especially the MRSA and ESBL strains. The prevalence of MDR organisms was alarmingly high in the diabetic foot patients in India because of indiscriminate use of antibiotics. The findings of the present study suggest that prospective multicentre studies are required to assess the appropriate empirical antibiotic regimen in diabetic foot ulcer infections. The study also directs us that proper management of diabetic foot ulcers with appropriate antibiotics such as carbapenems and chloramphenicol along with good glycemic control must be implemented to decrease the incidence of MDR organisms for better clinical outcome.

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Original article

The impact of creatinine clearance on the outcome of diabetic foot ulcers in north Indian tertiary care hospital

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ABSTRACT

Aim: Wound healing has been reported to be poor in diabetic patients with impaired kidney functions that usually accompanies retinopathy and neuropathy. The insensitive foot is vulnerable to repeated trauma and development of ulcer precedes 70–80% of non-traumatic lower extremity amputation. The present study was aimed to study the impact of creatinine clearance (CCre) on the outcome of diabetic foot ulcers (DFU).

Materials and methods: Data from 162 DFU patients admitted to Rajiv Gandhi Centre for Diabetes and Endocrinology of J.N. Medical College, Aligarh Muslim University, Aligarh, India, between December 2009 and March 2011 were analyzed. Detailed history and patient's profile, grade of DFU, co-morbidities and complications, laboratory data, microbiological profile and final outcome were collected. CCre was calculated according to MDRD formula.

Results: The study revealed that, DFU healing was worst in patients with decreased CCre than in those who had normal CCre. Other factors associated with poor outcome were, higher grade of ulcer, infection type (subcutaneous and osteomyelitis) and biofilm infection. Amputation rates were also found to be higher in those with poor renal functions.

Conclusions: The results suggest that CCre is an important factor affecting wound healing in patients with DFUs. The automatic reporting of eGFR each time a serum creatinine concentration is requested will increase the awareness of significant kidney dysfunction in clinical practice especially in DFU patients and appropriate measures will improve the outcome.

1. Introduction

Diabetic nephropathy is a major chronic complication of diabetes, which is associated with increased morbidity and reduced survival. It usually accompanies retinopathy and neuropathy [1]. Renal failure due to diabetic nephropathy is associated with an increased risk for developing a diabetic foot ulcer [2–4]. Diabetic foot ulcer (DFU) precedes 70–85% of non-traumatic lower extremity amputations [5,6]. The amputation rate is significantly higher among diabetic patients with impaired kidney functions than general diabetic population [7,8]. Diabetic neuropathy accounts for the majority of cases presenting with DFUs. The outcome is insensitive feet which are vulnerable to repeated traumas such as stepping on a sharp object or, simply, injury due to ill-fitting shoes [9]. Neuropathy is also associated with impaired innervation of foot muscles which leads to muscular atrophy and deformities, diminished sweating which results in dry skin and callus formation, and loss of proprioception, all of which are major risk factors for ulcer formation [10]. The increased risk of amputation has also been mostly associated with arterial calcifications, namely with the severity of peripheral artery disease (PAD) [4,11,12].

Animal studies also have shown that uraemia, per se, is significantly associated with poor wound healing [13,14]. Creatinine clearance (CCre) is widely used to estimate glomerular filtration rate (eGFR) in clinical practice [15]. The error margin of CCre is acceptable considering the easy use of its assay in routine clinical practice and diagnosis [16]. CCre can be simply calculated by the Modification of Diet in Renal Disease (MDRD) formula [17]. The aim of this study was to study the impact of creatinine clearance on the outcome of diabetic foot ulcer in north Indian tertiary care hospital.

2. Materials and methods

2.1. Study design

Prospective, hospital based study. Total of 162 DFU patients admitted in the Rajiv Gandhi Centre for Diabetes and Endocrinology (RGCDE), Jawaharlal Nehru Medical College Hospital (JNMCH), Aligarh Muslim University (AMU), Aligarh, India, had ulcer in their

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Table 1
The statistical analysis within different creatinine clearance groups (CCre).

Category	Difference mean	*t*-value	*p*-value (between groups)[#]	Critical level	*p*-value[*]
Category A vs. Category B	47.7	5.926	<0.001	0.017	<0.001
Category A vs. Category C	87.1	5.469	<0.001	0.025	
Category B vs. Category C	39.3	2.747	=0.009	0.050	

Category A: no nephropathy or microalbuminuria only; CCre ≥ 90 ml/min/1.73 m^2, Category B: mild to moderate reduced CCre, CCre: 30–89 ml/min/1.73 m^2, Category C: severe reduced CCre or need for renal replacement treatment, CCre < 30 ml/min/1.73 m^2, CCre: creatinine clearance.
[*] Kruskal–Wallis method.
[#] Holm–Sidak method.

foot during December 2009 to March 2011. CCre was calculated according to MDRD formula [17]. Because of our relatively small sample size (n = 162), categories of 0 and 1 (no nephropathy or micro-albuminuria only; CCre ≥ 90 ml/min/1.73 m^2; Category A), 2 and 3 (mild to moderate reduced CCre, CCre: 30–89 ml/min/1.73 m^2; Category B), and 4 and 5 (severe reduced CCre or need for renal replacement treatment, CCre < 30 ml/min/1.73 m^2; Category C) were combined (Table 1). Data were collected prospectively, and used retrospectively to compare the outcome of patients with various degrees of renal impairment. All the subjects gave informed consent and clearance was obtained from the Institutional Ethics Committee (IEC).

2.2. Clinical examination

The methodology for clinical evaluation of diabetic patients was adopted from our previous studies [3]. Ulcers were managed by the team of diabetic foot care and research of RGCDE, JNMCH, AMU. Standard treatment of the ulcers consisted of daily wound care, bed rest, proper off-loading, parenteral antibiotics, debridement, and amputation when indicated. Wound debridement was performed routinely to remove extensive callus and necrotic tissue. Patients with infected diabetic foot ulcers were treated with antibiotics. After obtaining culture specimens, empirical parenteral treatment was started; change in antimicrobial regimen was guided by culture results and clinical follow-up.

2.3. Microbiological methods

Method of culture of specimens and antimicrobial susceptibility testing was described elsewhere [2] and the biofilm production assay were adopted from our previous study [18].

2.4. Antibiotic treatment

Initially antibiotics regiment was selected according to published recommendation [19]. The treatment was modified in accordance with the culture results. The duration of the treatment was at least 4–6 weeks and prolonged in cases of osteomyelitis. All patients also received an intensive insulin treatment.

2.5. Statistics

Independent sample *t*-test for equality of variance was used to compare variables of patients. Differences in proportions were compared using chi-square test, Holm–Sidak method and Kruskal–Wallis method. Analyses were conducted on Sigma plot 11.0 software. Values were given as mean ± standard error (SE) and/or n (%) otherwise indicated. p values less than 0.05 were considered statistically significant.

3. Results

Males were predominant 105(64.8%) in the study subjects. All patients had ulcers graded 1–3 in the University of Texas classification system. Majority had type 2 diabetes 134(82.7%). The mean age of the subjects was 51.1 ± 11.4 years. The mean duration of diabetes was 13.5 ± 4.6 years, and nearly 111(68.5%) had the condition for ≤10 years. Eighty-two patients (50.6%) had neuropathy, 72(54.4%) nephropathy, 82(50.6%) retinopathy, and 92(56.7%) were hypertensive. Osteomyelitis was present in 20(12.3%) subjects. Nearly one third 60(37%) had ulcer for >1 month before presentation at the hospital. Majority of the ulcer was found on interdigits (43.2%), followed by plantar surface (28.4%), heel (29.5%), margins (12.6%), malleoli (16.7%), and on multiple areas (≥2 sites) was 31.4%. Size of ulcer ≤4 cm^2 was observed in 38(23.4%) patients and ≥4 cm^2 in 124(76.5%) in which majority of the patients were males. Grade I ulcer was found in 48(29.6%, Grade II in 94(58%) and Grade III in 20(12.3%) patients. Majority of the subjects were having poor glycemic control (A1c > 7%) in 147(90.7%) at the time of hospital admission. More than 63(38.8%) received surgical treatment, mainly in the form of debridement. 46(28.4%) patients were subject to amputation and 18(11.1%) died during the hospital stay (mean hospital stay 22.9 ± 15.5). The bacterial infection in ulcer was superficial in 48(29.6%) cases, subcutaneous 94(58%) and osteomyelitic in 20(12.3%) patients.

3.1. Microbiological observations

A total of 275 (aerobic + anaerobic) bacteria were isolated, averaging of 1.70 bacterial species per patient. 35% patients had monomicrobial infection and polymicrobial etiology was observed in 65%. Gram positive cocci comprised of 34.5% and gram negative bacilli for 65.4%. Gram positive to gram negative ratio was 1:1.8. The frequency of bacterial isolates from the DFU is shown in Table 1. *Escherichia coli* was the most common isolate, accounting for 27.8%; followed by *Staphylococcus aureus* 23.5%, *Pseudomonas aeruginosa* 15.6%, *Klebsiella oxytoca* 7%, *Klebsiella pneumonia* 5.8%, *Proteus vulgaris* and *Enterococcus faecalis* 3.5% each, *Acinetobacter* sp. 3.1%, *Coryneform* sp. 2.7%, beta hemolytic *streptococcus* spp. *and CONS* 2.3% each, *Proteus mirabilis* 1.5% *and Morganella morganii* 0.7%. Anaerobic gram-positive cocci were found in 10 patients, 5 patients had infection by gram-positive bacilli and only two patients had infection by gram negative bacilli. The remaining 145 patients (89.5%) were found negative for anaerobic culture. Among the anaerobic bacteria isolated, gram positive cocci comprised of 58.8%, gram positive bacilli 29.4% and gram negative bacilli for 11.7%. *Peptostreptococcus* sp. was the most common isolate, accounting for 35.2%; followed by *Peptostreptococcus anaerobius* 23.5%, *Propionibacterium* sp. 17.6%, *Bacteroides ureolyticus* 11.7%, *Clostridium perfringens* 5.8% and *Eggerthella lenta* 5.8% *were* isolated from DFU patients (Table 2).

3.2. Antibiotic resistance profile

The result of resistance studies are summarized in Fig. 1. High degree of antibiotic resistance were exhibited by *Proteus mirabilis* (67.4%) followed by *Pseudomonas aeruginosa* (67.1%), CONS (65.9%), *Acinetobacter* sp. (58.7%), *Proteus vulgaris* (55.1%), *Enterococcus faecalis* (54.0%), *Klebsiella oxytoca* (53.4%), *Klebsiella pneumoniae*

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Table 2
Frequency of distribution of isolates from 162 DFU patients in relation to treatment.

	Name of isolates	Total
AEROBIC		*n* (%)
	Gram positive cocci	**88 (34.5)**
1	*Staphylococcus aureus*	60 (23.5)
2	*Enterococcus faecalis*	9 (3.5)
3	*Beta hemolytic streptococcus*	6 (2.3)
4	*CONS*[*]	6 (2.3)
5	*Coryneform spp*	7 (2.7)
	Gram negative bacilli	167 (65.4)
6	*Escherichia coli*	71 (27.8)
7	*Pseudomonas aeruginosa*	40 (15.6)
8	*Klebsiella oxytoca*	18 (7.0)
9	*Klebsiella pneumonia*	15 (5.8)
10	*Proteus vulgaris*	9 (3.5)
11	*Proteus mirabilis*	4 (1.5)
12	*Acinetobacter spp*	8 (3.13)
13	*Morganella morganii*	2 (0.7)
	TOTAL AEROBIC	**255 (93.7)**
ANAEROBIC		
	Gram positive cocci	**10 (58.8)**
14	*Peptostreptococcus spp*	6 (35.2)
15	*Peptostreptococcus anaerobius*	4 (23.5)
	Gram positive bacilli	**5 (29.4)**
16	*Propionibacterium spp*	3 (17.6)
17	*Clostridium perfringens*	1 (5.8)
18	*Eggerthella lenta*	1 (5.8)
	Gram negative bacilli	**2 (11.7)**
19	*Bacteroides ureolyticus*	2 (11.7)
	TOTAL ANAEROBIC	**17(6.25)**
	TOTAL	**272**

[*] Coagulase negative *staphylococcus* spp.

(51.6%), beta hemolytic *streptococcus* (50.8%), *Staphylococcus aureus* (50.6%), *Escherichia coli* (50%), *Morganella morganii* (50%) and *Corynebacterium* sp. (39.5%). Higher percentage of resistance (75.2%) was shown among the Penicillin group followed by Lincosamide (71.7%), Macrolide (69.8%), Monobactams (63.5%), Aminoglycosides (61%), Quinolones and Fluoroquinolones (60.5%), Cephalosporin (57.2%), Chloramphenicol (50.8%), beta lactam inhibitors (21.7%) and Carbapenems (12.1%).

Table 3 shows the difference in clinical characteristics of patients in Category A, B and C. Patients in Category B (mild to moderate reduced CCre, CCre: 30–89 ml/min/1.73 m^2) and category C (severe reduced CCre or need for renal replacement treatment, CCre < 30 ml/min/1.73 m^2) were older in age than those in category A (no nephropathy or micro-albuminuria only; CCre ≥ 90 ml/min/1.73 m^2) [$p = 0.068$]. The significant difference of CCre category was observed in hemoglobulin [$p = 0.05$], serum creatinine [$p < 0.001$], LDL-C [$p < 0.001$], HDL-C [$p < 0.001$], total cholesterol [$p = 0.011$] and eGFR [$p < 0.001$]. The final outcomes of three category of CCre on DFU were presented in Table 4. The level of CCre were significantly correlated with the status of kidney function [$p < 0.006$], grades of foot ulcer (grade 2 and grade 3) [$p = 0.002$, $p < 0.001$], amputation [$p < 0.001$], infection type (subcutaneous, osteomyelitic) [$p = 0.002$, $p < 0.001$] and biofilm infection assay positivity by the bacterial isolated from foot [$p = 0.006$].

4. Discussion

In this present study, we found that reduced creatinine clearance was associated with impaired wound healing in diabetic foot ulcers. There was also an increase in amputation rates in patients with decreased CCre. Diabetic nephropathy has been proposed as a risk factor for diabetic foot ulcer development [20] and the same has been reported from our previous studies [2,3]. The frequency of foot ulcers increases with the progression of nephropathy [2,3,21–23]. Renal failure has been associated with poor outcome in patients with diabetic foot ulcers [2,4]. Aulivola et al. [24] reported that wound healing was inferior in patients with renal failure who underwent infra-popliteal angioplasty. Johnson et al. [25] reported that failure of foot salvage in patients with end stage renal disease and critical ischemia after surgical revascularization was due to wound healing problems rather than graft thrombosis. Another study showed a significant association between end stage renal disease and the failure of trans-metatarsal amputations to heal [26].

Although majority of evidence has pointed out the ischemia as the underlying problem which led to ulcer development and poor healing in the presence of the ulcer in diabetic patients with renal failure, neuropathy is particularly common, possibly aggravated by a component of uraemic neuropathy [11,12,27]. The present study clearly showed that wound healing was associated with the decrease in CCre in patients with diabetic foot ulcers. Experimental studies have demonstrated that uraemia impairs wound healing [7,13,14,28]. In the animal model study, it has been shown that acute uraemia delayed the healing of wound in rats [13]. In rats, Kursh et al. [14] showed that uraemia had a deleterious effect on wound healing which was demonstrated by measuring the tensile strength of skin wounds and the amount of collagen formation in polyvinyl sponges implanted subcutaneously. Reduced healing was noted in more severely uraemic rats indicating that the degree of uraemia was important. Another study showed that rats with renal failure formed less granulation tissue [28]. It has been shown that renal failure is associated with altered inflammatory response to wounding, which makes the wound more susceptible to infection. Impaired defence response in renal failure has been found to be manifested by depressed neutrophil function, leucopenia related to complement activation, diminished T and B lymphocyte function, and a reduction in natural killer cell activity [7,29].

Some other features of patients with renal failure may also be associated with the poor wound healing in diabetic foot ulcers

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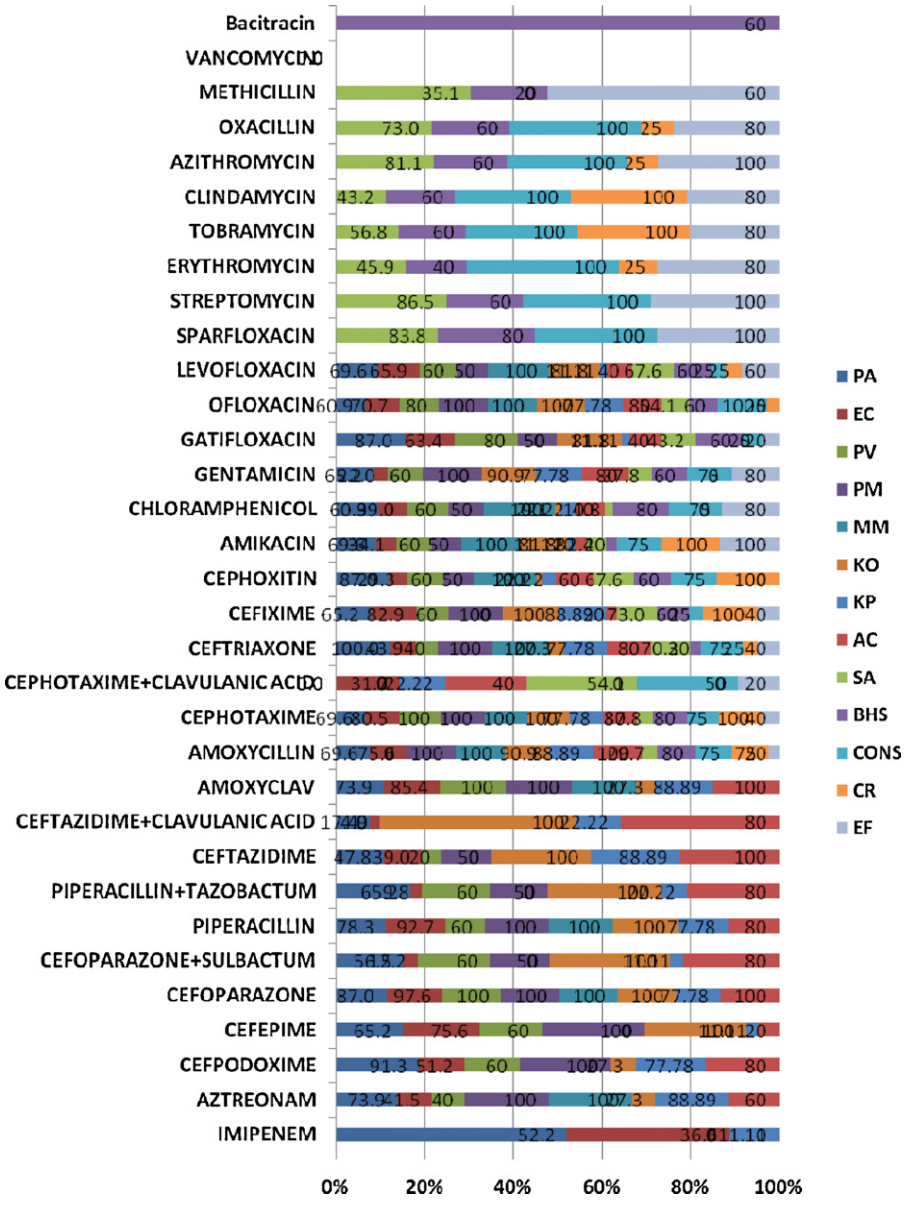

Fig. 1. Antimicrobial resistance patter bar graph of bacteria isolated from diabetic foot ulcers in diabetic patients. Ps: *Pseudomonas aeruginosa*, Ec: *Escherichia coli*, Pv: *Proteus vulgaris*, Pm: *Proteus mirabilis*, Mm: *Morganella morganii*, Ko: *Klebsiella oxytoca*, Kp: *Klebsiella pneumoniae*, Ac: *Acinetobacter* sp., Sa: *Staphylococcus aureus*, Bhs: Beta hemolytic *streptococcus*, CONS: Coagulase negative *staphylococcus* sp., Cr: *Coryneform* sp, En: *Enterococcus faecalis*.

leading to amputation was also reported in our study [3]. Autonomic neuropathy may accompany peripheral neuropathy in some diabetic patients with renal failure. Autonomic neuropathy results in dry and fragile skin, and hypotension, which may lead to repeating traumas [7]. Anaemia due to renal failure may lead to reduced tissue oxygenation [30,31]. Tissue oedema due to hypervolemia may also impair wound healing [7].

In the present study polymicrobial etiology was found in 65% and monomicrobial in 35% patients with the rate of isolation of about 1.70 bacteria per patient which is lower than the previous studies [32,33] which showed rate of isolation between 2.3 and 5.8%. We found gram negative aerobic bacteria were most frequently isolated which is in accordance with our previous reports [2,3]. The studies from western countries show that gram positive aerobes are the predominant organisms isolated from DFU [34–38]. The gram positive to gram negative ratio was 1:1.8 which is in similar to the findings reported earlier [2,3,32]. While, recent studies from India have shown a preponderance of gram negative aerobes [2,3,32,39]. Zubair et al., [3] in their study on 162 DFU patients, recovered 255 aerobic bacteria of which 65.4% were gram negative and only 34.5% gram positive. Gadepalli et al., [32] also reported gram negative aerobes to be most frequently isolated pathogens (28.7%), followed by 13.8% gram positive aerobes. Similar results were also reported by Shankar et al., [39]. Studies from Malaysia have also reported a predominance of gram negative bacteria (52%) in patients with DFU, the most common pathogens isolated being *Proteus* sp., *Klebsiella pneumonia*, *E coli* and *Enterobacter cloacae* [40]. In our anaerobic study, *Peptostreptococcus* sp. was the most predominant one which is in accordance to the previous studies [41,42]. *Clostridium* sp. was the most commonly isolated anaerobe, followed by *Bacteroides* [43], we recovered fewer anaerobic species compared with earlier culture reports [44,45], because most of our patients did not have chronic draining wounds. This may be an indication of fewer anaerobic species among non-threatening lower-extremity infections, which is also reported earlier by Lipsky and Berendt [46].

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Table 3
Baseline characteristics of diabetic foot ulcer patients with various levels of CCre.

	Category A	Category B	Category C	P-value
	N = 31	N = 123	N = 8	–
Sex (*Male*)	21(67.7)	79(64.2)	5(62.2)	0.926*
Age (*years*)	43.1 ± 14.6	48.4 ± 12.7	55.1 ± 8.3	0.068
Size of ulcer (cm^2)	10.5 ± 8.7	26.8 ± 56.5	17.3 ± 25.7	0.765
HbA1c (%)	9.1 ± 2.03	9.6 ± 2.0	9.9 ± 2.0	0.334
Smoking history	18(58.0)	64(52.0)	5(62.2)	0.732*
WBC (10^3/IU)	10.9 ± 3.6	10.3 ± 3.12	9.9 ± 4.2	0.132
Hb (mg/dl)	11.4 ± 1.3	10.4 ± 2.26	9.6 ± 1.76	0.050
S-creatinine (mg/dl)	0.83 ± 0.20	1.18 ± 0.44	1.65 ± 0.72	<0.001
SGOT/AST(IU/L)	21.7 ± 12.6	18.3 ± 9.0	15.9 ± 9.6	0.155
SGPT/AST(IU/L)	19.8 ± 14.8	16.9 ± 8.5	16.2 ± 10.4	0.655
LDL-C (mg/dl)	74.6 ± 25.2	91.7 ± 26.1	102.0 ± 9.5	<0.001
T-cholesterol (mg/dl)	146.5 ± 21.1	168.5 ± 40.12	169.3 ± 5.50	0.011
HDL-C (mg/dl)	42.3 ± 7.8	41.3 ± 4.7	35.7 ± 5.16	<0.001
Triglycerides (mg/dl)	127.6 ± 28.3	150.2 ± 89.9	169.9 ± 109.1	0.972
TSP (gm%)	6.6 ± 0.75	6.5 ± 1.0	6.5 ± 0.73	0.881
Total Albumin (gm%)	3.16 ± 0.57	3.26 ± 0.69	3.2 ± 0.56	0.659
Total Globulin (gm%)	3.86 ± 1.99	3.30 ± 0.97	3.5 ± 0.63	0.907
eGFR	111.34 ± 43.22	64.36 ± 12.53	25.7 ± 3.76	<0.001
Hospital Stay (days)	23.7 ± 11.98	25.12 ± 18.57	14.75 ± 10.4	0.096
Ulcer duration (days)	16.7 ± 10.3	27.6 ± 12.3	38.1 ± 13.3	<0.001

Category A: no nephropathy or microalbuminuria only; CCre ≥ 90 ml/min/1.73 m^2, Category B: mild to moderate reduced CCre, CCre: 30–89 ml/min/1.73 m^2, Category C: severe reduced CCre or need for renal replacement treatment, CCre < 30 ml/min/1.73 m^2. Data are mean ± sd or *n* (%) unless otherwise indicated. WBC, white blood cells, Hb, haemoglobulin; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; SGOT/AST, serum glutamic oxaloacetic transaminase/aspartate transaminase; SGPT/AST, serum glutamate-pyruvate transaminase/aspartate transaminase, TSP, total serum protein.
* Difference in proportions was compared using Kruskal-Wallis One way Analysis of variance and/or chi-square test.

The overall resistance in our patients was high. One possible reason could be that most of our patients received some antimicrobial treatment before presenting at our centre from the referring hospitals using a combination of different antimicrobials empirically. The resistance pattern in our study was similar to the recent studies done in India and outside [32,39,40,47].

Table 4
Outcome of DFU in patients with various levels of CCre.

	Category A	Category B	Category C	P-value
	N = 31	N = 123	N = 8	–
Complications				
Hypertension	10(32.2)	76(61.7)	6(75)	**0.015**
Retinopathy	16(51.6)	62(50.4)	4(50)	0.995
Neuropathy	9(29.0)	66(53.6)	7(87.5)	**0.049**
Nephropathy	18(58.0)	47(38.2)	7(87.5)	**<0.006**
Texas Grade				
1	11(35.4)	30(24.3)	4(50)	0.166
2	10(32.2)	77(62.6)	6(75.0)	**0.002**
3	4(12.9)	10(8.1)	6(75.0)	**<0.001**
Hospital Stay (days)	23.7 ± 11.98	25.12 ± 18.57	14.75 ± 10.4	0.096#
Amputation	3(9.6)	36(29.2)	7(87.5)	**<0.001**
Site of ulcer				
Interdigit	18(58.0)	47(38.2)	5(62.5)	0.072
Planter	18(58.0)	22(17.8)	6(75)	**<0.001**
Heel	19(61.2)	26(21.1)	3(37.5)	**<0.001**
Margins	3(9.6)	16(13.0)	2(25)	0.516
Malleoli	7(22.5)	19(15.4)	7(87.5)	**<0.001**
Multiple site(>2)	17(54.8)	28(22.7)	6(75)	**<0.001**
Ulcer duration (days)	16.7 ± 10.3	27.6 ± 12.3	38.1 ± 13.3	**<0.001**#
Infection type				
Superficial	11(35.4)	30(24.3)	4(50)	0.166
Subcutaneous	10(32.2)	77(62.6)	6(75.0)	**0.002**
Osteomyelitis	4(12.9)	10(8.1)	6(75.0)	**<0.001**
Biofilm Infection	12(38.7)	85(69.1)	6(75.0)	**0.006**

Category A: no nephropathy or microalbuminuria only; ≥ 90 ml/min/1.73 m^2, Category B: mild to moderate reduced CCre, CCre: 30–89 ml/min/1.73 m^2, Category C: severe reduced CCre or need for renal replacement treatment, CCre < 30 ml/min/1.73 m^2. Data are mean ± sd or *n* (%) unless otherwise indicated.
Difference in proportions was compared using chi-square test and/ or Kruskal-Wallis One way Analysis of variance.

The biofilm infection in foot ulcer was also found to be an independent and the most important significant factor for CCre study. According to our literature search, this was the first report of biofilm production as risk factor for decrease CCre level. Because the resistance in the biofilm producing bacteria is high as compared to non-biofilm producers as reported in our previous studies [18]. The biofilm producing bacteria have close cell–cell contact that permits easy transfer of plasmids to one another than in the planktonic state. It can force bacteria into a slow-growing state when placed in an environment with adverse growth condition. In this state of intermission, bacteria are less susceptible to antimicrobial attack [48]. The biofilm also provides a physical protection to bacteria because antimicrobial agents are also ineffective at penetrating the biofilm, decreasing the concentration acting on the bacterial cells within the biofilm and as a consequence their efficacy [49]. In addition, biofilm also appear to have an antiphagocytic property within the biofilm, which renders leukocytes present within the matrix ineffective [50]. Additionally, there appears to be a component within the polysaccharide that inactivates and traps both complement and host antibodies. These factors lead to an accumulation of host immune factors that can lead to host tissue damage and eventually chronic inflammation [51].

There are some limitations in the present study. A small number of patients with severe renal failure (severe reduced CCre or need for renal replacement treatment, CCre < 30 ml/min/1.73 m^2; group C) and significant differences between groups in both age and disease duration.

4.1. Conclusion

The observation of the present study are important, showing an association between moderate CCre (30–90 ml/min/1.73 m^2) and severe CCre (≤30 ml/min/1.73 m^2), and an increased risk for the onset of diabetic foot ulcer, poor healing and amputation. It is important to note that demonstrating an association is not the same as showing causation, which often requires an experimental design such as a randomized clinical trial and the demonstration of a common mechanism that causes CKD and failure of the skin to

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heal. It is likely that chronic kidney disease (CKD) and DFU among those with diabetes are associated more tightly than was recognized previously. Clinicians should be aware of the fact that diabetic patients with foot ulcers who have impaired kidney functions are at increased risk for poor wound healing and amputation even in absence of limb ischemia. Noted that, introduction of automatic reporting of eGFR each time a test for serum creatinine concentration is requested will increase the awareness of significance of kidney dysfunction in clinical practice and appropriate measures will help in improving the prognosis.

Conflict of interest

The authors declare that there was no conflict of interest between them.

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