Ozdemir et al. BMC Medical Imaging (2025) 25:20
https://doi.org/10.1186/s12880-025-01558-8

BMC Medical Imaging

RESEARCH

Ultrasonographic examination of the maturational effect of maternal vitamin D use on fetal clavicle bone development

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Abstract

Aim This study aimed to evaluate the effect of maternal vitamin D use during intrauterine life on fetal bone development using ultrasonographic image processing techniques.

Materials and methods We evaluated 52 pregnant women receiving vitamin D supplementation and 50 who refused vitamin D supplementation. Ultrasonographic imaging was performed on the fetal clavicle at 37–40 weeks of gestation. The fetal clavicle images were compared with adult male clavicle images. The texture features obtained from these images were used for analysis.

Results No difference was observed in bone formation and destruction markers between the two groups. However, the texture analysis of ultrasonographic images revealed similarities in the characteristics of fetal clavicles in pregnant women receiving vitamin D supplementation and those of adult male clavicles.

Conclusions Vitamin D supplementation in pregnancy has significant positive effects on fetal bone maturation besides contributing to maternal bone health. Texture feature analyses using ultrasonographic images successfully demonstrated fetal bone maturation.

Keywords Fetal clavicle, Fetal clavicle ultrasonography, Image processing, Vitamin D

Introduction

Today, many organizations recommend vitamin D supplementation during pregnancy [1] due to the high prevalence of vitamin D deficiency in the population [2]. Vitamin D deficiency during pregnancy is a significant public health concern and it has been associated with various adverse maternal and fetal outcomes, including reduced fetal growth and impaired skeletal development [3–5]. Several studies have demonstrated the importance of maternal vitamin D status during pregnancy for fetal bone development [6–10]. For instance, lower levels of maternal 25-hydroxyvitamin D in late pregnancy are associated with reduced whole-body and lumbar spine bone mineral content in children at 9 years of age [6].

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Maternal bone resorption increases significantly in the second half of pregnancy. The levels of bone resorption markers are low in the first trimester but double at term [11]. Additionally, vitamin D deficiency during pregnancy is linked to adverse pregnancy outcomes including preeclampsia, gestational diabetes, impaired fetal growth, and low birth weight [7, 9, 10].

Insufficient calcium intake in pregnancy has rarely led to spontaneous vertebral compression fractures in late pregnancy or puerperium likely due to maternal skeletal calcium mobilization to meet fetal demands [12]. These findings underscore the critical role of vitamin D in maternal and fetal skeletal health.

Vitamin D supplementation studies have demonstrated improvements in bone mineral density and a decrease in bone turnover and fracture incidence [13]. This effect is mediated through the Nuclear factor-kappa B (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) pathway, which regulates bone resorption. RANK, expressed on pre-osteoclast membranes and secreted by osteoblasts, is a well-established marker of bone resorption, while OPG, a decoy receptor for RANKL, blocks the interaction between RANK and RANKL, thereby preventing bone resorption [14, 15].

Although much research focuses on maternal bone health, studies on fetal bone development remain limited. Moreover, unresolved questions persist regarding the optimal serum levels of vitamin D and the recommended intake during pregnancy to achieve these levels [8, 16]. Vitamin D is recognized as essential for bone health; however, some studies have reported no significant effects of vitamin D insufficiency on bone density [17]. Further research is needed to clarify these discrepancies and explore the role of vitamin D in both bone and non-bone health outcomes during pregnancy and lactation.

One notable gap in the literature is the lack of studies investigating the effects of maternal vitamin D status on the development of specific fetal bones such as the clavicle. A crucial component of the fetal skeleton, the clavicle, undergoes early and significant mineralization, which may be influenced by maternal vitamin D levels [9, 18]. Addressing this gap could provide valuable insights into the role of vitamin D in the comprehensive assessment of fetal skeletal development.

Ultrasonographic imaging has become an increasingly popular tool for evaluating bone diseases due to technological advancements facilitating the diagnosis of various conditions [19]. It is widely used to monitor fetal bone growth and development, including parameters such as biparietal diameter and abdominal circumference for small-for-gestational-age screening [20]. Beyond traditional imaging, texture analysis of ultrasound images offers a novel method to quantitatively assess fetal bone characteristics. Texture features describing the distribution and relationship of pixel intensity distribution can provide objective measures that may be more sensitive to subtle changes in bone development associated with maternal vitamin D status [21, 22].

This study aimed to address these gaps using texture analysis and comparing and analyzing fetal clavicle images in pregnant women who received vitamin D supplementation and those who did not. The quantitative insights obtained could enhance understanding of the clinical significance of maternal vitamin D supplementation during pregnancy and its impact on fetal skeletal health. Additionally, focusing on fetal clavicle development, the study contributes to the ongoing discussions on optimal vitamin D intake and its broader implications during pregnancy.

Materials and methods

This study was conducted at the perinatology clinic of the Erciyes University School of Medicine and approved by the Ethics Committee of Erciyes University (approval no: 2019/145). All the participants provided informed constant form.

Participants and image acquisition

In this study, we evaluated 295 volunteers who were at the beginning of the first trimester of pregnancy. A total of 43 volunteers were excluded based on the exclusion criteria and 244 volunteers were offered vitamin D supplementation. Then the patients were divided into two groups according to their vitamin D use. After further patient exclusion during the follow-up, experimental groups comprised 102 pregnant women: 52 volunteers who used vitamin D and 50 volunteers who did not use vitamin D. A total of 50 adult male volunteers aged 25 years formed the control group. The flowchart of the study is shown in Fig. 1.

Exclusion and inclusion criteria

This study included 152 participants divided into three groups. Group 1 comprised 52 pregnant volunteers with male fetuses and who received 1200 IU vitamin D supplements. Group 2 comprised 50 pregnant volunteers with male fetuses, who refused to receive 1200 IU vitamin D supplements. Group 3 comprised 50 male volunteers aged 25 years who completed clavicle ossification. Male embryos and adult males were selected based on a deliberate methodological approach to ensure consistency and minimize variability introduced by sex-based differences in skeletal development and metabolism. This approach allowed us to focus on the specific effects of vitamin D supplementation on clavicle development without interference from pregnancy

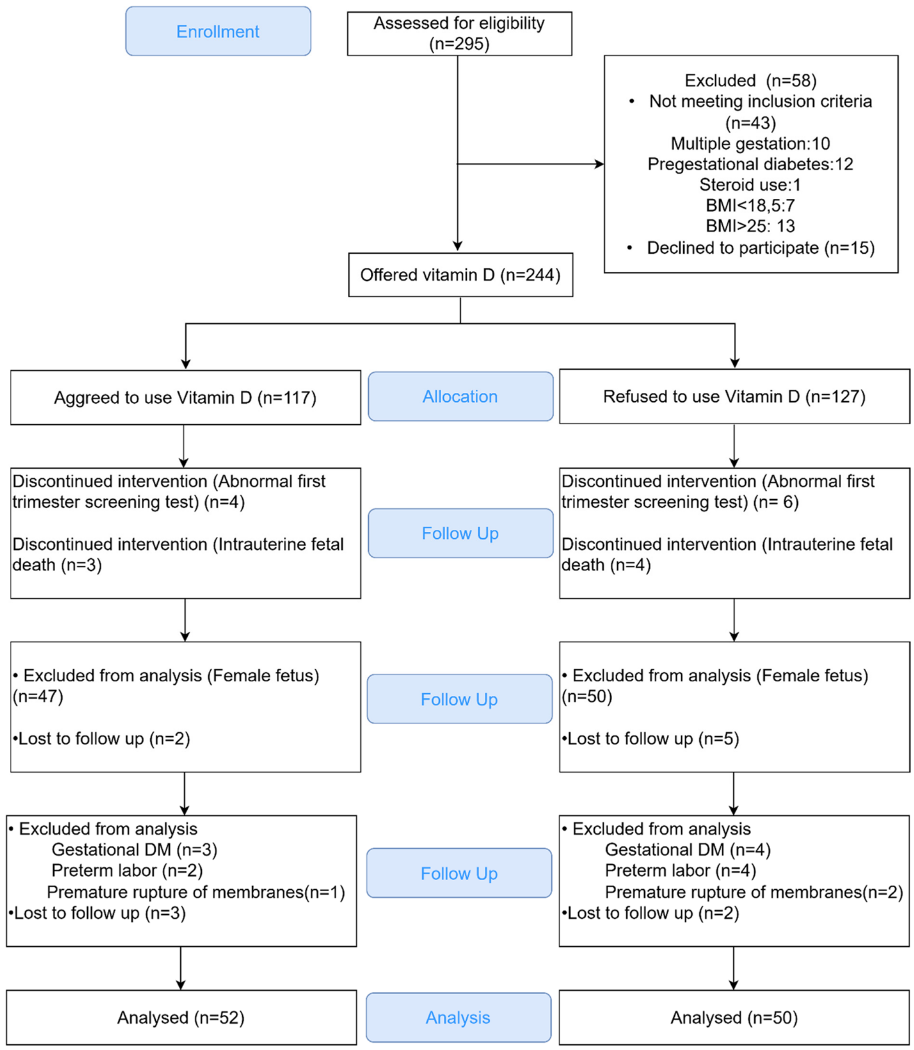

Fig. 1 The flowchart of the study

or lactation-related confounding factors in females. The adult clavicle bone was used as a reference because it represents the final stage of ossification and structural organization.

The exclusion criteria were as follows: gestational or pre-gestational diabetes mellitus, pregnant women with known osteoporosis, female fetus, twin or multiple gestations, abnormal prenatal screening tests, and/or any abnormality in the second-trimester ultrasound screening, collagen tissue diseases, osteomalacia, use of drugs (steroids and bisphosphonates) effective against bone metabolism for any reason, prior history of cholecystectomy, injury or fracture of the clavicle bone during delivery, malabsorption, ulcerative colitis, Chron's disease, and body mass index (BMI) less than 18.5 kg/m^2 or over 25 kg/m^2.

Clinical interventions

Both pregnant volunteers and young male volunteers were Caucasian. Pregnant volunteers were followed up at the perinatology clinic of Erciyes University from the diagnosis of pregnancy to childbirth. All pregnant volunteers underwent both first- and second-trimester screening tests. Those with abnormal results or any abnormality were excluded from the study. We included only patients with a BMI between 18.5 and 25 in the study.

Bone selection

All pregnant volunteers in the study were examined between the 37th and 40th weeks of gestation because human fetal bones accumulate 80% of calcium in the third trimester [23, 24]. The clavicle bone was selected for this study because it is the bone in which ossification begins the earliest among all bones, and its ossification is complete at the age of 25 [25]. In other words, the clavicle is almost always metabolically active. Moreover, the clavicle is situated on the anterior side of the body, which contains less fat and creates a bulge in the skin. Thus, the clavicle was the most appropriate bone for examination.

Ultrasonographic examinations

The ultrasonographic examinations were performed by a single author (F.O.) between 37th and 40th weeks of gestation using a Voluson E6 ultrasound device. The aim was to view a coronal plane of both the clavicle and the fetus. Therefore, the convex probe was moved on the volunteer's abdomen until a perfect view was achieved. The measurements were done keeping all sonographic settings and gain properties constant for participants. The records of the left clavicle achieved under the coronal plane and were stored.

Delivery of pregnant

All pregnant volunteers delivered in our clinic. After delivery, the cord blood samples were used for measuring the cord blood pH and the levels of RANK, RANKL, OPG, and vitamin D.

The ultrasound images of the clavicle bone were systematically analyzed using a combination of preprocessing, segmentation, and feature extraction techniques, as outlined in the flow chart in Fig. 2.

Image preprocessing and image segmentation

Image segmentation is a process in which pixels with similar characteristics such as intensity or texture are grouped into meaningful regions to identify specific structures within an image. In this process, an image is divided into meaningful regions with different features. These features may be, for example, similar density within the image, which may represent objects in various regions of the relevant image [26].

Preprocessing and segmentation methods were applied to the clavicle ultrasonographic images before the feature extraction phase of the study. As illustrated in Fig. 2, preprocessing steps such as Gamma correction and region of interest (ROI) cropping improve image quality by enhancing contrast and reducing noise. Figure 3 demonstrates the segmentation process using the Fast Fuzzy c-Means (FFCM) algorithm to extract the clavicle contour precisely. The ultrasonographic images were preprocessed to adjust their contrast and reduce noise. The input images were standardized using the gamma correction method which adjusts the brightness and contrast of an image non-linearly, enhancing its visual quality. Then, the images were cropped according to their ROI. The FFCM algorithm clusters image pixels based on their similarity, allowing precise segmentation of regions such as the clavicle contour [27, 28]. Gamma correction was chosen due to its robust performance in enhancing ultrasound image quality, while the FFCM algorithm was selected for its proven accuracy in segmenting biomedical images with irregular boundaries. Alternative methods such as Otsu's thresholding or K-means clustering were considered; however, these were found to be less effective for this specific dataset. Figure 3 shows the image processing and clavicle bone segmentation steps.

Feature extraction

The quantitative feature metrics and texture features were extracted from the ROIs of clavicle ultrasonographic images to analyze clavicle bone characteristics in volunteers using or not using vitamin D. Feature extraction involves analyzing specific visual patterns, such as texture or intensity, to generate quantitative metrics that summarize critical characteristics of the image. The shape, color, texture, and other types of information are frequently used to extract features.

Intensity-based and texture-based features were specifically chosen due to their ability to capture both global and local structural variations in the clavicle bone, which are critical for identifying potential differences between individuals who use vitamins and those who do not. Moreover, the gray-level co-occurrence matrix (GLCM) was obtained to extract the texture properties of the clavicle ultrasonographic images [29]. GLCM is a matrix reflecting how often different combinations of gray levels occur in an image. It is widely used to extract features, especially while analyzing biomedical images. In other words, it shows the relationship between two neighboring pixels [29, 30]. In this study, the GLCM was obtained in the 0° direction in the lateral plane [31]. This direction was selected to align with the predominant orientation of

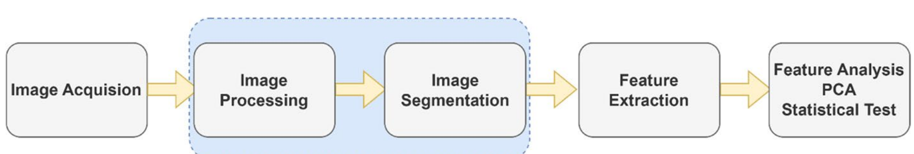

Fig. 2 Steps for the image analysis of the study

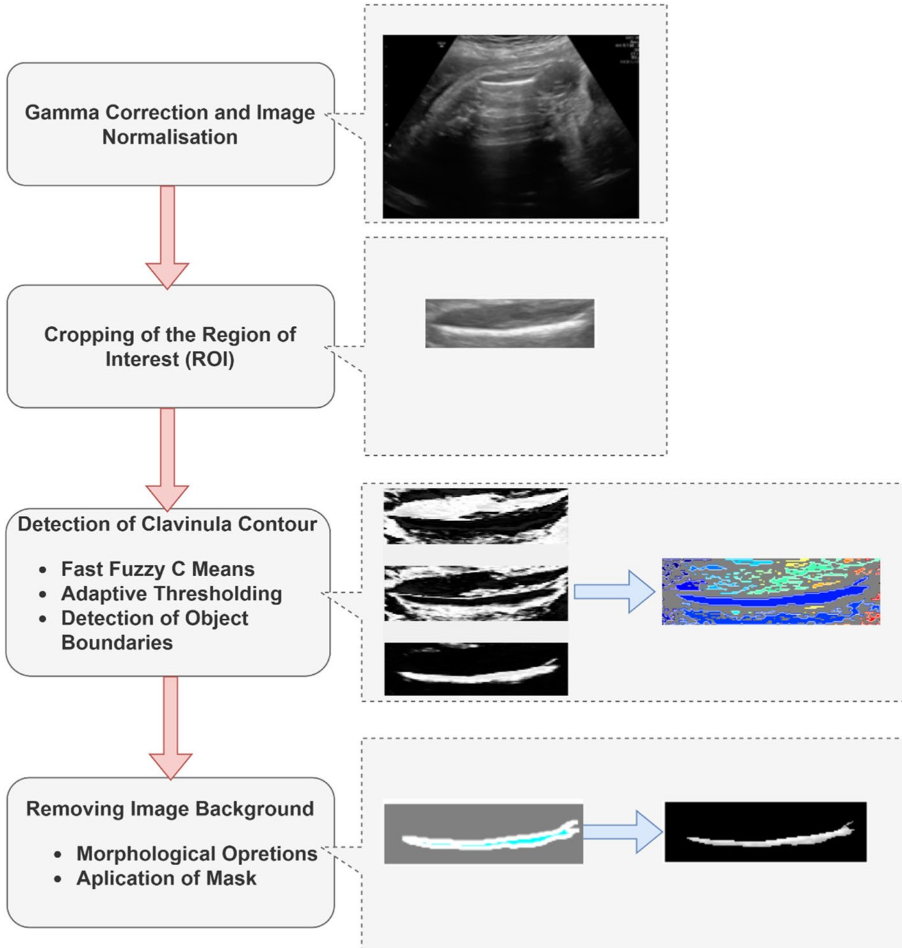

Fig. 3 Image processing and clavicle bone segmentation steps

clavicle features in ultrasound images, ensuring consistent feature extraction across samples.

Texture features are important low-level attributes used to describe the content of an image, such as its perceived texture.

Intensity features

Intensity features characterize the intensity of each region of an image. Image histograms, on the contrary, are widely used in image analysis because they are an important way to obtain a global and compact representation of a set of values [32]. Histogram analyses provide a global summary of pixel intensity distributions, capturing key statistical properties such as mean, variance, and entropy, which are crucial for differentiating image regions. A histogram analysis is performed using the intensity values of all or part of the image. Intensity in image pixels is defined by the mean of pixel values, standard deviation, variance, and asymmetry. These statistical parameters provide a comprehensive summary of the image intensity distribution, enabling robust differentiation of subtle structural differences between study groups.

In this study, the mean and standard deviation values, which are the basic statistical parameters, were calculated as the intensity properties of ultrasonographic images. In addition, the entropy value of these images was calculated. Further, the variance, skewness, kurtosis, and entropy values were calculated from the image histogram [33].

Texture features

In statistical texture analysis, the texture features are calculated using the statistical distribution of intensity combinations observed at specific positions relative to each other in the image. The texture features are classified as first-order, second-order, and higher-order statistical features depending on the number of intensity values (pixels) in each case. The GLCM features (Haralick features) are commonly used in texture feature analysis [29, 31].

The second-order statistical texture features containing information about the positions of pixels with similar gray-level values are extracted using the GLCM method. The image texture can be characterized using statistical parameters extracted from the GLCM that is extensively used in medical imaging for its capability to quantify spatial relationships between pixel intensities, offering insights into tissue structure and texture variability. Therefore, many applications use tissue-based identification, quality analysis, and classification and segmentation algorithms in the medical image analysis of GLCM properties [34, 35]. The detailed mathematical formulations of the GLCM features, including contrast, correlation, and entropy, are provided in supplementary data for further reference [29, 36]. Principal component analysis (PCA) is a well-known approach for reducing dimensionality. It reduces a large number of correlated variables into a smaller set of uncorrelated variables termed principal components (PCs), which still preserves the majority of the variability seen in the original data. PCA was chosen due to its ability to handle high-dimensional, correlated data efficiently, making it particularly well-suited for biomedical image analysis where quantitative features are often interdependent. It may alternatively be viewed as a rotation of the axes of original variables to a new set of orthogonal axes (the primary axes). As a result, the new coordinate system now contains the direction of the largest variations of the original variables [37]. In this study, PCA was applied to each of the features from each group to visualize the similarity in the computed features between the groups. The PCs of each feature from the groups were plotted with a spider plot. The most essential PC (PC1) was used to represent the PC of each feature and PC1s were shown with a spider plot.

Biochemical analysis

For biochemical analysis, 10 mL of the blood sample was taken from the umbilical cord following the delivery of the fetus and centrifuged. The RANK, RANKL, and OPG expression levels in the serum were obtained by centrifuging the collected blood using the enzyme-linked immunosorbent assay kit (Wuhan USCN Business Co., Ltd., China). Additionally, the cord blood pH and vitamin D levels were measured.

Statistical analysis

The Shapiro–Wilk test was used to examine the normality assumption of the data. The variance homogeneity assumption was tested with the Levene test. The data were expressed as mean ± standard deviation, median (25th percentile – 75th percentile), or *n* (%). Parametric comparisons were made with *t* test or *Z* test. Nonparametric comparisons were performed with the Mann-Whitney *U* test. PASW Statistics 18 program was used for all comparisons. A *p*- value < 0.05 indicated a statistically significant difference.

Results

As described in the experimental design (Sect. 2.1), the study was conducted on three groups: vitamin D supplement users, vitamin D supplement nonusers, and the control group (adult males). The aim of the study was to analyze the effect of maternal vitamin D levels on fetal clavicle development using ultrasonographic images, feature extraction, and biochemical markers.

The demographic values for vitamin D users and nonusers were similar. All pregnant volunteers were term and gave birth between the 37th and 40th weeks of gestation. Both maternal and cord vitamin D levels were significantly high in the vitamin D supplement user group. The levels of bone resorption marker RANK were high in vitamin D nonusers, whereas the levels of bone resorption inhibitor OPG were high in vitamin D users. However, the differences in OPG and RANK levels were not statistically significant. The demographic values are presented in Table 1.

Maternal and cord vitamin D levels were significantly higher in the vitamin D supplement user group compared with the nonuser group ($p < 0.001$ and $p = 0.033$, respectively). The RANK levels increased in the nonuser group and OPG levels were higher in the supplement user group, indicating that the differences were not statistically significant. No significant differences were observed in other demographic parameters.

Statistical analysis results of features obtained from ultrasonographic images, histogram of image features, and GLCM features are shown in Table 2.

There were significant differences in various features among the study groups. Specifically, mean intensity values showed a significant difference between adult volunteers and vitamin D nonusers ($\boldsymbol{p} = 0.045$). Additionally, entropy values revealed significant differences between the users and nonusers of vitamin D ($\boldsymbol{p} = 0.0285$) as well as between adult volunteers and vitamin D nonusers ($\boldsymbol{p} = 0.0109$).

Among GLCM-based texture features, several notable findings were observed. The correlation feature exhibited significant differences both between the users and nonusers of vitamin D ($\boldsymbol{p} = 0.0100$) and between adult volunteers and vitamin D nonusers ($\boldsymbol{p} = 0.0135$). The energy feature showed a significant difference between the users and nonusers of vitamin D ($\boldsymbol{p} = 0.0069$). Furthermore, the Information Measure of Correlation feature demonstrated significant differences between the users and nonusers of vitamin D ($\boldsymbol{p} = 0.0108$). Similarly, the Maximum Probability feature displayed significant

Table 1 Demographic values, the timing of delivery, birth weight, gravidity, parity, abortion, maternal and cord vitamin D levels, cord pH and RANK, RANKL, and OPG levels in pregnant participants

	Vitamin D supplement users (*n* = 52)	Vitamin D supplement nonusers (*n* = 50)	*p*- value	Significance
Age	30.02 ± 4.15	29.44 ± 3.86	0.467	
Gravidity	2 (2–3.75)	2.50 (2–4)	0.569	
Parity	1 (0.25–2)	1 (1–2)	0.265	
Abortion	0 (0–1)	0 (0–1)	0.533	
Timing of delivery (day)	287 (273–301)	280 (273–301)	0.940	
Delivery method (vaginal)	40 (%77)	39 (%78)	0.897	
Birth weight (g)	3145(2950–3488.75)	3287.50 (3073.75–3478.75)	0.232	
Maternal vitamin D level	17.45(9.74–27.75)	10 (5.78–14)	**< 0.001**	significant
Cord vitamin D level	16 (10.45–25.75)	13.05 (8.23–17.05)	**0.033**	significant
Cord RANK level	15.49 (9.86–21.86)	22.05 (7.58–26.75)	0.126	
Cord RANKL level	70.25 (35.90–121.95)	74.33 (47.92–135.41)	0.505	
Cord OPG level	1.43 (0.74–2.28)	1.38 (0.77–2.09)	0.815	
Cord pH level	7.36 (7.34–7.38)	7.35 (7.32–7.38)	0.404	

Note: *p*-values < 0.05 are considered statistically significant.

$p < 0.05$ indicates significance.

$p < 0.01$ indicates high significance.

differences between the users and nonusers of vitamin D (***p*** = 0.0059).

These findings highlighted significant variations in texture and intensity features, particularly in the vitamin D nonuser group compared with the other groups. This underscored the potential influence of maternal vitamin D supplementation on fetal clavicle bone characteristics.

Also, PC1 values for mean, entropy, and variance features calculated from ultrasonographic images, the principal component of the histogram of ultrasonographic image features, and the GLCM of ultrasonographic image features of each group are shown in Fig. 4.

Discussion

The GLCM feature analysis offers a novel approach to understanding the evolution of texture features in bone ultrasonographic images, offering insights into ossification patterns during clavicle development [31, 38, 39].

This study demonstrated significant differences in entropy features between the 25-year-old male adult volunteers and the vitamin D nonusers. Lower entropy values in the control group suggest a more homogenous image texture, indicative of a well-organized calcification pattern. Entropy, which measures the randomness of image pixel intensities, reflects the structural organization of ossification, particularly during the critical period of 37th – 40th weeks of gestation [40]. The mean intensity values observed in ultrasonographic images can indicate mineralized osteoid clusters, further supporting this finding.

Energy, another GLCM-derived feature, was significantly different between the control and vitamin D nonuser groups, with no notable differences between the control and vitamin D user groups. Energy, which quantifies the sum of squared elements in the GLCM, was higher in groups with repetitive pixel intensities, aligning with the orderly calcification pattern seen in vitamin D users and the control group [36]. This highlights the potential of vitamin D supplementation in promoting structured ossification.

Osteoblasts, derived from neural crest mesenchyme, play a pivotal role in bone formation by secreting osteoid clusters, where calcium precipitates to form ossification centers [41]. These centers contribute to both trabecular and cortical bone development, with osteoblasts on the inner surface of the periosteum generating osteoid layers parallel to the bone matrix, creating a compact bone structure [42]. The osteogenic properties of the periosteum and its alignment with the bone's shape suggest that texture features in ultrasonographic images can serve as markers of calcification orderliness. The resemblance of clavicle texture features in vitamin D users to those of 25-year-old adults underscores the role of vitamin D in fostering structured ossification patterns.

Correlation, a measure of linear relationships between pixel intensities, reflects the spatial distribution of white points in the ultrasound image [43]. The control group had the highest correlation values, signifying a highly organized calcification pattern. The correlation values of vitamin D users were closer to those of the control group,

Table 2 *P* values for ultrasonographic image features

Feature	Adults (*n* = 50)	Vitamin D users (*n* = 52)	Vitamin D nonusers (*n* = 50)	*p*-Value (Adult volunteers-Vitamin D users)	*p*-Value (Adult volunteers-Vitamin D Nonusers)	*p*-Value (Vitamin D users-nonusers)	Significance
Image Intensity Features							
Mean	12.83 ± 5.7	13.37 ± 6.5	15.77 ± 7.9	0.6326	0.0450	0.0955	Significant
Entropy	0.8252 ± 0.34	0.803 ± 0.28	1.011 ± 0.47	0.7110	0.0109	0.0285	Significant
Standard Deviation	46.89 ± 12.8	46.08 ± 12.44	49.0 ± 12.7	0.7173	0.2551	0.4101	Not Significant
Histogram of Image Features							
Variance	217,987 ± 201,265	180,638 ± 59,787	165,507 ± 14,406	0.2184	0.0741	0.0924	Not Significant
Skewness	15.905 ± 0.0011	15.901 ± 0.0015	15.902 ± 0.0019	0.1670	0.0150	0.1062	Significant
Kurtosis	253.9 ± 0.01	253.9 ± 0.012	253.8 ± 0.08	0.1583	0.0162	0.1177	Significant
Entropy	0.9 ± 0.1	0.89 ± 0.1	0.91 ± 0.1	0.5217	0.7702	0.2935	Not Significant
GLCM features							
Auto Correlation	3.66 ± 1.35	3.77 ± 1.38	3.92 ± 1.55	0.6868	0.3780	0.5978	Not Significant
Cluster Prominence	789.76 ± 493.12	812.06 ± 436.84	764.02 ± 433.89	0.8011	0.7649	0.5959	Not Significant
Contrast	0.22 ± 0.10	0.23 ± 0.11	0.23 ± 0.12	0.7333	0.5089	0.7345	Not Significant
Correlation	0.93 ± 0.02	0.94 ± 0.037	0.90 ± 0.09	0.0744	0.0135	0.0100	Significant
Difference Entropy	0.12 ± 0.03	0.12 ± 0.044	0.13 ± 0.05	0.9538	0.4810	0.5473	Not Significant
Difference Variance	0.22 ± 0.10	0.24 ± 0.11	0.23 ± 0.13	0.7358	0.5183	0.7432	Not Significant
Dissimilarity	0.054 ± 0.02	0.055 ± 0.02	0.058 ± 0.023	0.7976	0.3432	0.4893	Not Significant
Energy	0.85 ± 0.05	0.86 ± 0.079	0.80 ± 0.11	0.3995	0.0068	0.0069	Significant
Entropy	0.41 ± 0.12	0.42 ± 0.16	0.44 ± 0.18	0.8729	0.2089	0.2945	Not Significant
Homogeneity	0.985 ± 0.05	0.984 ± 0.006	0.983 ± 0.008	0.5219	0.1685	0.4977	Not Significant
Information Measure of Correlation	−0.75 ± 0.04	−0.76 ± 0.05	−0.72 ± 0.08	0.2933	0.0316	0.0108	Significant
Maximum Probability	0.92 ± 0.02	0.94 ± 0.05	0.87 ± 0.11	0.1698	0.0048	0.0059	Significant
Sum Entropy	0.39 ± 0.12	0.40 ± 0.15	0.43 ± 0.17	0.8768	0.2138	0.2943	Not Significant
The sum of Squares Variance	1.87 ± 0.94	1.93 ± 0.92	2.01 ± 0.98	0.7338	0.4751	0.6906	Not Significant

indicating that vitamin D supplementation enhances the calcification process. Conversely, the vitamin D nonuser group demonstrated less organized patterns, consistent with lower ossification quality.

Ogata and Uhthoff [44] identified two ossification centers in the fetal clavicle at the 7.5th week of gestation, which subsequently fused by the 11th week. These findings align with the observations of organized ossification patterns in the control and vitamin D user groups in this study. Similarly, Baumgart et al. [45], using computed tomography (CT) imaging, described linear growth patterns in the clavicle with no sex-related and laterality differences. While CT provides detailed structural analysis, its radiation risks limit its applicability in the uterus. Ultrasonographic imaging, combined with advanced texture analysis, offers a safer and equally informative alternative.

The increasing use of ultrasonographic imaging for musculoskeletal evaluations underscores its value in detecting bone-related abnormalities. Studies have shown that ultrasound surpasses conventional radiography in detecting bone erosions, making it a reliable tool for skeletal assessments [45, 46]. The development of colored nomograms for fetal bones could be made possible by integrating artificial intelligence (AI) into ultrasound analysis, which could further improve diagnostic accuracy. These advancements may allow for the simultaneous evaluation of the physical and biochemical properties

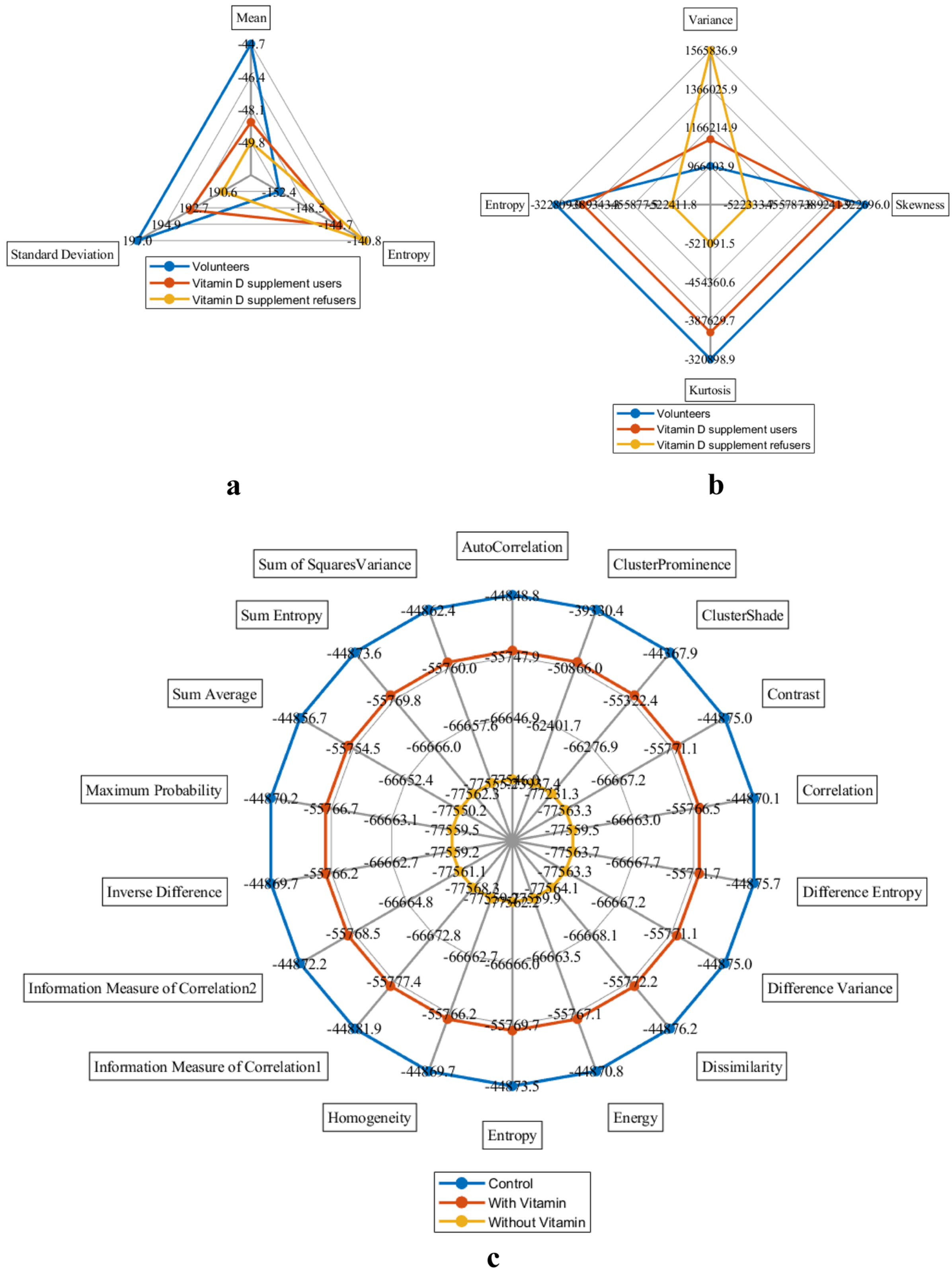

Fig. 4 **a** Spider plot for the first principal component of ultrasonographic image features, **b** Spider plot for the first principal component of the histogram of ultrasonographic image features, **c** The first principal component of GLCM of ultrasonographic image features

of ossification, offering a comprehensive approach to diagnosing and monitoring skeletal disorders.

This study highlighted the potential of GLCM-based textural analysis in assessing ossification patterns and the role of maternal vitamin D supplementation in promoting organized calcification in fetal clavicles. Future research should focus on integrating AI with ultrasonographic imaging to establish reliable and non-invasive diagnostic tools for skeletal abnormalities during pregnancy.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12880-025-01558-8.

Additional file 1.

Acknowledgements
Not applicable.

Clinical trial number
Not applicable.

Authors' contributions
F.O.: Conceptualization, Data curation, Ultrasonographic examination, B.A.: Methodology, Project administration F.L.: Analyzing the texture feature of images S.M.: Biochemical analyses, N.T.O.: Statistical analyses, G.A.: Writing-Original draft preparation, I.I.M.: Writing- Reviewing and Editing.

Funding
This study was supported by the Erciyes University Scientific Research Projects Unit (Project number: TSA-2020-10207).

Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the Ethics Committee of Erciyes University (approval no: 2019/145). All methods were carried out in accordance with relevant guidelines and regulations of the Declaration of Helsinki. All informed consent was obtained from the participants and/or their legal guardian(s).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 17 October 2024 Accepted: 11 January 2025
Published online: 16 January 2025

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