

# Human Papillomavirus DNA Typing in the Cervical Specimens among Women of Split and Dalmatian County

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## ABSTRACT

*Human papillomavirus (HPV) infection is the main cause of cervical cancer, the second most common cancer in women worldwide. More than 200 types of HPV have been described, and within this range more than 40 types attack epithelium of genital tract. The types that were most commonly related to the development of cervical cancer are called high-risk types (HR HPV). There are very few studies about HPV prevalence in Croatia and there is an absolute lack of data for Split and Dalmatian County. Therefore, during a 6 month period, we routinely screened 570 women for HPV DNA at the Educational Public Health Institute of Split and Dalmatian County. HR HPV was detected in cervical exfoliated cells, by using Hybrid Capture 2 HPV DNA test. Out of the total number of tested women, 200 (35%) of them were positive to HR HPV. Polymerase chain reaction (PCR) based assays were employed for HR HPV genotyping in positive specimens. The following frequency was observed: HPV 16 in 10%, HPV 18 in 6.1%, HPV 31 in 2.6%, HPV 33 in 1.9%, HPV 52 in 1.4%, HPV 59 in 0.7%, HPV 45 in 0.4% specimens, while 11.9% of tested specimens currently remained untyped. It is necessary to expand this study to a larger number of women, in order to better evaluate genital HPV types distribution among women in this region.*

**Key words:** Human papillomavirus (HPV), high-risk genotypes, Split and Dalmatian County, Croatia

## Introduction

Cervical cancer is the second most common cancer in women worldwide. Infection with Human papillomavirus (HPV) is the main cause of cervical cancer<sup>1</sup>. HPV is the most common sexually transmitted viral disease and one of the most frequent causal agents of sexually transmitted diseases. Genital HPV infection is rarely reported (there is no legal obligation). While its prevalence is assumed to be higher than 20 million, the incidence of HPV infection only in the United States ranges from one million to 5.5 million per year<sup>2</sup>. The prevalence of HPV DNA in cervical cancer cases is 99.7%<sup>3</sup>, while in cervical specimens of women without cervical cancer ranges from 5% to 20%<sup>4-6</sup>. More than 200 types of HPV are known, and within this range more than 40 attack epithelium of gen-

ital tract. The types that are most commonly related to the development of cervical cancer, so called oncogenic or high-risk types (HR HPV), are: 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 70<sup>2,7</sup>. The most common HPV types in cervical cancer are: HPV type 16 (46% to 63%) and HPV type 18 (10% to 14%)<sup>7</sup>. There are very few studies in Croatia about HPV prevalence related only to cervical specimens of women with abnormal Papanicolaou (Pap) smear, with the most common types: HPV type 16 (11.4% to 20.2%) and HPV type 31 (5% to 17.8%)<sup>8-10</sup>. Concerning the absolute lack of data for Split and Dalmatian County, we initiated a testing for HPV in order to evaluate the prevalence in our region.

## Materials and Methods

In the Educational Public Health Institute of Split and Dalmatian County, during the 6 months period in 2006, HPV screening tests were done in 570 women. The age range of the study was 18 to 62 years (the average age 40 years). The study population included all women referred by their gynecologists for HPV DNA testing to our laboratory, as a survey of general population (with unknown cytological diagnosis). HR HPV was detected in cervical exfoliated cells, by using Hybrid Capture 2 (HC2) HPV DNA test (Digene Corporation, Gaithersburg, MD USA). HC2 test was used in screening specimens for high-risk types of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), which had been approved by Food and Drug Administration (FDA, USA) for detection of HPV DNA in cervical specimens for diagnostic purpose. This test cannot differentiate individual types of HPV within the group<sup>2</sup>.

Only HR HPV positive specimens analyzed by HC2 test were sent for further genotyping. DNA was extracted from cervical cells, by using reagents Nucleo Spin Tissue (Machery-Nagel, Düren, Germany). Genotypes were distinguished by polymerase chain reaction (PCR) based assays using primer sequences for HR HPV types: 16, 18, 31, 33, 45, 52, and 59 (Table 1). Positive and negative controls were included in each reaction. PCR reactions were performed according to the manufacturer instruction on PCR system (Applied Biosystems, Foster City, USA). PCR products were detected using agarose gel electrophoresis (3% agarose gel). The specific primers for amplification of the sequence containing E6 region of HPV types: 16, 18 and 33 were used (TaKaRa, Bio. Inc., Japan)<sup>11-12</sup>. Amplification products yield DNA fragment of 140 bp for HPV types: 16, 18 and 141 bp for HPV type 33. The presence of HPV types: 31, 45, 52 and 59 was detected by using primers for amplification of the sequence containing E7 region, described by Walboomers et al.<sup>3</sup>, yielding a PCR product of 100 bp.

**TABLE 1**  
PRIMER SEQUENCES USED FOR HIGH-RISK HPV GENOTYPING

HPV Type	Primer sequence (5'-3')
HPV 16	GTTTGCAGCTCTGTGCATA
HPV 16	CATTTTATGCACAAAAGAGAAGCTGCAATG
HPV 18	GTGTTTCAGTTCCTGCACA
HPV 18	TGAGAAACACACCACAATACTATGGCGCGC
HPV 33	GTCTCCAATGCTTGGCACA
HPV 33	CATTTTGCAGTAAGGTACTGCACGACTATG
HPV 31	GGGCTCATTTGGAATCGTGTG
HPV 31	AACCATTGCATCCCCTCCC
HPV 45	CCCACGAGCCGAACCACAG
HPV 45	TCTAAGGTCTCTGCCGAGC
HPV 52	GCAGAACAAGCCACAAGCAA
HPV 52	TAGAGTACGAAGGTCCGTCG
HPV 59	CTCCGAGAATGAAAAAGATGAA
HPV 59	GCTGAAGTTGATTATTACA

HPV – Human Papillomavirus

## Results

During routine HPV testing in Split and Dalmatian County, total of 570 women were tested, and 200 (35%) of them were positive to HR HPV, when samples were assessed by HC2 test. Only HR HPV positive specimens by HC2 were further genotyped by PCR based assays. Our analysis revealed the frequency of HPV 16 in 10%, HPV 18 in 6.1%, HPV 31 in 2.6%, HPV 33 in 1.9%, HPV 52 in 1.4%, HPV 59 in 0.7%, HPV 45 in 0.4% specimens (Table 2). Among HPV positive sample, HPV 16 was the more abundantly found types in 28.5% cases, followed by HPV 18 (17.5%), 31 (7.5%), 33 (5.5%), 52 (4%), 59 (2%) and 45 (1%), while 68 (34%) specimens remained with unresolved genotype (Table 2). Multiple HPV infections were found in two cases out of 200 (1%): HPV 16 and 33, and HPV 18 and 31, respectively.

**TABLE 2**  
HIGH-RISK HPV FOUND IN THE CERVICAL SPECIMENS AMONG WOMEN IN SPLIT AND DALMATIAN COUNTY

HPV Type	Number	% of tested samples (N=570)	% of positive specimens (N=200)
16	57	10.0	28.5
18	35	6.1	17.5
31	15	2.6	7.5
33	11	1.9	5.5
52	8	1.4	4.0
59	4	0.7	2.0
45	2	0.4	1.0
others	68	11.9	34.0

HPV – Human Papillomavirus

## Discussion

Having in mind the absolute lack of data for Split and Dalmatian County, we screened HPV prevalence in our region. Our preliminary study included 570 women who underwent routine screening as a survey of general population with either normal or abnormal Pap smears. Our results showed that 35% of them were positive for HR HPV. These positive samples detected by HC2 were further genotyped by type-specific primer-directed PCR for 7 most common types of HR HPV (Table 1). In our study, among HPV positive sample the most commonly found HPV types were: HPV 16 in 28.5%, HPV 18 in 17.5%, and HPV 31 in 7.5% specimens, while 68 (34%) specimens currently remained undetermined (Table 2).

The prevalence of HR HPV in cervical specimens of women without cervical cancer ranges from 5% to 20% worldwide, while in healthy women with normal Pap smears it is approximately 10%<sup>4-6</sup>. Among European women with low grade lesions found in Pap smears, HPV prevalence in exfoliated cells is 67.8%, with the most common HPV types: HPV type 16 in 19.4% specimens

and HPV type 31 in 10.4% specimens. Prevalence of HPV type 18 in Europe is 5.1%, but ranges from 0% to 31.4%, compared by region<sup>13</sup>. Very few studies about HPV prevalence in Croatia were conducted. The studies presented by Grce et al. analyzed only women with abnormal Pap smears. According to these studies in Croatia, HPV prevalence in cervical specimens of women with abnormal Pap smears ranges from 43% to 64%, with the most common types: HPV type 16 (11.4% to 20.2%) and HPV type 31 (5% to 17.8%)<sup>8–10</sup>. In our study, we had unexpectedly high number of specimen without genotype (34%), in comparison to studies by Grce et al. that reported from 23.7% to 49% of untyped HPV DNA positive samples<sup>9,10</sup>. This result might be due to presence of other rare types of HPV, insufficient quantity of DNA in the specimen or due to differences between detection methods (HC2 and PCR). The integrity of DNA from 68 specimens with unresolved HPV genotype was further tested for  $\beta$ -actin by PCR, and 13 (19.1%) did not yield amplification product.

Our results of HR HPV genotyping are slightly different from those found in other studies conducted in Croatia due to several reasons. In previous studies regarding HPV prevalence in Croatia, mainly women with abnormal Pap smears were tested for the presence of HPV and typed for a limited number of HPV types (HPV 6/11, 16, 18, 31 and 33)<sup>8–10</sup>. In our study, population included women who were referred by their gynecologists for HPV DNA testing to our laboratory (with unknown cytological diagnosis). Furthermore, in this study HPV DNA positive samples by HC2 were further genotyped for more HR HPV types (HPV 16, 18, 31, 33, 52, 59 and 45) than those reported in previous studies. The frequency of

HPV types in the study of Grce et al. 2001<sup>9</sup> conducted on 1,874 cervical specimens collected in the region of Zagreb and this study are similar for HPV types 16 (12 versus 10%) and 33 (2.7 versus 1.9%), but discordant for HPV 18 (2.4 versus 6.1%) and 31 (5.1 versus 2.6%). The reason for these discrepancies could be (1) small number of analyzed sample in this study, (2) the choice of type-specific primers for DNA amplification, and (3) different distribution of HR HPV types in both Counties. Split and Dalmatian County is an important tourist region of Croatia with a high number of tourists passing through. It is also a maritime centre with the port and significant number of seamen that are carriers of HPV types gained from all over the world and spread within this County. Due to these reasons, it is important to continue this study on larger number of specimens and to enlarge the spectrum of genotyping of HR HPV in order to evaluate HPV genital types distribution among women in this region, especially in the context of HPV vaccine application.

## Conclusion

The data obtained from our study indicate that HPV 16 and 18 are the most common HPV types found in the cervical specimens among women of Split and Dalmatian County as in most studies worldwide. However, low abundant HR HPV types differ between regions in Croatia. In our opinion, it is necessary to expand this study to a larger number of women in order to obtain a better distribution of HR HPV types in Split and Dalmatian County.

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## **RASPODJELA POJEDINIH TIPOVA HUMANOGA PAPILOMA VIRUSA U UZORCIMA OBRISKA VRATA MATERNICE ŽENA SPLITSKO-DALMATINSKE ŽUPANIJE**

### **S A Ž E T A K**

Infekcija uzrokovana human papillomavirusom (HPV) je glavni čimbenik razvoja karcinoma vrata maternice, drugoga po učestalosti među karcinomima u žena u svijetu. Poznato je više od 200 tipova HPV-a, od kojih više od 40 tipova napada epitel spolnoga sustava. Tipovi koji se najčešće povezuju s razvojem karcinoma vrata maternice nazivaju se visokorizični tipovi (HR, engl. *High Risk* HPV). U Hrvatskoj je, do sada, napravljeno svega nekoliko studija o prevalenciji HPV-a, a ne postoje nikakvi podatci za Splitsko-dalmatinsku županiju. Zbog toga smo, u Nastavnom zavodu za javno zdravstvo Splitsko-dalmatinske županije, napravili probir 570 žena na HPV DNK, u razdoblju od 6 mjeseci. HR HPV dokazan je u obriscima vrata maternice, metodom HC2 (Hybrid Capture 2, Digene). Od ukupnoga broja testiranih žena njih 200 (35%) je bilo pozitivno na visokorizične tipove HPV-a. Daljnjom genotipizacijom HR HPV pozitivnih uzoraka, metodom lančane reakcije polimerazom (PCR, engl. *Polymerase Chain Reaction*), utvrđena je učestalost tipa 16 u 10% uzoraka, tipa 18 u 6,1%, tipa 31 u 2,6%, tipa 33 u 1,9%, tipa 52 u 1,4%, tipa 59 u 0,7%, tipa 45 u 0,4% uzoraka, dok su 11,9% uzorka, za sada, ostala netipizirana. Smatramo da je neophodno proširiti studiju na veći broj ispitanica, te nastaviti praćenje proširenosti pojedinih tipova HPV-a u žena ove regije.