

## HUMAN PAPILLOMAVIRUS GENOTYPE SPECTRUM IN STUDIED GROUP OF MONTENEGRIN WOMEN

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**Summary:** The risk of high-grade lesions and carcinoma is known to correlate with specific high-risk HPV genotypes. The distribution of HPV types varies between countries and little is known about HPV distribution in our country. Therefore, the purpose of this study was to determine the range and frequency of HPV genotypes in studied group of women in Montenegro. HPV genotypes were determined using the method of enzyme restriction of PCR products amplified with group-specific primers MY09/MY11 and restricted with seven different restriction endonucleases. Out of the total number of women HPV infection was found in 1/5 of participants (20%). Genotyping performed in HPV DNA positive women shows that the HPV genotype 16 is dominant and present in more than 1/3 of the participants (36.8%). The second most frequent HPV infection is with HPV genotype 58 and it is found in 10.5% of participants. HPV 31 and HPV 6 infections are present in 7.9% of women, while infections with other genotypes were demonstrated individually by 2.6%. Multiple HPV infection was demonstrated in 18.4% and they are dominant in younger women (aged 25 to 30 years).

**Key words:** HPV genotype; PCR; RFLP; Multiple HPV infection

### Introduction

Infection with high-risk genotypes of human papillomaviruses (HPV) is the main etiological agent of cervical cancer, the second most common form of cancer in women worldwide (8, 17). More than 100 different HPV genotypes have been identified (and their number is still growing), out of which approximately 45 were detected in anogenital region (4, 16). Among these, potentially high-risk HPV genotypes, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 (and probably high-risk types 26, 53, 66, 68, 73, and 82), are defined based on their prevalence in cervical intraepithelial neoplasia (CIN) lesions and cervical cancers (5, 6). The results of recent studies have shown that the prevalence of high-risk HPV genotypes increases with increasing degree of CIN and that the prevalence of infection with HPV in women with invasive form of cervical cancer is 99.7% (2, 14).

The distribution of HPV types varies between countries (5) and little is known about HPV distribution in our country. The main objective of this study was to determine the range and frequency of HPV genotypes in studied women in Montenegro. Modern techniques of molecular virology were used, which is the first and only study of its kind in Montenegro by now days.

### Material and Methods

A total of 189 cervical specimens obtained from the same number of women were involved in the study. Cervical smears were taken from women during a routine gynecological examination at the Clinical Center in Podgorica. Sampling was done using a commercial diagnostic kit HC II (Digene Specimen Collection Kit, Silver Spring, Maryland, USA). Diagnostic was performed with PCR method by detection of DNA HPV in cervical materials at the Laboratory for Molecular Microbiology and Diagnostics of AIDS, the Institute of Microbiology and Immunology, Faculty of Medicine in Ljubljana. DNA from cervical smears samples was isolated by a procedure previously developed in that laboratory (10).

For HPV DNA amplification two group-specific primers MY09 and MY11 were used. Primers MY09 and MY11 allow amplification of 450 bp large, very conservative part of the L1 gene. More than 50 different HPV genotypes in a one PCR reaction can be covered by using these primers. After completion of PCR, specificity of PCR products (amplicons) was determined by gel-electrophoresis by previously described procedure (12).

For to determination HPV genotype, restriction fragment length polymorphism (RFLP) method was performed by using seven different restriction endonucleases: *Bam*HI, *Dde*I, *Hae*III, *DNA* *Hinf*I digest, *Pst*I, *Rsa*I, and *Sau*3AI

(Gibco-BRL, Bethesda, USA). Forty-four different restriction patterns of MY09/MY11 PCR products have been described. Individual sample restriction is characteristic for the certain HPV genotype. Patterns of enzyme restriction of PCR products were compared to predefined standard patterns of restriction and in this way defined genotype (1, 12).

## Results

Out of 189 women included in the survey about 4/5 women (79%) belong to a group of women from 20 to 45 years of age, which indicates that these are participants in full sexual maturity and sexual activity. Average age of participants was 38 years. The youngest woman was 23 and the oldest 68 years old.

HPV infection was found in 1/5 of our participants (20%, 38/189). Genotyping performed in 38 HPV DNA positive women showed that the HPV genotype 16 was present in 36.8%; HPV 58 infection was at the second place and it is found in 10.5%; HPV 31 and HPV 6 infections were present in 7.9% of HPV positive women, while infections with other genotypes were demonstrated individually by 2.6% (Table 1).

At the end of the study a total of 16 different HPV genotypes in 38 HPV positive women were detected. HPV infection with one genotype was dominant (Table 1). HPV single infection is present in 4/5 patients (81.6%, 31/38). Infection caused by two different HPV genotypes was found in 15.8% (6/38) of women, while in one woman (2.6%) infection caused by three different HPV genotypes was found. Multiple infections were demonstrated in seven (18.4%) HPV infected women (Table 1).

**Tab. 1:** Distribution of HPV genotypes in 38 HPV infected women in Montenegro

HPV genotype	No. of women	Percent of women (%)
6	3	7.9
16	14	36.8
31	3	7.9
33	1	2.6
51	1	2.6
52	1	2.6
54	1	2.6
58	4	10.5
61	1	2.6
66	1	2.6
MM7	1	2.6
6+M109+58	1	2.6

16+6	1	2.6
16+52	1	2.6
16+CP8304	1	2.6
18+53	1	2.6
26+X*	1	2.6
58+52	1	2.6
Total	38	100

\*undefined HPV genotype

Analyzing 16 detected HPV genotypes, in 4/5 patients (81.6%, 31/38) the presence of high-risk HPV genotypes was confirmed. In two women (5.2%) the presence of probably high-risk genotypes was found, whereas the low-risk HPV genotypes were present in 5 women (13.2%). In our study out of 38 HPV DNA positive participants the infection was most common in women aged 20–30 years, even 65.8% (25/38), while the remaining 13/38 (34.2%) was detected in women aged 40–50 years. Multiple infections were found in younger women, aged 25 to 30 years.

## Discussion

HPV infection is associated with increased risk of developing cervical cancer (17). In this regard, accurate detection of HPV infection is clinically important and requires a reliable diagnostic method. We can say that the sensitive detection of HPV and accurate genotyping are crucial. Early detection of HPV, followed by treatment of cervical dysplasia, can significantly reduce the risk of developing cervical cancer (15).

In our study infection with 16 different HPV genotypes was found. The most common HPV genotypes were HPV 16 (36.8%) and HPV 58 (10.5%). In other words half of the studied HPV positive women have infection with these two genotypes. These results suggest that it is necessary to do a broader study of HPV infection and genotyping, especially since there are deviations from the common HPV genotypes. The establishment for further observation of our population of women is the fact that meta-analysis conducted based on studies published from 1995 to 2009 in 1,016,719 women, showed that five types of HPV (16, 18, 31, 52, and 58) are most frequent genotypes (3). The study included only those researches based on PCR technology.

In our study we find out that HPV infection are more common in younger women (68.5% in women ages 20 to 30 years). Also, it was found that mixed HPV infection, with more than one HPV genotype is dominant in younger women and with at least one high-risk or probably high-risk HPV genotype. These findings correlate with HPV prevalence among young women worldwide (18). Castel P et al concluded that HPV16 is more strongly associated with cervical precancer diagnosed in younger women

(vs. older women). HPV18 infections were linked to precancerous lesions that were missed by cytology (19). Considering that young women with persistent high-risk HPV infection, specially types 16 and 18, are more susceptible for developing a precancerous cervical lesions (20, 21).

## Conclusions

In our study, we found the presence of different HPV genotypes, total 16. In our country, the same as in the world, infection with genotype HPV 16 is most common, followed by infection with HPV 58, HPV 31 and HPV 6.

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