

THE FREQUENCY AND DISTRIBUTION OF HUMAN PAPILLOMAVIRUS TYPES IN THE MALE POPULATION

UČESTALOST I DISTRIBUCIJA TIPOVA HUMANIH PAPILOMA VIRUSA U MUŠKOJ POPULACIJI

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Abstract

Introduction: One of the leading causes of sexually transmitted infections is human papillomavirus (HPV). Depending on the population, the anatomical location of sampling, and the techniques utilized for detection and typing, genital HPV infections can be identified in 16% to 69% of cases in healthy men.

Aim: This research aimed to determine the frequency and distribution of HPV types in the genital tract of men using molecular methods in four cities in the Republic of Serbia.

Materials and methods: This retrospective study included 468 men with suspected genital HPV infection from Belgrade, Kragujevac, Niš, and Novi Sad. The data were collected in the period from 2017 to 2021. Detection and genotyping of the virus was done using the Real-Time PCR method and Sanger DNA sequencing. Descriptive statistics and the χ^2 test were used in the statistical significance testing.

Results: The presence of HPV was proven in 77 out of 468 samples (16%). The highest HPV prevalence of 35.2% was detected in men older than 45 years of age. The percentage of positive samples was highest in men from Niš (24%), followed by Kragujevac (22.1%), Belgrade (20%), and Novi Sad (11.6%). The difference in HPV positivity in men between cities was found to be statistically significant ($p=0.02$). A total of 16 different HPV types were detected. Type 16 HPV is the most frequently detected genotype (30%), followed by HPV 31 (14%), HPV 52 (14%), while types 56, 11, 33, 39 and 45 were identified with lower frequency. Also, HPV 16 was the most often found type in all cities, except for Kragujevac, where HPV 52, 56, and 59 were the most frequently identified.

Conclusion: The results show the presence of 16 different genital HPV genotypes in men with the highest frequency of HPV 16. The HPV infection was detected in 16% of cases, where the highest prevalence was observed in older men.

Keywords:

human
papillomavirus,
genotyping,
men,
genital infections

Sažetak

Uvod: Humani papiloma virus (HPV) jedan je od najčešćih uzročnika polno prenosivih infekcija. U zavisnosti od populacije, anatomskog mesta uzorkovanja i tehnika koje se koriste za detekciju i tipizaciju, genitalne HPV infekcije mogu se identifikovati u 16% do 69% slučajeva kod zdravih muškaraca.

Cilj: Cilj ovog istraživanja je bio utvrditi učestalost i distribuciju HPV genotipova u genitalnom traktu muškaraca pomoću molekularnih metoda u četiri grada sa teritorije Republike Srbije.

Materijal i metode: Ova retrospektivna studija je obuhvatila 468 muškaraca sa sumnjom na prisustvo genitalne HPV infekcije iz Beograda, Kragujevca, Niša i Novog Sada. Podaci su prikupljeni u periodu od 2017. do 2021. godine. Detekcija i genotipizacija virusa urađene su pomoću metode polimerazne lančane reakcije u realnom vremenu (engl. *Real-Time Polymerase Chain Reaction* - PCR) i Sanger DNK sekvenciranja. U testiranju statističke značajnosti korišćene su deskriptivna statistička metoda i χ^2 test.

Rezultati: Prisustvo HPV-a je dokazano kod 77 od 468 uzoraka (16%). Najveća prevalencija pozitivnih uzoraka od 35,2% detektovana je u grupi starijih od 45 godina. Najveća učestalost pozitivnih uzoraka je bila u Nišu (24%), zatim u Kragujevcu (22,1%), Beogradu (20%) i Novom Sadu (11,6%). Uočena je statistički značajna razlika u HPV pozitivnosti između različitih gradova ($p=0,02$). Ukupno je detektovano 16 različitih tipova ovog virusa. Tip 16 je najčešće detektovan genotip (30%), zatim HPV 31 (14%), HPV 52 (14%), dok su genotipovi 56, 11, 33, 39 i 45 bili identifikovani u manjem broju. Najčešće detektovan tip virusa u svim gradovima bio je HPV 16, sem u Kragujevcu, gde su HPV 52, 56 i 59 bili prvi po učestalosti.

Zaključak: Rezultati ove studije pokazuju prisustvo 16 različitih genitalnih HPV genotipova kod muškaraca, gde je najučestaliji genotip HPV 16. Infekcija HPV je otkrivena u 16% slučajeva, pri čemu je najveća prevalencija zabeležena kod starijih muškaraca.

Ključne reči:

humani papiloma virus,
genotipizacija,
muškarci,
genitalne infekcije

Introduction

Human papillomavirus (HPV), a member of the *Papillomaviridae* family, is an epitheliotropic DNA virus (1). It is transmitted by direct contact and the establishment of infection occurs when the virus penetrates the epithelium due to damage to the skin or mucous membrane (2). More than 200 HPV genotypes have been recognized (3). About 60 genotypes are known to infect the genital tract (4). The International Agency for Research on Cancer has characterized 13 HPV genotypes as group 1 carcinogens (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66), often known as "high-risk HPV" (HR-HPV), with HPV 16 being the most commonly detected in cancer tissue and precancerous lesions. Infections with high-risk genotypes are strongly related to the development of cervical, vulva, and vaginal tumors in women, penile cancer in males, anal canal tumors, and head and neck cancer (mostly oropharynx) in both sexes. Types 6, 11, 40, 42, 43, 44, and 54 HPV are classified as "low-risk HPV" genotypes (LR-HPV) for their epidemiological association with benign proliferations such as skin warts or papillomas on the mucosa (5).

According to the data from the World Health Organization (WHO), between 9% and 13% of people worldwide, or around 630 million people, are estimated to have genital HPV infections. The epidemiology of genital HPV infections in men is relatively poorly understood as compared to cervical HPV infections. It is assumed that men can represent a reservoir for unintentional transmission of

infections to women and, due to their asymptomatic nature, contribute to the existence of infection and the occurrence of cancer. These HPV infections in men are linked to lower rates of death and morbidity. Still, ongoing research is essential due to their association with genital warts, penile cancer, anorectal and oropharyngeal cancer, and the involvement of infected males in the transmission of HPV to female partners (6).

The frequency of genital human papillomavirus infection in men is comparable to that in women, ranging from 3% to 40%, depending on demographic and age group (7). The estimates of HPV prevalence in men have been published in several studies, with range from 0% to 73%. These differences are thought to be the result of inconsistencies in the sampling technique and the variety of combinations of sampling sites and samples themselves, as well as the testing method for different HPV types (4). According to the estimates, the overall prevalence of HPV infections in men is 20%, but it is as high as 70% in some age groups, particularly in those aged from 15 to 24 years (6).

This research aimed to determine the frequency and distribution of HPV types in the urogenital tract of men using molecular methods in four cities from the territory of the Republic of Serbia.

Material and methods

Patients and clinical samples

This retrospective study included the data about HPV testing of 468 men with suspected genital HPV infection from 4 centers in the Republic of Serbia. This study was implemented following the International Code of Medical Ethics of the World Medical Association (Declaration of Helsinki). This study was approved by the Ethical Committee of the Faculty of Medicine, University of Belgrade (decision number 1550/V-4; date of approval: 31 May 2019).

The data about HPV testing were collected for 75 men (16.03%) from the Institute of Microbiology and Immunology of the Faculty of Medicine of the University of Belgrade, 95 (20.30%) from the Institute for Public Health Kragujevac, 50 (10.68%) from the Institute for Public Health Niš and 248 (52.99%) from the Institute for Public Health of Vojvodina in Novi Sad. Data from Novi Sad were collected in the period from 2017 to 2019, while data for other cities were collected in the period from 2017 to 2021. The information that was collected from the subjects was age, year of sampling, type of sample, method of virus detection in the samples, name of the kit, and types of viruses that the test can detect. The types of samples used in the research are urethral swabs, anal swabs, penile glans swabs, and semen samples. Detailed indications for testing and sample selection were not available. The most common sample was a urethral swab, which was taken from 428 subjects, followed by an anal swab from 36 subjects, a penile glans swab from 2 subjects, and a sperm sample taken from 2 subjects.

Methods

The detection and genotyping of the virus was done using different molecular methods, namely the Real-Time PCR method and Sanger DNA sequencing, which made it possible to detect different HPV genotypes. The used methods and the range of detected genotypes proven by centers are shown in **table 1**.

The determination of the frequency of HPV types was done according to the number of positives in relation to the total number of patients to whom the method for proving an individual type was applied.

Statistical analysis

Descriptive statistical methods were used for frequency analysis. The statistical significance was evaluated using the χ^2 test. A p value of less than 0.05 was considered statistically significant. Data processing was done in the software program IBM SPSS 26.0 (IBM Corporation, Chicago, USA).

Results

The age of the subjects ranged from 17 years to 63 years (29.12 ± 8.00 years ($\bar{x} \pm SD$)). According to age, respondents were classified into 4 groups: 17 to 25 years, 26 to 35 years, 36 to 45 years, and older than 45 years. For 96 out of 468 respondents, age data were not available. The presence of HPV infection was detected in 77 samples out of 468 samples (16%). The age group over 45 years had the highest percentage of positive samples (35.2%). The prevalence of positive samples decreased in younger age groups, with the lowest frequency of 16.1% found in the group from 17 to 25 years of age (**figure 1**).

The frequency of positive HPV samples among defined age groups was not statistically significant ($\chi^2 = 5.6953$; $p > 0.05$). The highest HPV frequency was observed in males from Niš (24%), followed by Kragujevac (22.1%), Belgrade (20%), and Novi Sad (11.6%) (**figure 2**). The differences in HPV positivity between cities were statistically significant ($\chi^2 = 9.0531$; $p = 0.02$).

Infections with one HPV type were proven in 51% of those examined, coinfections with two HPV viruses in 33 cases (43%), while coinfections with three HPV viruses were detected in 5 cases (6%).

A total of 16 different types of human papillomavirus were detected (6, 11, 16, 18, 31, 33, 39, 45, 51, 52, 56, 58, 59, 61, 66 and 91). Type 16 HPV is the most frequently detected genotype, which was identified in 23 examined samples (30%). Next in frequency are HPV 31 (14%), HPV 52 (14%), HPV 56 (9%), HPV 11 (8%), HPV 33 (8%), HPV 39 (8%), HPV 45 (8%). Other genotypes had a lower frequency (**figure 3**).

Table 1. The used methods and range of detected genotypes by centers.

Medical center	Number of samples	Methods	HPV genotypes
Institute of Microbiology and Immunology, Faculty of Medicine in Belgrade	75	Sanger DNA sequencing (MY09/MY11, GP1/GP2 primers)	All genotypes
Institute for Public Health, Kragujevac	28	Real/Time HPV DNA (Sacace)	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 57, 59, 66 and 68
	67	RealLine HPV HCR Genotype (Bioron)	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59
Institute for Public Health, Niš	9	Real/Time HPV DNA (Sacace)	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 57, 59, 66 and 68
	41	RealLine HPV HCR Genotype (Bioron) and RealLine HPV 6/11 (Bioron)	6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59
Institute for Public Health of Vojvodina in Novi Sad	248	RealLine HPV HCR Genotype (Bioron) and RealLine HPV 6/11 (Bioron)	6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59

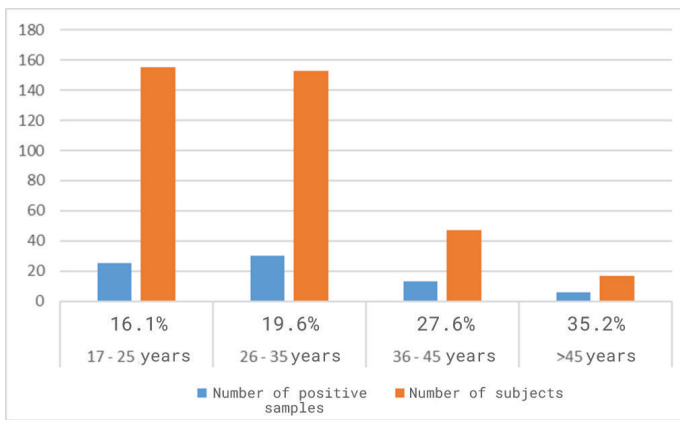


Figure 1. Presentation of the total frequency of HPV-positive samples by different age groups ($\chi^2=5.6953$, $p>0.05$).

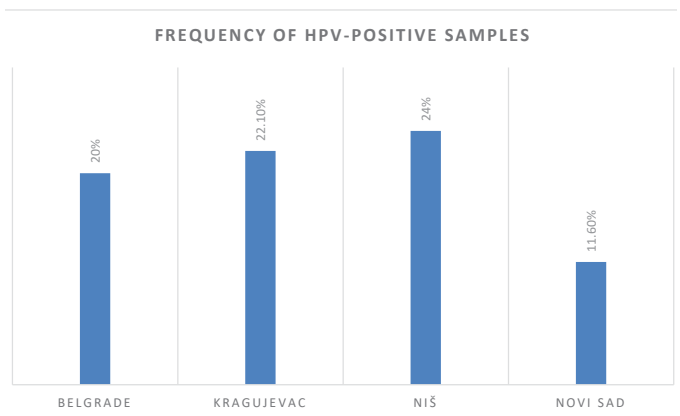


Figure 2. Presentation of the frequency of HPV-positive samples in Belgrade, Kragujevac, Niš, and Novi Sad ($\chi^2=9.0531$, $p=0.02$).

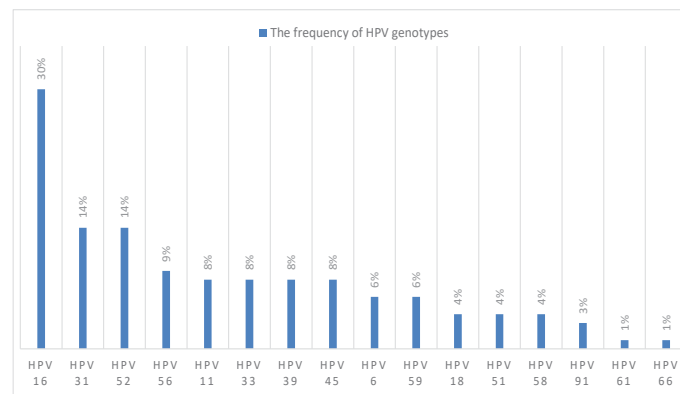


Figure 3. The frequency of all detected HPV genotypes.

In all cities, the presence of infection with high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56 and 59) was tested. It was observed that there is a variance in the distribution of highly oncogenic genotypes among cities. Type 16 was the most frequently detected type of virus in all cities, except in Kragujevac, where it was the second most frequent. In Kragujevac, HPV 52, 56, and 59 were the most frequent. Type 31 was the second most frequent in Novi Sad, Niš, and Kragujevac, while it was not detected in Belgrade. The highly oncogenic type HPV 35 was not detected in any sample. Other genotypes were present in a smaller percentage and they also showed a different frequency in the cities (figure 4).

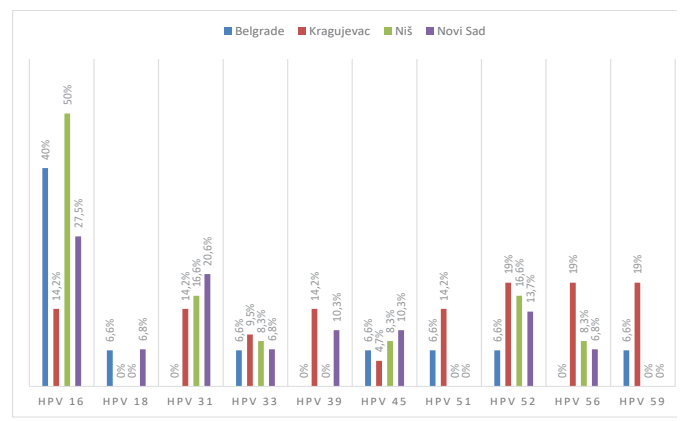


Figure 4. Frequency of different HR-HPV genotypes in Belgrade, Kragujevac, Niš, and Novi Sad.

Discussion

Human papillomavirus infection of the genital tract is one of the most often diagnosed sexually transmitted infections worldwide (8). The data from STD clinics throughout various nations have reported the rates of HPV infection in males between 13% and 45%. The reported prevalence among healthy young males was 16% in Finland, 35% in Seattle, United States, and 49% in Mexico. The reason for the variation in the prevalence of HPV in men may also depend on the population that is more often tested for STDs, such as sexually active men and men who have sex with other men, the age distribution of the particular study, variations in the anatomical site of sampling, the sensitivity of the HPV detection technique, and the endemic presence of specific HPV strains can all be used to explain this discrepancy (9).

High-risk HPV types are linked to high-grade dysplasia and different malignancies (8). The most common genital lesions that may occur during HPV infection in men are genital warts (*condyloma acuminata*) and penile intraepithelial neoplasia (PeIN) which is considered to precede penile cancer. Both LR-HPV 6 and 11 are detected in 90% of genital warts (5). According to published data, HPV DNA was detected in about 50% of penile cancer tissue, where HPV 16 and 18 are the most often found (10). In a study published in 2024 in Guangzhou, South China, in the period from 2012 to 2023, 1814 (48.54%) men out of 3737 had asymptomatic HPV infection, while 1675 (44.82%) had anogenital warts, inflammation, and male infertility. Also in the same study, an increased frequency of HPV was observed in men (42.15%) compared to women (21.66%) at the same time and in the same city. Men are usually tested for HPV infection when typical symptoms of the disease appear, which is the reason for the increased positivity of HPV tests in men compared to the systematic screening HPV test of the cervix in women (14). A large percentage of asymptomatic carriers have a role as the virus's reservoirs and vectors (11). Due to the easy transmission of the virus between sexual partners, high levels of HPV infection in men in one geographic region play an important role in the presence of infection in women in the same region (14).

In Serbia, the data about HPV frequency in the male population are scarce. In this research, the presence of HPV was proven in 16% of the samples. It was observed that there is a statistically significant difference in the frequency between different cities in Serbia, where the highest frequency of positive samples was in Niš, followed by Kragujevac, Belgrade, and the lowest in Novi Sad. Different prevalences of positive samples between different cities and countries have been observed in other studies. In a global meta-analysis, it was shown that the pooled prevalence of genital HPV infection among men is 31%, and for HR-HPV types is 21%. This was established based on 65 studies (including 44 769 men) conducted between Jan 1, 1995, and June 1, 2022. It has been shown that the most common genotypes are HPV-16 and HPV-6 (15). A study looking at HPV prevalence in Europe from nine general population studies found that the overall HPV prevalence was 12.4%, but that HPV prevalence in individual studies varied from 0% to 33.8%. It was noted that the pooled prevalence was significantly higher in studies published after 2000 (28.5%) than in earlier studies (8.8%), where the PCR method of detection was used in only 4 of 7 studies. It is believed that this change in prevalence in earlier studies was precisely due to the use of a less sensitive method of virus detection. Although the prevalence of HPV in Northern and Southern Europe was higher than in Western Europe, this difference in prevalence was not statistically significant. In studies investigating multiple HPV genotypes, HPV 16 was among the most common (12). In one study, the initial HPV prevalence was found to be 34.8% for high-risk HPV and 23.9% for low-risk HPV in a group of healthy soldiers in Mexico (8). The most prevalent non-oncogenic HPV types in this study were HPV 84 (7.1%), HPV 6 (4.3%), and HPV 54 (3.9%), while the most common oncogenic HPV types were HPV 59 (8.8%), followed by HPV 16 (6%) and HPV 52 (5.9%) (8). In a study where men from Spain, Colombia, Thailand, the Philippines, and Brazil were tested, HPV was detected in 16% of the samples. The most common HPV genotypes were HPV 16 (24.7%), HPV 18 (4.9%), HPV 6, 11 (3.3%), HPV 53 (3.3%), HPV 31 (2.7%), and HPV 33 (2.2%). While HPV types 35, 39, 45, 51, 52, 54, and 59 accounted for less than 1.5% of positive samples (13). When data were analyzed separately by geographic region in one study, the prevalence of HPV infection was higher among men tested in Brazil (72.3%) compared to that in the United States (61.3%) and Mexico (61.9%). Types 16, 51, and 59 were the most frequently detected HR-HPV types, and HPV 84, 62, and 6 were the most abundant LR-HPVs, although some regional differences in the distribution of virus types were observed (5). In our research, HPV 16 was most often detected in 30% of cases, followed by HPV 31 (14%), HPV 52 (14%), HPV 56 (9%), HPV 11 (8%), HPV 33 (8%), HPV 39 (8%), HPV 45 (8%). Genotypes HPV 6, 18, 51, 58, 59, 61, 66 and 91 were detected with low frequency. Type 16 was the most frequently detected type of virus in all cities, except in Kragujevac where HPV 52, 56, and 59 were the first in frequency.

In one study, the prevalence of positive men was 28.2%, while 6.1% of those surveyed tested positive for multiple types. The majority of individuals who tested positive for multiple HPV types were infected with both oncogenic and non-oncogenic strains (11). The presence of infection with one HPV type in our research was proven in 51% of the examined, coinfections with two HPVs were present in 43% of cases, while coinfections with three HPVs were detected in 6% of cases.

In our research, the frequency of positive samples was the lowest in the age group of 17 to 25 years (16.1%), while the highest frequency was in the group of men over 45 years of age (35.2%). In a study from Guangzhou, China, the most frequently detected genotype was HPV 6, 11, and 52 with distinct age-specific distribution patterns. Two peaks with an increased frequency of the virus were observed, one was in men older than 25 years and the other in men aged 40-44 years. They also noted that this phenomenon of two peaks is also seen in women from the same region (14). While a global meta-analysis showed a high prevalence in young men, which is maintained throughout life, this suggests that sexually active men are at risk for HPV infection regardless of their age (15). This information may be significant in considering the need for new vaccination strategies beyond the male adolescent period.

Differences in the frequency of positive HPV samples may also be due to the type of sample used in the research. Studies have shown that the prevalence of the virus after sampling the swabs from glans, coronal sulcus, and urethra is only 28.2%, while other studies in which samples were taken from multiple places on the genitals show the presence of infection in 45% of subjects (9). In our research, the urethral swab was the most frequently tested sample. Therefore, to obtain more informative data on HPV frequency in men in future studies, different samples from one subject should be used.

HPV infection in men is a serious healthcare problem, considering its link with numerous cancers in men, including anal, penile, and oral cancers (8). While HPV has been extensively studied in women, information on male infections is lacking. Additional research in this area is needed to address the shortcomings of this study, such as retrospective data collection that affects the quality of the information collected and makes generalization difficult, which should be supplemented in further research with a larger number of subjects from several different cities and information on different clinical conditions of subjects with different HPV genotypes. For further epidemiological and public health purposes, it would be very useful to follow up on the same patients and assess the risk of cancers caused by oncogenic HPV viruses. Also, further prospective studies would help us monitor changes in the distribution and incidence of HPV infection in the population and how the growth of interest and use of HPV vaccination affects it over time.

Studies on HPV in men are crucial to a better knowledge of HPV transmission, HPV-related

carcinogenesis, and disease prevention in both men and women (11). Knowledge of the frequency of different genotypes of HPV infection in men, especially oncogenic types, is essential for the success of future prevention strategies for HPV-related diseases, including expanded access to HPV prophylactic vaccines for boys and young men.

Conclusion

The results of this study show the prevalence of genital HPV infection in 16% of cases in the male population. The presence of 16 different HPV genotypes was proven, of which the most common was HPV 16 (30%). It was noticed that there is a statistically significant difference in frequency between different cities in Serbia. For a more detailed assessment of the frequency and distribution of HPV infections in men, additional research is needed on a larger cohort of subjects.

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