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Original scientific paper

Prevalence and Genotype Distribution of High-risk Human Papillomavirus (HR HPV) in Male Genital Samples of Osijek-Baranja County

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A B S T R A C T

This is a first cross-sectional study on the prevalence and distribution of HPV infection in asymptomatic, heterosexual men from Osijek-Baranja County, Croatia. Between 2009 and 2011, 330 men tested for sexually transmitted diseases (STDs) were recruited. Their genital swabs were tested for high-risk HPV (HR HPV) infection by the AMPLICOR HPV test and further genotyped by the Linear Array HPV Genotyping Test (both by Roche). Infection with a single HR HPV was detected in almost one third of men (39%) whereas multiple HPV types, in more than a half of HR HPV-positive men (61%). The highest HR HPV prevalence was detected in those younger than 20 (37.5%) and lowest in 31–35 year old men (27.8%). The most common genotypes were HPV 6 (24%), 16 (17.8%), 51 (9%), 52 (6%), 35, 55, 66, 84 (each 5%), 31, 62 (each 4%), 39, 58, 59, 83 (each 2.5%), and finally 56, 18, 53, and 54 (each 1.3%). Having more than one sexual partner per year was significantly associated with HR HPV infection in age group between 26 and 30 years (p=0.001). Due to the high prevalence of HR HPV infection in men of this County and its risk of transmission to women, we recommend more public awareness about this particular STD and initiating vaccination programs of young men and women.

***Key words**: High-risk HPV genital infection, HPV genotype, male genital sample, Osijek-Baranja County*

Introduction

Genital infection with the human papilloma virus (HPV) is a common sexually transmitted disease (STD). Out of 118 known HPV types, more than 40 affect the genitals and specifically 8 of them called the high-risk HPVs (HR HPVs) cause almost all cervical cancers. In Croatia with a population of 4.4 million, 355 newly cases of cervical cancers are registered each year[1].

Genital HR HPV infections in men, however, are often asymptomatic and rarely cause malignancies. For example, in Osijek-Baranja County, when compared to 362 cases of cervical cancer, only 11, 29 or 44 cases of the HPV-related anal, penile, and oropharyngeal cancers respectively were reported in men between 1998 and 2011 (Epidemiology Department, Institution of Public Health of the Osijek-Baranja County, unpublished). Because HR HPV infections are asymptomatic in the majority of men, they get unknowingly transmitted to women and this is a major cause of concern[2,3].

Prophylactic programs are one solution to reduce the prevalence of HR HPV infections. In Australia, the implementation of a routine and government-funded vaccination of 12-year old girls since 2007 diminished the incidence of HPV-related genital warts and is expected to dramatically reduce HR HPV infection in both women and men[4]. Furthermore, HPV vaccination of young men has now been proposed. In Croatia, however, the quadrivalent recombinant vaccine against HPVs 6, 11, 16 and 18 (Gardasil) has been rarely used: no men and only 50 women were immunized against HPV in Osijek-Baranja County between 2007 and 2011 (Epidemiology Department, Institute of Public Health, Osijek, 2011).

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There is a lack of information on HPV infection prevalence and genotype distribution in men. When compared to similar studies done in women, the epidemiology and pathogenesis of HR HPV infection in Croatian men have been less investigated[5–7] and not at all in men from Osijek-Baranja County, Croatia. Our objective was thus to report on HPV infection prevalence and genotype distribution in men from north-east Croatia.

In this work, genital swabs of 416 sexually active and mostly asymptomatic men from Osijek-Baranja County who attended an STD testing between 2009 and 2011 were analysed for HPV. A sensitive, Federal Drug Administration (FDA)-approved polymerase chain reaction (PCR)-based assay was employed to detect for 15 HR HPVs, 3 intermediate risk HPVs (IR HPV) and 19 low-risk HPV types (LR HPVs). We hope that our evidence of a relatively high HR HPV prevalence among men in north-east Croatia (32%), including their high rate of multiple HPV infections (61%), will increase public awareness especially among men and initiate vaccination programs for young women and men.

Methods

Study design and population

This cross-sectional study included 416 sexually active and mostly asymptomatic men from Osijek-Baranja County tested for sexually transmitted diseases (STDs) between March 2009 and December 2011. The mean age of participants was 32±7.9 years (ages 17 to 60). Informed consent was obtained from all HR HPV analyzed patients. Participants completed a questionnaire that included questions on marital and socio-economic status, education level, sexual and smoking habits. Eight incomplete questionnaires were excluded from the statistical analyses of lifestyle risk factors associated with HPV genital infection. The Ethics Committee of the Institute of Public Health of Osijek-Baranja County approved the study.

Genital samples for HPV testing

Genital wet swabs were collected either at our department at the Institute of Public Health of Osijek-Baranja County (216 samples, collected from the entire genital areas) or at outpatient clinics by general practitioners or other medical specialists (200 samples, collected mainly from urethral canals). Samples were stored in the Micro-Test M4RT transport medium (Remel, USA) until further analysis.

Detection and genotyping of HPV

DNA isolation from 416 genital swabs was performed with the High Pure PCR Template Kit (Roche Diagnostics GmbH, Germany). HR HPV presence was determined by the AMPLICOR HPV test (Roche Diagnostics GmbH, Germany) that detects 13 HR HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). Successful β-globin amplification served as DNA quality control. Genital specimens PCR negative for β-globin (86/416) were excluded from further analysis of HR HPV prevalence and risk factors for HPV infection. DNA from HR HPV positive specimens was stored at –20 °C until more detailed HPV typing. Out of 106 HR HPV-positive genital swabs, 47 were referred to our department for HPV genotyping by their general practitioners. Genotype was determined using the Linear Array HPV Detection and Linear Array Genotyping test (Roche Diagnostics GmbH, Germany) that detects 15 HR HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82), 3 IR HPVs (26, 53, 66) and 19 LR HPVs (6, 11, 40, 42, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 83, 84, IS39 and CP6108) genotypes. DNA isolation and amplification were performed according to the manufacturer's specifications.

HPV prevalence and statistical analysis

HR HPV prevalence was expressed as a percentage of HR HPV-positive samples against 330 genital swabs that

TABLE 1
STATISTICAL ANALYSIS OF POTENTIAL RISK FACTORS FOR HR HPV INFECTION IN MEN

HPV-related risk factors	HPV positive	HPV negative	Total	χ^2 (p value)
Age (years)				
21–25	24	42	66	0.8759
26–30	28	67	95	
31–35	20	52	72	
36–40	15	26	41	
≥41	16	32	48	
Current smoking				
Yes	40	51	91	0.8405
No	105	122	227	
No. of cigarettes/day				
≤5	12	38	50	0.2356
6–19	18	29	47	
≥20	16	46	62	
Marital status				
Unmarried	40	103	143	0.7434
Married	53	122	173	
No. of sex partners/year				
0	6	12	18	0.594
1	82	172	254	
>1	15*	22	37	
Condom use				
Yes	16	37	53	0.8437
No	87	179	266	
Monthly income				
<500	51	100	151	0.9252
500–1000	43	95	138	
1000–1500	6	12	18	
>1500	4	6	10	

*(χ^2=3.37; p=0.053)

TABLE 2
AGE-SPECIFIC HPV INFECTION IN RELATION TO THE NUMBER OF SEXUAL PARTNERS PER YEAR

Age groups/years	0 sex partner per year		1 sex partner per year		>1 sex partners per year	
	HPV (+)	HPV (–)	HPV (+)	HPV (–)	HPV (+)	HPV (–)
21–25	1	0	6	22	2	6
26–30	2	4	17	50	7*	2
31–35	0	5	18	5	3	7
36–40	2	2	20	50	3	3
≥41	1	1	21	45	0	4

*(χ^2=10.08; p=0.001)

passed quality control. Male participants in this study were classified according their age into six groups: under 20 (8 participants), 21–25 (66 participants), 26–30 (95 participants), 31–35 (72 participants), 36–40 (41 participants) and older than 40 (48 participants) years. Age-specific prevalence of different LR and HR HPV genotypes was calculated using the Microsoft Office Excel 2003 software (Microsoft). Multivariate analyses of age, HR HPV or various HPV genotypes in relation to possible lifestyle risk factors for genital infection with HPV were calculated using MedCalc 10.2 (MedCalc Software) and Statistica 8.0 (StatSoft). Analyses of risk factors excluded a number of subjects because of incomplete questionnaires (Tables 1 and 2). Results were considered significant for p values <0.05.

Results

HR HPV prevalence

Between March 2009 and December 2011, one third (106/330) of study participants whose genital swabs passed the PCR quality control (330/416) was infected with HR-HPV. Figure 1 shows that the highest infection rate, 37.5% (3/8), was found in men younger than 20 years. Similar rates were observed in majority of other age categories: from 36.6% (15/41) in 36–40 year old men to 36.4% (24/66) in 21–25 year old men. The lowest HR HPV prevalence was observed in 31–35 years old men (27.8% i.e. 20/72). Finally, there was no statistically significant association between age and HR HPV prevalence.

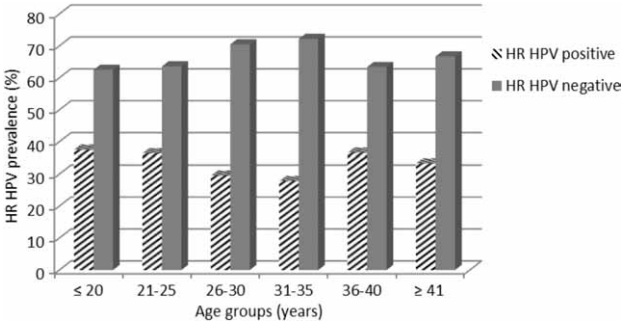

Fig. 1. Age-specific prevalence of HR-HPV infection in men from north-eastern Croatia.

Distribution of HPV Genotypes in HR-HPV infected men

Out of 47 HR HPV-positive genital swabs, 87.2% (41/47) were successfully genotyped while 12.3% (6/47) were positive for an unclassified HPV type. These unclassified specimens were PCR positive for β-globin gene and PCR negative for any of 37 HPVs due to insufficient amount of HPV DNA. Figure 2 shows that HPV 6 was the most prevalent genotype in HR HPV-infected men (24.2%). This was followed by HPV 16 (17.8%), HPV 51 (8.8%), HPV 52 (6.4%), HPVs 35, 55, 66 and 84 (5% each), HPVs 31 and 62 (3.8% each), HPVs 39, 58, 59, 83 (2.5% each), and HPVs 56, 18, 53 and 54 (1.3% each).

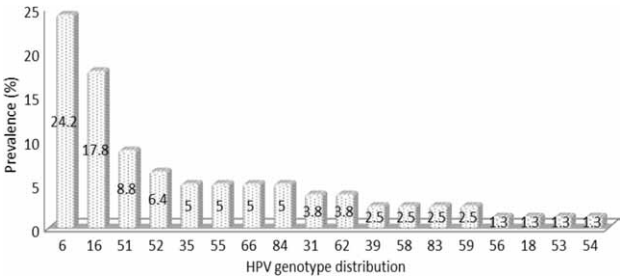

Fig. 2. HPV genotype distribution in men from north-eastern Croatia.

Age-specific distribution of different HPV genotypes is presented in Figure 3. HPVs 6 and 51 were mostly detected in 26–30 year old men 10/19 (52.6%) and 4/8 (50%), whereas HPV 16 was mostly detected in 31–35 year old men 7/15 (46.6%). Interestingly, some genotypes

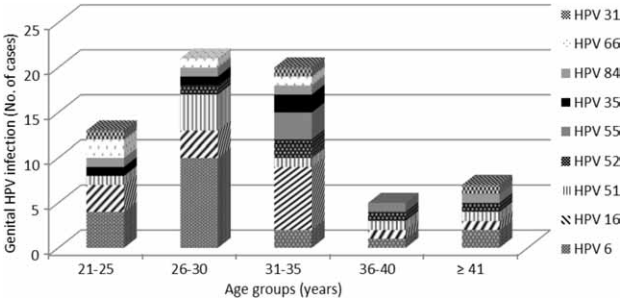

Fig. 3. Age-specific distribution of HPV genotypes in men from north-eastern Croatia.

were not detected in older age: HPVs 35, 18 and 66 were not found in men older than 36 while HPVs 35, 84, 66 and 31 were not found in 36–40 year old men.

Concomitant infections with multiple HPV genotypes were detected in 61% of HR HPV-infected participants of the study. The highest prevalence of multiple HPV infections was found in 31–35 years old and in men older than 41 years (80% and 60%, respectively). The latter age group also showed a significantly higher prevalence of multiple infections than single infections. Multiple infections were less frequent in other age categories: 57% in 21–25 year old men, 53% in 26–30 years old men and 50% in 36–40 year old men (Figure 4).

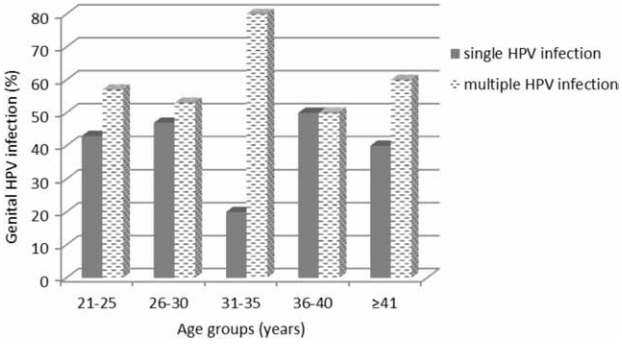

Fig. 4. Age-specific prevalence of single and multiple HPV infection in men from north-east Croatia.

Risk factors for HPV infection

Table 1 summarizes possible risk factors for HPV infection (age, marital status, smoking and sexual habits, and income) that were investigated in this study. Statistical analysis confirmed that one of them – having more than 1 sexual partner per year – was statistical marginally significant associated with HR HPV infection (χ^2= 3.37; p=0.053). When analysed by age group, 26–30 year old men with more than one sexual partner per year had significantly higher HR HPV infection rate (χ^2=10.08; p=0.001) when compared to men of the same age with one sexual partner/year (Table 2). Other risk factors such as age, marital status, smoking and income were not significantly associated with HPV infection in men from north-east Croatia.

Discussion

HR HPV Prevalence

Between 2009 and 2011, one third of 330 men from north-east Croatia whose genital swabs passed PCR quality control were infected with HR HPV. This was higher than in two previous studies of men in central and west Croatia: the first showed that 14.1% of 581 men from Zagreb and Rijeka were infected between 2006 and 2008[5], the second one, that 21% of 100 men from Zagreb were infected between 1996 and 2000[7]. Two reasons may explain the higher HR HPV prevalence in men from our study: firstly, a potentially higher risk of our study population for HPV infection as all participants underwent a self – initiated STD screening, and secondly a higher number of different HR HPV genotypes analysed[8].

Genital HPV prevalence in men across the world varies widely from 2 to 93%[9,10]. This variation is not only due to the true, region-specific HPV prevalence but also to differences in study design and methods. For instance, different sampling techniques give different HPV prevalences: more HPV can be detected in external genitals (glans/corona, shaft, and scrotum) than in anal samples, semen, or urine[11–14]. Moreover, samples from the urethral meatus can contain about half of HPV compared to those from external genitals[5]. Different approaches in genital sampling may also explain the relatively high--proportion of inadequate specimens (21%) in this study – all of the inadequate samples were swabs from urethral canals collected by general practitioners. Other studies confirmed that testing multiple genital sites compared to sampling from one genital site in men decreased the number of inadequate, β-globin negative samples for HPV testing by PCR technique[5,14,15].

Among studies that used similar methods and target populations-a PCR-based detection of HPV infection in the genital swabs of attendees of STD clinics – the prevalence of HR HPV in men in this study (32%) was comparable to men from Florida and Arizona (29.2% in heterosexual men including STD attendees) but higher than in STD attendees from British Columbia (24%), Denmark (12.6%), Arizona (12%), and China (4.3%)[12,16–21]. Moreover, as a relatively small number of men less than 20 years of age participated in our survey (12 *vs.* 51–117 participants in other age groups), the true HR HPV prevalence in STD attendees of our County may be even higher than 37.5%.

Age-specific distribution of HR HPV infection

Compared to the U-shaped age-specific HPV prevalence curve reported by others[17], the HR HPV prevalence in men from north-east Croatia showed the highest peak in men younger than 20 years (37.5%) and in 36–40 years old men (36.6%) followed by a slow decline with increasing age: from 36.4% (in 21–25 year old men) to 27.8% (in 31–35 year old men). Whether other regions in Croatia show a similar age-specific HPV distribution in men remains to be determined. Other studies showed similar age-associated peaks of HR HPV infection in men: in STD clinic attendees from Denmark, the highest HR HPV prevalence was detected in 18–24 year old men (23.3%); in men attending vasectomy clinics in Mexico, the highest prevalence was in men <25 years (10.6%); in HIV-negative homosexual men from several USA cities, the highest HR HPV prevalence was in 30–34 year old men (30%)[20,22,23]. Nevertheless, not all of these results may be comparable as different target populations were analyzed.

The highest prevalence of HR HPV in our study was detected in men ≤20 years (37.5%). One reason for this might be their low number – while we recruited between

51 to 117 men in other age categories, we only found 12 men younger than 20 years probably because they were less likely to visit general practitioners and STD clinics than older men. This small proportion of tested young men in our study may have resulted in an overestimation of HR HPV infection compared to other age groups. Additionally, one third of men ≤20 years inaccurately completed their questionnaires and were disqualified. Such behavior of young men has been reported before[24]. Thus, further studies are necessary to accurately estimate the true HR HPV prevalence in young men of north-east Croatia.

HPV Genotype Distribution

HPVs 6 and 16 were the predominant LR and HR HPV genotypes (24.2 and 17.8%, respectively) in men from north-east Croatia (Figure 2). These levels are similar to those found in other Croatian men[5–7,25]. However, HPV 6 prevalence in women from the same region was much lower (1.3%, Bošnjak et al, in preparation). Higher prevalence of HPV 6 in men may be caused by their hand-to-genital transmission of LR-HPVs, as previously reported[26]. Contrary to other studies in Croatia however, we detected less HPV 18 (1.3 vs. 12.2%), more HPV 31 (3.8 vs 1.8%) and no HPV 33 (0 vs. 3.1%)[5–7]. Whether this reflects a region-specific difference or is a consequence of different methods used for the HPV genotyping.

Compared to studies in other countries, the low prevalence of HPV 18 in our study (1.3%) was similar to that in heterosexual men in Mexico (1.7%) and USA (0.5%). Also our HPV 51 prevalence (8.8%) was similar to that in Mexico (6.6%) and France (8%)[27]. Similarly, the prevalences of HPVs 31, 35 and 66 (3.8%, 5% and 5%, respectively) were similar to those in Brazil (4.2%, 3.4% and 6.5%, respectively)[28].

Multiple HPV infections

More than half of HPV-positive men in our study (61%) were infected with multiple HPV types (Figure 4). This percentage was much higher than previously reported for Croatian men (3–13%)[5–7]. This difference may be in part caused by our more sensitive methodology which allowed detection of 37 HPV types. Interestingly, HPV-positive men between 31–35 years of age were significantly more infected with multiple HPV types (80%) than with single HPV types (20%). This may be a consequence of their higher susceptibility to novel HPV infections and/or higher persistence of multiple HPV infections, as suggested previously[29].

When compared to heterosexual men from other countries, the prevalence of multiple HPV infection in our region was higher to that of 18–24 year olds from Kenya (59%)[30], of 18–70 year olds from Brazil (36.2%) and Mexico (23.8%)[31], and higher than the 18–40 year olds from Florida (27.2%)[12]. Considering the high prevalence of multiple HPV types in 31–35 year old men from east-north Croatia, their sexual partners could have an increased risk for HPV-related malignancies.

Risk factors associated with HPV infection

Sexual activity is a well-known risk factor for HPV infection[32]. Indeed, we observed that having more than one sexual partner/year was a significant risk factor for HR HPV infection in men (Table 1). Contrary to other countries that reported the highest sexual activity in men of less than 25 years[33,34], our study observed it in the 26–30 year old men (Table 2). This correlated with the high peak of HR HPV prevalence in the same age category (Figure 1).

Other possible risk factors not significantly associated with HR HPV infection in men from Osijek-Baranja County were age, smoking, marital status, condom use, and income. This may be due to the relatively low number of participants in this study as smoking and condom use has been previously associated with HPV infection[35].

Conclusion

This was the first cross-sectional study that determined the age-specific prevalence and genotype distribution of HPV infection in men from north-east Croatia. There are several limitations to this study: a low number and potentially high-risk behavior of the participants, unknown medical history of participants and their sexual partners, and a relatively high proportion of inadequate samples. Nevertheless, this study showed strong evidence of high HPV prevalence in men from Osijek-Baranja County: between 2009 and 2011, one third of STD attendees were infected with at least one HR HPV and of these, more than a half suffered from multiple HPV infections.

The most efficient and cheapest way of decreasing the occurrence of HPV-related disease burden is vaccination. However, in Croatia, vaccination is expensive and not covered by state insurance programs: only 50 women in our County and no men were immunized against HPVs 6, 11, 16 and 18 between 2007 and 2011 (Epidemiology Department, Institute of Public Health, Osijek, 2011). For these reasons, asymptomatic infections with HR HPV, especially in 21–30 year old men, remain the main source of infection for their sexual partners. We hope that this study will initiate further research on HPV prevalence in Croatian men, increase public awareness of safe sex practices and make vaccination more accessible.

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PREVALENCIJA VISOKORIZIČNIH LJUDSKIH PAPILOMA VIRUSA (HR HPV) I RASPODJELA HPV GENOTIPOVA U GENITALNIH UZORAKA KOD MUŠKARACA OSJEČKO-BARANJSKE ŽUPANIJE

SAŽETAK

U Osječko-baranjskoj županiji ovo je prvo presječno istraživanje prevalencije visokorizičnih ljudskih papiloma virusa (HR HPV, prema engl. High risk Human Papillomavirus) i raspodjele HPV-a u populaciji asimptomatskih heteroseksualaca. U periodu od 2009. do 2011. godine, 330 muškaraca je ispitano na spolno prenosive bolesti. Brisevi spolnog područja su testirani AMPLICOR HPV testom i genotipizirani LINEAR ARRAY HPV testom za genotipizaciju. Kod trećine muškaraca utvrđena je infekcija s jednim HPV-om (32%) dok je u više od polovice utvrđena infekcija s više virusa HPV-a (61%). Najviša HR HPV prevalencija je utvrđena u dobnoj skupini mlađoj od 20 godina (37,5%), dok je najniža u dobnoj skupini od 31 do 35 godina (27,8%). Određena je raspodjela HPV genotipova HPV 6 (24%), 16 (17,8%), 51 (9%), 52 (6%), 35, 55, 66, 84 (svaki 5%), 31, 62 (svaki 4%), 39, 58, 59, 83 (svaki 2,5%), 56, 18, 53 i 54 (svaki 1,3%). Statistički značajna povezanost utvrđena je između rizičnog spolnog ponašanja (>1 spolnog partnera/godišnje) u dobnoj skupini od 26 do 30 godina starosti (p=0,001). Zbog visoke prevalencije HR HPV-a kod muškaraca Osječko-baranjske županije i rizika prijenosa na žene opravdano je sustavno provođenje informiranja javnosti o spolno prenosivom HPV-u kao i pokretanje programa cijepljenja mlađe populacije muškaraca i žena.