ANTICANCER RESEARCH *40*: 1597-1604 (2020)
doi:10.21873/anticanres.14107

Prevalence and Risk Factors for Oral HPV in Healthy Population, in Central Europe

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Abstract. *Background/Aim: The incidence of oropharyngeal tumours induced by human papillomaviruses (HPV) is ever increasing. Information about oral HPV prevalence and its risk factors are very important for future screening and early diagnosis of the disease. The present study aimed to assess oral HPV prevalence in healthy population and risk factors for HPV infection, since this data is scarce or even missing in Central Europe. Patients and Methods: HPV prevalence in oral rinse and HPV-specific antibodies in peripheral blood were investigated in two groups of healthy participants. Group I consisted of 294 students who reached sexual maturity after the HPV vaccine had been licensed with mean age 23.2 years, and Group II of 215 unvaccinated participants with the mean age 55.7 years. Additionally, the risk factors were evaluated. Results: In Group I, 2% of participants were positive for oral HPV DNA. A statistically significantly higher rate (8.8%) was found in Group II. The seropositivity rates for anamnestic HPV antibodies were comparable in both groups. None of the analysed risk factors was significantly associated with oral HPV positivity. Conclusion: The lower prevalence of oral HPV DNA in younger participants suggests the positive influence of vaccination against oral HPV.*

Human papillomaviruses (HPV) comprise a heterogeneous group with more than 200 genotypes classified into five genera. Alpha-PVs cause most HPV-associated mucosal lesions, both malignant and benign, while cutaneous beta-PV infections are common but mostly asymptomatic in immunocompetent patients. Alpha-PVs are categorized into low-risk (LR), which are involved in genital warts, premalignant lesions, and laryngeal papillomatosis, and high-risk (HR), including those associated with cervical, vaginal, penile, anal, and head and neck tumours. HPV16 is the most prevalent type found in cervical cancer cells and in all other malignancies associated with papillomaviruses (1).

The increasing incidence of oropharyngeal cancer in developed countries has been accompanied by an increasing number of patients with HPV-associated tumours (2, 3). HR HPV-associated head and neck cancers have been shown to occur in younger patients, who lack the common risk factors of alcohol and tobacco use, and moreover, have been associated with a better prognosis (2, 4).

Oral HPV prevalence in healthy individuals has been investigated in a number of studies. Comparable results have been reported on different continents, but only limited data is available from Europe (5). A review by Kreimer *et al*. on oral HPV prevalence in the US population included 18 studies with 4,581 healthy adults; it showed a prevalence of 4.5% for HPV, 3.5% for HR HPV, and 1.3% for HPV16 (6). A review based on 29 studies of Asian populations (22,756 individuals) has shown a comparable HPV prevalence of 5.5%, with 2.7% of HR HPV and 1.0% of HPV16 (7).

The most common route of HPV transmission is sexual. Oral infection most likely occurs through intimate contact. Several studies have found that sexual behaviour (8-11), smoking, and alcohol drinking (8) are significant risk factors for oral HPV infection, with smoking being a more significant factor in females. In their study, Gillison *et al*. have shown that HPV prevalence, although generally higher in males, is much higher in female than male smokers (8). However, smoking and sexual behaviour have only been identified as significant risk factors in one European study (12).

Recently, the effect of routine HPV vaccination on oral HPV prevalence has been observed in Sweden. Before routine

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Key Words: Oral HPV prevalence, HPV antibodies, healthy population, oropharyngeal cancer.

vaccination, the oral HPV prevalence was 9.3%, and after 73% of the population were vaccinated, it was decreased to 1.4% (13). A clinical trial in Costa Rica demonstrated that 4 years after the vaccination with a bivalent vaccine, oral HPV prevalence was much lower among vaccinated women compared to the non-vaccinated. The protection against oral HPV infection implies the importance of vaccination for the prevention of HPV-associated oropharyngeal cancer (14). The vaccination programs are expanded to include also boys in an increasing number of countries.

Because of its prognostic advantage for patients, early detection of HPV-associated oropharyngeal tumours is essential, and modification of therapy is considered. In some studies, detection of HPV-specific antibodies against viral oncogenes proved to be predictive of HPV-associated cancers diagnosed many years later (15, 16). Therefore, surveillance studies of oral HPV prevalence and HPV-specific seroprevalence in healthy population are crucial in view of the possible implication for oropharyngeal cancer screening. However, in Central Europe, such studies are still scarce.

This study aimed to contribute to the body of epidemiological data on oral HPV DNA prevalence and HPV seroprevalence in healthy adults in the region of Central Europe, as well as to investigate the risk factors of oral HPV infection. Moreover, oral HPV prevalence was compared between a younger group with higher vaccination rate and in group of older unvaccinated individuals.

Patients and Methods

Study design and population. Two groups of healthy participants were evaluated. Group I included 294 students of medicine in central European region with mean age of 23.2 years enrolled between September 2015 and December 2017. The exclusion criterion was a history of head and neck or cervical cancer. Students previously vaccinated against HPV provided only an oral rinse sample, while a blood sample was also taken from the unvaccinated students. Each participant was assigned a unique ID number to be used for marking the respective oral rinse and blood samples and questionnaire. The aim of the study was explained to the students, and all participants signed an informed consent. The study was approved by the local ethics committee. Group II consisted of 215 individuals with a mean age of 55.7 years, enrolled in previous studies (3, 17). All of them were unvaccinated as they had been sampled between 2000 and 2010, before HPV vaccines were licensed in the European Union. Information about their oral HPV DNA status and seropositivity was also taken from previous studies, with four samples being unavailable for serological analysis.

Questionnaires. All Group I participants were asked to fill in an anonymized online questionnaire to collect data on age, sexual history, education, HPV vaccination status (vaccine type, first dose in relation to the start of sexual life), history of tonsillectomy, tongue or lip piercing, method of birth, and use of tobacco, alcohol, and drugs. Other questions were related to sexual behaviour, *i.e.* to sexual orientation, age at first sexual intercourse, number of open mouth kissing partners, number of vaginal and oral sex partners in their lifetime and in the last 12 months, usage of condom during vaginal intercourse, and former and current genital infections. From 388 enrolled students only 294 filled in correctly the questionnaire and were further evaluated. The questionnaire for Group II, which was administered in a previous study (3), did not fully overlap with the new one.

HPV DNA detection and genotyping. Oral exfoliated cells were obtained after oral rinse by gargling with 10 ml of phosphate buffered saline (PBS) solution (pH 7.4; AccuGENE, Cambrex Bio Science, Verviers, Belgium) for 30-60 s. The samples were centrifuged for 7 min at 5,000 rpm, the pellet was washed twice with 30 ml and 10 ml of PBS, and the cells were resuspended in 5 ml of PBS. DNA for HPV detection was extracted using the Puregene Core Kit B (Gentra Qiagen, Hilden, Germany), according to the manufacturer's protocol, and dissolved in 10 mM Tris-Cl, pH 8.0 to the final concentration of 50 ng/μl. To check the DNA integrity, a 110-bp fragment of the human beta-globin gene was amplified as described previously (18).

HPV DNA detection and genotyping were performed by PCR using broad-spectrum GP5+ and 5'-end biotin-labelled GP6+ (BSGP5+/6+bio) primers, specific for the L1 region, and by the reverse line blot hybridization (RLB) method, which is able to identify 37 different HPV types in a single assay, as has been previously described (19).

The PCR products that did not hybridize in the RLB assay were purified from the 2% agarose gel (NuSieve GTG agarose, FMC BioProducts, Rockland, ME, USA) using the MinElute Gel Extraction kit (Qiagen, Hilden, Germany), and sequenced to determine the exact HPV genotype using the BigDye Terminator Primer Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA). The analysis was done on the automated ABI PRISM 3500 sequencing system (Applied Biosystems, Foster City, CA, USA). The results were analysed by the Chromas software and entered into BLAST® (http://www.ncbi.nlm.nih.gov/BLAST/) (19).

Serological assays. The presence of antibodies against the antigens derived from HPV-specific proteins was tested using an in-house ELISA as has been previously described (20). L1-based virus-like particles (VLP), mimicking HPV6, 11, 16, 18, 31, 33, 45, 52, and 58 capsids, were prepared using the recombinant baculovirus expression system and served as antigens to detect antibodies to viral capsids (21). The presence of antibodies against HPV16 E6 and E7 oncoproteins was tested using the fusion proteins GST-E6 and GST-E7, in the glutathione-S-transferase (GST) capture ELISA (22). Sera samples known to be positive and negative were tested on each plate as a control. The cut-off value was calculated for each antigen and plotted separately as the mean absorbance of each sample plus 2 standard deviations (SDs) for VLP ELISA and 3 SDs for E6/E7 ELISA. Optical densities were recalculated into ratios by division of the cut-off value (OD index). Samples with an OD index lower than 1 were considered non-reactive. Samples with an OD index between 1 and 1.1 and about one third of all samples were retested. The result was acceptable when concordant results were obtained in two runs. In case of discordant results between the first two runs, the sample was retested for the third time.

Statistical analysis. The *t*-test and Fisher's exact test were used for comparison of HPV positive and HPV negative individuals within

each group. The significance level was set to 5%. Statistics were calculated in R version 3.3 Core Team (23) using the psych package (24). Data are presented as percentages or mean±SD.

Results

Demographic characteristics. In Group I, 294/388 volunteers (response rate 76%) correctly filled in the questionnaire and were enrolled in the study. The mean age of participants from Group I was 23.2±1.24 years and most of them were females (71.4%), non-smokers (75.2%), regular consumers of alcohol (64.0%), and HPV-unvaccinated (59.4%). The vaccinated students (n=119) were recipients of a tetravalent vaccine 39.5% (47/119), a bivalent vaccine 24.4% (29/119), or an unknown type of vaccine 36.1% (43/119). In Group II (n=215), the mean age was 55.7±10.63 years. Most of the participants were males (69.3%) and smokers (61.4%), and slightly more than half of the participants were currently regular consumers of alcohol (51.2%). Since these participants had been enrolled before HPV vaccines were licensed, their vaccination status was not established. Characteristics of the study population are summarized in Table I.

Characteristics of sexual behaviour. In Group I, most participants had a heterosexual orientation (96.3%). Six participants were men who have sex only with men, and five were women who have sex with men and women. The mean age at first sexual intercourse was 17.3±1.97 years. Most had between 1 and 5 lifetime vaginal sex partners (67.7%) and between 1 and 2 lifetime oral sex partners (46.3%). Only 11.9% of participants used condom for every sexual intercourse and 9.2% admitted to having a sexually transmitted disease in the past. The characteristics available for Group II were limited to the number of vaginal sex partners, which was between 1 and 5 for 58.6% of participants. All data are summarized in Table II.

Prevalence of oral HPV infection. Out of 294 participants in Group I, only 2.0% (6/294) were HPV-positive, while in Group II, 8.8% (19/215) turned out to be HPV-positive. Multiple HR HPV types were detected in oral rinses (HPV16, 45 and 59 in Group I, and 16, 18, 33, 39, 45, 51, 56 and 58 in Group II). The LR HPV types 30, 44, and 54 were found in Group I, while types 11, 61, 70, and 118 in Group II. The characteristics and sexual behaviour of the 6 oral HPV-positive participants from Group I are summarized in Table III.

Prevalence of HPV-specific antibodies. The seropositivity rates were comparable in both groups (p=0.758); 63.7% of unvaccinated participants in Group I (n=168) and 61.6% in Group II had antibodies against any HPV type tested. There was no difference between the two groups in the prevalence of antibodies against the vaccine HPV types either. Of the unvaccinated students from Group I, 15.5% (26/168) were positive for HPV16-specific antibodies and 16.7% (28/168)

Table I. *Demographic and virological characteristics of the study population*.

Characteristic	Group I (n=294), n (%)	Group II (n=215), n (%)
Age, mean±SD[1]	23.24±1.24	55.67±10.63
Gender		
Female	210 (71.4)	66 (30.7)
Male	84 (28.6)	149 (69.3)
Smoking		
Non-smoker	221 (75.2)	83 (38.6)
Current or ex-smoker	73 (24.8)	132 (61.4)
Alcohol		
No	106 (36.0)	105 (48.8)
Yes	188 (64.0)	110 (51.2)
Marijuana[2]		
No	235 (79.9)	NA
Yes	58 (19.7)	
HPV vaccine[2]		
No	174 (59.4)	NA
Yes – bivalent	29 (9.9)	
Yes – tetravalent	47 (16.0)	
Yes – no information about vaccine type	43 (14.7)	
Tonsillectomy		
No	272 (92.5)	NA
Yes	22 (7.5)	
Tongue piercing[3]		
No	283 (96.3)	NA
Yes	9 (3.1)	
Delivery		
Caesarean section	24 (8.2)	NA
Spontaneous vaginal	181 (61.6)	
No information	89 (30.3)	
Seropositivity for any HPV[4]		
Yes		
107/168 (63.7)		
130/211 (61.6)		
Oral HPV-positive (any type)		
Yes	6 (2.0)	19 (8.8)
Types	16, 30, 44, 45, 54, 59	11, 16, 18, 33, 39, 45, 51, 56, 58, 61, 70, 118
Oral HR HPV-positive		
Yes	3 (1.0)	15 (7.0)
Oral LR HPV-positive		
Yes	3 (1.0)	4 (1.9)

The percentages do not add up to 100% for some variables due to missing data: [1]Data missing for 74 participants in Group I; [2]Data missing for 1 participant in Group I; [3]Data missing for 2 participants in Group I; [4]Samples for serological testing were unavailable for 6 participants in Group I (out of 174 unvaccinated) and four participants in Group II; NA: Not available.

for HPV18-specific antibodies. In Group II, the respective rates were 16.1% (34/211) and 18.0% (38/211). Antibodies against at least one of the HR HPV types tested (16, 18, 31, 33, 45, 52, and 58) were detected in 38.1% (64/168) and 46.9% (99/211) of participants from Group I and II, respectively. The prevalence of antibodies against proteins E6 and E7 was very low in both groups; 1.8% (3/168) and 3.6% (6/174), respectively, in Group I, and 2.8% (6/211) for each of the proteins in Group II. No statistically significant difference was found between the two groups in the prevalence of any of the tested antibodies (Table IV).

Risk factors associated with oral HPV infection. We further investigated risk factors for oral HPV infection in Group I where detailed information about potential risk behaviours of the participants was collected (Table II). The main focus of the analysis was on the association of each risk factor with HPV DNA status. However, when oral HPV positive and negative participants were compared, none of the variables was statistically significantly associated with the infection in our study population (Table V). Only 6 oral HPV positive cases were available and none of the considered factors was significantly associated with HPV DNA status and therefore a multivariate analysis was meaningless in this case. This is mainly due to the categorical nature of the considered factors and the low observed number of cases.

Table II. *Sexual behaviour characteristics*.

Characteristic	Group I	Group II
Sexual orientation		
Heterosexual	283/294 (96.3%)	NA
Homosexual	6/294 (2.0%)	
Bisexual	5/294 (1.7%)	
Number of open-mouth kissing partners		
Lifetime[1]		
1-5	92/294 (31.3%)	NA
5-10	74/294 (25.2%)	
10-30	96/294 (32.7%)	
More than 30	26/294 (8.6%)	
Last 12 months[2]		
1	178/294 (60.5%)	
≥2	113/294 (38.4%)	
Age at first sexual intercourse		
Mean (SD)	17.3 (1.97)	NA
Number of vaginal sex partners		
Lifetime		
None	16/294 (5.4%)	2/215 (0.9%)
1-5	199/294 (67.7%)	126/215 (58.6%)
≥6	79/294 (26.9%)	81/215 (37.7%)
Last 12 months		
None	30/294 (10.2%)	NA
1	187/294 (63.6 %)	
≥2	77/294 (26.2%)	
Condom usage[3]		
Always	35/294 (11.9%)	NA
Usually/sometimes	205/294 (69.7%)	
Never	43/294 (14.6%)	
Number of oral sex partners		
Lifetime		
None	22/294 (7.5%)	NA
1-2	136/294 (46.3%)	
3-5	78/294 (26.5%)	
≥6	58/294 (19.7%)	
Past 12 months		
None	41/294 (14.0%)	
1	188/294 (64.0%)	
≥2	65/294 (22.1%)	
Sexually transmitted disease in the past		
No	267/294 (90.8%)	NA
Yes	27/294 (9.2%)	

The percentages do not add up to 100% for some variables due to missing data: [1]Data missing for 6 participants in Group I; [2]Data missing for 3 participants in Group I; [3]Data missing for 11 participants in Group I; NA: Not available.

Discussion

In this study, the prevalence of oral HPV infection was determined in healthy population of Central Europe. Specifically, two groups were examined; Group I consisted of young medical students who reached sexual maturity after the HPV vaccine had been licensed, and Group II included older individuals who were unvaccinated. Moreover, the possible association of different risk factors with HPV infection was investigated in Group I. Identifying the population at risk is important for targeted screening programs and for defining the impact of routine vaccination on oral HPV prevalence. Although oral HPV prevalence was significantly lower in Group I (2.0%) than in Group II (8.8%), the prevalence rates of antibodies against the HPV types tested were comparable.

We achieved an overall participation rate of 76%, which is higher than that of 67% reported in the US oral prevalence study (8) and much better compared to other studies (33% and 10%, respectively) (25). Conceivably, medical students appear to be more responsive and motivated in comparison to the randomly selected groups targeted in other studies.

In our study, we sampled oral rinse from the participants. It has been shown that oral rinse provides higher yield of HPV positivity in comparison to oral and oropharyngeal brushings, probably due to the lower amount of material obtained by the latter approach (26). Oral HPV prevalence in youth was recently followed in several studies from other parts of Europe and the world. Reviews over the last ten years have shown an oral HPV prevalence of 4.5-5.5%, HR HPV prevalence of 2.7-3.5%, and HPV16 prevalence of 1.0-1.3% (6, 7). The most recent review (28), which included 66

Table III. *Characteristics of oral human papillomavirus (HPV) DNA- positive (HPV+) Group I participants.*

Genotype	Gender	Smoking	Age at the first intercourse	Vaginal sex partners (N) LT	Vaginal sex partners (N) 12M	Condom usage	Oral sex partners (N) LT	Oral sex partners (N)12M	HPV vaccine
LR HPV+									
HPV 30	Female	Past	17	≥6	≥2	Always	≥6	≥2	No
HPV 44	Female	No	18	1-5	0-1	Never	1-2	1	Yes
HPV 54	Female	No	17	≥6	≥2	Usually/sometimes	≥6	≥2	Yes
HR HPV+									
HPV 16	Female	No	15	1-5	1	Usually/sometimes	3-5	1	No
HPV 45	Female	No	17	≥6	1	Usually/sometimes	3-5	1	Yes
HPV 59	Female	No	16	1-5	1	Usually/sometimes	3-5	1	No

N: Number; LT: lifetime; 12M: last 12 months; LR: low-risk; HR: high-risk.

studies conducted between 1995 and 2017 with a total sample size of 56,600, has reported an oral HPV prevalence of 7.7%, HR HPV prevalence of 3.5%, and HPV16 prevalence of 1.4%. It also pointed to geographical variability in oral HPV prevalence, being the highest in South America (12.4%) and the lowest in Asia (2.6%). In European studies, the median of oral HPV prevalence was 9.9%. This result is comparable to the HPV prevalence found in Group II (8.8%).

The age-specific oral prevalence of HPV has been determined in a US study involving 5,579 individuals aged 14-69 years. The highest prevalence was observed in the age groups 30-34 (7.0%) and 60-64 years (11.0%). The authors hypothesized that the high rates of HPV infection could be attributed to the increased sexual activity in the first peak and the reduced effectiveness of immune mechanisms due to aging in the second peak (8). Similarly, the age difference could explain the difference in the prevalence between our two study groups. Recent meta-analyses have reported lower oral HPV prevalence in females than in males (7, 28). In the present study, all six positive participants from Group I were females, but the association with gender was not statistically significant. This contrasts with the results of other studies (9); however, the facts that most of the participants in Group I were females and that the numbers of participants were low may have biased the outcome of this study.

Several studies have shown a considerable drop in the prevalence of oral HPV infection in the vaccinated population. In Europe, studies of Grun *et al*. (13) and Nordfords *et al*. (29) in populations with vaccine coverage rates of 73% and 64% have found oral HPV prevalence rates to be 1.4% and 1.8%, respectively. In the present study, a comparable oral HPV prevalence was observed, despite the lower vaccine coverage (40.5%). The difference in oral HPV prevalence between the two study groups in the present study may be explained by the vaccine uptake in Group I. Although half of the HPV-positive participants (3 out of 6) were vaccinated (Table III), none of the vaccine HPV types was identified in their oral rinse samples. HPV type 16 was detected in oral rinse of only one individual. Despite the fact that only few of the study participants were positive for HPV DNA in their oral rinse, the results agree with those from larger studies, which have shown that HPV vaccination leads to a decrease in oral HPV DNA prevalence (14).

Table IV. *Prevalence of human papillomavirus (HPV)-specific antibodies.*

	Group I Number (%)	Group II Number (%)	*p*-Value
Any VLP+	107/168 (63.7)	130/211 (61.6)	0.758
HR VLP+ (16, 18, 31, 33, 45, 52, 58)	64/168 (38.1)	99/211 (46.9)	0.105
VLP16+	26/168 (15.5)	34/211 (16.1)	0.978
VLP18+	28/168 (16.7)	38/211 (18.0)	0.837
E6+	3/168 (1.8)	6/211 (2.8)	0.740
E7+	6/168 (3.6)	6/211 (2.8)	0.915

VLP: Virus-like particles.

The prevalence of HPV-specific antibodies against VLPs did not statistically differ for any HR VLP (Table IV) or VLP16. In our previous study (20), antigens for only four instead of seven HR HPV types were used. The prevalence rates for these four HR HPV-specific antibodies were calculated to be 32% and 30% in Group I and II, respectively. When compared with the corresponding data from a cross-sectional study in the general population, no statistically significant difference was observed either for the younger or for the older populations (Group I *vs*. age group 18-35 from the cross-sectional study, 30% *vs*. 26%; Group II *vs*. age group 35-88 from the cross-sectional study, 32% *vs*. 27%) (20). Similar data were obtained for comparisons of the prevalence of HPV16-specific antibodies (Group I *vs*. age group 18-35 from the cross-sectional study, 16% *vs*. 12%; Group II *vs*. age group 35-88 from the cross-sectional study, 16% *vs*. 13%).

Table V. *Association of risk factors with oral human papillomavirus (HPV) DNA status in Group I.*

	Oral HPV-positive (N=6) n (%)	Oral HPV-negative (N=288) n (%)	*p*-Value
Age (years), mean (SD)	22.5 (1)	23.25 (1.24)	0.229
Gender			0.188
Female	6 (100.0)	204 (70.8)	
Male	-	84 (29.2)	
Smoking			1.000
Yes	1 (16.7)	72 (25.0)	
No	5 (83.3)	216 (75.0)	
Alcohol			0.424
Yes	5 (83.3)	183 (63.5)	
No	1 (16.7)	105 (36.5)	
Marijuana*			0.055
Yes	3 (50.0)	55 (19.1)	
No	2 (33.3)	233 (80.9)	
Vaccinated*			0.690
Yes	3 (50.0)	116 (40.3)	
No	3 (50.0)	171 (59.4)	
Tonsillectomy			1.000
Yes	-	22 (7.7)	
No	6 (100.0)	266 (92.4)	
Piercing*			1.000
Yes	-	9 (3.1)	
No	6 (100.0)	277 (96.2)	
Delivery			0.068
C section	2 (33.3)	22 (7.6)	
Spontaneous vaginal	2 (33.3)	179 (62.2)	
Unknown	2 (33.3)	87 (30.2)	
Sexual orientation			1.000
Heterosexual	6 (100.0)	277 (96.2)	
Bisexual	-	5 (1.7)	
Homosexual	-	6 (2.1)	
Age at first sexual intercourse (years), mean (SD)	16.67 (1.03)	17.27 (1.99)	0.221
STI in the past			
Yes	-	27 (9.4)	1.000
No	6 (100.0)	261 (90.6)	
Condom usage*			0.817
Never	1 (16.7)	42 (14.6)	
Usually/sometimes	4 (66.7)	201 (69.8)	
Always	1 (16.7)	34 (11.8)	
Kissing partners in lifetime*			0.262
1-5	-	92 (31.9)	
5-10	2 (33.3)	72 (25.0)	
10-30	3 (50.0)	93 (32.3)	
More than 30	1 (16.7)	25 (8.7)	
Kissing partners in last year*			0.681
1	3 (50.0)	175 (60.8)	
2 or more	3 (50.0)	110 (38.2)	
Vaginal sex partners (lifetime)			0.542
None	-	16 (5.6)	
1-5	3 (50.0)	196 (68.1)	
≥6	3 (50.0)	76 (36.4)	
Vaginal sex partners (last year)			1.000
None	-	30 (10.4)	
1	4 (66.7)	183 (63.5)	
≥2	2 (33.3)	75 (26.0)	
Oral sex partners (lifetime)			0.287
None	-	22 (7.7)	
1-2	1 (16.7)	135 (46.9)	
3-5	3 (50)	75 (26.0)	
≥6	2 (33.3)	56 (19.4)	
Oral sex partners (last year)			0.701
None	-	41 (14.2)	
1	4 (66.7)	184 (63.9)	
≥2	2 (33.3)	63 (21.9)	

*The percentages do not add up to 100% for some variables due to missing data. See individual variables in Tables I and II for details.

In the younger group, in which the detailed questionnaire was used, we were not able to identify any risk factors associated with the infection. Previously, several studies have found that sexual behaviour, smoking, and drinking (8, 10) are significant risk factors for oral HPV infection. An American study of the association between oral HPV infection and sexual behaviour has reported a higher oral HPV prevalence in individuals who had oral sex in the last three months (11). In this study, all individuals with positive oral samples had more than one oral sex partner in the last 12 months; however, the result is not significant due to the small number of HPV-positive individuals.

The major limitation of this study is the non-randomized selection of the participants of Group I; medical students have good knowledge of sexually transmitted diseases and barrier contraception. Moreover, the small number of participants could impact the statistical significance of association of HPV infection with the risk factors; however, it is comparable to that in other studies (9). Another limitation, similar to other studies, is the fact that oral HPV prevalence is generally low. Therefore, the importance of some risk factors may have been underestimated. Additional larger studies with longer follow-up periods could reveal the prognostic significance of positivity for both HPV DNA and HPV-specific antibodies.

In conclusion, epidemiology of oral HPV infections is currently intensively studied. HPV is known to be the causative agent of a number of carcinomas, and better understanding of its distribution and risk factors is crucial for an efficient targeting of preventive measures. To our knowledge, this is one of the first observational

epidemiological studies of oral HPV prevalence and serology in healthy population in Central Europe. A significantly lower prevalence of oral HPV DNA was detected in the group of younger participants, the majority of whom were vaccinated against HPV, compared to the group of older unvaccinated participants, suggesting that vaccination against HPV might reduce oral HPV DNA prevalence, and the HPV-associated cancers as well.

Conflicts of Interest

None of the Authors declare any conflicts of interest.

Authors' Contributions

S. Malerova designed the questionnaire, sampled the participants, performed molecular biological testing and participated in data analyses and evaluation, and preparation of the manuscript; A. Hejtmankova participated in data analyses and evaluation, and preparation of the manuscript; E. Hamsikova performed the serological testing and data analyses, and participated in the manuscript preparation; J. Smahelova and M. Salakova participated in the molecular testing, data evaluation and manuscript preparation; J. Klozar helped with the design of the study, data analyses and manuscript preparation; R. Tachezy participated in the study design and implementation, data analyses and manuscript preparation.

Acknowledgements

The study was supported by research grants nos. 114216 from the Grant Agency of the 1st Faculty of Medicine, Charles University in Prague, #17-28055A from the Agency for Medical Research of the Ministry of Health of the Czech Republic, and LQ1604 from the Ministry of Education, Youth and Sports of the Czech Republic within the National Sustainability Program II (BIOCEV-FAR project), Charles University, Prague. The authors acknowledge Mgr. Ondřej Vencálek Ph.D. for the consultations of the statistical analysis.

Data Availability Statement

The data have been submitted to Mendeley database and are publicly accessible.

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Received November 21, 2019
Revised January 31, 2020
Accepted February 4, 2020