

Anal HPV infection in Slovenian men who have sex with men

Research Article

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Abstract: Anal human papillomavirus (HPV) infection commonly affects men who have sex with men (MSM) and is associated with the development of anal cancer, with the highest prevalence in MSM who are positive for human immunodeficiency virus (HIV). The purpose of this study was to determine the prevalence of anal HPV infection and genotype distribution in Slovenian MSM and its correlation with behavioral patterns in order to predict the possible impact of quadrivalent HPV vaccine in this population. To our knowledge, this is the first study of prevalence and genotype distribution of anal HPV infection in MSM from any of the Eastern European countries. The study included 136 MSM who visited two outpatient offices from January 2007 through December 2008. All participants were clinically examined and tested for HIV, and anal swabs were taken for HPV testing. The results showed a high prevalence of anal HPV infection in both HIV-negative (75%) and HIV-positive (95%) MSM. Promiscuity and use of "poppers" (alkyl nitrites taken for recreational purposes through direct inhalation) were clearly associated with a higher prevalence of anal HPV infection. The four most common HPV genotypes were 6, 11, 16, and 18. The prevalence of anal HPV infection in Slovenian MSM is higher in comparison with other studies. Quadrivalent HPV vaccine could have great potential in this population.

Keywords: HPV • Anal infection • Men who have sex with men • HIV • Poppers • Promiscuity

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Abbreviations

MSM – men who have sex with men

1. Introduction

Human papillomavirus (HPV) infection is one of the most common sexually transmitted diseases; it commonly affects the anal region in men who have sex with men (MSM) and is clearly associated with the development of anal cancer [1,2]. There are more than 30 HPV genotypes that may cause infection of the anogenital

mucosa, [3-9] and they are classified according to their carcinogenic potential as high-risk (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82), probable high-risk (HPV 26, 53, 66), low-risk (HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108), and unknown-risk genotypes (all others) [10,11].

The prevalence of anal HPV infection in MSM is high, with estimates >86% in MSM who are positive for the human immunodeficiency virus (HIV) and >57% in HIV-negative MSM [12-17].

Previous studies in HIV-positive MSM showed a clear association between multiple-type infections and high-grade anal intraepithelial neoplasia (AIN) [12]. A high prevalence of anal HPV infection and AIN was

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proven in all age groups of sexually active, HIV-negative MSM, and thus, a high proportion of HIV-negative MSM may be at risk of developing anal cancer [13].

The incidence of anal cancer among men and women in the general population has increased [18,19]. In addition, certain subpopulations, such as MSM, HIV-positive men and women, transplant recipients, and women with cervical squamous intraepithelial lesions, are at even higher risk [20,21].

Before the HIV epidemic, the estimated incidence of anal cancer among MSM was as high as 35 per 100,000 [22,23]. The incidence of anal cancer in HIV-positive MSM was reported to be two times higher than in their HIV-negative counterparts [24,25], whereas the relative risk of anal cancer among HIV-positive MSM was 59 times higher than in the general population [26]. In fact, the rates of anal cancer among HIV-infected men are similar to the rates of cervical cancer in women prior to the initiation of the Pap-smear screening program [27].

In this study, we analyzed the prevalence of anal HPV infection and distribution of HPV genotypes among Slovenian MSM and tried to determine its correlation with their sexual behavior. Patients were recruited through two proctologic outpatient offices, which deal with the majority of the Slovenian MSM population. We believe our data to be unique, since, to our knowledge, no study published to date has revealed the prevalence and genotype distribution of anal HPV infection in MSM from any of the Eastern European countries.

2. Material and Methods

2.1. Study population

We prospectively recruited 136 men with a history of receptive anal sexual intercourse from January 2007 through December 2008 through two proctologic outpatient offices in the Rožna dolina Surgical Centre, Ljubljana, Slovenia and in the Surgical Centre Zdrav Splet, Maribor, Slovenia. At enrolment, each participant provided written, informed consent. The Medical Ethics Committee of Slovenia approved the study.

2.2. Data collection

As a part of a standard proctologic exam, we collected anal samples for testing for HPV infection. All participants were asked to complete a questionnaire regarding demographic data and sexual behavior. Blood samples were drawn from all participants with unknown HIV status and were tested for HIV infection via a standard procedure.

2.3. Anal sample collection and HPV testing

The physician performing the proctologic examination collected anal samples by rotating a water-moistened swab in the anal canal, without direct visualization. The samples were collected in Specimen Transport Medium – STM (Digene Corporation, Gaithersburg, MD) and transported to the laboratory. After isolation of DNA from 200 µl of non-denatured STM by a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), the presence of HPV DNA was detected according to the manufacturer's instructions with the commercially available Linear Array HPV Genotyping Test (Roche Diagnostics GmbH, Mannheim, Germany), which is capable of recognizing 37 different alpha-HPV genotypes.

2.4. Statistical analysis

Univariate analysis of variance was used for testing the differences in variance between groups. We expressed mean values with a standard error of the mean value. A null hypothesis was disproved with $p < 0.05$. We tested the correlation of parametric and nonparametric variables with the Pearson and Spearman correlation tests.

Data was analyzed with SPSS 16.0 software (SPSS Inc., Chicago, Illinois, USA).

3. Results

The study included 136 male participants with a history of receptive anal sexual intercourse, with a median age of 30 years (range, 16–80). Twenty participants (14.7%) were HIV-positive. Of the 136 participants, 102 (75.0%) completed the questionnaire. Demographic data revealed that the participants came mainly from urban areas (85 subjects, 83.3%) and were, on average, well educated (41 subjects [46.6%] had completed high school, and 37 subjects [42.0%] had a college or post-graduate degree). Most subjects were employed ($n=69$, 67.6%), and 27 (26.5%) were students.

At enrolment, most participants ($n=94$, 69.1%) had some clinically evident anal disorder. The most common clinical findings were anal warts ($n=77$, 56.6%), followed by hemorrhoids ($n=5$, 3.7%) and anal fissure ($n=3$, 2.2%).

As shown in Table 1, we detected HPV DNA in the anal samples of 106 subjects (77.9%). HPV DNA was detected in 19 (95.0%) out of 20 HIV-positive men, and in 87 (75.0%) out of 116 HIV-negative men ($p < 0.05$). The distribution of HPV genotypes among all participants is shown in Figure 1.

Most of the HPV genotypes detected (53.3%) were classified as high-risk or probable high-risk; 32.8%

Figure 1. Frequency of HPV genotypes among study participants (white columns: low-risk HPV genotypes; gray columns: probable high-risk HPV genotypes; black columns: high-risk HPV genotypes; dashed columns: unknown-risk HPV genotypes).

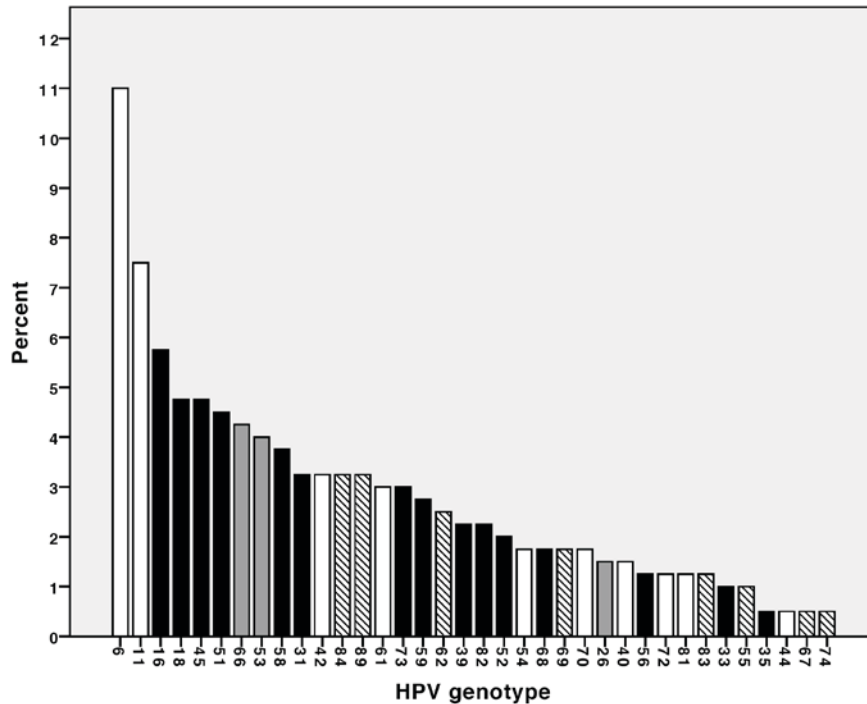


Table 1. HPV infection with regard to HIV status.

	Overall (n = 136)	HIV-negative (n = 116)	HIV-positive (n = 20)	statistical significance
Age (years), median (range)	30 (16 – 80)	29 (16 – 80)	32.5 (20 – 57)	n.s.
HPV infection (incidence; mean number of genotypes)				
Any HPV	106 (77.9%)	87 (75.0%)	19 (95.0%)	p<0.05
(number, mean $\pm$ S.E.)	2.94 $\pm$ 0.225	2.53 $\pm$ 0.221	5.30 $\pm$ 0.629	p<0.05
(median count)	2.00	2.00	5.00	
Multiple HPV infection (> 2 isolates of any type)	88 (64.7%)	69 (59.5%)	19 (95.0%)	p<0.01
High-risk HPV	77 (56.6%)	60 (51.7%)	17 (85.0%)	p<0.01
(number, mean $\pm$ S.E.)	1.28 $\pm$ 0.128	1.09 $\pm$ 0.128	2.35 $\pm$ 0.379	p<0.05
High-risk and probable high-risk HPV	82 (60.3%)	65 (56.0%)	17 (85.0%)	p<0.05
(number, mean $\pm$ S.E.)	1.57 $\pm$ 0.150	1.35 $\pm$ 0.146	2.80 $\pm$ 0.490	p<0.05
Low-risk HPV	94 (69.1%)	75 (64.7%)	19 (95.0%)	p<0.01
(number, mean $\pm$ S.E.)	0.96 $\pm$ 0.084	0.82 $\pm$ 0.081	1.80 $\pm$ 0.258	p<0.05

were low-risk, and others were unknown risk genotypes (Table 1). The distribution of HPV genotypes associated with HIV status is shown in Table 1. The distribution of HPV genotypes associated with the clinical presence of anal condyloma is shown in Table 2.

Our data show that fewer HPV genotypes were detected in subjects who practiced homosexual or bisexual intercourse for less than 10 years than in those with a longer period of practice (2.59 ± 0.345 versus 3.66 ± 0.392 , $p<0.05$). In those who engaged in such a practice for less than 10 years, fewer high-risk genotypes were detected (1.08 ± 0.183 versus $1.70 \pm$

0.231 , $p<0.05$), as were fewer high-risk and probable high-risk genotypes (1.29 ± 0.216 versus 2.04 ± 0.268 , $p<0.05$) and fewer low-risk genotypes (0.78 ± 0.110 versus 1.20 ± 0.162 , $p<0.05$).

Important epidemiological characteristics of the 102 participants (75%) who completed the questionnaire on their sexual behavior are shown in Table 3.

Our data reveal that the number of homosexual partners during the course of a lifetime correlated with the number of HPV genotypes ($p<0.01$), high-risk HPV genotypes ($p<0.01$), high-risk and probable high-risk HPV genotypes ($p<0.01$), and low-risk HPV

Table 2. HPV infection with regard to clinical presence of anal condiloma.

	Overall (n = 136)	Without clinically evident anal condiloma (n = 59)	With clinically evident anal condiloma (n = 77)	Statistical significance
Age (years), median (range)	30 (16 – 80)	29 (16 – 64)	31 (19 – 80)	n.s.
HPV infection (incidence; mean number of genotypes)				
Any HPV	106 (77.9%)	39 (66.1%)	67 (87.0%)	p<0.01
(number, mean $\pm$ S.E.)	2.94 $\pm$ 0.225	2.53 $\pm$ 0.343	3.26 $\pm$ 0.295	n.s.
(median count)	2.00	2.00	3.00	
Multiple-type HPV infection(> 2 isolates of any type)	88 (64.7%)	31 (52.5%)	57 (74.0%)	p<0.01
High-risk HPV	77 (56.6%)	29 (49.2%)	48 (62.3%)	n.s.
(number, mean $\pm$ S.E.)	1.28 $\pm$ 0.128	1.20 $\pm$ 0.195	1.34 $\pm$ 0.170	n.s.
High-risk and probable high-risk HPV type	82 (60.3%)	32 (54.2%)	50 (64.9%)	n.s.
(number, mean $\pm$ S.E.)	1.57 $\pm$ 0.150	1.51 $\pm$ 0.226	1.61 $\pm$ 0.201	n.s.
Low-risk and unknown risk HPV	94 (69.1%)	32 (54.2%)	62 (80.1%)	p < 0.01
(number, mean $\pm$ S.E.)	1.38 $\pm$ 0.111	1.02 $\pm$ 0.165	1.65 $\pm$ 0.144	p < 0.05

Table 3. Sexual behavior data.

Age (years), median (range)	30 (16 – 80)
Age at first homosexual intercourse (years), median (range)	19 (6-43)
Sexual orientation (number)	
Homosexual	75, 73.5%
Bisexual	14, 13.7%
Homosexual, with occasional heterosexual partners	7, 6.9%
Heterosexual, with occasional homosexual partners	3, 2.9 %
Could not declare	3, 2.9%
Drug use during sexual intercourse (number)	
Tobacco	38, 38.8%
Alcohol	53, 55.2%
Marihuana	22, 22.7%
Methamphetamine ("Speed")	4, 4.2%
Alkyl nitrites ("Poppers")	33, 34.0%
Ketamine	1, 1.1%
Ecstasy	8, 8.6%

genotypes ($p<0.05$) determined in a single person. We also found that the number of homosexual partners in the last 6 months correlated with the total number of HPV genotypes and with the number of low-risk HPV genotypes from a single person ($p<0.05$).

The number of heterosexual partners during the course of a lifetime correlated with the number of heterosexual partners in the last 6 months ($p<0.01$), but it did not correlate with any HPV findings. Our data show a correlation between the number of homosexual partners in a lifetime and the participant's age ($p<0.01$); there was also a correlation with age at first homosexual intercourse ($p<0.05$) and with the number of homosexual partners in the last 6 months ($p<0.01$).

We detected more HPV genotypes in patients who used "poppers" (slang for various alkyl nitrites taken for recreational purposes through direct inhalation), during sexual intercourse (3.94 ± 0.469 , $p<0.05$) than in those who did not use them (2.80 ± 0.328). A significantly higher burden ($p<0.01$) of low-risk HPV genotypes was observed among those who used "poppers" (1.39 ± 0.204) compared with those who did not (0.80 ± 0.107). Our data suggest that the use of crystal methamphetamine during anal intercourse promotes infection with low-risk HPV genotypes ($p<0.11$). Use of other substances during anal intercourse did not affect the risk of anal HPV infection ($p>0.05$).

4. Discussion

In this first study analyzing the prevalence of anal HPV infection and genotype distribution in MSM originating from Eastern Europe, we found a high prevalence of HPV infection in both HIV-negative and HIV-positive MSM, 75.0% and 95.0%, respectively, which is comparable to data from some previous studies from Western Europe and the United States [12-17,28], but data from other studies has shown a lower prevalence [13,29]. Multiple-genotype anal HPV infection was detected in 95% of HIV-positive participants (median 5.0 isolates) and in 59.5% of those who were HIV-negative (median 2.0 isolates), which is a much higher prevalence than observed in other similar studies [14,29].

Most of the isolates were high-risk and probable high-risk HPV genotypes (53.3%) and, as expected, were more frequently detected in HIV-positive MSM (85.0%). The prevalence of high-risk HPV genotypes

in HIV-negative subjects was 51.7%, which is twice as high as that reported by Chin-Hong, et al. [13].

We assume that a higher prevalence of anal warts at the time of enrolment was responsible for the higher HPV prevalence in our study compared with that in a previous study [28]. As expected, we found more low-risk HPV infections of the anal region in patients with clinically evident anal warts than in those who did not have such warts ($p < 0.01$), although there was no significant difference between groups in regard to the overall number of HPV isolates nor to the number of high-risk HPV isolates. Patients with normal findings (without anal warts) at proctoscopy, therefore, probably do not have less risk of the development of anal dysplasia and anal cancer than do patients with anal warts. We conclude that the same screening for anal health is needed in both groups of MSM, and it should include HPV sampling in addition to proctoscopy, which appears to be insufficient as the sole screening method.

Overall, HPV 6, 11, 16, and 18 were most commonly detected, representing 29.0% of all isolates (Figure 1). Twenty-eight percent of all HPVs in HIV-positive MSM were HPV 6, 11, 16, and 18, which is a higher proportion than that found in a recent study (22.6%) in which the spectrum of most common HPV genotypes was slightly different [15,17]. According to our data, we conclude that use of a quadrivalent HPV vaccine (against HPV 6, 11, 16, and 18) could be beneficial in all MSM who are not yet infected. Data from a recent study show that only 30% of MSM were aware that a vaccine is available for protection against infection with certain HPV genotypes, but once they were informed of the increased risk of

anal cancer among MSM, the participants were strongly in favor of receiving the HPV vaccine [30]. We conclude that young MSM should be educated properly to promote HPV vaccination.

We found that promiscuous homosexual behavior, which was demonstrated in young MSM and in those who began having homosexual intercourse early in life, promotes anal HPV infection; this finding is similar to that of a previous study [13]. Heterosexual promiscuity does not influence anal HPV infection in MSM.

Among all substances MSM used during sexual intercourse, only "poppers" showed an influence on the prevalence of anal HPV infection in MSM. Chin-Hong, et al showed an independent association of the use of "poppers" with the risk of AIN, for which the mechanism remains unknown [31]. However, research has shown that the effect of "poppers" has been associated with a decrease in condom use and that "popper" use suppresses the natural killer cell function, which increases vulnerability to infectious agents and produces sustained alterations in the immune system [32]. Our data suggest that the use of crystal methamphetamine during sexual intercourse could promote anal HPV infection with low-risk genotypes, although the data in our study were insufficient to allow proper statistical evaluation.

In conclusion, our study showed that the MSM population in Slovenia is highly exposed to HPV, and we demonstrated a potentially high impact for quadrivalent HPV vaccination in this population.

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