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ORIGINAL ARTICLE

Prevalence of Anal Human Papillomavirus Infection in Hungarian Men Who Have Sex with Men

Eszter Szabó[1,2] · **Csaba Kósa**[3] · **Edit Babarczi**[4] · **Mihály Sulyok**[5,6] · **Eszter Ujhelyi**[1] · **Dénes Bánhegyi**[3] · **István Vályi-Nagy**[7]

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Abstract Anal cancer is one of the leading causes of death in non-AIDS defining cancers. Most of these cancers are associated with high risk HPV infection. So far, the prevalence and the significance of anal HPV infection have not been studied in the Hungarian MSM population. The main objective of our study was to determine the prevalence and associated risk factors of HPV-infection in the Hungarian MSM community, particularly in HIV-infected MSM. Out of 109 examinations 92 samples (80 HIV-infected and 12 HIV-negative MSM) were evaluated for both cytological abnormalities and HPV genotyping PCR. Using a questionnaire all enrolled individuals were interviewed about their sexual behavior, socioeconomic factors, drug use and other known or suspected risk factors. In the HIV-infected cohort 97.5% of the examined individuals were positive for any HPV type. In this group we detected high risk (HR) HPV in 88.8%, low risk (LR) HPV in 75.0% and probably high risk (PHR) HPV in 47.5% and multiple HPV infection was absolutely common (82.5%). In the HIV-negative MSM group the incidence of HPV-infection was 58.3%. The respective rate of HR-HPV, LR-HPV and PHR-HPV genotypes were 33.3%, 58.4%, and 16.7%. In the HIV-negative group both HPV infection frequency and the prevalence of the pertinent genotypes were much lower. The Hungarian MSM population is severely infected with HPV and HR-HPV. High-risk sexual behaviors are strong predictors for acquiring HR-HPV co-infections. Our results underline the necessity of anal cancer screening and the introduction of the vaccination program in the high-risk population.

Keywords Anal cancer screening · HIV-infected MSM · HPV · HPV genotype · MSM · Risk factor

✉ Eszter Szabó
eszter.szabo321@gmail.com

[1] Molecular Biology Laboratory, St. István and St. László Hospital, Albert Flórián str. 5-7, Budapest 1097, Hungary

[2] Institute of Biology Department of Immunology, Eötvös Loránd University, Pázmány Péter str. 1/c, Budapest 1117, Hungary

[3] 3th Department of Infectology, St. István and St. László Hospital, Albert Flórián str. 5-7, Budapest 1097, Hungary

[4] Department of Pathology, St. István and St. László Hospital, Nagyvárad square 1, Budapest 1097, Hungary

[5] Doctoral School for Clinical Medicine, Semmelweis University, Üllői str. 26, Budapest 1085, Hungary

[6] Institute for Tropical Medicine, Eberhard Karl University, Wilhelmstr. 27, 72074 Tuebingen, Germany

[7] Hepatology Center, St. István and St. László Hospital, Nagyvárad square 1, Budapest 1097, Hungary

Abbreviations

AIDS	acquired immune deficiency syndrome
AC	anal cancer
AIN	anal intraepithelial neoplasia
CIN	cervical intraepithelial lesion
cART	combination antiretroviral therapy
HAV	hepatitis A
HBV	hepatitis B
HCV	hepatitis C
HR	high-risk
HIV	human immunodeficiency virus
HPV	human papillomavirus
LSIL	low grade SIL
LR	low-risk
MSM	men who have sex with men
NADM	non-AIDS defining malignancies
PCR	polymerase chain reaction
PHR	probably high-risk

STD sexually transmitted diseases
SIL squamous intraepithelial lesion

Introduction

Anal cancer (AC) is a rare disease [1, 2] but its occurrence is higher among men undergoing anal intercourse (men who have sex with men, MSM) as compared to the general population [3–6]. In the MSM group its incidence has been estimated to be 17–35 fold higher [4, 7] and an additional two- or threefold higher risk is estimated in MSM individuals living with HIV [5–8]. Recently, with the availability of effective combination anti-retroviral therapy (cART) in the MSM group the number of AIDS-defining deaths has decreased [9] and non-AIDS-defining malignancies (NADM) have become the main causes of lethality [9, 10], AC being each of the most frequent among these [10, 11].

Human papillomavirus (HPV) infection has been identified as a major risk factor for anal cancer and the virus can be detected in 80–90% in these malignancies [12–14]. In HIV-infected patients HPV infection persists and induces the development of anal condylomas (warts) and anal intraepithelial neoplasias (AIN) with higher frequency than in the uninfected population [15–17]. Moreover, AIN may progress to invasive AC with a greater chance and within a short period of time [13]. According to several studies anal cancer screening in the high-risk population, particularly in HIV-infected MSM is necessary and justified.

Objectives

Our study is the first in Hungary that analyses the prevalence of HPV infection and the diversity of HPV genotypes in MSM with an emphasis on HIV-infected individuals. Parallel to the analysis of viral infection, cytological evaluation of samples was performed and known and suspected risk factors for HPV-infection were assessed.

The objective of this article is to draw attention to the high prevalence and serious consequences of HPV-infection in a group of Hungarian MSM. We also emphasize the importance and necessity of anal cancer screening in such high-risk populations.

Materials and Methods

Study Design

Recruitment of Participants and Data Collection

Examinations were performed in the Saint István and Saint László Hospital, Budapest, Hungary with the permission of the Ethics Committee of the Hospital (Appr. Number: 24/EB/2010). Anal samples were collected from HIV-1-infected MSM treated in the HIV outpatient unit of the 3th Department of Infectology of the Hospital and from HIV-negative MSM.

Prior to sample collection all enrolled individuals read and signed a patient information leaflet and an informed consent form and filled out a detailed anonymous questionnaire that contained inquiries regarding sexual behavior, substance use, sexually transmitted diseases (STD) or social and living conditions. We had access to the complete case histories of HIV-infected MSM.

HIV-negative patients were tested for HIV-1/2 and all participants were tested for hepatitis C (HCV), hepatitis B (HBV), hepatitis A (HAV) and syphilis.

Sample Collection

For HPV analysis and pathological examination (slide smears) anal samples were taken from the area of the anal canal, specifically from the transformation zone, using a cytobrush (Medical Wire & Equipment CO. LTD). The swab was inserted 3–5 cm into the anus, rotated for 30 s and slowly removed. Following sample collection cytobrushes were stored dry at 2–8 °C in 2 ml tubes and processed within 1 week. Immediately before the HPV analysis each sample was dissolved in 2 ml Cobas® polymerase chain reaction (PCR) Cell Collection Media (Roche Molecular Diagnostics, Inc) by using strong vortex. Each anal sample was tested for HPV in the Molecular Biology Laboratory and was subjected to cytological examination in the Surgical Pathology Laboratory of the hospital. Sampling for both examinations was performed simultaneously. A total of 109 anal samples were derived from 92 HIV-infected and 17 HIV-negative MSM.

HPV Detection and Genotyping

HPV testing was performed by using the Roche Linear Array HPV Genotyping Test that includes the Amplilute Liquid Media Extraction Kit, the Linear Array HPV Genotyping Test Kit and the Linear Array Detection Kit (all from Roche Molecular Diagnostics, Inc.). This is a Communantè Europèenne and an In Vitro Diagnostic labeled test that allows qualitative in vitro detection of HPV in clinical samples [18, 19]. The test was carried out according to the instructions of the manufacturer. It works on the principle of a method denoted as the Linear Probe Assay. In this procedure, isolated PCR amplified and biotinylated specific single strain DNA were subjected to reverse-line blot hybridization for detection of 37 different HPV genotypes. The assay is able to detect all epidemiologically classified high risk (HR) HPV (HPV16, 18,

31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82), probably high risk (PHR) HPV (HPV 26, 53, 66) and the majority of low risk (LR) HPV (HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108) [20].

That HPV test does not directly detect HPV52 but combines a set of probes that detects HPV33, 35, 52, and 58 (HPV52mix). Specimens that test negative for HPV33, 35, and 58 but are positive for the HPV52mix are considered to be HPV52 positive. Specimens that test positive for HPV33, 35, and/or 58 and the HPV52mix have an uncertain HPV52 status and for this analysis these specimens were considered to be HPV52 negative [19].

Statistical Analysis

Categorical variables were studied by using the two-sided Chi-square test, whereas quantitative variables were analysed using the Student t-test. All statistical analyses were performed using SPSS v.11.5 for Windows. A p-value of 0.05 determined with Bonferroni correction was considered to indicate statistical significance.

Results

A total of 109 anal samples were collected from MSM. As a result of inadequate sampling or improper patient compliance, 17 (15.6%) samples were negative for cellular DNA control, therefore these samples were excluded from our analysis.

HPV-Infection

HIV-infected MSM: the 92 evaluable anal samples were derived from 80 HIV-infected and 12 HIV-negative MSM.

HPV infection of any kind was detected in 97.5% of the HIV-infected individuals. In this cohort we detected HR-HPV in 88.8%, LR-HPV in 75.0% and PHR-HPV in 47.5% (Table 1).

Single HR-HPV infection was found in 20.0% of the cases. In contrast, LR-HPV was present only in 5.0% (Table 1). Multiple HPV infection was absolutely common, 82.5% (Table 2). Only 15.0% of the samples contained only one genotype. Prevalence of either double or triple HPV infections (both 17.5%) was the highest and higher than those infected by only one HPV type. The maximum number was 12 types in one sample.

Diversity of HPV genotypes was very high in the HIV-infected MSM and a total of 34 different genotypes were detected. The prevalence of HPV6, HPVCP6108, HPV16, HPV18, HPV52 and HPV59 was above 20%, whereas, the prevalence of HPV11, HPV55, HPV61, HPV62, HPV84, HPV53, HPV66, HPV45, HPV58, HPV68 and HPV73 was above 10%. The most frequent HR genotype was HPV 16 (42.5%) and HPV6 and HPVCP6108 types were the most frequently detected LR-HPV genotypes (23.8%). HPV 6, 11, 16, 18 – the genotypes in the quadrivalent vaccine – are apparently frequently detected in the HIV-infected MSM.

HIV-negative MSM: HPV infection of any kind was detected in 58.3% of HIV-negative MSM (Table 1). We found HR-HPV genotypes in 33.3%, PHR-HPV genotypes in 16.7% and LR-HPV genotypes in 58.35% of the patients.

There were no individuals who were infected with only HR-HPV strains and LR-HPV was always present in the positive samples. The most characteristic was triple infection (33.3%) (Table2).

Fourteen different genotypes (HPV 6, 11, 42, 54, 61, 70, 83, 84, CP6108, 53, 16, 33, 59, 73) were detected in HIV-negative patients (Table 3). The most frequently detected genotypes (25.0%) were HPV16, HPV 61 and 84. Genotypes 6 and 11 were also detected in these patients. Due to the low number of enrolled individuals further studies are necessary to assess the HPV infection paradigm of Hungarian HIV-negative MSM. It has to be emphasized however, that in the HIV-negative cohort both the frequency of HPV infection and the prevalence of certain HPV genotypes were much lower

Table 1 Prevalence of HPV infection in the different examined groups

	HIV-positive (N = 80)		HIV-negative (N = 12)	
	n	%	n	%
Any HPV	78	97,5	7	58,3
Any low-risk HPV	60	75,0	7	58,3
Any probably-high-risk HPV	38	47,5	2	16,7
Any high-risk HPV	71	88,8	4	33,3
Only low-risk HPV	4	5,0	2	16,7
Only probably high-risk HPV	1	1,3	0	0,0
Only high-risk HPV	16	20,0	0	0,0
Low-risk and high-risk HPV	54	67,5	4	33,3
Low-risk and probably low-risk and high-risk HPV	14	17,5	1	8,3

Table 2 Prevalence of genotypes in one anal samples of HIV-infected MSM and HIV-negative MSM at the same time

Number of genotypes	1	2	3	4	5	6	7	8	9	10	11	12
Anal samples of HIV-infected MSM (%)	15.0	17.5	17.5	13.7	7.5	6.3	5	8.7	3.7	0	1.3	1.3
Anal samples of HIV-negative MSM (%)	8.3	8.3	33.3	0	0	8.3	0	0	0	0	0	0

than those in the HIV-infected group. Differences were found in diversity of genotypes (Table 3). In HIV-infected patients 34 and in HIV-negative patients 14 different genotypes were detected. HPV 26, 64, 67 genotypes could not be detected in any samples. HPV 16 was the most frequent type in both of groups. Quadrivalent vaccine types were detected in the two groups except the HPV 18 in the HIV-negative.

HPV prevalence on HIV-positive and negative MSM are summarized in Table 1.

Cytological Examination

The Bethesda System was used to evaluate cytological samples. Out of 92 anal samples 82 (70 HIV-infected and 12 HIV-negative) could be evaluated.

In 18.5% of HIV-infected individuals squamous intraepithelial lesion (SIL) could not be detected, 72.9% had low grade SIL (LSIL), 5.7% had uncertain high grade squamous intraepithelial lesion (HSIL) and 2.9% had HSIL. In the HIV-negative MSM group 66.7% were SIL negative, 33.3% had LSIL and 0% had HSIL.

HPV-induced cellular abnormalities were found uncertain in 12.9% in the HIV-infected and in 16.7% in the HIV-negative patients. Cytological abnormalities were detected in 67.1% in the HIV-infected MSM and only in 16.7% in the HIV-negative MSM samples. Parakeratosis or hyperkeratosis was present in almost all specimens (85.7% in the HIV-infected and 91.7% in the HIV-negative participant).

Characteristics of the Study Population

In the HIV-infected MSM group the average age was 39 years and the age range in this sexually active group was 20–70 years. No correlation between HPV infection (common HPV/HR-HPV/LR-HPV/PHR-HPV) and age could be observed in either the HIV-infected or the HIV-negative patient cohort.

Based on questionnaire surveys, potential risk factors for HPV infection are summarized in (Table 4). As a result of the homogeneity of the investigated population it is difficult to establish a significant correlation between the high HPV positivity rate and particular risk factors. Nevertheless, there were some correlations between certain HPV types and certain characteristics of MSM (Table 5). Smoking, anal bleeding during sex, fisting and passive role were independent risk factor for HR-HPV (Odds ratio (OR): 1.89, 1.66, 1.18, 1.14 respectively). A history of intravenous drug use and use of poppers did not increase the risk of HPV-infection. According to already published data the number of $CD4^+$-cells and simultaneous multiple HR-HPV infection was in negative correlation in HIV-infected MSM (not shown).

Discussion

Our results demonstrate the high prevalence of HPV infections in HIV-infected MSM study population. Also, an extremely high diversity of genotypes in individual patients was observed. The most frequent genotype was HPV 16 but as many

Table 3 Distribution of the detected HPV genotypes in the examined groups

	HPV genotypes and frequency
HIV-infected	6 (23.8%), 11 (16,3%), 40 (2.5%), 42 (1.3%), 54 (6.3%), 55 (18.8%), 61 (10.0%), 62 (15.0%), 69 (3.8%), 70 (8.8%), 71 (1.3%), 72 (5.0%), 81(5.0%), 83 (3.8%), 84 (17.5%), IS39 (1.3%), CP6108 (23.8%), 53 (12.5%), 66 (10.0%), 16 (42,5%), 18 (22.5%), 31 (6.3%), 33 (7.5%), 35 (5.0%), 39 (6.3%), 45 (13.8%), 51 (21.3%), 52 (23.8%), 56 (1.3%), 58 (11.3%), 59 (22.5%), 68 (11.3%), 73 (15.0%), 82 (3.8%)
HIV-negative	6 (8.3%), 11 (8.3%), 42 (8.3%), 54 (8.3%), 61 (25.0%), 70 (8.3%), 83 (8.3%), 84 (25.0%), CP6108 (8.3%), 53 (16.7%), 16 (25.0%), 33 (8.3%), 59 (8.3%), 73 (8.3%)

Table 4 HPV infection and the sexual behaviour in HIV-infected MSM

Risk factor	HIV-infected MSM (N 80)		HIV-negative MSM (N 12)	
	N	%	N	%
Sexual relationship in past 3 month	75	93.8	11	91.6
≥ 3 different sexual partner in last 3 month	22	27.5	2	16.7
≥ 15 sexual partner in their life	56	70	8	66.7
Use of condom:				
Never	5	6.3	0	0
Not always	32	40.0	2	16.7
Always	42	52.5	9	75.0
Passive role[1]	64	80.0	10	83.3
Oral sex	42	52.5	8	66.7
Fisting sex	10	12.5	1	8.3
Bleeding during the anal intercourse	14	17.5	2	16.7
Smoking (>5/day)	19	23.8	4	33.3
Poppers, rush	27	33.8	2	16.7
Intravenous drug	4	5	1	8.3
Perianal wart	19	23.8	2	16.7
STD infection (HBV, HCV, syphilis, gonorrhea)	37	46.3	3	25.0
History of the $CD4^+$-cells < 200/mm^3	21	26.3		
HIV- infected on cART	41	51.3		
Actual percent of the $CD4^+$-cells	24.48 +/−1.94 (CI 95%)			
Duration of HIV infection (years)	5.7 +/− 1.42 (CI 95%)			

as 35 different genotypes with high frequency values (above 20%) were detected. These results strongly reflect the previously published data [6, 12, 21].

HPV positivity in HIV-infected MSM is approximately twice as high as that in HIV-negative individuals (97.5 and 47.5%). As the number of enrolled individuals was low we intend to conduct further examinations on the HIV-negative patient cohort.

Table 5 As correlation analysis (Chi-squared test) results significant correlations were between the certain HPV genotypes and the risk factors

Risk factor	HIV-infected MSM (N 80)		HIV-negative MSM (N 12)	
	HPV genotype	p	HPV genotype	p
$CD4^+$-cells number	HPV 18	0.05	-	-
	HPV 51	0.05	-	-
≥ 15 sexual partner in their life and ≥3 different sexual partner in last 3 month	HPV 31	0.05	-	-
Poppers	HPV 39	0.05	-	-
	HPV 82	0.05	-	-
	HPV 45	0.05	-	-
	-	-	HPV 33	0.05
	positivity for at least 2,3 or 5 LR-HPV genotypes	0.05	-	-

No significant association between various risk factors and the frequency of HPV infection could be observed. Information collected from our questionnaire, however clearly indicates that promiscuity and the practice of unprotected intercourse as well as frequent STIs are characteristic and a more intensive sex education would be necessary in the MSM group of individuals.

There is a higher probability for a more rapid histological progression of AIN in HIV-infected individuals [13, 17]. Across the cohort, different grades of cytological lesions were observed (72.9% LSIL, 5.7% uncertain HSIL, 2.9% HSIL). However Kreuter and et al. [13] results show higher frequent high grade lesion, but these results derived from histological examinations. Follow-up examinations with HRA and histology examinations will be needed to determine the exact significance of these lesions in the pathogenesis of anal cancer.

It would be ideal to use a molecular triage test (e.g. p16 immunohistochemistry) for differential diagnosis but for now such test is not routinely available in Hungary. Based on the actual cytology test of the patient we recommend control cytology test in 3 to 6 months.

Based on the high rate of HPV infection and cytopathologic abnormalities the HIV-infected MSM group is at high risk for developing anal tumors. Introduction of regular anal cancer

screening in this cohort will be needed because the regular clinical control and early treatment of high grade AIN would be prevent the developing of anal cancer [11, 13, 22–24]. The question is that what it would be the optimal screening strategy which is appropriate sensitive and specificity. Sensitivity and specificity of cytological examination are wide range (69–93% and 32–59%) [22, 25, 26]. As the high prevalence of HPV-infections in MSM-group HPV PCR test is not suitable for HSIL anal screening, however its negative predictive value is very high. Cost-effectiveness analyses show that routine anal cancer screening of MSM is more cost-effective in the prevention of anal cancer than that in routine cervical cancer screening of women [25, 27]. Anoscopy may be the most appropriate as it is a cost-effective, sensitive and specific method for screening [27]. The importance of self-examination should be emphasized as well [28, 29].

According to our results on the high genotypic diversity of virus infection we believe that individuals in the high risk group of HIV-infected MSM should be vaccinated [2, 30]. Both the quadrivalent and the newly available nonavalent vaccine will be appropriate for primary prevention. The quadrivalent vaccine can be used safely in HIV-infected individuals [31]. The quadrivalent vaccine can be effective in reducing the persistent HPV infection of vaccine types thereby it can prevent the majority development of genital warts and HSILs [30, 32].

In summary, a more intensive education on medical risks and an introduction of regular anal screening is needed in HIV-infected MSM in Hungary. More data should be collected in order to establish a precise risk assessment for the HIV-negative MSM cohort.

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