

RESEARCH ARTICLE

Open Access



Human papillomavirus infection in anal intraepithelial lesions from HIV infected Cuban men

Celia M. Limia^{1†}, Yudira Soto^{1*†}, Yanara García², Orestes Blanco¹, Vivian Kouri¹, María V. López², María E. Toledo¹, Lissette Pérez¹, Yoanna Baños¹, Yaniris Caturla¹ and Francisco Aguayo³

Abstract

Background: An association between HPV infection and progression to anal squamous intraepithelial lesions (ASIL) has been established, specifically in high-risk populations such as HIV-infected men. In this population, anal cancer is one of the most common non-AIDS-defining malignancies.

Methods: A cross-sectional study to detect anal lesions and HPV infection was performed. Anal mucosa samples were collected from 56 HIV-infected men from Cuba. The cytological diagnosis was done according to Bethesda 2001 System. HPV DNA detection was determined by qPCR for six high-risk HPV types and end point PCR for low-risk HPV types (6 and 11). The end point PCR with nucleotide sequencing technique was achieved to detect other genotypes of HPV not included in the qPCR in those samples negative for HPV- 6 and 11 or negative for the six genotypes identified in the qPCR.

Results: Cytological diagnosis identified 53 of 56 (95%) men with abnormal anal cytology. Among those, 26% (14/53) had atypical squamous cells of undetermined significance (ASC-US), 4% (2/53) had atypical squamous cells of undetermined significance cannot exclude high-grade lesions (ASC-H), 64% (34/53) had low-grade squamous intraepithelial lesions (LSIL), and 6% (3/53) had high-grade squamous intraepithelial lesions (HSIL). HPV DNA was detected in 89% (50/56) of men and 79% had at least one of the high-risk HPV types. HPV- 16 was the most common genotype (52%), while HPV-18 was the most frequently detected genotype in men with HSIL. We found statistically significant differences in the HPV viral loads with respect to the cytology results ($p = 0.0006$) and that the practice of receptive anal sex was a risk factor for anal HPV infection ($p = 0.032$).

Conclusion: This study shows a high prevalence of ASIL and high-risk HPV infections in the study group and is the first study showing the distribution of HPV genotypes in HIV infected Cuban men with abnormal anal cytology. This information may be of importance for local decision makers to improve prevention strategies, including the introduction of HPV vaccine in Cuba.

Keywords: HPV, Anal squamous intraepithelial lesions, HIV, Anal cancer

* Correspondence: yudira@ipk.sld.cu

†Equal contributors

¹Institute of Tropical Medicine "Pedro Kouri", Autopista Novia del Mediodía
Km 6 ½, P.O.Box. 601, Marianao 13, La Habana, Cuba

Full list of author information is available at the end of the article



Background

While anal carcinoma is rare in the general population [1], it is one of the most common non-AIDS-defining malignancies in the era of combination antiretroviral therapy [2]. It is more frequent among individuals with human immunodeficiency virus (HIV) infection, particularly in men who have sex with men (MSM) [3]. In the past decades, the incidence of anal cancer has been increasing in both general MSM and HIV-infected MSM [4]. The Cuban AIDS epidemic has a predominance of male HIV-infected individuals (81%), of which 89% are MSM [5].

Persistent human papillomavirus (HPV) infection, especially high-risk HPV (HR-HPV) viral types such as HPV-16 and HPV-18, is associated with anal cancer and its precursor lesions, high-grade anal squamous intraepithelial lesions (HSIL) [6]. Recent studies have demonstrated that more than 90% of HIV-positive MSM were also infected with HPV [2, 7]. This particular group has a very high risk of developing ASIL and anal cancer [8]. The development of anal squamous intraepithelial lesions (ASIL) is associated with HPV infection, as well as other risk factors such as receptive anal intercourse, history of sexually transmitted diseases, number of lifetime sexual partners, HIV status, lower CD4 cell count, and current cigarette smoking [9]. Screening of anal precancerous lesions in HIV-infected MSM has been suggested to be important for cancer prevention [10].

Little is known about anal HPV infection or anal cytological abnormalities in HIV-infected men in Cuba. The objective of this study was to investigate the distribution of HPV genotypes, and anal cytological abnormalities in HIV-infected men in Havana, Cuba.

Methods

Study design and population

We performed a cross-sectional study to detect ASIL and HPV infection. Study participants were outpatients of the sexually transmitted diseases (STD) clinic at the Institute of Tropical Medicine "Pedro Kouri" and were recruited between April and August 2012. Those eligible to participate were HIV-positive males with a history of a previous or current STD, willing to give informed consent to an interview and tests for anal HPV infection and anal cytology. The inclusion criteria also included having an anal cytology useful for diagnosis.

Cytological analysis and HPV genotyping

Prior to the sample collection, the anal canal was cleaned carefully twice using a saline-moistened cotton-flocked swab. The cytology specimens were collected by means of a pre-scored brush (QIAGEN, Hilden, Germany), placed on a glass slide, stained with a Papanicolaou stain and classified using the Bethesda system criteria for evaluation of cervical cytological results [11, 12].

After the cytology, another sample was collected for DNA extraction. The brush was then placed in a 1 mL Specimen Transport MediumTM (QIAGEN, Hilden, Germany). DNA was obtained using the QIAamp DNA Mini kit (QIAGEN, Germany) following the manufacturer's protocol.

HPV DNA detection was determined by real time polymerase chain reaction (qPCR) for six HR-HPV types (HPV-16, -18, -31, -33, -45 and -58), as described below. HPV-6 and 11 were detected using type specific end point PCR with L1 primers [13]. The end point PCR with nucleotide sequencing technique was achieved to detect other genotypes of HPV not included in the qPCR in those samples negative for HPV-6 and 11 or negative for the six genotypes identified in the qPCR.

Real time polymerase chain reaction

The methodology used in this study is based on a protocol previously published by Schmitz et al. in 2009 [14] and it has already been described in detail in a previous article by our group [15].

PCR primers and corresponding TaqMan probes were used for HPV types -16, -18, -31, -33, -45, and -58 for target amplification of the LCR/E6/E7 regions of HPV genome. To identify and quantify viral load of those HR-HPV types, single PCR reactions were performed for each HPV type. To control for DNA quality, β -globin was amplified for each sample, in one of the reactions. Sequences of primers and probes are listed below (Table 1).

HPV-16 and 18 standard curves were obtained from purified genomic DNA of SiHa and HeLa cell lines, respectively. HPV16-positive cervical carcinoma cell line SiHa (2 genome copies of HPV-16) and the HPV18-positive cell line HeLa (10–50 genome copies of HPV-18) were used to generate standard DNA for measurements of viral load (tenfold genome dilutions between 10^6 and 10 copies/ μ L).

The pattern curves were constructed on the basis of each of the resulting standard DNA, which showed good linear correlation ($r: 0,99$) and low error values throughout 6 target DNA concentrations. The system had a lower detection limit of 10 copies for HPV-16 and other genetically related genotypes such as HPV-31, -33 and -58. The lower detection limit for HPV-18 and other genetically related genotype, HPV-45, was also of 10 copies of viral genome. No cross reactions between HPV types and other DNA viruses were observed [14, 16].

PCR was performed in a Light Cycler 1.5 platform (Roche Molecular Biochemical of Indianapolis, USA). The PCR reaction comprises 5 μ L of DNA (up to 50 ng), 4 μ L of Quantitative PCR TaqMan Master Mix (Roche Molecular Biochemical of Indianapolis, USA), 10 pmol of each primer and 1 to 5 pmol of each probe in a final

Table 1 Primer and probes for real time polymerase chain reaction

LCR/E6/E7 regions primers and TaqMan probes	Sequences 5'- 3'
HPV 16 +	5' gaa ccg aaa ccg gtt agt ata a 3'
HPV 16 -	5' atg tat agt tgt ttg cag ctc tgt 3'
HPV 16 Probe	5' cat ttt atg cac caa aag aga act gca atg ttt c 3'
HPV 18 +	5' gga ccg aaa acg gtg tat ata a 3'
HPV 18 -	5' cag tga agt gtt cag ttc ggt 3'
HPV 18 Probe	5' atg tga gaa aca cac cac aat act atg gcg cg 3'
HPV 31 +	5' gaa ccg aaa acg gtt ggt ata ta 3'
HPV 31 -	5' atc gta ggg tat ttc caa tgc 3'
HPV 31 Probe	5' cat agt att ttg tgc aaa cct aca gac gcc atg t 3'
HPV 33 +	5' gca tga ttt gtg cca agc at 3'
HPV 33 -	5' ctc aga tcg ttg caa agg ttt 3'
HPV 33 Probe	5' act ata cac aac att gaa cta cag tgc gtg gaa tgc 3'
HPV 45 +	5' cag tgt aat aca tgt tgt gac cag 3'
HPV 45 -	5' aca gga tct aat tca ttc tga ggt 3'
HPV 45 Probe	5' caa gaa aga ctt cgc aga cgt agg gaa aca c 3'
HPV 58 +	5' cac gga cat tgc atg att tgt 3'
HPV 58 -	5' tca gat cgc tgc aaa gtc ttt 3'
HPV 58 Probe	5' ttt caa ttc gat ttc atg cac 3'
β globin +	5' aca caa ctg tgt tca cta gc 3'
β globin -	5' caa ctt cat cca cgt tca cc 3'
β globin Probe	5' tca aac aga cac cat ggt gca tct gac tcc 3'

volume of 25 μL. The initial denaturation step at 94 °C for 10 min was followed by 45 PCR cycles at 94 °C for 15 s, 50 °C for 20 s and 60 °C for 40 s each.

HPV viral load was measured using absolute quantification. The viral load values were expressed as copies/μL. Cases with more than one HPV type were reported as multiple infections.

End point PCR and sequencing

The amplification reactions included a set of degenerated primers MY09/MY11 [17]. Each amplification reaction was performed in a total volume of 50 μL. The reaction mixture contained nuclease-free water, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 6 mM MgCl₂, 200 μM of each dNTP, 5 μM of MY primers, 2,5-U Taq Polymerase (Roche, Diagnostics, Indianapolis, USA), and 100 ng of each DNA. Each PCR was performed with first denaturation step at 95 °C for 10 min, followed by 45 PCR cycles at 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min. A final extension at 72 °C for 10 min was included.

Polymerase chain reaction products of positive samples were purified using QIAquick® PCR purification kit (QIAGEN, Hilden, Alemania). These were subsequently sequenced using 1 μL of 5 μM of MY09 and MY11 as the sequencing primers, 8 μL of sequence reaction mixture DTCS Quick Star Master Mix supplied with the Dye Terminator Cycle Sequencing (DTCS) Quick Start Kit (Beckman Coulter, Fullerton, CA), 5 μL of purified DNA and 6 μL of water to complete 20 μL of reaction mixture. The sequencing reaction consisted of 2 min denaturation at 96 °C, followed by 50 cycles of denaturing at 96 °C for 20 s, 20 s of hybridization at 50 °C, and 4 min of extension at 60 °C. Once the sequence reaction was concluded, it was purified, after the protocol described in the DTCS Quick Star Master Mix commercial kit (Beckman Coulter). The sequencing fragments were run on the genetic analysis system CEQ 8800 (Beckman Coulter). Finally, the sequences were edited and assembled using Sequencher, version 4.10 (Gene Codes Corporation, Ann Arbor, MI). Human papillomavirus genotype was determined using Basic Local Alignment Search Tool (BLAST, [www/ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) and confirmed by manual phylogenetic analysis, using CLUSTAL-X and the neighbor-joining method in MEGA version 5 (Kimura's 2-parameter correction, bootstrap 1000) [18].

Statistical analysis

Data were processed using IBM SPSS Statistics 19. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated, using univariate logistic regression, as estimates of the association of HPV infection and anal cytology results with potential risk factors. Comparisons between viral loads from groups of patients with different cytological classifications were made using the Kruskal-Wallis and Mann–Whitney tests. All statistical tests were considered to be significant at a *p* value of < .05.

Results

A total of 56 eligible participants consented to be included into this study. The socio-demographic, epidemiological, and clinical characteristics of the participants are shown in Table 2. The median age of the participants was 35 years (range, 20–61 years). The majority of individuals (89%) described themselves as MSM, and 66% of these had their first intercourse at 15 years old or later. Around 84% of the participants have STD other than HIV at the time of this study, and 44 (79%) were diagnosed for anogenital condyloma. Thirty (54%) participants had a recent CD4 cell count between 200 to 500 cells/mL of blood, and 79% participants ever had highly active antiretroviral therapy (HAART). About 34% of the participants reported ≥5 anal sex partners during the last two years.

Overall, HPV DNA was detected in 89% (50/56) men and 79% (44/56) had at least one of the six HR-HPV

Table 2 Relationship between anal HPV infection and socio-demographic, epidemiologic and clinical risk factors in the studied population

Variables	N (%)	HPV-positive N (%)	HPV-negative N (%)	p value	OR (CI 95%)
Total	56 (100)	50 (89)	6 (11)		
Age, median (IQR)	35 (27–46)	–	–		
Age (years)				0.862	
20–29	22 (39)	20 (40)	2 (33)		2.50 (0.30–20.92)
30–39	11 (20)	11 (22)	0 (0)		—
40–49	13 (23)	11 (22)	2 (33)		1.38 (0.16–11.94)
≥50	10 (18)	8 (16)	2 (33)		1 (Reference)
Education				0.766	
Primary and junior high school	15 (27)	14 (28)	1 (17)		2.55 (0.20–31.86)
High School	28 (50)	25 (50)	3 (50)		1.52 (0.22–10.38)
University	13 (23)	11 (22)	2 (33)		1 (Reference)
Marriage status				0.999	
Married	9 (16)	8 (16)	1 (17)		1 (Reference)
Unmarried	43 (77)	38 (76)	5 (83)		0.95 (0.10–9.27)
Divorced	4 (7)	4 (8)	0 (0)		—
Self-reported sexual orientation				0.084	
MSM	50 (89)	46 (92)	4 (67)		5.75 (0.79–41.69)
Heterosexual	6 (11)	4 (8)	2 (33)		1 (Reference)
Alcohol consumption				0.196	
Yes	23 (41)	19 (38)	4 (67)		0.31 (0.05–1.84)
No	33 (59)	31(62)	2 (33)		1 (Reference)
Smoking				0.229	
Yes	24 (43)	20 (40)	4 (67)		0.33 (0.6–2.00)
No	32 (57)	30 (60)	2 (33)		1 (Reference)
CD4+ T cells count				0.465	
<200	17 (30)	16 (32)	1 (17)		4.57 (0.35–59.11)
200–500	30 (54)	27 (54)	3 (50)		2.57 (0.36–18.49)
>500	9 (16)	7 (14)	2 (33)		1 (Reference)
Age of first sexual intercourse (years)				0.362	
<15	19 (34)	18 (36)	1 (17)		2.81 (0.30–25.98)
≥15	37 (66)	32 (64)	5 (83)		1 (Reference)
Sexual practice				0.492	
Genital	5 (9)	4 (8)	1 (17)		1 (Reference)
Oral/Genital	51 (91)	46 (92)	5 (83)		2.30 (0.21–24.80)
Anal receptive sex				0.032	
Yes	47 (84)	45 (90)	3 (50)		9.00 (2.42–57.12)
No	9 (16)	5 (10)	3 (50)		1 (Reference)
Number of sexual partners during the last two years.				0.756	
1	19 (34)	16 (32)	3 (50)		1 (Reference)
2–4	18 (32)	17 (34)	1 (17)		3.19 (0.30–33.89)
5–10	8 (14)	8 (16)	0		—
>10	11 (20)	9 (18)	2 (33)		0.84 (0.12–6.03)

Table 2 Relationship between anal HPV infection and socio-demographic, epidemiologic and clinical risk factors in the studied population (Continued)

HAART					0.459	
	Yes	44 (79)	40 (80)	4 (67)		1 (Reference)
	No	12 (21)	10 (20)	2 (33)		0.50 (0.08–3.13)
STD at the time of the study (except HIV)					0.031	
	Yes	47 (84)	44 (88)	3 (50)		7.33 (1.20–44.96)
	No	9 (16)	6 (12)	3 (50)		1 (Reference)
History of STD (except HIV)					0.690	
	Yes	43 (77)	38 (76)	5 (83)		0.63 (0.07–5.97)
	No	13 (23)	12 (24)	1 (17)		1 (Reference)

The values with statistical significance appear in italic letters

HPV Human papillomavirus, OR Odd ratio, IQR Inter-quartile ratio, CI Confidence interval, MSM Men who have sex with men STD Sexually transmitted diseases, HIV Human immunodeficiency virus, HAART High activity anti-retroviral therapy

types that we sought. The most common types detected were HPV-16 (52%), HPV-11 (48%), HPV-6 (41%) and HPV-18 (30%). Infection with multiple HPV types was detected in 71% of the participants (40/56), (Table 3). We also found two cases positive for HPV-53 (possibly carcinogenic genotype) and HPV-83 (genotype of unknown carcinogenicity), through generic PCR and DNA sequencing.

Concerning cytological findings, 53 of 56 (95%) men had abnormal anal cytology. Among those with abnormal cytology, 26% (14/53) had ASC-US, 4% (2/53) had atypical ASC-H, 64% (34/53) had LSIL and 6% (3/53) HSIL.

HPV prevalence was remarkably high in both normal and abnormal anal cytology. All cases with normal anal cytology had HPV infection (3/3). Among those with abnormal anal cytology, 89% (47/53) were positive for at

least one of HPV type and 74% (39/53) were infected with multiple HPV types. Among the HPV-positive cases with abnormal anal cytology, 87% (41/47) had at least one HR-HPV genotype and 55% (26/47) had more than two HR-HPV types. The most prevalent HR-HPV types among cases with abnormal cytology were HPV-16 (51%, 27/53) and HPV-18 (28%, 15/53). HPV-16 was the major genotype in men with ASC-US, LSIL or ASC-H, followed by HPV-33 in men with ASC-US, HPV-18 and 33 in men with LSIL and HPV-18 and 45 in ASC-H. HPV-18 was the most frequently detected genotype in men with HSIL, followed by HPV-16 and 58. In men with normal cytology, HPV-16 and 58 were the most prevalent types detected, (Table 3).

Viral load analysis was detected for different anal cytological classifications. HPV viral load levels were significantly different among those with different anal cytology results ($p = 0.0006$). Those classified as negative and ASC-US showed lower viral load (median: 8.5×10^3 copy/ μL , ranges 3.2×10^2 to 4.6×10^6 copy/ μL and median: 3.7×10^2 copy/ μL , ranges 9.4×10^1 to 5.1×10^5 copy/ μL , respectively). In contrast, higher viral load was detected in cases with LSIL, ASC-H and HSIL (median: 6.8×10^5 copy/ μL , ranges 2.1×10^2 to 1.6×10^8 copy/ μL , median: 3.8×10^7 copy/ μL , ranges 1.1×10^6 to 2.0×10^8 copy/ μL and median: 8.5×10^5 copy/ μL , ranges 3.8×10^2 to 1.4×10^7 copy/ μL , respectively) (Fig. 1).

Table 2 shows the association between HPV infection and socio-demographic, epidemiological, and clinical variables analyzed in the studied population. The participants who had receptive anal sex as a regular sexual behavior were at higher risk to HPV infection ($p = 0.032$; OR = 9.00 CI 95% 2.42–57.12). Sexually transmitted diseases were also a risk factor for viral infection ($p = 0.031$; OR = 7.33 CI 95% 1.20–44.96). Regarding HPV infection, according to age distribution, viral infection was not associated with age but a slight pick in prevalence for the 20- to 29-years old individuals was observed.

Table 3 HPV types distribution in HIV positive men according to anal cytology classification

HPV type	Cytology classification					
	NLIM N=3 n (%)	ASC-US N=14 n (%)	LSIL N=34 n (%)	ASC-H N=2 n (%)	HSIL N=3 n (%)	Total N=56 n (%)
HR-HPV types	3 (100)	9 (64)	27 (79)	2 (100)	3 (100)	44 (79)
HPV-16	2 (67)	6 (43)	17 (50)	2 (100)	2 (67)	29 (52)
HPV-18	1 (33)	1 (7)	11 (32)	1 (50)	3 (100)	17 (30)
HPV-31	0	1 (7)	5 (15)	0	1 (33)	7 (12)
HPV-33	1 (33)	3 (21)	11 (32)	0	0	15 (27)
HPV-45	1 (33)	2 (14)	5 (15)	1 (50)	0	9 (16)
HPV-58	2 (67)	0	8 (24)	0	2 (67)	12 (21)
HPV 16–18 only	1 (33)	0	4 (12)	0	1 (33)	6 (11)
HPV 16–18 and other HR-HPV	1 (33)	0	7 (21)	1 (50)	2 (67)	11 (20)
Multiple infections	3 (100)	7 (50)	26 (76)	1 (50)	3 (100)	40 (71)

HR-HPV types (16, 18, 31, 33, 45 and 58)

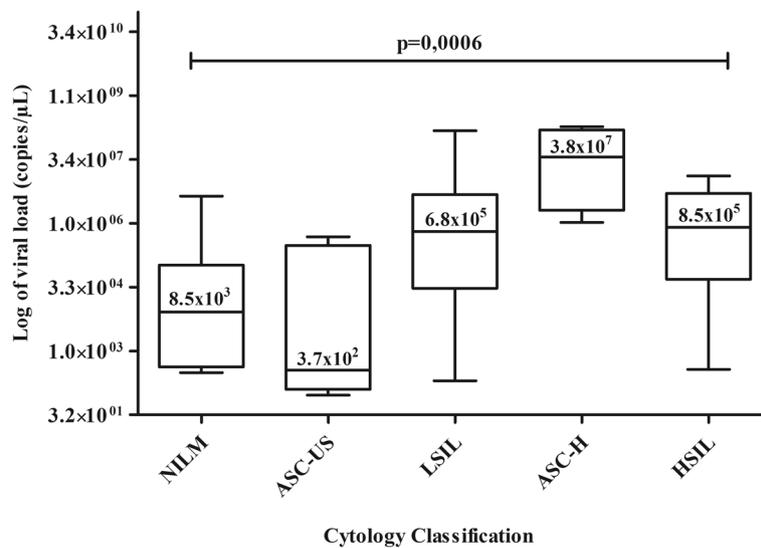


Fig. 1 Comparison of the medians of HPV viral load among each anal cytology classification. Abbreviations: ASC-US: atypical squamous cells of undetermined significance, ASC-H: atypical squamous cells of undetermined significance cannot exclude high-grade lesions, LSIL: low-grade squamous intraepithelial lesions, HSIL: high-grade squamous intraepithelial lesions, NILM: negative for intraepithelial lesion and malignancy

The population with ASIL/ASC-H was compared with those with normal anal cytology and ASC-US. A higher prevalence of ASIL/ASC-H was observed among those having oral/genital sex as a regular sexual behavior ($p = 0.026$; OR = 11.69 CI 95% 1.20-114.31). In addition, a positive diagnosis of STD, predominantly anal condyloma, was found to be related with an increased risk of ASIL/ASC-H ($p = 0.017$; OR = 6.55 CI 95% 1.40-30.58).

Discussion

In the past decade, multiple studies have reported that anal HPV infection is common among HIV infected men [19–21]. In Cuba, there are few data about HPV prevalence in HIV-infected men [22]. In the present study a very high prevalence of HPV infection was found in both, individuals with abnormal and normal anal cytology. This high prevalence had been also found in various studies developed in HIV-infected men [7, 8, 23]. Torres et al. found a global HPV prevalence of 95.8% (95% CI, 94.6 to 96.7). They also found that patients with abnormal cytology presented a higher prevalence of any HPV type (99.7%), than those with negative cytology results. However, patients with negative results also had a very high prevalence of any HPV type (94.1%) [7]. Sahasrabudde et al. detected that the prevalence of any HPV genotype was 94.4% (95% CI, 91.4%–96.6%), and the prevalence of any carcinogenic HPV genotype was 75.4% (95% CI, 70.5%–79.9%). They found anal HPV infection in 98.4% of patients with HSIL and in 89.2% of men with nondysplastic biopsy findings [8]. These high prevalence rates could be explained by the effect of chronic HIV infection on normal immune function, which increases susceptibility to

HPV infection and retention [24]. The mechanisms that facilitate this are still unknown.

The most common types detected were HPV-16, HPV-11, HPV-6, and HPV-18, which are included in the currently used tetravalent HPV vaccine. HR-HPV types were frequently detected for both, individuals with abnormal and normal anal cytology. These results are similar to studies from different geographic areas [7, 23]. However, Colon-Lopez et al. detected mostly HPV-58, 31 and 51 in high-risk men attending a sexually transmitted infection clinic in Puerto Rico [25]. The different prevalence and distribution of anal HPV subtypes in different study populations is of interest since it might reflect different geographic distributions of HPV genotypes or heterogeneous host susceptibility to the pathogens.

Our cytology results show that the prevalence of abnormal anal cytology overall was remarkably high, primarily with LSIL. Only 5.4% (3/56) of the participants had normal anal cytology.

It is difficult to directly compare the results from different studies due to the various characteristics of the study participants including age, stage of disease, status of immune system, and history of HAART. Salit and colleges, in 2010, evaluated the prevalence of abnormal anal cytology based on a cross-sectional study of 401 HIV-positive MSM from Canada. They found the highest prevalence of LSIL (43%). In a study of 247 HIV-Infected MSM, Pokomandy et al. found a prevalence of abnormal anal cytology of 80.9% [26]. In the present study, the prevalence of abnormal anal cytology was higher. According to these figures, further investigations

are needed to confirm the high prevalence of cytological anal abnormalities in Cuban HIV positive men.

Our results showed receptive anal sex and other STD, specifically anal warts as risk factors for HPV infection in the studied population. A past history of receptive anal intercourse and recent receptive anal intercourses are the most consistently reported risk factors for prevalent anal HPV infections [27]. A recent history of anal warts and others STD have been described as important risk factors associated with the prevalence of anal HPV infections and anal cancer precursor lesions [21, 28].

Among MSM, there are few data on age-specific HPV prevalence. In our study HPV detection was very high and viral infection was not associated with age. However, we observed a slightly high prevalence of anal HPV in men aged 20- to 29- years.

The majority of studies found a high prevalence of anal HPV that did not decline with age [29]. Contrary, in a study by Gao et al. of 578 MSM (528 HIV-negative and 50 HIV-positive) recruited from two cities in China, a decrease in anal HPV prevalence was observed with increasing age (71% for ≤ 19 years, 62% for 20–29 years, 64% for 30–39 years and 54% for ≥ 40 years); however, this was not statistically significant [30]. In another study by Nyitray et al. of 179 HIV-negative MSM recruited through advertisements and from a genitourinary clinic in three sites in Brazil, Mexico and the US, a significantly decreasing age-specific prevalence of anal HPV was observed, with the highest HPV rates reported in the 18- to 24-year-old age group. These age-specific HPV infection prevalence trends may result from age-specific sexual behavior [31]. Difference source populations may explain the different age-specific estimates [29].

The significance of the high incidence of HPV detection in this population is uncertain. One potential explanation for the high prevalence of anal HPV infection among MSM and the lack of association with age is that HPV infection in the anus is more persistent than in the cervix. However, the persistence of some HPV types may not reflect the true persistence but rather clearance of an HPV type followed by subsequent exposure or reinfection with the same type [29].

In our study, higher levels of HPV viral load were associated with abnormal anal cytology. Tamalet et al. reported that HPV 16 viral load was an independent factor for abnormal anal cytology [32]. Rodel and colleagues found that HPV 16 DNA load and p16 (INK4a) expression were significant prognostic factors for local tumor control and overall survival of patients with anal cancer following chemo-radiotherapy [33]. However, Poizot-Martin et al. found that there was not association between HPV 16/18 viral loads and abnormal anosopic results [34]. Longitudinal studies are needed to evaluate the link between high anal HPV DNA load

and progression to anal squamous intraepithelial lesions and anal cancer.

Some limitations of this study should be considered. The small sample size of this study limited the analysis when interpreting the prevalence data, mainly to perform statistical association analysis. On the other hand, even when anal cytology results were confirmed by biopsy using standard anoscopy and patients were treated properly, this study had no access to biopsy results and to the outcome of each patient, because they were attended at coloproctology consults from different hospitals.

Conclusion

In this study we found for the first time in Cuba, a high prevalence of HR-HPV infection in anal mucosa of HIV infected men. Because a large number of cases have shown cytological abnormality, we suggest the possibility of including a screening for HPV detection and genotyping in anal cancer precursor lesions of HIV seropositive individuals. This would facilitate the clinical management of these patients in the presence of anal cancer precursor lesions, taking into account the increased incidence of this disease in this risk group. Moreover, the information reported here may be useful to consider the possibility of including HPV vaccination in Cuba.

Abbreviations

ASC-H: Atypical squamous cells of undetermined significance cannot exclude high-grade lesions; ASC-US: Atypical squamous cells of undetermined significance; ASIL: Anal squamous intraepithelial lesions; CIs: Confidence intervals; HAART: Highly active antiretroviral therapy; HIV: Human immunodeficiency virus; HPV: Human papillomavirus; HR-HPV: High risk-human papillomavirus; HSIL: Had high-grade squamous intraepithelial lesions; LSIL: Low-grade squamous intraepithelial lesions; MSM: Men who have sex with men; ORs: Odds ratios; qPCR: Quantitative real time polymerase chain reaction; STD: Sexually transmitted disease

Acknowledgements

We thank Dr. Joel M. Palefsky, University of San Francisco, California, for the revision of the manuscript and his useful comments about this work. We also thank the patients who participated in this study.

Funding

This study was supported by Cuba Ministry of Public Health and Fondecyt Grant 1161219 to FA, Santiago, Chile.

Availability of data and material

All data have been included in the manuscript.

Authors' contributions

CML and YS had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: CML, YS and VK. Epidemiological information and clinical specimens for analysis: OAB, YG and MVL. Cytological analysis: MVL. Experiments and samples processing: CML, YS, YG, YB and YC. Analysis and interpretation of data: YS, CML, YG and VK. Drafting of manuscript: CML and YS. Critical revision of the manuscript for important intellectual content: VK, LP, and FA. Statistical analysis: CML, YS and MET. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Institute of Tropical Medicine “Pedro Kouri” and complied with the principles laid down in the Declaration of Helsinki. Written informed consent was obtained from each study participant before the interview, sample collection and testing. Socio-demographic, epidemiological and clinical data were collected using one-to-one interviews in a separate room and a standardized questionnaire.

Author details

¹Institute of Tropical Medicine “Pedro Kouri”, Autopista Novia del Mediodía Km 6 ½, P.O.Box. 601, Marianao 13, La Habana, Cuba. ²General Hospital “Enrique Cabrera Cossío”, Calzada Aldabó 11117 esquina E, Boyeros 10800, La Habana, Cuba. ³Virology Program, I.C.B.M., Faculty of Medicine, University of Chile, Independencia 1027, Chile.

Received: 30 September 2016 Accepted: 11 January 2017

Published online: 17 January 2017

References

- Abbas A, Yang G, Fakhri M. Management of anal cancer in 2010. Part 1: overview, screening, and diagnosis. *Oncology (Williston Park)*. 2010;24:364–9.
- Tong WW, Hillman RJ, Kelleher AD, Grulich AE, Carr A. Anal intraepithelial neoplasia and squamous cell carcinoma in HIV-infected adults. *HIV Med*. 2014;15:65–76.
- van Aar F, Mooij SH, van der Sande MA, Speksnijder AG, Stolte IG, Meijer CJ, et al. Anal and penile high-risk human papillomavirus prevalence in HIV-negative and HIV-infected MSM. *AIDS*. 2013;27:2921–31.
- Palefsky JM, Rubin M. The epidemiology of anal human papillomavirus and related neoplasia. *Obstet Gynecol Clin North Am*. 2009;36:187–200.
- Aragones C, Sanchez L, Campos J, Perez J. Antiretroviral therapy adherence in persons with HIV/AIDS in Cuba. *MEDICC review*. 2011;13:17–23.
- Palefsky JM, Holly EA, Efricit JT, Da Costa M, Jay N, Berry JM, et al. Anal intraepithelial neoplasia in the highly active antiretroviral therapy era among HIV-positive men who have sex with men. *AIDS*. 2005;19:1407–14.
- Torres M, Gonzalez C, del Romero J, Viciana P, Ocampo A, Rodriguez-Fortunez P, et al. Anal human papillomavirus genotype distribution in HIV-infected men who have sex with men by geographical origin, age, and cytological status in a Spanish cohort. *J Clin Microbiol*. 2013;51:3512–20.
- Sahasrabudhe VV, Castle PE, Follansbee S, Boronovo S, Tokugawa D, Schwartz LM, et al. Human papillomavirus genotype attribution and estimation of preventable fraction of anal intraepithelial neoplasia cases among HIV-infected men who have sex with men. *J Infect Dis*. 2013;207:392–401.
- Schwartz LM, Castle PE, Follansbee S, Boronovo S, Fetterman B, Tokugawa D, et al. Risk factors for anal HPV infection and anal precancer in HIV-infected men who have sex with men. *J Infect Dis*. 2013;208:1768–75.
- Lam JM, Hoch JS, Tinmouth J, Sano M, Raboud J, Salit IE. Cost-effectiveness of screening for anal precancers in HIV-positive men. *AIDS*. 2011;25:635–42.
- Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, et al. The 2001 Bethesda system. Terminology for reporting results of cervical cytology. *JAMA*. 2002;287:2114–9.
- Park IU, Palefsky JM. Evaluation and management of anal intraepithelial neoplasia in HIV-negative and HIV-positive men who have sex with men. *Curr Infect Dis Rep*. 2010;12:126–33.
- Seaman WT, Andrews E, Couch M, Kojic EM, Cu-Uvin S, Palefsky J, et al. Detection and quantitation of HPV in genital and oral tissues and fluids by real time PCR. *Virology*. 2010;7:194.
- Schmitz M, Scheungraber C, Herrmann J, Teller K, Gajda M, Runnebaum IB, et al. Quantitative multiplex PCR assay for the detection of the seven clinically most relevant high-risk HPV types. *J Clin Virol*. 2009;44:302–7.
- Soto Y, Kouri V, Martinez PA, Correa CB, Torres G, Goicolea A, et al. Standardization of a real-time based polymerase chain reaction system for the quantification of human papillomavirus of high oncogenic risk. *Vaccinemonitor*. 2012;21:30–7.
- Takacs T, Jeney C, Kovacs L, Mozes J, Benczik M, Sebe A. Molecular beacon-based real-time PCR method for detection of 15 high-risk and 5 low-risk HPV types. *J Virol Methods* 2008;149(1):153–62.
- Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Coutlee F, Hildesheim A, et al. Improved amplification of genital human papillomaviruses. *J Clin Microbiol*. 2000;38:357–61.
- Tamura KPN, Stecher G, Nei M, Kumar S. MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*. 2011;28:2731–9.
- Panther LA, Wagner K, Proper J, Fugelso DK, Chatis PA, Weeden W, et al. High resolution anoscopy findings for men who have sex with men: inaccuracy of anal cytology as a predictor of histologic high-grade anal intraepithelial neoplasia and the impact of HIV serostatus. *Clin Infect Dis*. 2004;38:1490–2.
- van der Snoek EM, Niesters HG, Mulder PG, van Doornum GJ, Osterhaus AD, van der Meijden WJ. Human papillomavirus infection in men who have sex with men participating in a Dutch gay-cohort study. *Sex Transm Dis*. 2003;30:639–44.
- Vajdic CM, van Leeuwen MT, Jin F, Prestage G, Medley G, Hillman RJ, et al. Anal human papillomavirus genotype diversity and co-infection in a community-based sample of homosexual men. *Sex Transm Infect*. 2009;85:330–5.
- Blanco OA, Soto Y, Blanco B, Acosta S, Capó de Paz V, Toledo ME. Detection of Human Papillomavirus (HPV) in anogenital condyloma from HIV-1 infected Cuban men. *Rev Biomed*. 2011;22:21–30.
- de Pokomandy A, Rouleau D, Ghattas G, Vezina S, Cote P, Macleod J, et al. Prevalence, clearance, and incidence of anal human papillomavirus infection in HIV-infected men: the HIPVIRG cohort study. *J Infect Dis*. 2009;199:965–73.
- Palefsky J. Biology of HPV in HIV infection. *Adv Dent Res*. 2006;19:99–105.
- Colon-Lopez V, Ortiz AP, Del Toro-Mejias L, Clatts MC, Palefsky JM. Epidemiology of anal HPV infection in high-risk men attending a sexually transmitted infection clinic in Puerto Rico. *PLoS One*. 2014;9:e83209.
- de Pokomandy A, Rouleau D, Ghattas G, Trottier H, Vezina S, Cote P, et al. HAART and progression to high-grade anal intraepithelial neoplasia in men who have sex with men and are infected with HIV. *Clin Infect Dis*. 2011;52:1174–81.
- Goldstone S, Palefsky JM, Giuliano AR, Moreira Jr ED, Aranda C, Jessen H, et al. Prevalence of and risk factors for human papillomavirus (HPV) infection among HIV-seronegative men who have sex with men. *J Infect Dis*. 2011;203:66–74.
- Schlecht HP, Fugelso DK, Murphy RK, Wagner KT, Doweiko JP, Proper J, et al. Frequency of occult high-grade squamous intraepithelial neoplasia and invasive cancer within anal condylomata in men who have sex with men. *Clin Infect Dis*. 2010;51:107–10.
- Machalek DA, Grulich AE, Jin F, Templeton DJ, Poynter IM. The epidemiology and natural history of anal human papillomavirus infection in men who have sex with men. *Sex Health*. 2012;9:527–37.
- Gao L, Zhou F, Li X, Yang Y, Ruan Y, Jin Q. Anal HPV infection in HIV-positive men who have sex with men from China. *PLoS One*. 2010;5:e15256.
- Nyitray AG, da Silva RJ C, Baggio ML, Lu B, Smith D, Abrahamson M, et al. Age-specific prevalence of and risk factors for anal human papillomavirus (HPV) among men who have sex with women and men who have sex with men: the HPV in men (HIM) study. *J Infect Dis*. 2011;203:49–57.
- Tamalet C, Oby-Roguet V, Ressiot E, Bregigeton S, Del Grande J, Poizot-Martin I. Distribution of human papillomavirus genotypes, assessment of HPV 16 and 18 viral load and anal related lesions in HIV positive patients: a cross-sectional analysis. *J Med Virol*. 2014;86:419–25.
- Rodel F, Wieland U, Fraunholz I, Kitz J, Rave-Frank M, Wolff HA, et al. Human papillomavirus DNA load and p16(INK4a) expression predict for local control in patients with anal squamous cell carcinoma treated with chemoradiotherapy. *Int J Cancer*. 2015;136:278–88.
- Poizot-Martin I, Henry M, Benhaim S, Oby-Roguet V, Figarella D, Tamalet C. High level of HPV 16 and 18 DNA load in anal swabs from male and female HIV-1 infected patients. *J Clin Virol*. 2009;44:314–7.