LA INFECCIÓN POR EL VIRUS DEL PAPILOMA HUMANO EN LA MUCOSA BUCAL Y OROFARÍNGEA EN UNA COHORTE DE INDIVIDUOS CON VIH/SIDA.

I D Ó N E A C O M U N I C A C I Ó N D E R E S U L T A D O S

QUE PARA OBTENER EL GRADO DE

MAESTRA EN PATOLOGÍA Y MEDICINA BUCAL

P R E S E N T A

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Resumen

Introducción. Evidencia reciente sugiere que los individuos con VIH/SIDA tienen mayor riesgo de presentar cáncer de cabeza y cuello (CCyC). Adicionalmente, la persistencia de VPH-AR se ha asociado con un riesgo incrementado para el desarrollo de CCyC, específicamente de orofaringe.

Objetivo. Establecer la persistencia, incidencia y resolución de VPH en la mucosa bucal y orofaríngea (MByOF) en una cohorte de pacientes con VIH/SIDA.

Material y método. Estudio longitudinal, observacional y analítico, que incluyó pacientes con infección por VIH (01/2014 a 02/2015). Previo consentimiento informado, se obtuvieron datos demográficos y clínicos. En la visita inicial y a los tres meses, se registraron lesiones bucales, y se realizaron 3 cepillados citológicos de MB y OF, que fueron introducidos en un preservador celular. Se extrajo y cuantificó DNA, se amplificó el gen β–globina, y secuencias consenso de la región L1 de VPH (MY09/11, GP05+/06+). Los productos de PCR fueron purificados, secuenciados y comparados con las bases de datos en línea. Para el análisis estadístico, se utilizaron los programas JMPv.11 y STATA v. 20.

Resultados. Se incluyeron 97 pacientes (91% hombres, mediana edad 36 años), 59 (61%) de ellos completaron la visita de seguimiento (3 meses). La prevalencia de infección por VPH fue similar en la visita basal (59%) y en la de seguimiento (51%), el VPH tipo 18 fue el más identificado en la visita basal. A los tres meses, en 58.3% de los pacientes persistió la infección, en 41.7% se resolvió y se observó nueva infección en 9 (39.1%) pacientes. El consumo de tabaco, la carga viral-VIH >20,000 copias/ml y la terapia antirretroviral altamente activa (TARAA) fueron factores de riesgo asociados a la infección por VPH.

Discusión. Si bien se identificó una alta prevalencia puntual de VPH en ambas visitas, las secuencias de bajo riesgo representaron casi la mitad de los casos. La persistencia, resolución e infección incidente por VPH se presentó en frecuencias comparables con las reportadas en la literatura.

Conclusiones. Los factores de riesgo asociados con la infección por VPH, particularmente el consumo de tabaco, son características de gran relevancia en el seguimiento de individuos con VIH/SIDA y alto riesgo de CCyC.

Abstract

Background. HIV/AIDS patients present an elevated risk for head and neck cancer (HNC). On the other hand, human papillomavirus (HPV) persistence has been associated with a higher risk to develop HNC, particularly oropharyngeal cancer.

Objective. To establish the persistence, incidence and clearance rates of HPV infection in oral (OM) and oropharyngeal mucosa (OPhM), in a cohort of HIV/AIDS patients.

Methods. Longitudinal, observational and analytical study. Patients with confirmed HIV/AIDS infection, who attended the Oral Pathology and Medicine Clinic were consecutively included (January, 2014 - February, 2015). In each patient, prior informed consent, demographic and clinic characteristics were registered. At baseline and 3-month visits, diagnosed oral lesions were photographed and recorded. Three cytobrush were taken from oral (2 samples) and oropharyngeal (1 sample) mucosa, and then, placed in a polypropylene tube with a preservation cell solution. DNA was extracted and quantified, and PCR reactions were done in order to amplify β–globin gen, and consensus sequences from the L1 region of HPV (MY09/11, GP5+/6+). Previously purified, PCR products were sequenced and then, compared with the genome databases available online. Statistical analysis was done in JMP and STATA programs.

Results. Ninety-seven patients were included (91% males, median age 36 years-old), 59 (61%) completed the follow-up visit. HPV punctual prevalence was comparable in the baseline visit (59%) and in the 3-month visit (51%), being HPV-18 the most frequent type identified in the initial visit. Low risk sequences were identified in 27.8% at the baseline visit and in 23.7% at the 3-month visit; even though only 8.1% of the patients presented HPV-oral lesions. In the follow-up visit, 9 patients (39%) presented newly detected HPV infection; HPV persistence was identified in 58% of the patients, and clearance in 42%. Tobacco smoking, HIV-viral load (> 20,000 copies/ml), and HAART were risk factors for OM and/or OPhM HPV presence.

Conclusions. While a high punctual prevalence of HPV was identified in the individuals included at both visits, the percentage of low risk types contributes in almost a half of the patients. The rates of persistence were relatively high, and clearance was present in a quarter of the population. It is important to consider the HPV risk factors identified in the present study, particularly tobacco consumption, to the close monitoring of high risk HNC cancer populations.

Introduction

Worldwide studies have been reported the prevalence of oral human papillomavirus infection (HPV) in healthy individuals with values from 2% (Lima et al., 2014) to 25% (Beachler et al., 2012) in contrast with HIV/AIDS individuals, in whom, according to various cross-sectional studies (Kahn et al., 2015; Gaester et al 2015; Muller et al., 2015; Colon-López et al., 2014; Mooij et al., 2013; Beachler et al., 2012; Steinau et al., 2012; Del Mistro et al., 2010; D'Souza et al., 2009; Adamapoulou et al., 2008; Marais et al., 2008; Richter et al., 2008; Fakhry et al., 2006; Cameron et al., 2005; Kreimer et al., 2004) oral HPV prevalence varies from 3.5% (Gaester et al., 2015) to 56.7% (Mooij et al., 2013); being HPV-16 the most frequent genotype identified, representing from 9.5% (Mooij et al., 2013) to 42.8% (Adamapoulou et al., 2008) of all head and neck infections.

Currently, in HIV/AIDS individuals, there is enough evidence related to the clearance of oral and oropharyngeal (OOPh) HPV infection within the first year, independently of the HPV-type (Beachler et al., 2015; Kreimer et al., 2013). Interestingly, recent studies have recognized sex (male), older age and tobacco consumption, as factors associated with reduced rates of oral HPV clearance (Beachler et al., 2015; Mooij et al., 2013; Kreimer et al., 2013; D´Souza et al., 2011, 2007).

However, in HIV-infected patients, OOPhHPV infection persists for longer than in healthy population (Beachler et al., 2015; Beachler et al., 2012; D´Souza et al., 2007), probably due to a local immune dysfunction (Bere et al., 2014; Beachler et al., 2011; Gillison et al., 2009; Cameron et al., 2008; Palefsky et al., 2006). Some authors have reported an association between HPV-infection and HIV-viral load levels (Beachler et al., 2015; Beachler et al., 2012), lower CD4+ counts (Darwich et al., 2014; Beachler et al., 2012; Kreimer et al., 2004)

and the use of highly active antiretroviral therapy (Videla et al., 2013; Gillisonet al., 2009; Cameron et al., 2005).

Recent longitudinal studies (Beachler et al., 2015; Ong et al., 2014; Van Aar et al., 2014; Beachler et al., 2013; Darwich et al., 2013; Videla et al., 2013; Parisi et al 2011; Fakhry et al., 2010; D´Souza et al., 2007) have approached to the natural history of OOPh HPV infection. As respect, while a cumulative incidence of 11% is estimated (Darwich et al., 2013), the OOPh HPV infection could persist for 6 months in 46% (Beachler et al., 2013) to 78% of the infected individuals (Fakhry et al., 2010); however this persistence decrease to 29% at 12 months of follow (Beachler et al., 2013). Considering that the persistence of HPV infection has been associated with the development of a subset of head and neck cancer and HIV-infected individuals present an elevated risk (2-6 fold) to develop HPV-associated cancer, mainly oropharyngeal cancer (Shiels et al., 2011; Dubrow et al., 2012; Palefsky & Rubin et al., 2009; Gillison et al., 2009), it is essential to distinguish the role of the virus in the oral mucosa, particularly of high risk (HR) types.

Thus, we conducted a prospective cohort study, to identify the persistence, incidence and clearance of OOPh HPV infection in a group of HIV-infected patients in Mexico City.

Material and methods.

Descriptive longitudinal study conducted in the Oral Pathology and Medicine Clinic of the Universidad Autónoma Metropolitana (UAM-X) located in an HIV/AIDS referral center (Clínica Especializada Condesa [CEC]) in Mexico City, from January 2014 to February 2015. The study was approved by the review board and each individual approved its participation in the study.

Adults HIV-infected patients (≥18 years old) were included and followed for 3 months. Exclusion criteria considered patients who received antiviral treatment (Aciclovir, Imiquimod) in the 3 months prior the basal visit. Demographic and clinical data included age, sex, schooling, occupation and tobacco and alcohol consumption. Tobacco and alcohol use was categorized considering the categorization consumption previously described (Anaya-Saavedra et al., 2008). Clinical and laboratory data obtained from the medical records comprised HIV-transmission category, CDC (Clinical Disease Center) clinical stage (Thompson et al., 2010), lymphocyte $CD4^{+}$T-cell count, HIV viral load, and highly active antiretroviral therapy (HAART) use (type and duration) were obtained from the medical records. Patients were considered to be on HAART if they received ARV drugs for more than 30 days.

A systematic oral exam was carried out in each visit (basal and 3-months). HIV associated oral lesions (HIV-OLs) were identified according to OHARA system (Shiboski et al., 2009). Each HIV-OL identified was carefully described and registered; when necessary, a tissue biopsy was obtained using a disposable punch, fixed in a 10% formalin solution, and processed. The histopathologic diagnosis was performed by the Oral Pathology and Medicine Department staff of the UAM-X.

At each visit, three cytobrush samples were taken in each patient: two from the oral mucosa (OM) and one from the oropharyngeal mucosa (OPhM). A sterile cytobrush was deeply rotated (against clockwise) in the mucosa, and introduced in a labeled polypropylene tube containing 5 ml. of a preservative cell solution (PreservCyt, Cytyc Co. Marlborough MA, USA).

The molecular analysis was performed at the Virus and Cancer Laboratory of the Biomedical Research Unit of the Mexican National Autonomous University, placed at the National Cancer Institute. The DNA was extracted using the Wizard Genomic ADN Purification kit (Promega, Wisconsin, USA), according with the manufacturer's instructions. The total DNA concentration was determined by spectrophotometry, and the 260/280 ratio was measured using a NanoDrop 2000 Spectrophotometer (ThermoScientific/Waltham, MA, USA). A randomized group of samples were selected to cell quantification using a Neubauer chamber (Hausser Scientific, Horsham PA, USA) and an optical microscope; the number of cells were calculated by the next formula:

Concentration= number of cells x10000/ number of fields x dilution.

HPV testing was performed through polymerase chain reaction (PCR), following strict procedures in order to avoid false-positive reactions due to contamination. In every reaction, water and DNA from CasKi cell line were used as negative and positive control, respectively; all the reagents used were prepared and stored in a PCR-amplified products free area. PCR was performed in a programmable thermal cycler (Mastercycler gradient; Eppendorf, Westbury, NY). Sensibility curves to determine the optimal amount of DNA for MY09/11 PCR reaction were carried out using DNA from HeLa and SiHa cell lines.

In order to demonstrate the DNA integrity, a β-globin gene PCR assay was performed, using the PCO4/GH20 primers, at a 55°C (TM). The PCR products (280 bp) were placed on a 2% agarose gel for electrophoresis, and visualized by UV light.

Genomic HPV-DNA amplification was carried out using MY09 (5′-CGT CCM ARR GGA WAC TGA TC-3′, 20 pmol) and MY11 (5′-GCM CAG GGW CAT AAY AAT GG- 3′; 20 pmol) primers that amplify L1 region of HPV with a TM of 55°C. Negative samples were subsequently processed with GP5+ (5′-TTT GTT ACT GTG GTA GAT ACT AC-3′; 20 pmol) and GP6+ (5′-GAA AAA TAA ACT GTA AAT CAT ATT C-3′; 20 pmol) primers with annealing temperature of 51°C. PCR products were analyzed by 2.5% electrophoresis, stained with gel Red and visualized with ultraviolet light.

Positive PCR products were purified using ExoSap and the Big Dye protocol (Zymo Research Corp. USA.), subsequently sequenced using one of the PCR primers as a sequencing primer (MY11 and GP6+). The obtained sequences were matched and compared with the GenBank database sequences (National Center for Biotechnology Information, Bethesda, MD, USA) using the BLAST program (http://blast.ncbi.nlm.nih.gov/), blinded to the histopathological results (Figure 1).

Patients were categorized as positive to HPV when at least one of the obtained samples (oral or oropharyngeal) amplified HPV-DNA. HPV-genotypes were classified as high risk (HR) and low risk (LR) according with Muñoz et al. (2003). The presence of multiple infections was defined as the presence of two or more different HPV types in the same sample or site.

The longitudinal pattern of HPV infection was evaluated among those HIV-infected patients who completed the 3-month follow-up. Persistent infection was defined when HPV was detected in the 3-month visit of patients with baseline HPV^+ sample; clearance when a baseline HPV+ patient presented HPV negative samples (OM/OPhM) in the 3-month visit. A newly detected HPV infection was defined in patients with baseline HPV negative samples but with a 3-month HPV^+ sample (OM or OPhM).

Statistical analysis. Descriptive data were summarized through medians and interquartile intervals. In order to determine the association between categorical variables and the dependent variables, the X^2 and Fisher's exact (when necessary) tests were used. For continuous variables, the Wilcoxon rank sum test was utilized. Risk factors were assessed using logistic regression models, odds ratios and its 95% confidence intervals for the corresponding categories were constructed. A 2-sided p value ≤ 0.05 was considered significant. The statistical analysis was done in the JMP (v.11) and STATA (v.20) program.

Results

A total of 97HIV-positive patients (90.7% men, median age of 36[Q_1-Q_3, 29-44] years) were included, 59 of them (60.8%) concluded the 3-month follow-up visit. The characteristics of patients (CD4+ count, HIV-viral load, and type and time of HAART use) who were lost at follow-up were similar to those who completed the 3-month visit.

Forty-six patients (47.4%) were current smokers and 40 (47.6%) referred alcohol consumption. Among males, the most frequent transmission category (76/88, 86.4%) was men who have sex with men (MSM) and heterosexual transmission for females. More than a half of the patients were in advanced stage of the disease (AIDS) (67/69.1%), and almost two thirds received HAART (72.2%), with a median time of use of 15.5 (Q_1-Q_3: 4-42.3) months. The median of lymphocyte CD4+ current count was 349 (Q_1-Q_3: 165-494.5) cells/µl, almost half of the patients (40/49.4%) had undetectable plasma HIV-viral load (<40 copies/ml). Patients with HIV viral load suppression had a longer median time under HAART that those with detectable viral load (25.5 *vs*. 2 months, p=<0.0001)

Forty six (47.2%) patients had at least one HIV-associated oral lesion at baseline, being oral candidiasis (16/16.5%) and hairy leukoplakia (13/13.4%) the most frequent manifestations; HPV oral lesions (HPV-OLs) were identified in 8 patients (8.2%), 2 corresponding to squamous cell papilloma and 6 to multifocal epithelial hyperplasia. All oral lesions were properly treated in each patient. No lesions were identified in the oropharyngeal region.

Molecular analysis.

All collected samples (194) were evaluable for HPV-detection after β–globin amplification. The median DNA yield obtained from OM was lower (58.5 [Q_1-Q_3: 35-152] ng/µl) than from OPhM samples (141.1[Q_1-Q_3:47- 353] ng/µl). As expected, in oropharynx samples, DNA quantity increases proportionally with the number of cells present (p=0.013).

The overall OOPh HPV baseline prevalence was 58.8%, 25 of 57 HPV^+ patients (43.8%) showed concurrent infection in both sites. The overall baseline prevalence in each anatomical site was 46.4% (45/97) in OM and 38.1% (37/97) in OPhM. In regards to DNA yield and HPV positivity, it is interesting to note that the HPV+ samples exhibited lower DNA amounts (ng) per µl than the negative ones, in both oral (54[Q_1-Q_3:36-137] *vs*. 84.9 [Q_1-Q_3:31-198], p=0.330) and oropharyngeal mucosa (126 [Q_1-Q_3: 62-322] *vs.*147 [Q_1-Q_3: 44-404], p=0.355).

As it is shown in Table 1, more than half of HPV+ patients were smokers (59.6%), in contrast with the low frequency (32.5%) found in the HPV negative group (p=0.004). Also, although the difference was not significant (p=0.069), higher viral load levels were identified in patients with HPV+ in any mucosa in comparison to HPV negative individuals.

The prevalence of OOPh HPV was comparable at the baseline (58.8%) and at the 3-month visit (50.8%) (Table 2); furthermore, the distribution of HR and LR-HPV types was similar in both visits. At baseline, the most frequent HPV types identified were HPV-18 (17/57, 29.8%), followed by the HPV-58 (7/57, 12.3%) and HPV-16 (5/57, 8.7%). Multiple infections were observed in seven individuals, all corresponded to HPV-18 in combination with other HR (HPV-16 and HPV-69) and LR types (HPV-13, HPV-70, HPV-72 and HPV-83). At the 3-month visit, HPV-16 and HPV-18 were the most frequent HR-HPV types (6/30, 20%, each one). Three individuals presented multiple infections (one HPV-18 &HPV-16; one HPV-18 &HPV-13, and another one with HPV-59 & HPV-72).

Fifty-nine patients completed the follow-up; at it is shown in Table 3, 21 (58.3%) of them presented HPV persistence; while 15 patients (41.7%) cleared of the HPV identified at the initial visit. In the 3-month visit, 9 (39.1%) of the patients presented newly detected HPV infection and the incidence density was 7.7/100 person-months. For anatomic site, there was a slight difference between OM (48%) and OPhM (41%) persistence and the rates of HPV clearance at both sites (52% for OM and 59% for OPhM). A discrete higher percentage of newly detected infections were identified in OPhM (33.3%) than in OM (23.3%) (Figure 2).

In the bivariate analysis (Table 1), only tobacco consumers showed a 3.4-fold risk to present HPV in OOPh mucosa in comparison to no-smokers (95% CI: 1.4 - 8.1, p=0.004). Multivariate analysis (Table 4) showed that tobacco consumption (p=0.017), high HIV-viral load (>20,000 copies/ml) (p=0.027) and use and duration of HAART (p=0.044) were factors independently associated with the risk of HPV infection at any site (OM or OPhM); moreover, in the bivariate analysis to HR-HPV infection, there were no risk factors independently associated. Only in the multivariate analysis the longer period of HAART use (>14-months) represented a risk factor for HR-HPV infection (OR= 4.3; 95% CI: 1.0- 19.2. p=0.054). Also, a borderline association between lower CD4+ counts (≤ 200 cells/mm^3) and HPV persistence was found (p=0.50).

Table1. Baseline characteristics in 97 HIV/AIDS individuals according to OOPh HPV status.

	HPV DNA+ (n=57)	HPV DNA- (n=40)	OR (95% CI)	*p*
	n (%)	n (%)		
Years of education				
≥ 10 years-old	40 (70.2)	30 (75.0)	1.0	0.601[a]
≤ 9 years-old	17 (29.8)	10 (25.0)	0.78 (0.3- 1.95)	
Tobacco use				
No	23 (40.4)	28 (67.5)	1.0	**0.004[a]**
Yes	34 (59.6)	12 (32.5)	3.4 (1.4-8.1)	
Alcohol use	n=52	n=32		
No	26 (50.0)	18 (56.3)	1.0	0.577[a]
Yes	26 (50.0)	14 (43.7)	1.2 (0.5-3.1)	
Clinical stage				
No-AIDS(A_1,A_2,B_1,B_2)	15 (26.3)	15 (37.5)	1.0	0.240[a]
AIDS(A_3,B_3,C_1,C_2,C_3)	42 (73.7)	25 (62.5)	1.6 (0.7-4.0)	
Md $CD4^+$ count (cells/ml)(Q_1-Q_3)	365 (138.8-499)	349 (186-496)		0.884[b]
VL≥ 20,000 copies/mL	n=50	n=31		
No	28 (56.0)	22 (71.0)	1.0	0.117[a]
Yes	22 (44.0)	9 (29.0)	1.9 (0.7- 4.9)	
Md HIV-VL (copies/ml)(Q_1-Q_3)	179,565 (58,771-565,371)	31,027 (6,541-42,542)		0.069[b]
HAART use				
No	13 (22.8)	14 (35.0)	1.0	0.187[a]
Yes	44 (77.2)	26 (65.0)	1.8 (0.7-4.4)	
Md HAART(Q_1-Q_3)months	12.0 (4-58.5)	18.5 (4-35.3)		0.533[b]

HAART=Highly active antiretroviral therapy; VL=viral load; MD=median; [a] Chi square, [b] Wilcoxon rank sum test.

Table 2. Distribution of HPV types identified in the baseline and 3-months visit in any site.

	Baseline visit (n=97)		**3-months visit (n=59)**	
	n	**(%)**	**n**	**(%)**
HPV negative	40	(41.2)	29	(49.1)
HPV positive	57	(58.8)	30	(50.8)
HR- HPV	30[a]	(30.9)	16[b]	(27.1)
LR-HPV	27	(27.8)	14	(23.7)

HR=high risk; LR=low risk; [a] In five patients, HR types were combined with LR sequences; [b] In two patients, HR were identified with LR types.

Table 3. HPV longitudinal patterns in 59 HIV/AIDS patients who completed the 3-monthsvisit.

	V I S I T		
	Baseline	**3-months**	
		HPV$^+$	**HPV$^-$**
	n (%)	**Persistence**	**Clearence**
		n (%)	**n (%)**
HPV$^+$	36 (61.0)	21 (58.3)	15(41.7)
		Incidence	**Negative**
HPV$^-$	23 (39.0)	9 (39.1)	14 (60.9)

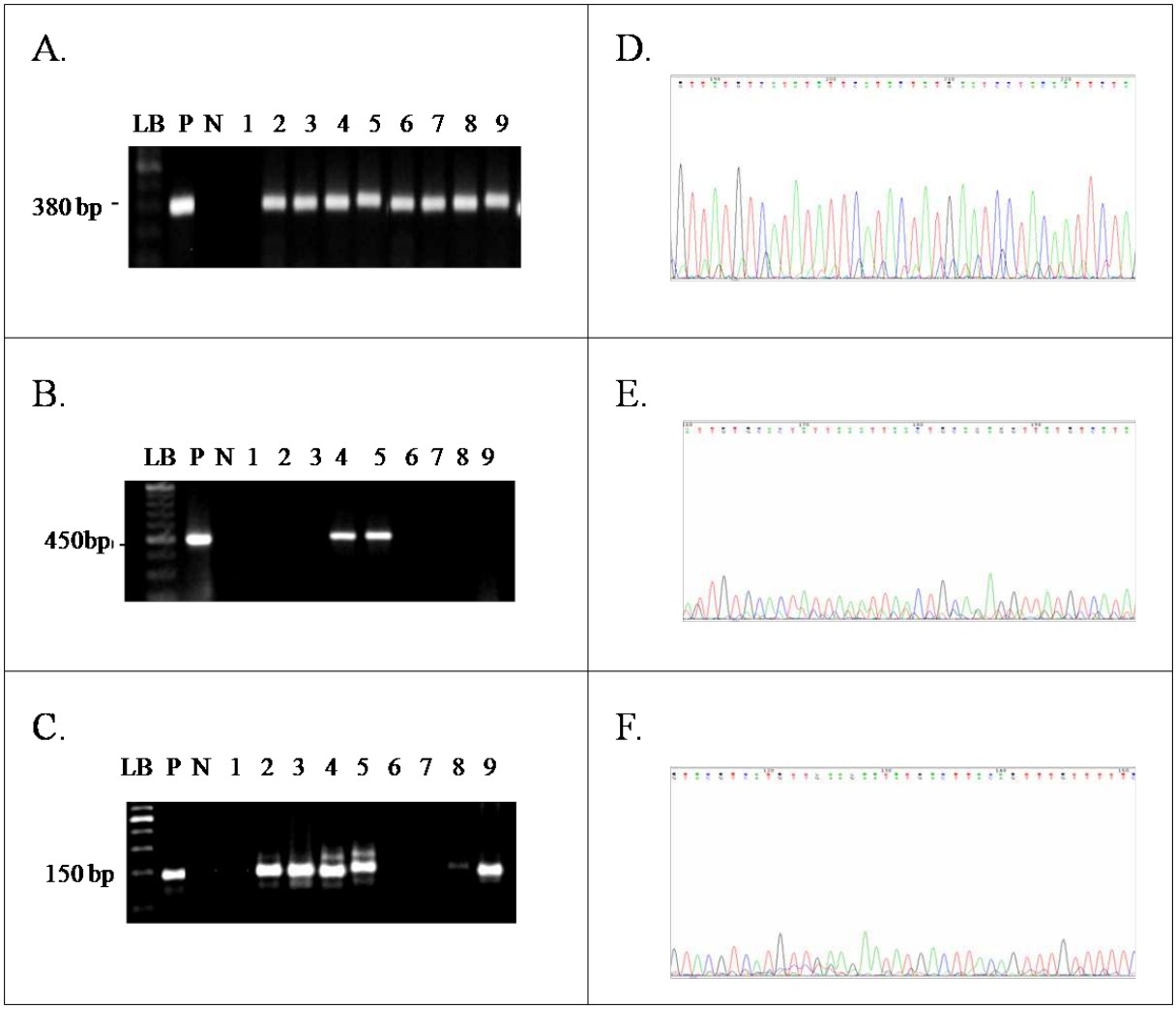

Figure 1. Representative figure of DNA electrophoresis (2.5% agarose gel) and electropherogram. L=loading buffer; P=positive control; N=negative control. Numbers (1 to 9) indicate the different samples. A) β-globin amplification gen showing the 380 pb. amplicons; B) MY09/11+ (L1-HPV fragment) showing 450 bp. amplicons; C) GP05/06+ (L1-HPV fragment) showing the 150 bp. amplicons; D) Representative image of an HPV-18 electropherogram; E) Representative image of an HPV-13 electropherogram; F) Representative image of an HPV-16electropherogram.

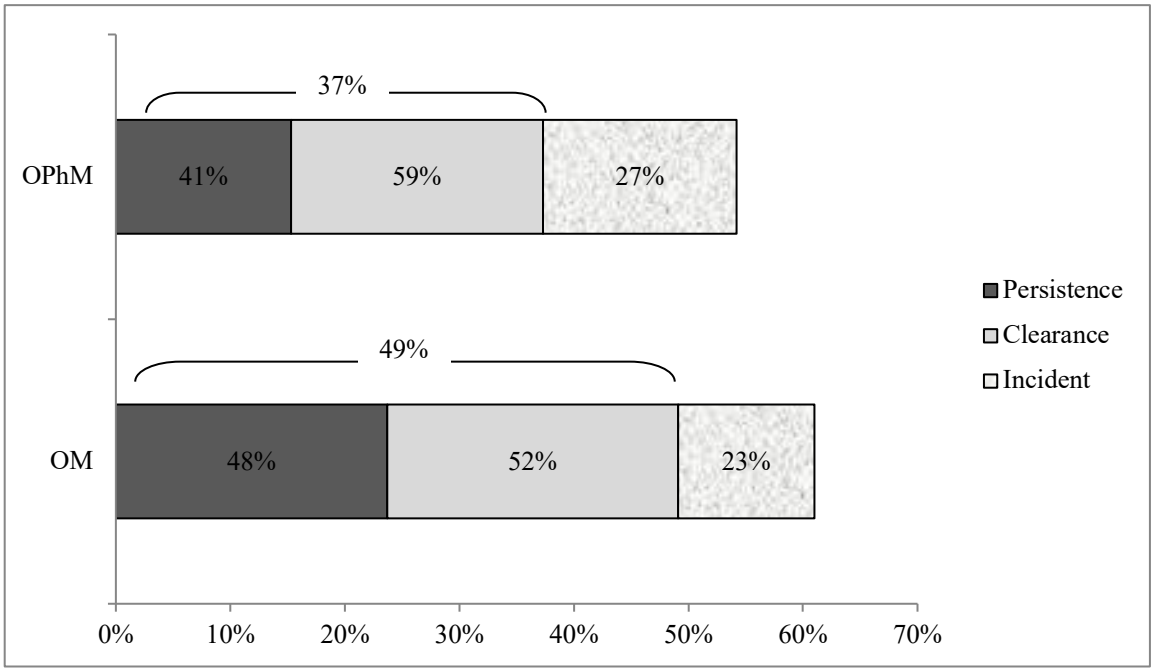

Figure 2. Persistence, clearance and newly detected HPV infection in 59 patients by location. The figure shows higher percentage of HPV^{+} samples in oropharyngeal mucosa (OPhM) that in oral mucosa (OM) at baseline visit (49% *vs*.37%). At the 3-month visit, a slightly higher percentage of HPV persistence was observed in OM (48%) that in OPhM (41%), In contrast, a lower percentage of clearance was detected in OM (52%) than in OPhM (59%) as well as HPV incidence (23% *vs*. 27%, respectively).

Table 4. Multivariate analysis of risk factors for prevalence in 57 OOPhM HPV^{+} patients at baseline.

	HPV DNA Positive		p
	Odds ratio	**(95% CI)**	
Tobacco use			
Yes	3.52	(1.25-9.94)	
No	1.0		**0.017**
VL≥ 20, 000 copies/ml			
Yes	3.60	(1.15-11.23)	
No	1.0		**0.027**
Time HAART use	1.02	(1.01-1.04)	**0.044**

* HAART use were included in the model as continuous variable

Discussion

In this longitudinal study, although a high point prevalence of HPV infection was found at baseline and at the 3-months visit (58.8% & 50.8%), only half of HPV infections corresponded to high risk sequences, and the rest of the patients presented LR-HPV. Important results of the study were the 58% of HPV-persistence and the 42% of clearance found in the 3-months study period. Additionally, tobacco consumption, higher HIV-viral load levels, as well as HAART use and duration were identified as factors associated with OOPh HPV infection.

The included patients were mainly MSM, in their fourth decade of life, comparable to the available data of HIV/AIDS patients in Mexico, that showed that in 2015 that 80.2% of HIV/AIDS individuals were males, at least 60% were men who have sex with men (MSM) (SS/DGE, 2013), and mostly (64.3%) in their third and fourth decade of life (SUIVE/DGE/SS, 2015).

Almost two thirds of the included patients were diagnosed in advanced stages of the disease (AIDS), and were receiving HAART (with a median time of use of one year), higher than the 51% of HAART coverage reported in Mexico (CENSIDA 2013).

At baseline, we identified that 47% of the patients had HIV-associated oral lesions (HIV-OL). In the last decade, different studies (Tamí-Maury et al., 2011; Khatibi et al., 2011; Lourenco et al., 2012; Nittayananta et al., 2010; Ortega et al., 2009; Ferreira et al., 2007) have reported frequencies of HIV-OL that ranging from 19.2% (Giuliani et al., 2008) to 74.5% (Khatibi et al., 2011). Our frequency is similar to the 46.6% informed in a cross-sectional study developed in 73 patients under HAART at least for 6 months (Aquino-Garcíaet al., 2008), and the frequencies found in two previous studies from our study group, 47.1% in Mexican

patients at the post-HAART era (Ramírez-Amador et al., 2006) and a 48.2% in patients attended the same referral center of the present study (Ramírez-Amador et al., 2003). The lack of changes in the frequencies of HIV-OL over time, despite the increase on HAART coverage represents an important topic to consider in future studies.

In our population, in concordance with most worldwide reports (Tamí-Maury et al., 2011; Lourenco et al., 2011; Khatibi et al., 2011; Ortega et al, 2009; Aquino-García et al, 2008; Ferreira et al., 2007; Ramírez-Amador et al., 2003), oral candidiasis (OC) and hairy leukoplakia (HL) were the most frequent HIV-OLs (16% and 13%, respectively). In recent years, OC prevalence has been reported from 3.0% (Khatibi et al., 2011) to 37.8% (Tamí-Maury et al, 2011), and HL from 1.4% (Aquino-García et al., 2008) to 13.4% (Ortega et al., 2009). In our country, a study carried out in the same referral center, with an 82% of HAART coverage (Ramírez-Amador et al., 2003) informed a similar prevalence of HL (14%), and a slightly higher frequency for OC (20.4%).

In regards to oral lesions for human papillomavirus (HPV-OLs), there are few studies about their prevalence/incidence in HIV/AIDS population (Anaya-Saavedra et al., 2012; Lourenco et al., 2011; Ortega et al., 2009; Giuliani et al.. 2008; Ferreira et al., 2007). Most of the published studies corresponded to case reports (Feller et al., 2010; Infante-Cossio et al., 2008; Martini et al., 2007; Aboulafia et al., 2002; Moerman et al., 2001). In the present study, the prevalence found for HPV-OL (8.2%) is higher than the 0.5% previously reported in two studies in HIV-adults (Lourenco et al., 2011; Ortega et al., 2009) and the 4.6% informed in a cross-sectional study (Giuliani et al., 2008), but slightly higher than the 6.9% reported previously (Anaya-Saavedra et al., 2012) in a comparable group of patients. The discrete increment could settle the rise in the prevalence of these lesions among HIV-treated people. (Patton et al., 2013; Patton et al., 2000; Greenspan et al., 2001; Ferreira et al., 2007) .

An interesting finding was the higher DNA quantity obtained in OPhM compared with OM samples, as well as the positive correlation between the number of cells obtained and the DNA yield in OPhM. The oropharyngeal mucosa, in contrast with oral mucosa, presents crypts covered by a reticulated epithelium, without a keratinized superficial layer (Westra 2012), thus, cells are more easily exfoliated, with the consequent obtention of more DNA. It has been postulated that the oropharynx, particularly the tonsils, is a natural reservoir of bacteria and viruses and due to the close contact with lymphoid tissue, could be more susceptible to HPV infection (Herrero 2013).

The prevalence of OOPh HPV infection in our patients (58.8 and 51%), is similar with the 56.7% of OOPh HPV infection (Mooij et al., 2013) informed in a comparable group of HIV^+ males (43% smokers and almost 80% under HAART) with a highly sensitive HPV assay (SPF10-PCR-DEIA-LiPA system). We used a GP5+/GP6+ nested PCR, an assay that increase the positivity, efficiency rate and sensitivity of HPV detection in oral samples (Jalouli et al., 2015). Lower frequencies of OOPhM infection (16%-47%) have been reported in studies among HIV patients with virological and immunological successful response (Kahn et al., 2015; Beachler et al., 2015; Gaester et al., 2015; Darwich et al., 2014; Beachler et al., 2013; Beachler et al., 2012; Parisi et al., 2011; Fakhry et al., 2010; Richter et al., 2008) (Appendix 1).

Almost a half of HPV positive patients had concomitant HPV infection in both OM and OPhM. This could be related to an autoinoculation process, since the virion present in the surface layers facilitate the transfer of infection between nearby surfaces. Also, we need to consider a possible locally impaired immune function in HIV/AIDS patients (Palefsky 2009), which may provide the ideal conditions to establish an infection in different sites.

High risk HPV infection was detected in 30.9 and 27.1% of the patients in baseline and 3-months visits, respectively, comparable with values reported in two cohorts of HIV males: 21% (Beachler et al., 2012) in a cohort with high percentage of smokers (61%) and HAART users (73%); and 26% (Cameron et al., 2005), in a population with moderate HAART use (62%). On the opposite, most studies have been found low frequencies of 10.9% (Fakhry et al., 2010) to 38.2% (Van Aar et al., 2014).

We identified HPV-18 as the most frequent HPV found, in contrast with most studies (Beachler 2015; Van Rijn et al., 2014; Darwich et al., 2014; Mooij et al., 2014; Beachler et al., 2012; Fakhry et al., 2010; D´Souza et al., 2007; Kreimer et al., 2004) that found HPV-16 as the commonest type, with frequencies from 9.5% (Mooij et al., 2013) to 42.8% (Adamapoulou et al., 2008). Few studies have found other HR-HPV types, such as HPV-66 (60% Gaester et al., 2015), HPV-55 (5.8% Fakhry et al., 2006; 4.3% Beachler et al., 2013); and LR-HPV types as HPV-74 (5.8% Mooij et al., 2013). The sequences found in the present cohort contribute with the wide spectrum of HPV types reported in oral and oropharyngeal mucosa of HIV/AIDS patients.

A higher proportion of HPV was found in the OM than in OPhM (46.4% *vs*.38.1%), but the frequencies did not vary when high risk types sequences were considered. As it has been demonstrated, the oropharyngeal mucosa anatomy facilitates HPV infection; hence, the low OPh-HPV prevalence found in the present study could be explained by the low sample size, and could be related with the number of viral copies of HPV at the time of sample collection, a variable that we did not analyze.

Our findings allow us to determine that HIV-patients with tobacco consumption have a 3-fold risk to present OOPh HPV infection. At this respect, recent studies (Muller et al., 2015;

Gaester et al., 2015; Read et al., 2012; Beachler et al., 2012) have confirmed a10-fold risk for OOPhM HPV infection in smoker HIV-patients (Gaester et al., 2015). Although we did not found this association with HR types, other studies have reported a3 to 8-fold risk for HR-OOPhM HPV infection in smokers (D´Souza et al., 2007; Mooij et al., 2013), and a higher risk of persistent infection (OR: 8.0, 95% CI:1.3-53, p=0.002) (D´Souza et al., 2007). Tobacco can stimulate the cell cycle and DNA replication (Merne et al., 2014) and cause local immunological deregulation (Kero 2014), representing a crucial factor to avoid the infection clearance and to facilitate the reactivation of latent infections (Arnson et al., 2010).The relation between tobacco consumption and HR-HPV infection should be observed, due to the important role of both factors in head and neck oncogenesis.

As others (Videla et al., 2013; Beachler et al., 2012; Cameron & Hagensse 2008; Cameron et al., 2005) we found an association between HAART use and OOPhM HPV infection, including the separated analysis for high risk types. Although the main objective of HAART are both viral suppression and immune reconstitution, the immune recovery could be incomplete, thus, it is possible that HPV infection represent the mucosal effect of a prolonged dysfunctional immune system (Patton et al., 2013; Anaya-Saavedra et a.,l 2012; Syrjänen 2011; Cameron and Hagensse 2008; D´Souza et al., 2007) in long term HIV-patients under HAART.

The association between OOPh HPV-infection and high HIV-viral load (HIV-VL) found (>20,000 copies/ml) has been previously reported. Patients with high VL levels (4,000 and 20,000 copies/ml) had at twice risk to HPV infection (95% CI: 1.5-3.5, p= 0.001) (Beachler et al., 2012; Muller et al., 2015). A reflect of HIV replication is the augmented mucosal expression of gap120 and Tat-1 proteins, that could induce disruption in the oral epithelial junctions, facilitating exposure to HPV (Tugizov et al., 2013). Also, HIV-1 Tat protein can

interact with the LCR region of HPV inducing promoter activation and increased expression of E6 and E7 genes, resulting in a loss of cell cycle control and enhances proliferation (Kim et al., 2009; Tornesello et al., 1998).

While in this study we did not found association between OOPhM HPV infection and other factors, other studies have informed that the older age (>50 years) could be a factor for OOPhM HPV (OR=2; 95% CI: 1.4-4.9) (Muller et al., 2015) and HR-HPV infection (OR=3.4; 95% CI: 1.6-7.2 p=0.002) (Mooij et al., 2013). Also, CD4+ counts below 200 cels/µl increased the risk two(95% CI, 1.7-4.1, p< 0.001) (Beachler et al., 2012; Kreimer et al., 2004) and four times (95%CI, 1.3- 15.5) (Muller et al., 2015), reflecting immunosuppression due to its role in coordinating the immune response is decreased by the effect of HIV (Konopnicki et al., 2013 Williams et al., 2002), increased susceptibility of infection , to facilitate replication and HPV establishment (Ferenczy et al ., 2003; Hille et al., 2002).

We found 58.3% (21/36) of HPV persistent infection in the HPV^{+} baseline patients who completed the 3-months visit. At this respect, similarly one study that evaluated viral persistence reported 60% at 6 months (D'Souza et al., 2007). However, a recent study informed 46% of persistence at 6-months (Beachler et al., 2013), that diminished to 29% when patients completed one year of follow-up. It is possible that the relative high frequency found in this study was related with the short time of evaluation, considering that 25 to 26.3 % of oral and oropharyngeal HPV infections disappear during the first six-months (Beachler et al., 2015; Fakhry et al., 2010) (Appendix 2).

The persistence of HPV infection has been associated with age (>45 years) (OR=20, 95%CI: 4.1-83, p<0.002), tobacco use (OR=8, 95% CI: 1.3-53, p=0.002), HAART time of use (>5

years) and viral load (>55,000 copies/ml) (OR=4, 95% CI: 0.2-46. p>0.05) (D'Souza et al., 2007). Whereas we identified a borderline association between HPV persistence and CD4+ counts <200 cell/mm^3, as previously reported (Beachler et al., 2013; D'Souza et al., 2007; Strickler et al., 2005).

On the other hand, 41.7% of the patients cleared HPV at the 3-months visit. Recent studies reported HPV resolution frequencies from 40% (D´Souza et al., 2007) to 54% (Beachler et al., 2013) at 6-months in HIV patients (Appendix 2). It has been hypothesized that the diminution of HPV clearance with older age may be related with age immunity changes (D'Souza et al., 2015; Gravitt et al., 2011). Also, it is possible that resolution represent a transient infection branded by low HPV-viral load (Beachler et al, 2013; Fakhry et al., 2010).

Likewise, in the 3-months visit, 9 (39.1%) of patients presented newly detected infections, higher than the found previously in studies that evaluated incidence in similar populations from 6 months to 2 years. (Beachler et al., 2015; Darwich et al., 2013; Videla et al., 2013; D'Souza et al., 2007). For HPV-16 has been reported a rate of 1.4-3.5/1,000 persons-months (Beachler et al., 2013; Van Aar et al., 2014); for HPV-18: 2.8/1,000 persons-months and LR-HPV: 9.2/1,000 persons-months (Van Rijn et al 2014). Some of the newly detected infections may actually be latent infections (Beachler et al., 2013).The results of the present study did not allow us to differentiate a new infection from the re-expression of a latent infection, besides the highest percentage of new infections detected in our study probably due to the short period of assessment. Similarly with HPV persistence, tobacco consumption has been associated with incident HPV infection (OR=3, 95% CI: 1.4-5.4, p=0.010) (Van Aar et al., 2014) and lower CD4+ (OR= 4, 95% CI: 1.8-6.5, p<0.007) (Beachler et al., 2015).

While we found higher rates of persistence in OM and OPhM, the newly detected infections of HPV was higher in OPhM than in OM. This variation could be taken with caution, considering the short time of evaluation of the present study and the low number of samples in each anatomical site with follow-up.

In conclusion, this study demonstrates a high prevalence of OM and OPhM HPV infection in HIV/AIDS patients, with both high and low risk genotypes. Tobacco consumption, high viral load and HAART were independently associated with any type of oral HPV infection in the multivariate logistic regression model. The high proportion of newly HPV infections, the relative high HPV persistence, and its association with tobacco consumption are important characteristics in the close monitoring of patients with HIV / AIDS, particularly in the new scenario that includes a high risk to develop head and neck cancer.

Limitations of this study are the small sample size and the loss of the patients for the second visit, which limited the follow-up period and analysis with variables of interest.

The longitudinal design of this study showed significant findings for knowledge of HPV in oral and oropharyngeal mucosa, so it could be a reference for future studies. Research involving more subjects and visits could clarify more risk factors for persistent HPV infection in HIV/AIDS patients.

References

Aboulafia DM. Condyloma acuminatum presenting as a dorsal tongue lesion in a patient with AIDS. *AIDS Read* 2002; 12(4):165-7, 172-3.

Adamopoulou M, Vairaktaris E, Panis V, Nkenke E, Neukam FW, Yapijakis C. HPV detection rate in saliva may depend on the immune system efficiency. *In Vivo* 2008; 22(5): 599-602.

Anaya-Saavedra G, Flores-Moreno B, García-Carrancá A, Irigoyen-Camacho E, Guido Jiménez Miriam, Ramírez-Amador V. HPV oral lesions in HIV-infected patients: the impact of long-term HAART. *Oral Pathol Med*. 2012; 42(6): 443-449.

Anaya-Saavedra G, Ramírez-Amador V, Irigoyen-Camacho ME, García-Cuellar CM, Guido-Jiménez M. High association of human papillomavirus infection with oral cancer: a case-control study. *Arch Med Res* 2008; 39(2):189-97.

Aquino-García SI, Rivas MA, Ceballos-Salobrena A, Acosta-Gio AE, Gaitan-Cepeda LA. Short communication: oral lesions in HIV/AIDS patients undergoing HAART including efavirenz. *AIDS Res Hum Retroviruses* 2008; 24: 815–820.

Arnson Y, Shoenfeld Y, Amital H. Effects of tobacco smoke on immunity, inflammation and autoimmunity. *J Autoimmun* 2010; 34: 258-265.

Beachler DC, Viscidi R, Sugar EA, Minkoff H, Strickler HD, Cranston RD, Wiley DJ, Jacobson LP, Weber KM, Margolick JB, Reddy S, Gillison ML, D'Souza G. A longitudinal study of human papillomavirus 16 L1, e6, and e7 seropositivity and oral human papillomavirus 16 infection. *Sex Transm Dis* 2015; 42(2): 93-7.

Beachler DC, Weber KM, Margolick JB, Strickler HD, Cranston RD, Burk RD, Wiley DJ, Minkoff H, Reddy S, Stammer EE, Gillison ML, D'Souza G.Risk factors for oral HPV infection among a high prevalence population of HIV-positive and at-risk HIV-negative adults. *Cancer Epidemiol Biomarkers Prev* 2012; 21(1):122-33.

Beachler DC, Guo Y, Xiao W, Burk RD, Minkoff H, Strickler HD, Cranston RD, Wiley DJ, Jacobson LP, Weber KM, Margolick JB, Sugar EA, Reddy S, Gillison ML, D'Souza G. High Oral Human Papillomavirus Type 16 Load Predicts Long-term Persistence in Individuals With or at Risk for HIV Infection. *J Infect Dis* 2015.

Bere A, Tayib S, Kriek JM, Massona L, Jaumdally S. Altered phenotype and function of NK cells infiltrating Human Papillomavirus (HPV)-associated genital warts during HIV infection. *Clinical Immunology* 2014; 15: 210–219.

Cameron JE, Hagensee ME. Oral HPV complications in HIV-infected patients. *Curr HIV/AIDS Reports* 2008; 5: 126–131.

Cameron JE, Mercante D, O´Brien M *et al*. The impact of highly active antiretroviral therapy and immunodeficiency on human papillomavirus infection of the oral cavity of human immunodeficiency virus-seropositive adults. *Sex Transm Dis* 2005; 32: 703-9.

CENSIDA. Vigilancia Epidemiológica de casos de VIH/SIDA en México. 2015.

CENSIDA. Vigilancia Epidemiológica de casos de VIH/SIDA en México. 2013.

Chaturvedi A, Engels E, Pfeiffer R, Hernández B, Xiao W, Kim E, Jiang B, Goodman M, Siburg-Saber M, Anderson W, Rosenberg P, Gillison M. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J ClinOncol* 2011; 29: 4294–4301.

Chung CH, Bagheri A, D'Souza G. Epidemiology of oral human papillomavirus infection. *Oral Oncol* 2014; 50(5):364-9.

Colon-López V, Quiñones-Avila V, Del Toro-Mejías LM, Reyes K, Rivera ME, *et al*. Oral HPV infection in a clinic-based sample of Hispanic men. *BMC Oral Health* 2014; 14(1):7.

Darwich L, Cañadas M, Videla S, Coll J, Molina-López R, Cobarsi P, Sirera G, Clotet B. Oral human papillomavirus type-specific infection in HIV-infected men: a prospective cohort study among men who have sex with men and heterosexual men. *Clin Microbiol Infect* 2014; 20(9): O585-O589.

Da Silva CA, Dourado I, Dahia SR, Harzheim E, Rutherford GW. Oral manifestations of HIV infection in patients receiving highly active antiretroviral therapy (HAART) in Bahia, Brazil. *J Public Health Dent* 2008; 68: 178–181

Del Mistro A, Baboci L, Frayle-Salamanca H, Trevisan R, Bergamo E, *et al*. Oral human papillomavirus and human herpesvirus-8 infections among human immunodeficiency virus type 1-infected men and women in Italy. *Sex Transm Dis* 2012;39(11):894-8

D'Souza G, Agrawal Y, Halpern J, Bodison S, GillisonML. Oral sexual behaviors associated with prevalent oral human papillomavirus infection. *J Infect Dis* 2009; 199(9):1263-9.

D'Souza G, Dempsey A. The role of HPV in head and neck cancer and review of the HPV vaccine. *Prev Med* 2011; 53(1): 5-11.

D´Souza G, Fakhry C, Sugar EA, *et al*. Six month natural history of oral versus cervical human papillomavirus infection. *Int J Cancer* 2007; 121 (1): 143-150.

Dubrow R, Silverberg MJ, Park LS, Crothers K, Justice AC. HIV infection, aging, and immune function: implications for cancer risk and prevention. *Curr Opin Oncol* 2012; 24(5): 506-16.

Fakhry C, Sugar E, D'Souza G, Gillison M. Two-week versus six-month sampling interval in a short-term natural history study of oral HPV infection in an HIV-positive cohort. PLoS ONE 2010; 5(7): e11918.

Fakhry C, D'souza G, Sugar E, Weber K, Goshu E, Minkoff H, Wright R, Seaberg E, Gillison M. Relationship between prevalent oral and cervical human papillomavirus infections in human immunodeficiency virus-positive and -negative women. *J ClinMicrobiol* 2006; 44(12):4479-85.

Feller L, Khammissa RA, Wood NH, Malema V, Meyerov R, Lemmer J. Focal epithelial hyperplasia (Heck disease) related to highly active antiretroviral therapy in an HIV-seropositive child. A report of a case, and a review of the literature. *SADJ*. 2001; 65(4):172-5.

Ferenczy A, Coutlée F, Franco E, Hankins C. Human papillomavirus and HIV coinfection and the risk of neoplasias of the lower genital tract: a review of recent developments. *CMAJ* 2003; 169(5):431-4.

Ferreira S, Noce C, Junior AS, Goncalves L, Torres S, Luiz R, Meeks V, Díaz E. Prevalence of oral manifestations of HIV infection in Rio De Janeiro, Brazil from 1988 to 2004. *AIDS Patient Care STDS* 2007; 21: 724–731.

Gaester K, Fonseca LA, Luiz O, Assone T, Fontes AS, Costa F, Duarte AJ, Casseb J. Human papillomavirus infection in oral fluids of HIV-1-positive men: prevalence and risk factors. *Sci Rep* 2015; 4:6592.

Gillison ML, Castellsagué X, Chaturvedi A, Goodman MT, Snijders P, Tommasino M, Arbyn M, Franceschi S. Eurogin Roadmap: comparative epidemiology of HPV infection and associated cancers of the head and neck and cervix. *Int J Cancer* 2014; 134(3):497-507.

Gillison ML. Oropharyngeal cancer: a potential consequence of concomitant HPV and HIV infection. *Curr Opin Oncol* 2009; 21: 439–44.

Giuliani R, Tortolero-Luna G, Ferre E. Burchell AN, de Sanjosé S, *et al*. Epidemiology of human papillomavirus infection in men, cancers other that cervical and benign coditions. *Vaccine* 2008. 26: 17-28.

Gravitt PE. The known unknowns of HPV natural history. *J Clin Invest* 2011; 121: 4593-4599.

Greenspan D, Canchola AJ, MacPhail LA, Cheikh B, Greenspan JS. Effect of highly active antiretroviral therapy on frequency of oral warts. *Lancet.* 2001; 357: 1411–1412

Hamza OJ, Matee MI, Simon EN *et al*. Oral manifestations of HIV infection in children and adults receiving highly active anti-retroviral therapy [HAART] in Dares Salaam, Tanzania. *BMC Oral Health* 2006; 6: 12.

Herrero R. Human Papillomavirus and Cancer of the Upper Aerodigestive Tract. *J Nat Cancer Inst Mon.* 2003; 31 (47): 47-51.

Hille JJ, Webster-Cyriaque J, Palefski JM, Raab-Traub N. Mechanisms of expression of HHV8, EBV and HPV in selected HIV-associated oral lesions. *Oral Dis* 2002; 8 (2):161-8.

Jalouli M, Jalouli J, Ibrahim SO, Hirsch JM, Sand L. Comparison between single PCR and nested PCR in detection of human papilloma viruses in paraffin-embedded OSCC and fresh oral mucosa. *In Vivo* 2015; 29(1):65-70.

Infante-Cossio P, Gonzalo DH, Hernandez-Gutierrez J, Borrero-Martin JJ. Oral inverted ductal papilloma associated with condyloma acuminata and HPV in an HIV+ patient. Int J Oral Maxillofac Surg 2008; 37(12):1159-61.

Kahn JA, Rudy BJ, Xu J, Secord EA, Kapogiannis BG, Thornton S, Gillison ML. Behavioral, immunologic, and virologic correlates of oral human papillomavirus infection in HIV-infected youth. *Sex Transm Dis* 2015 May; 42(5):246-52.

Kakabadze T, Rukhadze N, Mshvidobadze K, Lomtadze M, Kandelaki G. Oral lesions in HIV-positive patients in Georgia. *Georgian Med News* 2008; 12: 60–65.

Kero K, Rautava J, Syrjänen K, Grenman S, Syrjänen S. Oral mucosa as a reservoir of human papillomavirus: point prevalence, genotype distribution, and incident infections among males in a 7-year prospective study. *Eur Urol* 2012; 62: 1063–1070.

Khatibi M, Moshari A, Jahromi Z, Ramezankhani A. Prevalence of oral mucosal lesions and related factors in 200HIV+/AIDS Iranian patients. *J Oral PatholMed* 2011; 40: 659–664

Kim R, Yochim J, Kang M, Shin K, Christensen R, ParkN. HIV-1 Tat enhances replicative potential of human oral keratinocytes harboring HPV-16 genome. *Int J Oncol* 2008; 33:777–782.

Konopnicki D, Manigart Y, Gilles C, Barlow P, de Marchin J, *et al*. Sustained viral suppression and higher CD4+ T-cell count reduces the risk of persistent cervical high-risk human papillomavirus infection in HIV-positive women. *J Infect Dis* 2013; 207(11): 1723-9.

Kreimer AR, Pierce Campbell CM, Lin HY, Fulp W, Papenfuss MR, Abrahamsen M, Hildesheim A, Villa LL, Salmerón JJ, Lazcano-Ponce E, Giuliano AR. Incidence and clearance of oral human papillomavirus infection in men: the HIM cohort study. *Lancet* 2013; 382(9895):877-87.

Kreimer AR, Alberg AJ, Daniel R, Gravitt PE, Viscidi R, Garrett ES, Shah KV, Gillison ML. Oral human papillomavirus infection in adults is associated with sexual behavior and HIV serostatus. *J Infect Dis* 2004; 189(4):686-98

Kreimer AR, Villa A, Nyitray AG, Abrahamsen M, Papenfuss M, Smith D, Hildesheim A, Villa LL, Lazcano-Ponce E, Giuliano AR. The epidemiology of oral HPV infection among a multinational sample of healthy men. *Cancer Epidemiol Biomarkers Prev* 2011; 20(1):172-82.

Kreimer AR, Bhatia RK, Messeguer AL, González P, Herrero R, Giuliano AR.Oral human papillomavirus in healthy individuals: a systematic review of the literature. *Sex Transm Dis* 2010; 37(6):386-91.

Lima MD, Braz-Silva PH, Pereira SM, Riera C, Coelho AC, Gallottini M. Oral and cervical HPV infection in HIV-positive and HIV-negative women attending a sexual health clinic in São Paulo, Brazil. *Int J Gynaecol Obstet* 2014; 126(1):33-6.

Lourenco AG, Motta AC, Figueiredo LT, Machado AA, Komesu MC. Oral lesions associated with HIV infection before and during the antiretroviral therapy era in Ribeirao Preto, Brazil. *J Oral Sci* 2011; 53: 379–385.

Marais DJ, Passmore JA, Denny L, Sampson C, Allan BR, Williamson AL. Cervical and oral human papillomavirus types in HIV-1 positive and negative women with cervical disease in South Africa. *J Med Virol* 2008; 80: 953–959.

Martini S, Colella G, Masiello A, Lanza A, Pisapia R, Cascone A, Di Martino F, Filippini A, Filippini P. HPV oral infection. Case report of an HIV-positive Nigerian sex worker. *Infez Med* 2007; 15(2):115-8.

Merne M, Rautava J, Ruutu M, Syrjänen S. Smokeless tobacco increases aneuploidy in oral HPV16 E6/E7-transformed keratinocytes in vitro. *J Oral Pathol Med* 2014; 43(9):685-90.

Moerman M, Danielides VG, Nousia CS, Van Wanzeele F, Forsyth R, Vermeersch H. Recurrent focal epithelial hyperplasia due to HPV13 in an HIV-positive patient. *Dermatology* 2001; 203(4):339-41.

Mooij SH, Boot HJ, Speksnijder AG, Meijer CJ, King AJ, Verhagen DW, de Vries HJ, Quint WG, Molijn A, de Koning MN, van der Sande MA, van der Loeff MF. Six-month incidence and persistence of oral HPV infection in HIV-negative and HIV-infected men who have sex with men. PLoS One 2014; 9(6): e98955.

Mooij SH, Boot HJ, Speksnijder AG et al. Oral human papillomavirus infection in HIV-negative and HIV-infected men who have sex with men: the HIV & HPV in MSM (H2M) study. *AIDS* 2013; 27:2117–2128

Muller K, Kazimiroff J, Fatahzadeh M, Smith RV, Wiltz M, Polanco J, Grossberg RM, Belbin TJ, Strickler HD, Burk RD, Schlecht NF. Oral Human Papillomavirus Infection and Oral Lesions in HIV-Positive and HIV-Negative Dental Patients. *J Infect Dis* 2015; 212(5):760-8.

Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ; International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003; 348(6):518-27.

Nittayananta W, Talungchit S, Jaruratanasirikul S. Effects of long-term use of HAART on oral health status o fHIV-infected subjects. *J Oral Pathol Med* 2010; 39: 397–406.

Ong JJ, Read TRH, Vodstrcil LA, Walker S, Chen M. Detection of Oral Human Papillomavirus in HIV-Positive Men Who Have Sex with Men 3 Years after Baseline: A Follow Up Cross-Sectional Study. PLoS ONE 2014; 9(7): e102138.

Ortega KL, Vale DA, Magalhaes MH. Impact of PI and NNRTI HAART-based therapy on oral lesions of Brazilian HIV-infected patients. *J Oral Pathol Med* 2009 38: 489–494.

Palefsky J. Human papillomavirus –related disease in people with HIV. *CurrOpin HIV AIDS* 2009; 4: 52-6.

Palefsky J. Biology of HPV in HIV infection. *AdvDentRes* 2006; 19: 99–105.

ParisiSG, Cruciani M, Scaggiante R, Boldrin C, Andreis S, Dal Bello F, Paigni S, Barelli A, Sattin A, Mengoli C, Palú G. Anal and oral human papillomavirus (HPV) infection in HIV-infected subjects in northern Italy: a longitudinal cohort study among men who have sex with men. *BMC Infect Dis* 2011; 11: 150.

Patton L, Ramirez-Amador V, Anaya-Saavedra G, Nittayananta W, Carrozzo M, Ranganathan K. Urban legends series: oral manifestations of HIV infection. *Oral Diseases* 2013; 19: 533–550.

Patton LL. Sensitivity, specificity, and positive predictive value of oral opportunistic infections in adults with HIV/AIDS as markers of immune suppression and viral burden. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000; 90(2):182-8.

Ramírez-Amador V, Ponce-de-León S, Anaya-Saavedra G, Crabtree Ramírez B, Sierra-Madero J. Oral lesions as clinical markers of highly active antiretroviral therapy failure: a nested case-control study in Mexico City. *Clin Infect Dis* 2007; 45: 925–932.

Ramírez-Amador V, Esquivel-Pedraza L, Sierra-Madero J, Anaya-Saavedra G, González-Ramírez I, Ponce-de-León S. The changing clinical spectrum of human immunodeficiency virus (HIV)–related oral lesions in 1,000 consecutive patients: a twelve-year study in a referral center in Mexico. *Medicine* 2003; 82:39–50.

Read TR, Hocking JS, Vodstrcil LA *et al*. Oral human papillomavirus in men having sex with men: risk-factors and sampling. PLoS ONE 2012; 7(11): e49324.

Richter K, van Rensburg E, Van Heerden W, Boy S. Human papilloma virus types in the oral and cervical mucosa of HIV-positive South African women prior to antiretroviral therapy. *J Oral Pathol Med* 2008; 37(9): 555–559.

Shiboski CH, Patton LL, Webster-CyriaqueJY. The oral HIV/AIDS research alliance: updated case definitions of oral disease endpoints. *J Oral Pathol Med* 2009; 38(6): 481–488.

Shiels MS, Pfeiffer RM, Gail MH, *et al*. Cancer burden in the HIV-infected population in the United States. *J Natl Cancer Inst* 2011; 103:753–62.

Steinau M, Reddy D, Sumbry A. Oral sampling and human papillomavirus genotyping in HIV-infected patients. *J Oral Pathol Med* 2012; 41: 288–291.

Syrjänen S. Human papillomavirus infection and its association with HIV. *Adv Dent Res* 2011; 23: 84–89.

SUIVE/DGE/SS, SUIVE/DGE/SS. Sistema de Vigilancia Epidemiológica de VIH/SIDA. 2015.

Tami-Maury IM, Willig JH, Jolly PE. Prevalence, incidence, and recurrence of oral lesions among HIV-infected patients on HAART in Alabama: a two-year longitudinal study. *South Med J* 2011; 104: 561–566.

Thompson MA, Aberg JA, Cahn P *et al*. International AIDS Society-USA. Antiretroviral treatment of adult HIV infection: 2010 recommendations of the International AIDS Society-USA panel. *JAMA* 2010; 304: 321–33.

Strickler HD, Burk RD, Fazzari M, Anastos K, Minkoff H, *et al*. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women. J Natl Cancer Inst 2005; 97(8): 577-86.

Tugizov SM, Herrera R, Chin-Hong P, Veluppillai P, Greenspan D., *et al*. HIV-associated disruption of mucosal epithelium facilitates paracellular penetration by human papillomavirus. *Virology* 2013; 446: 378–388.

Van Aar F, Mooij SH, van der Sande MA, Speksnijder AG, Stolte IG *et al*. Twelve-month incidence and clearance of oral HPV infection in HIV-negative and HIV-infected men who have sex with men: the H2M cohort study. *BMC Infectious Diseases* 2014; 14:668.

Videla S, Darwich L, Ca~nadas MP *et al*. HIV-HPV Can Ruti Study Group. Natural history of human papillomavirus infections involving anal, penile, and oral sites among HIV-positive men. *Sex Transm Dis* 2013; 40: 3–10.

Van Rijn VM, Mooij SH, Mollers M, Snijders PJF, Speksnijder AGCL, *et al*. Anal, Penile, and Oral High-Risk HPV Infections and HPV Seropositivity in HIV-Positive and HIV-Negative Men Who Have Sex with Men. PLoS ONE 2014; 9(3): e92208.

Westra W. The Morphologic Profile of HPV-Related Head and Neck Squamous Carcinoma: Implications for Diagnosis, Prognosis, and Clinical Management. *Head and Neck Pathol* 2012; 6:S48–S54.

APPENDICES

Appendix 1. Prevalence of HPV infection in the oral and oropharyngeal mucosa of HIV/AIDS adult patients. Cross-sectional studies.

Author	Type of specimen	N	Gender	On HAART%	HPV %	HR-HPV %	HPV-16%
Gaester et al., 2015	Oral rinse	283	M	83.0	3.5	-	10.0*
Kahn et al., 2015	Oral rinse	272	M:F	-	19.5	11.0	17.0*
Muller et al., 2015	Oral rinse	161	M:F	-	32.0	17.0	23.5*
Van Rijn et al., 2014	Oral rinse	306	M	87.4	-	17.3	27.0*
Lima et al., 2014	Oral rinse	100	M	-	11.0	8.0	-
Colon – López et al 2014	Oral rinse	103	M	-	22.3	-	-
Fatahzadeh et al., 2013	Oral rinse	52	M:F	38.0	38.0	23.0	
Mooij et al., 2013	Oral rinse	314	M	87.3	56.7	24.8	9.5*
Beachler et al., 2012	Oral rinse	379	M	73.3	40.0	21.0	15.2*
Stenieu et al., 2012	Oral rinse	100	M	92.0	39.0	24.0	-
Del Mistro et al., 2010	Saliva	100	M:F	-	37.0	13.0	-
Adamapoulou et al., 2008	Saliva	34	-	-	35.3	20.6	42.8*
Marais et al., 2008	Cytobrush	37	F	-	33.0	12.1	9.0
Richter et al., 2008	Cytobrush	30	F	0.0	20.0	6.6	-
Fakhry et al., 2006	Oral rinse	143	F	-	25.2	14.0	5.1
Cameron et al., 2005	Saliva	95	M	62.0	37.0	26.0	-
Kreimer et al., 2004	Oral rinse	190	M:F	-	25.3	13.7	33.3*

G= gender; M= male; F= female; HR-HPV= High risk HPV infection. *The most common type found

Appendix 2. HPV infection in the oral and oropharyngeal mucosa of HIV/AIDS adult patients. Longitudinal

Author	Type of specimen	N	G	HAART %	HPV %	HR-HPV%	HPV-16%	P%	time	C%[1]	tim
Beachler et al., 2015	Oral rinse	761	F:M	79.0	35.0	22.0	13.6*	35.0	2 years	51.0	1 y
Ong et al., 2014	Oral rinse	249	M	94.0	17.0	9.0	26.6*	47.0 40.0[b] 50.0[c]	3 years	14.7/100 py 18.2/100 py 17.4/100 py	
Mooij et al., 2014	Oral rinse	276	M	87.8	-	23.9	-	48.5[b]	6 months	-	
Van Aar etal., 2014	Oral rinse	290	M	-	-	38.2	-	22.5[b]	1 year	53.2[b]	1 y
Beachler y cols., 2013	Oral rinse	404	M:F	68.0	28.0	13.0	8.3	46.0 29.0	6 months 12months	54.0	6 m
Darwich et al., 2013	Oral rinse	650	M	-	16.0	-	30.0*	-		44.0 18.9/1000 p	2 y
Videla et al., 2013	Cytobrush/ oral rinse	650	M	83.0	16.5	15.0	-	-		44.0	1 y
Read et al., 2012	Oral rinse	249	M	-	19.0	8.0	8.3	83.0	6 months	-	
Parisi et al., 2011	Oral rinse	166	M	63.0	20.1	11.1	3.7	-		-	
Fakhry et al., 2010	Oral rinse	112	M	-	45.0	10.9	21.4*	78.0 33.1	6 months 2 weeks	22.0 66.9	6 m 2 w
D´Souza et al., 2007	Oral rinse	136	M	65.0	24.0	15.0	23.3	60.0	6 months	40.0	6 m

G= Gender; P= Persistence; C= Clearance; I= Incidence; pm= persons-months; py= person-years
[1] Some included rates of clearance; [2]Some included rates of incidence * The most common type found; [c] Corresponds to HR-HPV; [c] Corresponds to HPV-16