Association between carcinogenic HPV and selected STIs and risk behaviors in HIV positive men who have sex with men in Lima, Peru

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BACKGROUND

Human Immunodeficiency Virus (HIV) infection in Peru is concentrated among men who have sex with men (MSM), with a seroprevalence which varies between 13.9% and 22.3% [1, 2]. Several risk factors for HIV transmission in this population have been described, such as co-infection with other sexually transmitted infections (STI) or practicing unprotected sex [1]. Among the STI that could co-infect an HIV positive individual, human papillomavirus (HPV) could be of increased importance given the potential carcinogenic impact of this infection upon the host.

Over the last 25 years, rates of anal cancer due to HPV have increased in the MSM population. Compared to the general population, HIV positive men are at 60.1 higher risk of developing anal carcinoma [3]. A study carried out in Spain found that HIV positive men without warts or visible lesions had high rates of anal HPV infection (78%) [4]. Studies in the U.S. have identified prevalences as high as 94% [5] and a recent meta-analysis on the topic showed an overall prevalence of 92.6% among HIV-positive individuals[6]. Although symptoms or macroscopic lesions may be absent, the presence of HPV can lead to anal cytology abnormalities such as anal intraepithelial lesions (AIL) or more developed neoplasias. Currently, fifteen types of HPV types have been described as carcinogenic or high-risk types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 [7, 8]; three have been described as probably of high-risk (26, 53, 66) and twelve were classified as low risk types (6, 11,40, 42, 43, 44, 54, 61, 70, 72, 81 and CP6108)[8].

Other viruses able to co-infect HIV positive individuals are the Human T-cell lymphotropic viruses type 1 and 2 (HTLV-1/2). Given the impact that these viruses have on the hosts' immune system, they could further increase the risk of developing neoplasias in those infected with HPV.

HTLV-1 and -2 were the first retroviruses described in humans [9, 10]; in Peruvian MSM, the seroprevalences of HTLV-1 and HTLV-2 are 6% [11, 12] and 1.3% [13, 14] respectively. As with HIV, risk factors for acquiring these retroviruses include unprotected anal intercourse and higher number of male sexual partners. However, contrary to what is seen with HIV infection, risk of HTLV-1/2 infection increases with age [13-15].

A previous study in a Peruvian MSM population described co-infection by these three retroviruses. This study found that 3.1% of participants had HTLV-1 and/or 2 infection, and 12.4% were co-infected with HIV [16].

Bacterial STI's have been previously described as an important gateway for [17] and strongly associated with HIV in the Peruvian MSM population. Among these, syphilis has proven to be one of the leading pathogens [1]. A previous study showed incidences as high as 8.4 per 100 person-years [2]. Chlamydial and gonococcal infections have also been extensively studied in this population, with prevalences ranging between 1.7% and 10%, depending on the sexual role of the individual (passive vs. active, respectively) [18].

All of the STI previously described are transmitted within complex sexual networks, which typically occur in individuals who exclusively play a receptive or an insertive sexual role. However, the MSM population in Lima is unique because most MSM have a versatile role (insertive and receptive), increasing the probability of identifying individuals with anal-rectal infection of HPV.

The aim of this study was to assess for an association between the presence of carcinogenic types of HPV and infection with selected STI's in HIV positive individuals.

HYPOTHESIS

- Null: There is no significant difference in the proportion of individuals with carcinogenic types of HPV between HIV positive individuals with and without co-infection with selected STIs.
- Alternative: There is a higher prevalence of carcinogenic types of HPV in HIV positive individuals co-infected with selected STIs.

STUDY OBJECTIVES

This study objectives were to define the prevalence of HPV (high-risk and low-risk types) in HIV-infected men who have sex with men, to determine if co-infection with selected STIs increased the risk of carcinogenic types of HPV, and to determine if some behavioral risks have an impact on the presence of carcinogenic types of HPV.

METHODS

Ethics

The data and samples presented here were obtained within the framework of the protocol "Determinants of HIV shedding in the anal-rectal canal in men who have sex with men with

attention to co-infection with Human T-cell lymphotropic virus I/II (HTLV- 1/2) and Human Papillomavirus (HPV)" – NAMRU6.2011.006. This protocol was reviewed and approved by the Institutional Review Boards of the Naval Medical Research Unit No.6 (NAMRU-6), the University of Washington and the NGO "Asociacion Civil Impacta".

Study Population

HIV-positive men who have sex with men (MSM) from Lima were invited to participate in this study at NGO and STI clinics providing healthcare to MSM. Only individuals over 18 years of age were enrolled. All participants underwent an informed consent process. As part of this process, each potential participant received an informed consent document which he had to sign in order to participate in the study. Individuals who had had any anal-rectal procedure in the previous two weeks were excluded from the study.

Study Design

Cross sectional study to assess the association between the presence of carcinogenic types of HPV and infection with selected STIs in HIV positive individuals

Study Activities

a) Enrollment

Individuals were approached at STI clinics and NGOs currently providing healthcare to the MSM population. Each potential subject was invited to participate by his treating physician. If a potential subject was willing to participate, he would go through the informed consent process. Several issues were addressed by the counselor during the process. It was made clear to all consenting participants that if he decided not to participate, he would not be jeopardizing any other right to health care to which he was entitled. The counselor also explained that he could also drop out of the study at any time without affecting the care he received.

Following the enrollment, the participants were asked to complete a questionnaire, give a blood sample of approximately 15 ml, and undergo a rectal examination with swabbing. The questionnaire assessed demographic data, medical health history and previous HIV diagnosis and antiretroviral treatment (ARV); it was administered by the treating physician. A blood sample of 15 ml was obtained by venipuncture from the participant's arm for serology testing after pre-test

counseling for HTLV was provided. This sample was used for the diagnosis of HTLV and syphilis. A urine sample was also requested to test for the presence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Those with symptoms related to either infection received treatment provided by the national STI program. Finally, a Dacron swab was inserted approximately 2-3 cm into the rectal canal to obtain a sample for HPV testing. Once the swab was inserted, it was turned clockwise 360°. Then, as the swab was withdrawn, it was turned counter clockwise 360°.

b) Results visits

Participants were asked to return to the health facility 2 weeks after the samples had been obtained to obtain results for syphilis, chlamydia and gonorrhea and receive counseling; individuals with positive results for any of these infections received treatment. Participants were also asked to return 3 months later to receive HPV and/or HTLV post-test counseling and their results. Those who were HPV and/or HTLV positive were referred for follow-up and eventual management.

Laboratory Methods

Serology: HTLV diagnosis was obtained by ELISA screening and Western Blot confirmation. For syphilis, screening was carried out using rapid plasma regain (RPR), and confirmation was through *Treponema pallidum* Haemagglutination assay (TPHA).

Urine: the APTIMA Combo 2 assay (Gen Probe), which is an amplification test, was used for the qualitative *in vitro* detection and differentiation of ribosomal RNA of *Chlamydia trachomaties* and *Neisseria gonorrhoeae.*

HPV diagnosis by PCR: rectal specimens were collected in 1.0 ml of specimen transport medium (Digene Diagnostics, Silver Spring, MD), HPV DNA was extracted and PCR analysis performed according to the single-hybridization, reverse line blot detection method as described by Gravitt, et al. [19]. This HPV assay can detect 27 individual HPV genotypes (HPV Types: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51 to 59, 66, 68, MM4, MM7, MM8, and MM9).

In accordance with previous publications, the following were considered high risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 [7, 8].

Statistical analysis

The data obtained using the questionnaire was entered into a database using Microsoft Access and analyzed using Stata/SE 10.0 for Windows (StataCorp LP; College Station, TX). T-test of means and Chi-square tests were used to compare means and assess associations between specific risk factors and presence of carcinogenic types of HPV, respectively. A multiple logistic regression model was applied to assess variables found to be associated to the presence of carcinogenic types of HPV in the bivariate analysis; p-values ≤ 0.05 were considered statistically significant.

RESULTS

Characteristics of study population. This cross-sectional study enrolled 152 HIV positive individuals between January 17 and March 19, 2012 in Lima, Peru. Their mean age was 33.4 years (SD 8.2; range: 20-58, median: 32); most participants were born in Lima (55.9%) the rest were evenly distributed in different regions of the country and only one participant came from abroad (Bolivia). Other demographic data is shown in Table 1.

The mean age for the first sexual intercourse was 15.4 years (SD 3.8; range: 3-25, median: 16), and currently all participants consider themselves either "gay" (115; 75.7%) or bisexual (37; 24.3%). Among the bisexual group, 14 had had at least one female sexual partner in the previous three months (8 had one female sexual partner, 5 had between two and five and 1 did not answer). When asked about the number of lifetime sexual partners (regardless of gender), 51 participants (33.6%) had 10 partners or less, 47 (30.9%) had between 11 and 50 partners, 28 (18.4%) had between 51 and 100, 10 (6.6%) between 101 and 200, and 16 (10.5%) over 201. During the 3 months prior to the study, 18 participants (11.8%) answered not having sexual partners, 57 (37.5%) referred having 1, 53 (34.9%) between 2 and 5, 8 (5.3%) between 6 and 10, 4 (2.6%) between 11 and 20, and 12 (7.9%) over 20 sexual partners. Most participants (131; 86.2%) had at least one male sexual partner in the 3 months previous to enrollment; 56 (42.8%) had one, 51 (38.9%) had between 2 and 5 and the remaining 24 (18.3%) had six or more. While over a third of the participants have a steady relationship (Table 1), 86 (56.6%) regularly had sex with someone other than their partner.

Regarding sexual practices, 142 participants (93.4%) reported having had receptive anal intercourse at least once, however around half of the participants (50.9%) reported having a

versatile role in bed (half the time insertive and half the time receptive). On both extremes, 10 (6.6%) and 22 (14.6%) participants described themselves as exclusively insertive and exclusively receptive respectively. The remaining participants were either mainly insertive (12.6%) or mainly receptive (15.2%). In the previous three months, 112 individuals (73.7%) practiced receptive anal intercourse, 47 (30.9%) with a single partner, 26 (30.3%) had between 2 and 5 partners, 9 (5.9%) between 6 and 10, 2 (1.3%) between 11 and 20, and 8 (5.3%) with more than 20 partners. Of these 112, 32 (28.6%) had unprotected receptive anal intercourse with one partner, 19 (16.9%) with between 2 and 5 partners, and 2 (1.8%) with between 6 and 10. However, all participants answered that they knew where to get condoms, but only 97 (64. 7%) answered that they always used a condom during anal sex. A similar proportion of participants did not use a condom in either their last insertive or receptive anal intercourse (18.4% and 17.9% respectively).

When asked about ever receiving money, food lodging or drugs in exchange for sex, 36 (23.7%) participants answered that they had, 17 of these in the last 6 months; all of them reported using a condom. Seven participants reported sex-work as their primary source of income, and the average number of clients of those who practiced sex-work was 25.9 per week (SD 20.9).

In the month prior to enrollment, most participants did not have alcohol or drugs before or during sex (105 (69.1%) and 147 (96.7%), respectively); 24% of those who consumed alcohol had some level of difficulty when using a condom, while those who consumed drugs did not report any difficulty.

The study survey contained several questions about signs and symptoms associated with STIs, health seeking behavior if an STI was identified, and HIV status. Eighty participants (52.63%) had one or more of the following signs in the six months prior to the study: secretion or pus discharge from the penis, genital or anal wounds, genital or anal warts, and/or pus or bloody discharge from the rectum (Table 2); 43 of these 80 participants (53.85%) sought treatment; most sought medical assistance at a private health facility (23; 53.49%), 12 went to a public health facility (27.9%), 6 (13.9%) consulted the pharmacy dispatcher, and 2 (4.7%) self-medicated.

HIV status. Regarding the HIV status of participants, the mean time from HIV diagnosis to enrollment was 4.4 years (SD 3.9 years; range: 0 – 26.6, median: 3.6). Although all participants were HIV positive, only 119 (78.3%) were receiving HAART at the time of enrollment. The

mean time on HAART was 2.6 years (SD 2.6; range: 0 – 13.3; median 2.1) and the most frequent regimen was zidovudine / lamivudine / efavirenz (75 participants (63.0%)); 139 and 137 individuals had their last CD4 cell-count and viral load information: mean values were 437.4 CD4 cells/mm^3 (SD 193.5) and 33120.9 copies /mL (SD 91756.6) respectively.

Prevalence of STIs. Laboratory testing showed that 4 participants (2.6%) had *Chlamydia trachomatis* and 1 (0.7%) had *Neisseria gonorrhoeae*. Regarding syphilis, 45 individuals (29.6%) had a reactive RPR test, with titers that ranged between 1:1 to over 1:512. All of them underwent confirmation by TPHA; 40 individuals (88.9%) had a positive result with titers that ranged between 1:80 and over 1:10,240, 2 were negative (4.4%) and 3 had an indeterminate result (6.7%), meaning that syphilis prevalence in this population was 26.3%.

Only one participant had a previous diagnosis of HTLV. Study participants were also tested for HTLV; 5 (3.3%) were reactive to HTLV ELISA testing, 3 were confirmed as HTLV-1 by Western Blot, while 2 were indeterminate (Table 3).

Forty-nine individuals (32.2%) had been diagnosed at least once clinically or through laboratory testing with HPV in the past by their regular treating physicians. HPV was assessed with PCR and 147 participants (96.7%) had at least one type of HPV; 133 of those (90.5%) had one or more carcinogenic types of HPV (Table 3). The prevalence of the different HPV types is shown in Figure 1.

Ninety-one participants had at least one carcinogenic type of HPV in absence of the studied STIs (chlamydia, gonorrhea or syphilis). At the moment of enrollment 2 (2.2%) had rectal bleeding and/or feeling of rectal fullness, 22 (24.2%) had anal warts and 4 (4.4) had genital warts.

Factors associated with HPV. The presence of carcinogenic types of HPV was assessed using several variables; some of the variables assessed are shown in Table 4. Other variables which did not show association with carcinogenic types of HPV were current relationship status, education level, number of lifetime sexual partners, number of female partners, sexual work as primary income, alcohol or drugs consumption. Multiple logistic regression adjusting for the following variables did not show an association either: ever practicing receptive anal intercourse, ever receiving money or goods in exchange for sex, condom use during the last time a participant who is a sex-worker received money in exchange for sex, gender of sexual partners (male, female or both), role in bed (exclusively insertive, mainly insertive, versatile, mainly receptive or

exclusively receptive), number of partners with whom the participant practiced receptive anal intercourse and the number of partners with whom the participant practiced receptive anal intercourse without a condom.

No statistical difference was found between the mean age of first sexual intercourse, mean number of sexual clients in those who practiced sex work, mean time since HIV diagnosis, mean time since HAART was initiates, mean value of last viral load, or mean value in last CD4-cell count (Figure 2), in those having a carcinogenic type of HPV and those who didn't (p-values: 0.71, 0.81, 0.53, 0.47, 0.66 and 0.78 respectively).

DISCUSSION

Previous studies have tried to assess risk factors, beyond acquiring HPV, for acquiring high-risk types of HPV[20, 21]. These attempts have included both male and female populations, HIV-infected and -uninfected. One of these studies, performed in HIV-infected and -uninfected women, assessed for associations between certain risk-factors and risky behaviors and the finding of high-risk HPV compared with low-risk HPV [20], and found a single variable associated with high-risk HPV, age. In the context of the HIV positive MSM population with multiple probable co-infections, assessing other risk factors and risky behaviors for high-risk HPV versus low-risk HPV acquisition is important, given that high rates of HPV infection has been described [22-24]

Although sub-Saharan Africa is the region most seriously affected by the HIV epidemic, Latin America contains an important proportion of the worldwide infection with very diverse patterns of transmission [25]. In the Andean region, the largest HIV-infected population is MSM [26], and particularly in Lima, where this study was developed, HIV prevalence increased between the mid 1990's and early 2000's [2]. Participants enrolled in this study were not significantly older than MSM enrolled in previous studies carried out in similar populations [1, 2], which may have been a reason for the higher education level found in the currently described participants [1]. However, these two factors don't seem to have an impact on the prevalence of STIs such as syphilis, gonorrhea, or HPV. The age of first sexual intercourse is in agreement with a previous study performed in Lima [27], and this factor is important for the risk of acquiring HPV, as will be discussed further. Over a third of the enrolled individuals defined themselves as bisexuals, having had at least one female partner in the previous three months; bisexuality is considered a

"bridge" for the transmission of HIV to heterosexuals [28], however this could be considered a "two-way bridge" of transmission for other STIs, such as HPV, since women can also have silent infections which can be introduced into the male partner's MSM community.

Although there is little information about the reported number of life-time sexual partners, over one third of MSM reported having over fifty partners, and 10% of the study population have hadreported over 200. However, during the study period, almost half of the participants reported not having sex or having only one sexual partner in the three months previous to their enrollment. Over 30% reported being in a steady relationship; however, this does not necessarily mean that the relationship was monogamous.

Role-based sexual identity provides an interesting framework for understanding transmission of STI, particularly HIV. MSM sexual conduct has traditionally been considered either active (associated with "insertive" sexual penetration, primarily anal; some "active" individuals consider themselves heterosexuals or at least non-gay) or passive (associated with receptive anal sex; these individuals are commonly considered the feminine part of the couple and are therefore homosexual) [29-31]. However, in Peru a versatile conduct, known as being *moderno* has altered this binary paradigm. The *moderno* population can have active and/or passive roles regardless of the masculine or feminine "tag" usually assigned to each conduct. In this context, over 50% of the study participants defined themselves as versatile or *moderno*. However, 12% and 15% defined themselves as "mainly active" or "mainly passive" respectively, meaning that they had performed both roles at a certain point in time. A study recently published found that 34% of participant considered themselves *modernos* [18]. This same publication showed that *moderno* individuals not only had higher rates of HIV infection than active and passive MSM, but also more syphilis than both and more chlamydial and/or gonococcal urethral infections than passive individuals. In this context, finding that versatile or "moderno" have higher odds of being infected by a high-risk HPV is understandable, given that previous publications show that individuals in the versatile-role sexual networks reported higher frequencies of unprotected insertive and receptive intercourse[18, 32]

Multiple interventions and widespread prevention messaging campaigns have emphasized the importance of condom use in the MSM population. Probably as a consequence of these, all study participants knew where to get condoms. Nevertheless, as in previous publications about this population [1], the use of a condom during the last sexual contact (regardless if it was receptive or

insertive anal intercourse) did not reach 20%. As previously mentioned this could be related to the sexual role of most of the study participants, having a direct impact on the infection rate of STIs and HPV.

HTLV has an impact upon the immune system. Due to this interaction, through the secretion of certain factors, HTLV (the first described retrovirus) can alter the development of HIV infection [33-36]. Furthermore, HTLV is associated with other infections such as *Strongyloides stercoralis* hyperinfection syndrome [37] or Norwegian scabies [38]. The prevalence of confirmed HTLV in this study was 2% (3.3% if we consider only the ELISA results). Although no association was found between positive HTLV serology and presence of high-risk HPV, a study carried out in Brazilian women found a higher HPV prevalence in HTLV-1 infected participants than in the HTLV- uninfected control group[39]. This difference may have occurred due to similar risk behaviors associated with acquiring either of the viruses, to the effect HTLV has on the carrier's immune system or a combination of both. No previous publications were found describing this co-infection in MSM, however, it is logical to assume that the same conditions can occur in both populations.

Bacterial STI are frequent in HIV-infected MSM. In this study we included testing for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Treponema pallidum* (syphilis). The prevalence of *C. trachomatis* in an MSM population in Argentina was 1.7% [40] while our study population had a prevalence of 2.63%. Despite the well known consequences of chlamydial infection, the mechanisms of pathogenesis are not well defined [41]. Although *in vitro* studies suggest that the response to this infection arises from epithelial cells, the role in the pathogenesis of CD4 T cells (targeted by HIV) remains unclear [41]. However, an association between *C. trachomatis* and female cervical cancer has been described [42]. The importance of *C. trachomatis* on HPV infection may be explained on two levels; the epithelial cells may be altered or microabrasions may be caused by this bacteria, facilitating HPV entry to the tissue; on the other hand, *C. trachomatis* may impair the host's capacity to resolve HPV infection through chronic inflammation of tissue and a shift in the host's immune response to T helper cell predominance [43, 44]. The second most frequently reported bacterial STI [45], *N. gonorrhoeae* (gonorrhea), most commonly infects the urogenital area. However, 50% of rectal infections remain asymptomatic [46]. The association between prior or current infection with *N. gonorrhoeae* and HPV has been described [47], and as with *C. trachomatis*, is probably related to epithelial alterations and inflammation. Although each of these bacterial STs has clinical characteristics, they usually co-

infect an individual and cause urethral signs and symptoms. For these reasons, some publications group them as a single entity. For example, Clark et al. describe prevalences of urethral chlamydial/gonorrhea infections of 1.7%, 6% and 10% in passive, *moderno*, and active sexual roles of the MSM studied population [18]. We found a prevalence of 3.3% among our study population regardless of their sexual role.

Another bacterial STI of well-known importance is syphilis. MSM have higher syphilis prevalences than other high-risk population [48]. Furthermore, a strong association has been described between HIV infection and evidence of syphilis [1]. A Chinese study assessing prevalence and risk behaviors for HIV, syphilis and HPV in MSM found a prevalence of syphilis among HIV-infected MSM of 27.6% [23]. A study of Peruvian MSM found a reactive RPR test in 21.6% of study participants [2]. Our study found 29.6% of MSM had a reactive RPR and 26.3% had a positive confirmatory TPHA. Of interest are the high titers for both tests: Sanchez et al. found that 7.8% of the individuals enrolled in his study had RPR titers over 1:8 [2]. Our population had 15.7% of participants with titers of at least 1:8, with some titers reaching 1: 512 or more; TPHA tests also titers ≥ 1:10, 240. Parisi *et al* reported a positive correlation between any diagnostic test for syphilis (namely RPR or TPHA) and the presence of high-risk HPV in anal swabs[49].

HPV productively infects keratinocytes of skin and mucous membranes. In the case of sexual transmission, the virus invades the membranes of the lower basal keratinocytes through abrasions occurring during sexual contact [7]. This tissue may be more vulnerable during adolescence, providing one of the most likely reasons for the increased risk of acquiring HPV when first sexual intercourse occurs early in life. Although some HPV types may be asymptomatic, others may cause warts, and a minority of typesare associated with cancer of the infected tissue [7] (cervix, oropharynx, rectum, penis, etc.). Nevertheless, longitudinal studies have shown that most infections clear within months or years after the virus was acquired [50, 51]. Still, some authors argue that the virus can remain hidden in stem cells even after the infection has apparently cleared [7]. As previously mentioned, some HPV types are associated with an increased risk of triggering neoplasias. These types are 16, 18 (these two have the highest risk), 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 73. Currently HPV is the leading cause of anogenital malignancies, particularly among HIV-infected individuals, MSM and transplant

recipients [24]. This study included individuals who belong to two of these high risk populations for anal cancer, as all HIV-infected individuals, even those receiving highly active antiretroviral therapy (HAART), appear to be at increased risk for development of HPV-related cancer [52]. Furthermore, HIV positive MSM have been described as particularly susceptible to concurrent infection of multiple HPV types [49], as was found in this study.
A study performed in Italy in HIV-positive homosexual men, described that 88.6% of participants had a positive rectal swab for HPV, and of those 24.1%, had at least one of the types considered high-risk for neoplasias[49]. Other studies from Western countries describe HPV prevalences of 90-95% in HIV positive MSM [53, 54]. Over 97% of the current study participants were found positive for at least one type of HPV using a PCR assay, while over 80% presented with at least one carcinogenic type.

Several variables were analyzed to assess the risk factors associated with the detection of at least carcinogenic HPV type, particularly those variables related to HIV infection. However, as will be discussed with the limitations of this study, only a few variables were found significant. As expected, ever practicing receptive anal intercourse carried a significantly higher risk of having a carcinogenic type of HPV. In addition, having a versatile (*modern)* or exclusively passive role was associated with significantly higher risk of having a carcinogenic type of HPV. This is consistent with versatile individuals having a higher risk of acquiring other STI, such as syphilis and even HIV [18], usually followed by passive individuals, added to the fact that an important proportion of the participants had some kind of rectal or anal symptom (bleeding, pressure or presence of anal warts).
We expected several assessed variables, such as having more than 20 partners with whom receptive anal intercourse was practiced or ever receiving money or goods in exchange for sex, to be associated with anal HPV infection. A factor that has been associated with higher rates of high risk HPV is younger age of sexual debut. Although carried out in Peruvian women, early age at first sexual intercourse significantly increased the risk of having a high risk HPV [55]. Another factor described in the same publication was the number of lifetime sexual partners; however one could argue that this is related to the age of first sexual intercourse, given that throughout life, the younger the age of first sexual contact, the higher the likelihood of having more partners. Nonetheless, as previously mentioned, the immature tissues of a teenager may facilitate the entry of the HPV or the capacity of the virus to “hide” in stem cells [7].

In our study population, another potential factor associated with carcinogenic HPV infection was CD4 count. Although we did not find a statistically significance association with this CD4 count, a previous study reported that a higher CD4 count in HIV positive individuals, reduced the probability of infection [49]. That same study also reported a positive association between the detection of high-risk HPV by anal swab and the patient's HIV viral load, and a negative association with antiretroviral therapy intake [49] (no statistical significance was found in this study).

The most prevalent type of HPV in our study population was type 16, which along with type 18, are the two types associated with highest risk for anal and oropharyngeal cancer. Furthermore, finding high-risk HPV types in rectal swabs has been independently associated with HIV acquisition [56, 57]. Currently available HPV vaccines protect against initial infection of these two types, and some vaccines also provide protection against types 6 and 11 (the types most commonly associated with genital warts) [58]. Our study was not designed to examine which came first, the HIV or the HPV infection. However, previous studies show that male HPV vaccination in naïve individuals could potentially reduce HIV acquisition by controlling HPV prevalence [53]. This strategy should be considered, particularly in male adolescents, taking into account that, once the vaccine has been properly explained, most Hispanic fathers (87.5%) are willing to vaccinate their sons [59] and most MSM individuals in this community have a versatile sexual role, which is associated with a higher risk of becoming infected.

This study suffered from a number of limitations. First, all but four participants were infected with HPV, and therefore the statistical power was not enough to identify differences between the groups. Second, given budget constraints, we were not able to examine the prevalence or effects associated with other important viral STI, such as herpes simplex virus (HSV). Several publications have mentioned the importance of HSV in the transmission of HIV and probably HPV [1, 2]. Third, socio-demographic data was obtained by a self-administered survey. Although participants were instructed to ask if a question was not clear, some information bias, or even recall bias associated with number of partners should be considered. Fourth, this study was carried out only in Lima, and therefore can't be used to draw conclusions about the country or region. Finally, a cross-sectional study design has limitations to infer temporal associations. The

next step would be to develop a longitudinal study in HIV-infected MSM to further assess risk factors for carcinogenic HPV.

This study reports an HIV-infected MSM population from Lima, with high prevalences of HPV and syphilis. Furthermore, among MSM who had positive testing for HPV, many had types considered at high risk for developing cancer (carcinogenic types). These findings illustrate that despite multiple prevention activities and interventions to promote consistent use of condoms, different strategies are needed to improve compliance towards safer sex in this population, including male adolescent HPV vaccination.

Table 1: Demographic and laboratory characteristics of HIV-positive MSM in Lima, Peru (n=152)

Age in years		
	Mean ± SD	33.43 ± 8.23
	Median [Range]	32 [20 – 58]
	≤ 30	66 (43.4)
	31 - 40	60 (39.5)
	41 - 50	21(13.8)
	>50	5 (3.3)
Social status		N (%)
	Married	1 (0.66)
	Co-habitant (regardless of gender)	22 (14.47)
	Divorced / Separated	2 (1.32)
	Single	97 (63.82)
	Partnered(regardless of gender)	30 (19.74)
Education level		N (%)
	Incomplete primary (lower school)	1 (0.66)
	Primary (lower school)	1 (0.66)
	Incomplete secondary (upper school)	8 (5.26)
	Secondary (upper school)	52 (34.21)
	Incomplete university	21 (13.82)

	University	29 (19.08)
	Technical training	40 (26.32)
Time since HIV Diagnosis		Years
	Mean ± SD	4.4 ± 3.9
	Median [Range]	3.6 [0 – 26.6]
Time in HAART		Years
	Mean ± SD	2.6 ± 2.6
	Median [Range]	2.1 [0 – 13.3]
CD4 count at enrollment		
	Mean ± SD	437.4 ± 193.5
	Median [Range]	418 [66 – 1273]
Viral load count at enrollment		
	Mean ± SD	33120.9 ± 91756.6
	Median [Range]	39 [19 – 540349]

Table 2: Signs associated with STIs

	N (%)
Participants with signs	80 (52.63%)
- Genital secretions or pus	7 (8.75%)
- Genital or anal wounds	26 (32.50%)
- Genital or anal warts	35 (43.75%)
- Pus or blood discharge from rectum	17 (21.25%)

Table 3: Prevalence of specific HPV types, syphilis, *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and HTLV

	N (152)	Percentage (%)
Human Papillomavirus		
Not infected*	4	2.6
Infected	147	96.7
- 1 type	10	6.6
- ≥ 2 types	137	90.1
Five Most Frequent HPV Types		
HPV 16	50	32.9
HPV 58	42	27.6
HPV 6	40	26.3
HPV 61	38	25.0
HPV 45	35	23.0
Individuals with High Risk Types		
Non carcinogenic types	14	9.2
Carcinogenic types	133	87.5
Other STIs		
Syphilis**	40	26.3
Neisseria gonorrhoeae	1	0.7
Chlamydia trachomatis	4	2.6
HTLV***	3	2

* One sample provided had insufficient DNA for diagnosis

** Screening by RPR, confirmation by TPHA

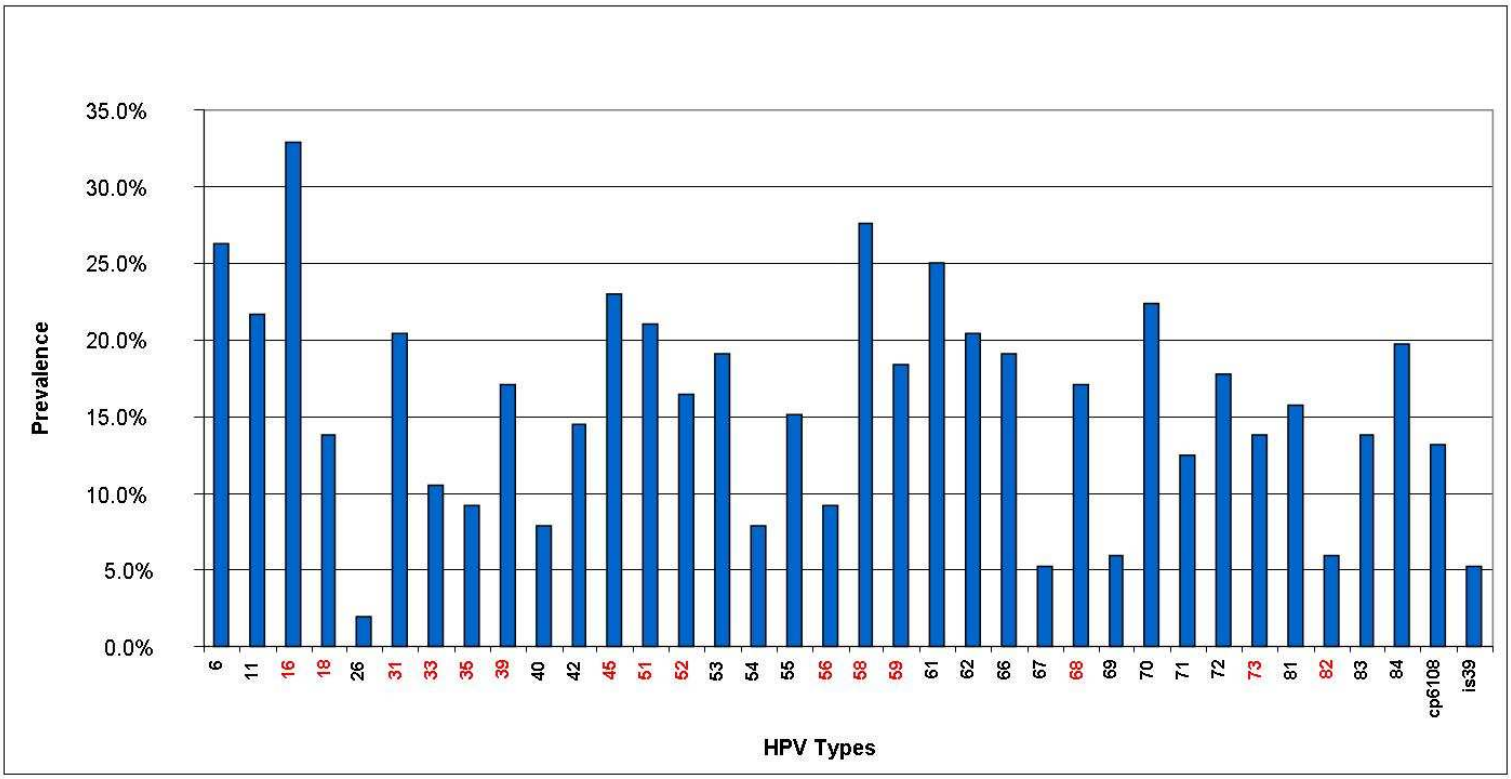
*** Screening by ELISA, confirmation by WB

Table 4: Selected variables for assessment of presence of carcinogenic types of HPV

	OR [95% CI]	P-value
Sex with people other than partner	0.75 [0 .27 - 1.99]	0.53
Sex partners:		
- Men	1.00 [ref.]	Ref.
- Women	0.58 [0.22 - 1.51]	0.26
- Both	0.48 [0.05 - 4.98]	0.53
Ever had receptive anal intercourse	**10.17 [2.12 - 52.61]**	**<0.01**
Main role in bed (previous 5 years):		
- Exclusively insertive	1.00 [ref.]	Ref.
- Mainly insertive	2.80 [0.52 – 15.06]	0.21
- Versatile	**5.91 [1.36 – 25.63]**	**<0.01**
- Exclusively receptive	**9.50 [1.07 – 84.09]**	**<0.05**
No. of partners (prev. 3 months) receptive anal intercourse:		
- 0	1.00 [ref.]	Ref.
- 1	**5.32 [1.27 – 22.12]**	**<0.05**
- 2 to 5	2.11 [0.72 – 6.23]	0.16
- 6 to 10	-------	-----
- 11 to 20	0.38 [0.02 – 6.95]	0.49
- >20	1.14 [0.19 – 6.64]	0.89

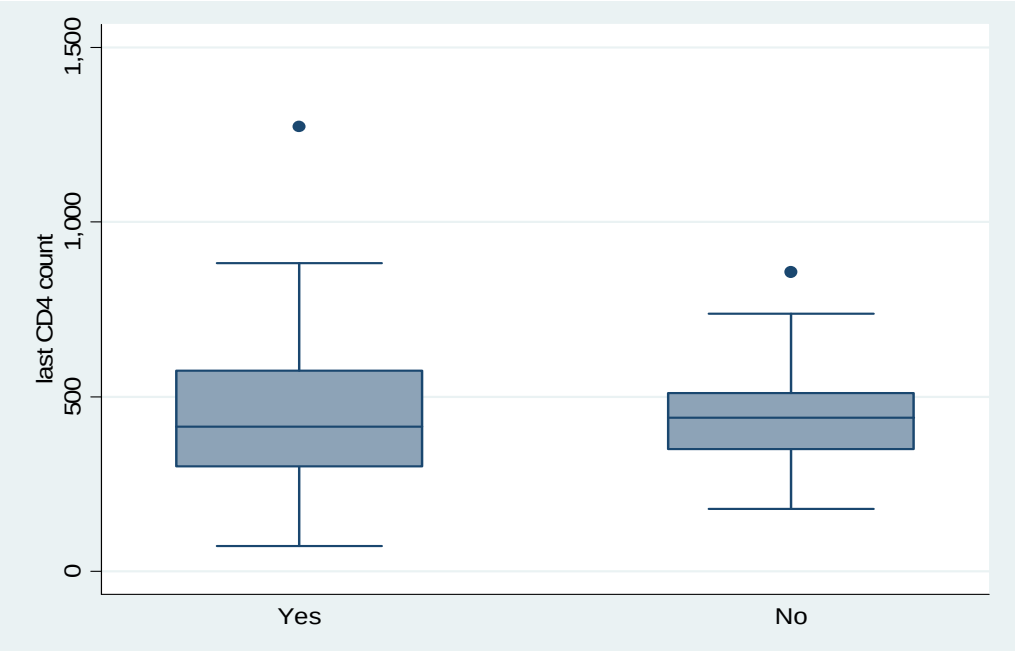
No. of partners (prev. 3 months) receptive unprotected anal intercourse:		
- 0	1.00 [ref.]	Ref.
- 1	1.04 [0.36 – 2.96]	0.94
- 2 to 5	1.13 [0.29 – 4.4]	0.85
- 6 to 10	0.19 [0.01 – 3.34]	0.20
Ever received money or goods for sex	1.24 [0.40 - 4.60]	0.69
Used a condom last time received money for sex	1.51 [0.36 - 6.84]	0.52
Positive Chlamydia test	0.56 [0.04 - 30.71]	0.62
Positive HTLV test	0.37 [0.019 - 22.83]	0.41
Positive syphilis test	0.65 [0.24 - 1.94]	0.37

Figure 1: Prevalence of HPV types



*Note: In red, high-risk HPV

Figure 2: Mean CD4-cell count in individuals with and without carcinogenic types of HPV



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