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Human papillomavirus infection in HIV-seropositive men who have sex with men in both the United States and India: prevalence, incidence, and risk factors for infection

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Human papillomavirus infection in HIV-seropositive men who have sex with men in both the United States and India: Prevalence, incidence, and risk factors for infection

by

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Abstract

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Doctor of Philosophy in Epidemiology

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Human immunodeficiency virus (HIV)-infected men who have sex with men (MSM) are at high risk of anal cancer compared with the general population. Human papillomavirus (HPV) infection, particularly HPV 16, causes anal cancer. The prevalence of anal HPV infection among HIV-infected men in the US is >90% but little is known about the risk factors for prevalence, and incidence of or the risk factors for type-specific anal HPV infection . There is also limited knowledge about anal HPV infection among HIV-infected MSM in India, although the background incidence of HPV-related cancers in both men and women is high in India. Indian HIV-infected MSM may be at especially high risk for anal HPV infection and HPV-associated disease.

My aim was to determine the prevalence and incidence of and risk factors for anal HPV infection among two populations of HIV-infected MSM, one from San Francisco and the second from India. The San Francisco population was two-year longitudinal study and allowed for both prevalence and incidence analysis; the India study was cross-sectional in design and allowed only for a prevalence analysis.

The prevalence of anal HPV infection among HIV-infected MSM in San Francisco was 92%, 80% had oncogenic types and 42% had HPV 16. A higher number of total life-time partners was associated with increased prevalence of HPV 16. The incidence of any anal HPV infection was 21.3 per 100 person-years (PY) and 3.5/100 for HPV 16. A higher number of recent partners with whom the participant was the receptive partner was associated with a higher incidence of any anal HPV infection. New receptive partners, more frequent receptive sex, and new oral-anal contact partners was also associated with a higher incidence of any anal HPV infection. The prevalence of anal HPV infection among HIV-infected Indian MSM was 71%, and ever having receptive anal intercourse and higher number of receptive partners increased prevalence. “Almost always” condom use with receptive anal intercourse decreased prevalence of anal HPV infection among HIV-infected Indian MSM.

In conclusions, HIV-infected MSM in both San Francisco and India have high prevalences of anal HPV infection and the incidence of anal HPV among these men in San Francisco is also high. The most important risk factors for infection are total number of male partners and receptive anal intercourse. Condoms may protect against anal HPV infection, but further research is needed to confirm results. HIV-infected MSM should be counseled on safe sex practices with all partners and encouraged to receive the recently recommended HPV vaccine.

Dedication

This dissertation is dedicated to my husband, Bhupendra Sheoran, and my daughter, Symiran Dalia Sheoran. Their love and support made this work possible.

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CHAPTER 1: Human Papillomavirus

1: Structure, virology and molecular biology

Human papillomaviruses (HPV) are a group of small non-enveloped, double-stranded, circular DNA viruses that infect the skin and mucosal membranes of humans [1, 2] . Papillomaviruses are members of the family *Papillomaviridae.* Genera, species and type are all determined by the amount of similarity in the open reading frame (ORF) of the HPV L1 gene, a gene that encodes the major structural viral protein. Genera share 60% identity with other genera, species share 60-70% with other species, and types share 71-89%. There are also subtypes sharing 90-98% identity and variants sharing more than 98% identity. There are more than 130 HPV types, and of those over 40 infect the ano-genital region. Different HPV types are responsible for a variety of clinical changes in the mucosal membranes, and several types are now classified as oncogenic and are associated with cervical, vaginal, vulvar, penile, and anal cancers, and a subset of oral cancers [1, 2]. The following types are considered oncogenic: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82 [3].

The HPV genome consists of eight ORF designated either as early (E), replicated early in the infection process, or late (L), replicated late in the infection process [1, 2]. L1 and L2 encode the two structural proteins of the virus and E1,E2, and E4-E7 encode for proteins responsible for other viral functions. There is also a non-coding region of the genome called the long control region (LCR), which has transcriptional control binding regions. The genes E1 and E2 function to establish viral transcription, viral DNA replication, and plasmid maintenance in the cell. E4 and E5 support virus amplification, may support immune evasion functions, and also may support transformation [1, 2].

In oncogenic HPV types the E6 protein is implicated in one of the major pathways that can lead to premalignant lesions and malignancies [1, 2]. Normally in the presence of HPV infection DNA damage and cellular stress lead to high levels of p53 expression, eventually leading to cell death. However, E6 complexes with p53 along with E6-associated protein and this complex is ubiquitinated and destroyed in protosomes. This leads to low levels of p53 and cell immortality. Similarly, the E7 protein is critical to oncogenesis. Retinoblastoma tumor-suppressor gene (pRB) inhibits cell cycle progression. E7 complexes pRB and inactivates its function, which induces DNA synthesis and cellular proliferation. E6 and E7 may also cause genomic instability in normal cells. Integration of both E6 and E7 into the host cell DNA may be partially responsible for high expression of both proteins and occurs in most tumors [1, 2]. Both E6 and E7 are multifunctional proteins and are involved with other viral functions, some of which may also promote cancer. For a full review, please see the International Agency for Research on Cancer's (IARC) monograph on HPV: the evaluation of carcinogenic risks to humans [1].

1A: HPV INFECTION

While most investigations of HPV infection and disease have focused on cervical HPV infection, the mechanisms of infection and disease are believed to be similar for all anatomical sites. Unless otherwise noted, the discussion that follows is based on observations made of cervical HPV infection.

HPV infection and replication are closely linked to the cell cycle[1, 2]. HPV can only infect basal squamous epithelial cells undergoing cell division. This implies that there must be a wound or abrasion of the skin to provide viral access to the basal cells through the upper layers of the epithelium. Once in the basal cell, HPV sheds its protein coat within an endosome and the genome then travels to the cytoplasm and then to the nucleus. Normally in cells not infected with HPV, the basal cell divides and the daughter cells migrate to the suprabasal compartment, withdraw from the cell cycle, differentiate, and no longer support DNA synthesis. However, in HPV-infected cells the daughter cells continue to proceed through the cell cycle and continue to support DNA synthesis. In the basal cell, there is production of some HPV early proteins (E1 and E2). At this stage of development the cell may proceed through several rounds of cell division, and multiple copies of HPV DNA plasmids will be replicated along with cellular DNA. The epithelial cell differentiates into a lower spinous layer cell and HPV now additionally express E4 and E5. It is in this layer where HPV also expresses the E6 and E7 proteins at high levels, and these proteins have been linked to the clinical manifestations of high-grade intraepithelial neoplasia as well as cancer. The epithelial cell further differentiates into the upper spinous layer and here HPV produces the late structural proteins (L1 and L2) and assembles virions. Mature HPV virions are located in the next level of the epidermis, the granular layer, and it is in the most superficial layer of the epidermis where sloughing is occurring, where HPV virions are finally shed[1, 2].

Most cervical HPV infections are self-limiting and are either cleared completely by the immune system, or are controlled to a level that they are no longer detectable on standard HPV DNA tests [1, 2]. Approximately 70% of cervical HPV infections cannot be detected one year after a positive HPV DNA test [1, 2]. There is some evidence that HPV becomes latent and is re-activated many years after the initial infection when a person's immunity is compromised in some way, such as during pregnancy, as one ages, or with HIV infection [1, 2].

Of the infections that remain detectable, 30% become persistently detectable, defined as testing, positive on two consecutive tests six months to a year apart [1, 2, 4]. As with the initial infection, most infections classified as persistent clear with no clinical intervention.

1B: HPV AND CANCER

The 2007 IARC monograph designated HPV as a necessary but insufficient cause of cervical cancer and it is also implicated HPV in other anogenital and oral cancers [1]. Although most HPV infections, even those of oncogenic HPV types, are self-limiting, a

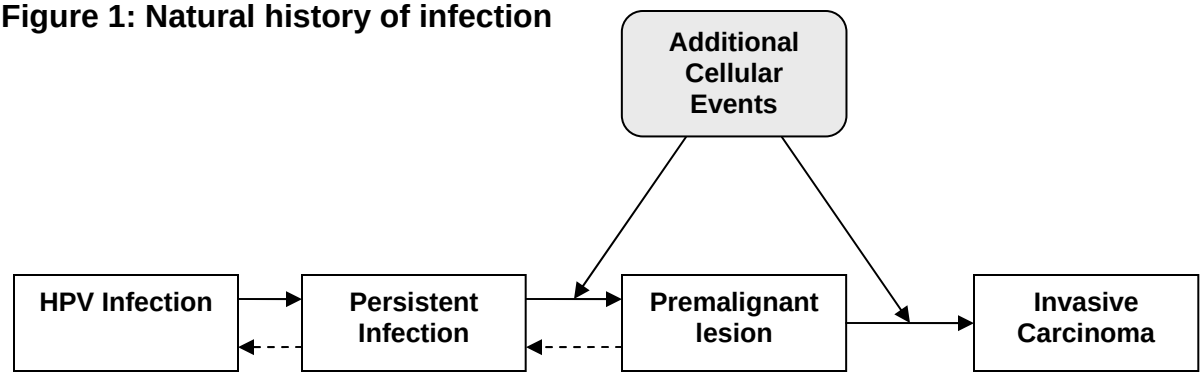
small but important percent do progress to cancer. This process may take as many as 20 years from initial infection to malignancy and both viral factors and host factors influence the outcome [1].

An infection with HPV of any type can cause a lesion in the epithelial tissue [1]. Non-oncogenic HPV types typically cause benign lesions such as genital warts. Oncogenic types cause lesions that range from benign (with very mild dysplastic changes) to severe dysplasia and finally to invasive carcinoma. Dysplastic changes are classified based on the amount of replacement of mature epithelial cells by immature basaloid cells in a lesion. These immature cells have lost their ability to differentiate into mature cells and therefore the virus cannot complete its life cycle in these cells.

In the Bethesda System[5] for classifying abnormalities, very mild and mild dysplastic changes are designated as low-grade squamous intraepithelial lesions (LSIL); these rarely progress to cancer. In contrast, high-grade squamous intraepithelial lesions (HSIL) are associated with progression to cancer with greater severity of dysplastic changes associated with a greater risk of cancer. These lesions can be diagnosed by biopsy as intraepithelial neoplasia, for example cervical intraepithelial neoplasia (CIN), and these are ranked from 1 to 3, indicating increasing severity of the lesion. More advanced diagnoses include *in situ* carcinoma and invasive carcinoma [1].

HPV is necessary for a lesion to progress to cancer, but it is clearly insufficient on its own, given the number of infections that do not progress to malignancy [1, 6]. Both HPV and additional cellular events are necessary to form a sufficient cause of cancer. These additional events can include the epigenetic silencing of numerous genes associated with tumor suppression, the loss of heterozygosity in some chromosome regions (loss of other tumor suppressor genes), a gain of chromosome 3q24-28 (promoting growth), and down-regulation of HLA antigen class I alleles (aiding immune system evasion). Some host factors have also been found to increase risk of cancer, including smoking, use of oral contraceptives, pregnancy, parity, nutrition, micronutrients and other sexually transmitted infections. It is unclear if these factors lower the host's immunity allowing an infection to become established and persist, thereby leading to an increased possibility of cancer, or if these factors induce mutations in HPV-infected cells, leading to the additional cellular events that contribute to the development of cancer [1, 6].

Figure 1: Natural history of infection



1C: IMMUNE RESPONSE TO HPV INFECTION

The close linkage of HPV infection with the cell cycle minimizes HPV's exposure to the immune system because mature virus is released only in differentiated squamous cells in the top layer of the epidermis [1, 2] The virus also causes little tissue damage, thereby limiting the danger signals to the immune system and antigen production for activation of the adaptive immune system. Nevertheless, the host immune response is important in the clearance, regression and maintenance of latency, and also provides some protection from future infection with the same HPV type [1, 2].

Type-specific neutralizing antibodies are formed against epitopes of L1 and the small portion of L2 that is exposed in the capsid [1, 2]. The neutralizing antibodies to L2 are less potent than those to L1. Most evidence implies that antibodies developed to a specific HPV type exhibit little if any cross-reactivity. Most studies of immune response have focused on HPV 16 infection, and they have shown that HPV 16 antibodies are slow to develop, reach low titers, and are not present in all individuals with incident infection[1]. These studies also show that persistent infection is associated with detection of antibodies. Additionally, detection of antibodies to E6 and E7 is associated with cervical cancer, but infection alone is not [1]. Not all women who test positive for cervical HPV DNA have detectable antibody, and some with no cervical infection detectable will have antibody present [1]. Therefore, antibody tests cannot be used as diagnostic markers for HPV infection or cervical cancer.

The details of how the immune system resolves an infection with anogenital HPV are not fully understood [7]. The basic process is known to be similar to the immune response to other viruses, and lesion clearance is associated with a T-cell response. The innate immune system senses tissue damage and its effector cells coordinate with the adaptive immune system's effector cells to protect mucosal tissue. Antigen-presenting cells in the cervical epithelium activate specific naïve T cells and shape their response to a type 1 response (cell mediated immunity (CMI)) or a type 2 response (stimulation of B cells). A type 1 response produces cytotoxic T lymphocytes that can kill cells infected with HPV. A low CMI response to HPV 16 has been associated with persistent HPV 16 infections and cervical cancer patients, whereas healthy subjects show a stronger CMI response [7].

1D: Detection of HPV infection

HPV-associated anogenital disease can be detected clinically by a variety of methods, including visual inspection under magnification assisted by topical acetic acid, which causes acteo-whitening of HPV-infected cells [1]. Colposcopy and high-resolution anoscopy (HRA) allow for the visual inspection of the cervix and anal canal, respectively, under magnification and again assisted with acetic acid. These methods are used to visualize lesions in order to perform a biopsy of suspicious lesions. Cytology (Pap smear for the cervix) is often used as a screening tool for recommending individuals with suspected lesions to have a colposcopy or HRA [1].

HPV infection can be accurately detected using methods that detect HPV nucleic acids [1]. There are a variety of methods available to detect HPV DNA or RNA, most having the ability to discriminate between the oncogenic and non-oncogenic types, and several of these methods providing type specific results. The detection techniques are broadly divided into methods that require polymerase chain reaction (PCR) amplification of DNA and those that do not [1].

PCR-based methods generally use consensus primers to a highly conserved region of the L1 gene that is homologous between different HPV types [1]. The most commonly used L1 consensus primers are the GP5+/GP6+, the MY09/11 and the PGMY09/11 (modified from MY09//11). Studies have also used consensus primers to the E1, E6, and E7 genes. The results of the amplification can then be used to detect more than 30 individual HPV types through hybridization with type-specific probes analyzed with restriction-fragment length polymorphism by gel electrophoresis, dot-blot hybridization, line-strip assays or microtitre-plate assays. These methods have comparable sensitivities for detecting overall HPV infection, but there are documented differences in their ability to detect individual HPV types. These methods have the disadvantage that the HPV viral load cannot be measured, although recently real-time PCR has detected HPV DNA and provided a qualitative measure of viral load [1].

The most widely used method for detection of HPV DNA is the commercially available Hybrid Capture 2 (HC2) assay [1]. This assay hybridizes synthetic RNA probes that are complementary to the genomic sequences of 13 oncogenic and 5 non-oncogenic HPV types. The DNA-RNA hybrids are then captured by antibodies bound to a microtitre plate. The intensity of emitted relative light units is proportional to the amount of DNA present and provides a semi-quantitative measure of HPV viral load [1].

Both PCR-based methods and HC2 are preformed on samples taken from swabs of anatomical sites such as a cervical swab or anal swab [1]. They do not have the ability to identify DNA in specific cells. In-situ hybridization, however, can be used to identify HPV DNA in specific tissues while conserving cell morphology. It can be used on fixed and processed tissues and can distinguish between plasmid and integrated HPV genetic material [1].

There are other detection methods for HPV infection, such as techniques that detect HPV proteins in tissue, Southern and northern blot hybridization, single-strand conformational polymorphisms, and DNA sequencing. However, these methods are difficult to perform and are rarely used in epidemiological studies [1].

1E: Prevention and the HPV Prophylactic Vaccine

Anogenital HPV is almost exclusively sexually transmitted through contact with infected cervical, vaginal, vulvar, penile or anal epithelium or through other sexual practices including oral sex, digital-vaginal/anal sex, and the use of objects in the vagina or anus [1]. Behaviorally, primary prevention of HPV infection would have to exclude almost all sexual activity. Interestingly, even open-mouth kissing would be precluded from because it has been associated with oral HPV infection [8]. Other prevention methods that have shown some level of protection against HPV infection include barrier methods (condoms [9], diaphragm [10]) and male circumcision [11-13].

In 2006, the US Food and Drug Administration (FDA) licensed a quadrivalent vaccine including HPV types 6, 11, 16, and 18 for use in women and girls aged between 9-26 to prevent genital warts, CIN 1-3 and vulvar intraepithelial neoplasia (VIN) 2-3 [14, 15]. A bivalent vaccine that includes only HPV types 16 and 18 was also recently approved for use in girls and young women[16]. In 2009, the same vaccine was approved for use in men and boys aged 9-26 for prevention of genital warts [17], and the FDA advisory committee recently approved prevention of anal intraepithelial neoplasia (AIN) and anal cancer as an indication for HPV vaccination in both men and women [18]. The vaccines are based on the recombinant expression and self-assembly of the L1 protein into virus-like particles (VLP) that lack any viral DNA. The VLPs elicit a strong type-specific neutralizing antibody response that is higher than the antibody response to natural infection. In phase 3 clinical trials, the quadrivalent vaccine has been shown to be almost 100% effective in preventing genital warts, cervical and vulvar, and vaginal intraepithelial neoplasia associated with HPV types 6,11,16, or 18 [14], and it has been found to be safe and tolerable in post-licensure studies [19] The current recommendation is to routinely vaccinate girls aged 11-12 years because the vaccine is most effective when administered before sexual initiation, but is licensed for children as young as nine years of age [17]. If not administered to pre-teens, it is recommended as a “catch-up” vaccine between the ages of 13-26 years. Three doses are recommended, with the second and third doses at one and three months after the first dose [17]. A second vaccine has also been recently approved by the FDA for prevention of cervical cancer and premalignant lesions. This bivalent vaccine includes VLP to HPV types 16 and 18. Although not included as a VLP, this bivalent vaccine has also been demonstrated to protect against HPV 31, another oncogenic type (http://www.gsk.com/media/pressreleases/2009/2009_pressrelease_10112.htm).

Cervical cancer screening is expensive and unavailable in most developing countries where cervical cancer rates remain high. The HPV vaccine will provide a more practical primary prevention strategy for cervical cancer as well as other HPV-associated cancers once the cost of vaccination is reduced. However, because the two oncogenic types

currently included in the both HPV vaccines (16 and 18) only account for approximately 70% of all cervical cancers, screening will still be needed to identify premalignant lesions or cancers that arise from other oncogenic types. A nine-valent HPV vaccine is currently in development which will include more oncogenic types and potentially prevent an even greater percentage of HPV-associated disease.

Vaccinating men with the quadrivalent vaccine could have the direct benefit of reducing genital warts in men. Both the quadrivalent and bivalent vaccines would reduce penile and anal cancer incidence in men who have sex with men (MSM), particularly HIV-infected MSM. There is also a potential additional benefit from reducing transmission from men to their female partners, thereby increasing "herd immunity" to the HPV types included in the vaccine, and thus reducing the incidence of cervical cancer [1, 20]

2: Epidemiology of anogenital HPV infection

2A: HPV INFECTION AND HPV-ASSOCIATED DISEASE OF THE CERVIX

HPV is the most common sexually transmitted infection (STI) [21]. In US women, the prevalence of cervical HPV infection is between 10 and 30% varying mostly by the age of the women sampled. In most Western countries, the peak of infection occurs between the ages of 15-25 coinciding with initiation of sexual activity, although other patterns have been noted, including a second peak around 45 years of age or a flat age curve (no relationship between age and infection rates) [22]. The incidence of infection is between 10-15% per year in women aged 15-25 years [21]. Risk factors for infection include age of first sexual contact, number of sexual partners, smoking, oral contraceptive use [21, 23] and HIV status[1, 24]. Condom use by male partners has been shown to reduce transmission of infection by 70% [25].

Between 70-90% of cervical HPV infections clear without causing clinically important disease and only 7% of US women have abnormal Pap smears each year [26]. HPV infection is the established necessary cause for cancer of the cervix[1]. Types 16 and 18 alone account for approximately 70% of these cancers, and the odds ratios for infection among cervical cancer cases are as high as 282 (for HPV 16) compared to healthy controls [27]. In the US, the incidence of cervical cancer has been decreasing since the implementation of standardized cervical screening programs approximately 40 years ago[28]. However, the incidence of this cancer remains high in developing countries, and each year there are approximately 500,000 new cases and 288,000 deaths reported worldwide[27].

The epidemiology of cervical HPV infection and HPV-associated disease has been well studied. A number of excellent reviews of the literature are available (zur Hausen (2009) [24], IARC 2007 HPV Monograph [1], and Castellsagué et al.(2008) [21]).

2B: HPV INFECTION AND HPV-ASSOCIATED DISEASE OF THE PENIS

The burden of HPV-associated disease is mostly borne by women. Cervical cancer is the most common cause of HPV-associated mortality. However, HPV infection is also important in men, both in that HPV causes disease in men and also because the primary mode of transmission to women is through sexual contact with an HPV-infected man. Prevention of HPV infection and associated disease in men benefits not only the men themselves, but may have a strong impact on transmission to their female partners.

The incidence of penile cancer in the US is low. The US National Cancer Institute reported that in 2010 there were 1250 new cases diagnosed and 310 deaths [29]. HPV is associated with a smaller proportion of penile cancers than it is with anal or cervical cancer, and estimates of penile cancers attributable to HPV vary from 40-50% [1, 27]. HPV is most associated with warty and basaloid carcinomas of the penis and less with verrucous and keratinizing carcinomas. There is also evidence that warty and basaloid cancers are preceded by penile intraepithelial neoplasia (PIN), just as cervical and anal cancers are preceded by CIN and AIN, respectively. In common with anal and cervical cancers, HPV 16 is the HPV type detected most often in warty and basaloid penile cancers, but two HPV types designated as non-oncogenic (6 and 11) also have occasionally been associated with penile cancers [1, 27].

HPV infection of the penis and its consequences have not been nearly as well studied as cervical HPV infection. A 2006 systematic review of the literature identified only 40 studies that included men (excluding studies including HIV-infected individuals)[30]. The authors found that the reported prevalence of penile HPV varied from 6.5-50% among heterosexual men. The studies varied in the anatomical locations sampled (corona, glans, penile shaft, prepuce, scrotum, urethra, semen, and urine), although the wide variation in prevalence estimates was present even when comparing the same anatomic sampling location. The variation in HPV prevalence estimates may be explained by the evolving methods available to sample and detect HPV DNA from the penis [31]. Additionally, the study populations included differed in important respects. Studies of men whose partners were HPV-infected (e.g. cervical cancer patients, HPV-infected women, women with CIN) not surprisingly, found higher prevalences of HPV than studies of men who were not selected based on that criterion. Men who were selected based on their presentation to a sexually transmitted infection clinic also had a higher prevalence then men who were recruited from the general population or from military recruits. A recently published study not included in the review found an even higher prevalence of penile HPV infection of 65% [32], and the higher prevalence may be explained by improved DNA detection methods. Only four studies included in the published review were longitudinal, following men from 6 months to 1.3 years. They found the annual cumulative incidence of penile HPV acquisition was between 14 and 23% [33-36]. A study published after the systematic review reported an even higher annual cumulative incidence of 62% [37]. There were differences among studies both in the methods used to calculate incidence and the methods used to detect HPV DNA that may explain the apparent differences. Two recent studies of the same US study population reported a period prevalence of 53% during follow-up [38, 39]. Also, among men positive for any HPV at baseline 75% no longer had detectable HPV DNA 12

months later [38, 39] a rate of clearance similar to that seen in cervical HPV infection in women. This same study found no association between age and prevalence, acquisition, or clearance of HPV infection, although the authors urged caution in interpreting these results due to the small sample size in the age strata [38].

Fewer studies have focused on HPV infection of the penis among MSM. One report from the Netherlands found the prevalence of penile HPV among MSM to be 16% [40]. A recent study of young MSM with fewer than five partners reported a prevalence of 18.5%. These HPV prevalence estimates for MSM are lower than the estimates for heterosexual partners of HPV-infected women, but higher than estimates from heterosexual men from the general population. Although estimating the prevalence and incidence of penile HPV among MSM based on existing studies is difficult, given the variation in the anatomic areas sampled, the populations studied, and the methods used, it is clear that the prevalence and incidence of penile HPV among both heterosexual and MSM is as high, if not higher, than that of cervical HPV in women.

As with HPV infection in women, the primary route of transmission in men is sexual contact. Penile HPV can be contracted through contact with the infected epithelium of a woman (vaginal, vulvar, cervical, or anal) or man (penile, perianal, anal) [1]. Few studies have evaluated risk factors for penile HPV infection, but those that did identified risk factors similar to those found to be risk factors for cervical HPV infection in women[1]. These include younger age of first sexual intercourse [41], greater number of partners [39, 42], having a new sex partner [37], and smoking [37, 42]. Condom use was evaluated in six studies, but only two studies showed protection with consistent use [34, 43]. A study of transmission to women by male partners found that consistent condom use reduced risk of transmission to women by 70%[25]. Another protective factor that has been evaluated is male circumcision. Observational studies have found that the reduction in risk of incident penile HPV infection ranges from 20-50% [44, 45]. Three recent randomized controlled trials conducted in Africa to evaluate the effect of circumcising adult men in reducing the incidence of HIV and other sexually transmitted infections found statistically significant RRs of 0.66-0.72 for acquisition of HPV infection associated with circumcision[11-13].

2C: HPV INFECTION AND HPV-ASSOCIATED DISEASE OF THE ANUS

Another important anatomical location where HPV infection can have serious consequences in both men and women is the anus [46]. The anus and the cervix share several biological factors that make the anus vulnerable to both HPV infection and associated disease. The structures share a common histology, including a transformative zone where cylindrical epithelium transitions into squamous epithelium, and it is in this zone that most serious HPV infections take root [46].

Anal cancer is preceded by anal intraepithelial neoplasia (AIN) just as cervical cancer is preceded by CIN. Anal cancer is rare with an age-adjusted incidence of about 1.7 per 100,000 person years in men and women combined [47] but the incidence has been increasing, approximately doubling from in the US 1973 to 2000. Approximately 90% of

anal cancers have detectable HPV DNA. HPV 16 is responsible for a greater proportion of anal cancers than of cervical cancer (76% vs. 65%) [3] and HPV 18 is the next most common type detected at 9%. Other oncogenic HPV types have also been detected in anal cancers, but at very low proportions [1].

In the general population, anal cancer is almost twice as common among women as among men[48]. Not many studies have evaluated anal HPV among HIV-negative women. A 2001 study including 68 HIV-uninfected women found anal HPV infection in 42%, abnormal anal cytology in 8%, and HSIL in 2% [49, 50]. In these women, anal HPV infection was more common than cervical HPV infection (42% vs. 24%) [50]. A study in 2009 found a prevalence of anal HPV infection of 50% and of AIN of 4% among [51]. Both anal HPV infection and disease are more common among HIV-infected women (see Section 3).

Few studies have included measurements of anal HPV infection or disease in heterosexual men (Table 1). Van Doornum (1994) found a prevalence of anal HPV of 1.2% in heterosexual STI clinic attendees [36] and Nicolau (2005) found that 8% of partners of women with HPV infection had anal HPV infection [52]. Nyitray (2010) evaluated 902 self-identified heterosexual men from Brazil, Mexico and the US and found a higher prevalence of 12%, although a risk factor for HPV infection in this group was a history of oral or anal sex with a man [53].

The incidence of anal cancer is much higher among MSM than among either women or heterosexual men. Even before the HIV epidemic, the incidence of anal cancer among MSM was estimated to be as high as 37 per 100,000 [54], which is close to the incidence rate for cervical cancer in Western countries before routine screening was introduced, and as high as the incidence of cervical cancer today in many developing countries [1].

Estimates of the prevalence of anal HPV infection are also higher among MSM than among either women or heterosexual men (Table 1). Van de Snoek evaluated MSM from the Netherlands and reported a prevalence of anal HPV infection 33% [40]. A study of San Francisco MSM reported an even higher prevalence of anal HPV infection of 61% [55], while a study that recruited HIV-negative MSM from four US cities had a similarly high prevalence of 57% [56]. A recent study evaluated 176 MSM from three countries found that 47% had anal HPV. One longitudinal study followed MSM for HPV infection for a median of 29 months [57]. The baseline prevalence of anal HPV infection among the HIV-negative men was 66% and the incidence was 39/100 person-years [57]. MSM who are infected with HIV have an even higher rate of anal HPV infection (See Section 3).

Several studies have examined risk factors for anal HPV infection and disease among MSM. Those that evaluated receptive anal intercourse all found it to be predictive of anal HPV infection in MSM [53, 55, 57-59]. Other risk factors include a higher number of male partners [53, 55, 58] and indicators of rectal inflammation or damage [55]. Several studies have also identified cigarette smoking as a risk factor for both anal HPV

infection and anal cancer [60, 61]. Carcinogens in cigarette smoke may provide the additional cellular-level event that is needed to propel anal HSIL to cancer. Although the studies consistently show that the level of anal HPV infection is high among MSM, more research is needed to determine the incidence of anal HPV infection in this population. Also, in is important to distinguish between risk factors for acquisition of infection and those for persistence of infection. In cross-sectional studies these two measures are combined and therefore longitudinal studies are needed to determine both risk factors for acquisition of HPV infection and risk factors for persistence of HPV infection.

3: HPV infection among HIV-infected Individuals

3A: Prevalence and Incidence of HPV Infection and associated disease among HIV-infected individuals

Individuals infected with HIV have higher prevalences and incidences of HPV infection than HIV-uninfected individuals. In recent studies, the prevalences of cervical HPV infection among HIV-infected women were in the range 60-70%[62, 63]. The prevalence of anal HPV infection among HIV-infected women may be even higher (>70%), and one study found that in the same study sample, HPV was detected more often in the anus than in the cervix [50]. Having a low CD4 T-cell count and a high HIV serum viral load are associated with higher prevalence of HPV infection [62-64], greater persistence of HPV infection [65, 66], less frequent clearance of prevalent HPV infection [65], and higher HPV viral loads [67, 68]. The prevalence and incidence of premalignant cervical lesions are also higher among HIV-infected women than among HIV-uninfected women [69-72]. Premalignant anal lesions are also more common among HIV-infected women [49, 73]. The incidences of both cervical cancer and anal cancer in women are also elevated among those who are HIV-infected compared to those who are not [74].

The association between HIV infection and HPV infection is also seen in men, particularly for anal HPV among MSM. In the pre-highly active antiretroviral therapy (HAART) era, almost all HIV-infected MSM studied were positive for at least one type of anal HPV [55, 57]. The advent of HAART has not had a great impact on estimates of prevalence of HPV in this population and recent studies of HIV-infected MSM report high prevalences. De Pokomandy et al (2009) found a prevalence of anal HPV of 98% in their study of MSM positive for HIV [75]. A study from Australia detected anal HPV infection in 95% of HIV-infected MSM [76] and a recent French study of 146 HIV infected MSM found that 75% of participants were positive for at least one type of HPV, and of the 146 men 65% had an oncogenic HPV type [77].

Additionally, HIV-infected MSM have 59 times the risk of developing anal cancer compared with men in the general population and twice the risk of HIV-negative MSM [74]. The prevalence and incidence of the precursor lesions to anal cancer, anal HSIL, are also highest among HIV-infected MSM, followed by HIV-uninfected MSM[31].

Fewer data exist on penile HPV infection among HIV-infected men. One study analyzed previously collected penile smears from HIV-infected and HIV-uninfected men and found HPV DNA in 50% and 30%, respectively, and PIN 2-3 in 67% and 17%, respectively [78]. In a mixed sample of HIV-infected heterosexual men and MSM, Sirera et al. found that 36% had detectable penile HPV DNA [79], which is similar to the prevalence among HIV-negative men. A recent study from Silva et al (2011) found no difference in persistence or clearance of HPV infection of the penis between HIV-infected and HIV- uninfected men [80]. However, the risk of penile cancer is seven times higher in HIV-infected men when compared to HIV-uninfected men[74]. It remains unclear to what extent HIV infection influences HPV infection and disease of the penis. Perhaps in part because the biology of penile cancer and its association with HPV infection is less clear than that of both cervical and anal cancer. Further studies are needed to evaluate the relationship between HIV infection and both HPV infection and HPV-associated disease.

3B: Mechanism of interaction between HIV and HPV

The effect that HIV has on HPV infection is likely modulation of the immune response. Although not fully understood, HPV infection is believed to be resolved largely through the action of activated cytotoxic T-cells. This immune response is weak compared with the response to systemic viruses [4, 6]. A low number of circulating HPV-specific memory cells may make HPV-specific immunity more vulnerable to the effects of HIV-induced immune suppression [4, 6]. HIV affects CD4+ T-cells as well as local immune responses and may reduce overall HPV-specific immunity [4, 6]. Under the pressure of immune suppression, an individual is at greater risk of acquiring a new HPV infection and less likely to resolve an existing infection. Because persistent HPV infection is a prerequisite for development of HSIL, HIV-infected individuals are more likely to develop premalignant lesions and to have these persist long enough to accumulate the genetic changes necessary to develop into cancer [4, 6]. This is consistent with the findings described above showing HPV-associated disease is more common among HIV-infected individuals. Lower CD4+ T-cell counts are the main risk factor for HPV-associated disease.

There is also some evidence that the two viruses may directly interact, although they do not infect the same cells. The HIV-1 *tat* protein increases expression of the E6 and E7 genes *in vitro*, and this protein is able to diffuse through cell membranes [81]. However, the HIV-1 *tat* protein has not been found in HPV-infected epithelial cells or in cervical tissue, making it unlikely that direct interaction of the viruses is important for HPV-associated disease outcomes [6].

3C: The effect of Highly Active Antiretroviral therapy (HAART) on HPV infection and HPV-associated disease

Surprisingly, HAART has not been shown to have a substantial impact on the natural history of HPV-associated disease [4, 6, 82, 83]. Although the evidence is mixed, the

consensus is that HAART may help protect against acquisition of new HPV infection and it may increase the likelihood of regression of LSIL. HAART does not, however, appear to increase the likelihood of regression of HSIL or prevent the progression of HSIL to cancer [6]. The incidences of both cervical cancer and anal cancer have been increasing in the post-HAART era [74, 84].

The immune reconstitution that follows initiation of HAART includes both memory and naïve CD4 T-cells and a reduction in the occurrence of opportunistic infection follows [85]. The immune response to infections in the presence of HAART requires infection and exposure to antigen under inflammatory conditions [85]. As described above, in mucosal infection HPV is not as exposed to the immune system. Additionally, once a HSIL is established, immune function may have a limited role in control of HPV-disease progression. Given that HAART is initiated in patients who are already immune suppressed (CD4+ T-cell counts below 350/mm^3) many will have already developed HPV-associated disease and HAART will have a limited opportunity to have an effect [6, 85]. Many individuals are now living with HIV infection as a chronic, manageable disease, and will now live long enough to develop HPV related diseases if infected with HPV. It is crucial that we continue to study the relationship between these two viruses to identify potentially modifiable risk or prognostic factors.

4: HPV INFECTION IN INDIA

4A: HPV INFECTION AND DISEASE AMONG INDIAN MEN

Penile cancer is more common among Indian men than it is among men in the US. Data from the Indian Council for Medical Research show that the incidence in urban areas was from 0.7 to 2.3 /100,000 and as high as 3/100,000 in rural areas [86]. Penile cancer represents more than 6% of all malignant cancers in Indian men [86]. It is unknown what percentage of these cases of penile cancer in India can be attributed to HPV infection, although there is no reason to believe that it would be different from the percentages in the US and other western countries, where 40-50% of men with penile cancer have detectable HPV DNA [84].

There is little information available on penile HPV infection among Indian men. One study from a pathology department in Mumbai tested penile cancer samples from seven men and none were positive for HPV [87]. The authors state that the results should be interpreted with caution, however, because of the small sample size. A second study conducted in male partners of women with cervical cancer found that 67% of the 30 men studied had penile HPV detectable [88]. The higher rate of penile cancer in India suggest that more investigation of penile HPV infection among men is necessary.

There is less information on anal cancer or other HPV-associated anal disease among men in India. India's cancer registry does not report anal cancer separately. The IARC publication "Cancer Incidence in 5 Countries, Vol IX" reports age-adjusted incidence rates of anal cancer among men in India ranging from 0.0 to 0.8 per 100,000 per year [89]. A study from the Tata Memorial Hospital (TMH) in Mumbai comparing the

incidence of cancer among HIV-infected and HIV-uninfected individuals suggested that anal cancer may be elevated among HIV-infected Indian men, with a proportional incidence ratio of 10.3 (95% CI 4.3-24.8) [90]. Given that 90% of anal cancers are attributed to HPV infection, this finding implies that anal HPV infection is an important factor among HIV-infected Indian men. The best established route of transmission for anal HPV is receptive anal intercourse and Indian MSM should also be a focus of investigation to determine if HPV infection and disease is increased in this group.

4B: HPV AND CERVICAL CANCER IN INDIAN WOMEN

Cervical cancer is the most common cancer among Indian women [91]. There are an estimated 132,000 new cases of cervical cancer diagnosed each year and 74,000 deaths [91]. India has more than 25% of all cases of cervical cancer worldwide [92]. However, there is substantial regional variation in the incidence of cervical cancer; the highest reported incidence is in the south Indian city of Chennai at 30 per 100,000 [91].

Several studies using sensitive PCR based HPV DNA detection methods have evaluated HPV infection in either biopsy samples or cervical smears of women diagnosed with cervical cancer or premalignant lesions (Table 2). Most of the reported prevalences of HPV infection in these samples are consistent with what is seen worldwide, ranging from 82% to 100% positive for at least one type of HPV. The type distribution of HPV found in these samples is also consistent with what is seen worldwide, with the largest proportion having HPV-16 detectable, followed by HPV-18. After these two types, HPV-33, -31, -35, and -45 are the most common types, although they are ranked differently in different studies. Several studies have also found a high proportion of other high-risk HPV types. For example, Sowjanya (2005) found that HPV-52 was present in 2.8% of cancers, HPV-58 in 2.8%, HPV -59 in 2.8% and HPV -73 in 2.8% in women in a regional cancer center [93]. Gnanamony (2010) reported 3% of HPV-52, and 1.4% each of HPV -51, HPV -58, HPV -73 in their sample of cervical biopsy samples from women with CIN 2/3, cancer *in situ,* or invasive cervical cancer [94]. A meta-analysis of eight studies from India reported the adjusted prevalence of HPV infection across all included studies was 94.6% (95% CI 94.0%-95.3%) and the most common types seen in these eight studies were 16, 18, 45, 33, and 35[95]. Overall, the existing findings suggest that HPV vaccines containing the high-risk types 16 and 18 that have been recently approved for use in women in India could prevent cervical cancer if there is wide uptake of the vaccine.

Of some concern is that a few recently published papers from India suggest that HPV is not a necessary cause for cervical cancer [96-98]. Authors of these papers cite detection of HPV in a low proportion women with cervical cancer in selected studies and the association of cervical cancer with other factors (such as other sexually transmitted infection). If such views are common among medical professional in India, education regarding the established causal relationship between HPV infection and cervical cancer may not be reaching all physicians in India. This has implications for any HPV vaccination effort made in India. If physicians are not convinced of a causal relationship, they may be hesitant to recommend HPV vaccine.

4C: HPV INFECTION IN INDIAN WOMEN WITHOUT CERVICAL CANCER

There have been several studies of the prevalence of HPV infection in either women with normal cervical cytology or women being screened for cervical cancer in India (Table 3). The studies are exclusively cross-sectional in nature and enrolled diverse sample populations, differing in age, marital status, disease status of the cervix, history of commercial sex work, and region of India from which they came. Of the studies that tested for many HPV DNA types combined, the prevalence of any HPV infection varied from 7% to a high of 37%. Many studies, however, tested only for HPV 16 and 18 or only for oncogenic HPV types. In these studies the prevalences of oncogenic HPV types ranged from 3% to 63%. Two studies included only commercial sex workers (CSW)[98, 99]. These studies, both from West Bengal, found the highest prevalences of the studies reviewed. Chaterjee (2001) tested only for HPV 16 and 18 using in-situ hybridization in 27 women and found that 63% were positive for either HPV 16 or 18 and these women also had a high prevalence (42%) of CIN 1-3 [98]. This study also reported a statistically significant association between HPV 16/18 cervical infection and Herpes Simplex virus type 2 (HSV-2) infection in their study population (unadjusted OR: 18 (1.8-184, p=0.01)[98], consistent with findings from other studies of sexually transmitted infection among commercial sex workers who have a higher prevalence of STIs than other women and are likely to have multiple STIs [100, 101]. The second study including 229 women working in brothels, tested for oncogenic HPV types through Hybrid Capture 2 (HC-2) and 25% of the sample was positive for an oncogenic HPV type[99]. This study found that younger age, number of years working as a sex worker, and number of daily clients were all associated with a higher prevalence of infection with oncogenic HPV [99]. These associations are consistent with studies that correlate younger age of first sex and number of partners as with cervical HPV infection [1].

As expected, the majority of the studies of HPV in healthy Indian women who were not commercial sex workers found prevalences lower than those seen in commercial sex workers. These women were largely recruited from women taking part in cervical cancer screening or women presenting to gynecology clinics for family planning services or because of vaginal symptoms (e.g. vaginal discharge). Figure 1 presents the reported prevalence of cervical infection with oncogenic HPV by Indian state. Where there were multiple studies in a single state, the combined prevalence weighted by study population is presented. The most striking feature of this map is the general lack of information on the prevalence of cervical HPV in India. In the states for which information is available, in most instances the data come from a single study from a single town (although one study did sample more widely [102]). The highest prevalence of cervical HPV infection of any type was 36% [103], in a study that enrolled women with normal cervical cytology attending a gynecology outpatient clinic in the northern region of Chandigarh. This prevalence is substantially higher than the 8-16% reported elsewhere in many parts of India. The women in the study were not exclusively young (mean age 38 years), and they had a high prevalence of vaginal discharge. The authors cite “poor hygiene” as the reason for the higher overall prevalence of HPV infection and the high prevalence of vaginal symptoms as evidence of poor hygiene.

Although there is no known relationship between hygiene and HPV infection, vaginal symptoms may be evidence of other sexually transmitted infections and thereby indicators of sexual behaviors that could lead to a higher prevalence of HPV infection. The authors did not query their participants about their sexual behaviors or the sexual behavior of their spouses. Additionally, the study did not use the standard HPV DNA PCR primers, instead using a primer directed at the E1 gene which may have different sensitivity and/or specificity compared with the standard primers. Most other studies from India found prevalences of cervical HPV infection that were lower than 36%. Franceschi et al. present prevalences that are age-adjusted to the world standard population (17.7% for overall HPV and 15.2% for oncogenic HPV) [102], and a 2005 publication from the same group reported the results of population-based cervical HPV prevalence surveys from 11 countries from Europe, Asia, Africa, and South America. The age-adjusted prevalence of cervical HPV infection for India was 14.2% (95% CI 12.0-16.4) [104], the highest prevalence among the Asian counties and the third highest of all countries included in the survey (behind Nigeria and Argentina) [104].

The highest separate prevalence of oncogenic HPV infection was in a study from rural Tamil Nadu in 2005 [102]. This study which enrolled 1891 married women sampled from 113 villages, detected HPV DNA through GP5+/GP6+ PCR primers followed by hybridization of products in EIA. Twelve and a half percent of the women had oncogenic HPV and 16.9% had any HPV. Other studies have reported very low prevalences of HPV infection including a study of women recruited from community cervical cancer screening programs in the North East, including prevalences of 3.5% in Manipur and of 5.3% in Sikkim [105]. These investigators tested only for HPV 16 and 18; the combined prevalence is still low, however, when compared to other studies that include only these two types. The variation seen in the prevalence of HPV infection across India does not seem to be explained by differences in regional prevalence, but at present, there is not enough published information available to draw any definitive conclusions.

One potential reason for the variation in the reported prevalences of cervical HPV infection in India could be the age of the study sample. However, the trends seen with HPV infection and age in studies from India do not follow the pattern seen worldwide. For example, a study in 2010 that included only women aged 16-24 years of age found a low prevalence of 8.4% for any HPV infection and a prevalence of 7.2% of oncogenic HPV types [106]. Another study that enrolled only women above 30 years of age old found a higher overall prevalence of HPV(15.5%) and a 13.2% prevalence of oncogenic types [107]. The higher prevalence of HPV infection among older women differs from what has been observed in western countries where the prevalence of cervical HPV infection is higher in younger women. Variation in the age of participants may explain some of the variation in reported prevalences of HPV infection among Indian women, but other factors are likely involved.

Another interesting observation related to age and HPV infection in India is that the age curve typically seen in Western countries (higher prevalence in younger women, with a peak at 15-25 years of age) is not always seen in Indian studies. For example, a survey of almost 2000 women from rural Tamil Nadu (southern India) found a

prevalence of cervical HPV infection in married women of 16.9%; the prevalence did not decrease with increasing age [102]. Franceschi et al. (2006) compared their results from India to results from 15 other countries and found that several other countries had different age curves, some showed no associations between HPV infection and age as with India, and some counties had age curves with two peaks one at 15-25 and one at older ages[22]. Duttagupta (2004) also found an unchanging prevalence of HPV infection with age but only among the Muslim women in the study[108]. Among Hindu women there was a linear trend of decreasing prevalence with increasing age (although the authors report that there was no difference in overall prevalence of HPV infection by religious group). Aggarwal (2006) found the same lack of association between prevalence of HPV and age but a much higher overall prevalence of 36.8% [103]. Gupta (2009) found that the prevalence of HPV 16/18 was highest among 40-45 year old women with normal cervical findings from Delhi [88]. A study of married women from three states in North-eastern India found differing age curves for each state [105]. In Manipur, the HPV prevalence did not change with age; women in Sikkim had an increasing prevalence of HPV with increasing age, and women in West Bengal had a decreasing prevalence with age. Of the three states, the women from West Bengal had the youngest age of marriage, the youngest age of first birth, and the highest number of total births per women, all factors cited by the authors as associated with both a higher prevalence of HPV and cervical cancer. These other factors may also have a role to play in the shape of the age curve.

In western countries, two reasons have been offered for the peak in the 15-25 age group. The first is that in the cervix of young women the transformative zone between the squamous and cylindrical epithelium is more superficial and moves deeper into the cervix as a woman ages. The second reason is that most young women become sexually active in this age group and quickly get exposed to HPV infection. Most women with HPV infection resolve the infection and few have instances of re-activation (except in the presence of immunosuppression). There is no reason to believe that the anatomy of Indian women is different from that of women in the west, therefore there must be differences in either sexual behavior or the natural history of infection. Indian women could be acquiring new HPV infections in older age through new sexual exposure. There is some data implying that married women may have exposures to new HPV infections through their husbands. Franceschi et al. (2003) and others have previously reported that a high proportion of husbands of women with cervical cancer patients have extramarital sex partners [109]. There is also a high reported rate of sex with CSWs among married Indian men [110]. Given the high prevalences of HPV infection in female CSWs discussed above, these men may be at high risk of acquiring and transmitting an HPV infection to their wives. Alternatively, Indian women could have lower rates of clearance of HPV infection (or high rates of persistence) compared with western women, or they could have higher rates of reactivation of an old infection. However, in the context of a cross-sectional study it is not possible to determine if prevalent infections are new, persistent, or re-activated. More research is needed to determine the reason for the different pattern of association between age and cervical HPV infection among Indian women.

The majority of studies of HPV infection in India included only married women. The authors report that unmarried women usually will not consent to a gynecological exam in India. Additionally, most studies have not asked about premarital sex or any kind of sexual behavior history because of concerns about the cultural appropriateness of asking these kinds of questions. Many authors used age of marriage as a surrogate for age of sexual initiation. Little work has been done on premarital sexual behavior of women in India, particularly in rural areas, and it is therefore difficult to know if age of marriage is an appropriate surrogate for sexual initiation. This limits the ability of investigators to estimate when women had their first exposure to HPV. If women are having their sexual debut at the time of marriage, it may be more culturally acceptable to recommend HPV vaccination to women shortly before marriage, instead of to adolescent girls, as is currently recommended. This would move the discussion of sexual behavior and cervical cancer from the pediatricians' office to the gynecologist office as part of discussions of marriage and family planning. This may be more culturally acceptable to both Indian women, and their parents. However, it is not known what percentage of Indian women are engaging in sex before marriage and more work is needed in this area before changing the recommendation.

4D: Risk factors for cervical HPV infection among Indian women

Most of the studies of HPV infection among Indian women did not examine risk factors for cervical HPV infection. Those that did reported that low socio-economic status (SES), as measured by income or education, was associated with an increase in the odds of HPV infection [88, 99, 103], consistent with studies of HPV infection and cervical cancer worldwide. Low SES may be a surrogate for poor nutrition, and lower levels of nutrients have been associated with persistent infection although the evidence for these associations is mixed [1]. Low SES has been consistently found to be a risk factor for cervical cancer, and this association is usually attributed to low levels of cervical cancer screening in low-income populations. However low SES could also be confounded by sexual behaviors of either women or their husbands, leading to increased HPV infection; currently, there are no data available to explore this possibility.

Studies in India have also found that women living in rural areas have a higher prevalence of HPV infection than women living in cities. Women in rural areas may have lower income and education and the association with HPV may be confounded by SES. Having a husband who travels for work may also be a confounding factor in this association. Men from rural areas are likely to travel to a city to find work and often remain separated from their wives for months at a time; as a result they may be more likely to visit commercial sex workers during these times [110]. Given the high prevalence of HPV infection among commercial sex workers in India, it is likely the migrant workers are a bridge from commercial sex workers to their wives. Future studies of rural women should include information on occupation and travel of the spouse in order to explore this possibility.

Several Indian studies have identified an increased risk of HPV infection in women of higher parity [88, 102]. Worldwide, studies have also shown a link between parity and

cervical cancer, but it is unclear if parity is a surrogate for sexual exposure or if the hormonal and physical changes that accompany pregnancies are associated with cancer [1].

4E: Discussion and Recommendations for Future Research

Given the high incidence of cervical cancer, it is crucial to understand the epidemiologic features of HPV infection in Indian women. There are several areas that warrant further research. First, studies of the prevalence of HPV among women throughout the country are needed. In many areas of India no studies have been conducted. It is important to sample from both urban centers and rural areas because their prevalences have been shown to differ. It may be that regional differences are actually a result of the rural/urban make-up of the study participants. It is also possible that regions differ not only in prevalence but in HPV type distribution. It is important that studies test for more than just HPV types 16 and 18 because several studies have found high proportions of other oncogenic types of HPV. Having an accurate assessment of the prevalence of HPV infection throughout India will help focus prevention efforts, both vaccination and behavioral interventions, as well as identify populations at high risk for cervical cancer in need of cervical cancer screening.

There is also a need in for studies of the incidence of HPV infection among Indian women. The possibility that women in India have high prevalences of HPV infection throughout life has important implications for both cervical cancer screening programs and for vaccine recommendation strategies. However, it is currently unclear if the relationship seen between age and HPV infection in some studies in India is due to bias, random error, or if there is truly no association between age and HPV infection, unlike in Western countries. If the Indian studies are accurate and there is no association, the reason is currently unknown. Cross-sectional studies cannot address this question because they cannot distinguish between new, persistent, or re-activated infection, and therefore large, well-designed cohort studies of Indian women are needed. To evaluate the association of age and HPV infection women should be enrolled by different age strata and measurement should be made in each stratum of incidence of new infection, clearance of prevalent infection, and persistence of prevalent infection.

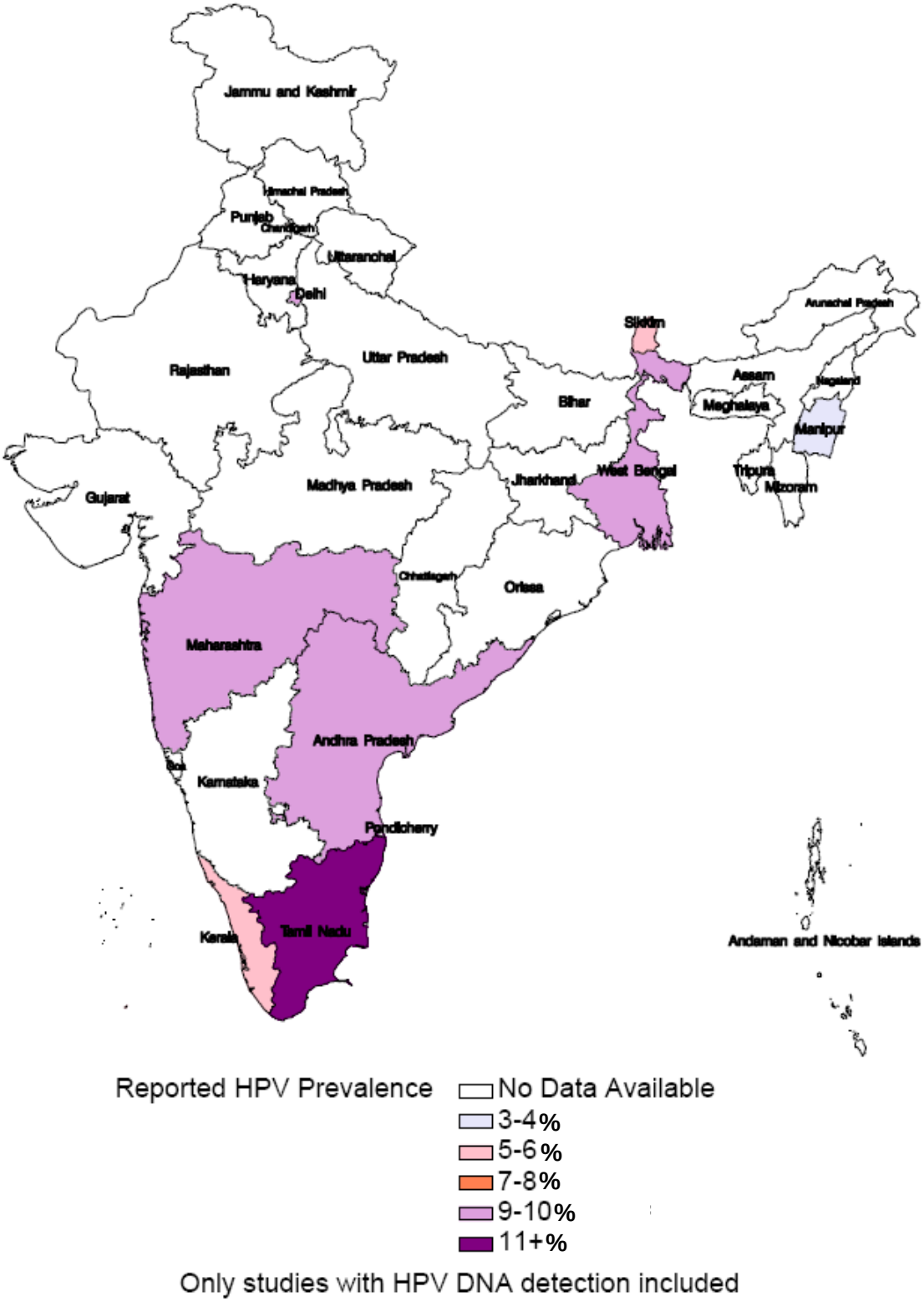
Another missing piece of information from India is an accurate sexual risk history in association with HPV infection. Many of the studies collected minimal if any data on sexual history, including sex before marriage; age of first sex,; number of partners; and any potential preventative methods used such as condoms or diaphragms. These authors cite the very real problem that it is culturally inappropriate to ask these questions of Indian women. There will undoubtedly be many barriers to conducting studies that include such questions, or that attempt to include unmarried women in study samples. However, I believe an effort should be made in this direction while remaining as culturally sensitive as possible. Perhaps some of the same strategies used by investigators working with other behaviors that are stigmatized could be utilized to do sexual health research in Indian women. For example, research is conducted with

men who have sex with men in communities where sexual behavior with other men is illegal [111, 112]. Research strategies could include nesting the sexual behavior study within a study of general women's health. Also, the consent for the sexual behavior piece of the study could be separate from the general study and administered in a private setting rather that in a participant's home or in a community setting. Another strategy could be to have interviewers and other staff trained to be sensitive to participant's potential embarrassment, hesitation, or concerns. Again, there may good examples from researchers working with populations of men who have sex with men. Several studies from India collect detailed sexual histories from Indian men who have sex with men [110, 113, 114], behaviors that are also highly stigmatized in India. Conducting preliminary focus groups with women, community members, and local non-governmental organizations on acceptable methods for enrollment, study procedures, and questionnaire text, would be an invaluable first step in undertaking research on this sensitive subject.

There are no published studies on the prevalence of anal HPV from women in India. It would be important to understand the prevalence rates for anal HPV in Indian women given that HPV infection is associated with anal HPV-associated disease. Sexual behavior among Indian women may be different from their western counterparts and it would be important to know if, as in the West, the anus is an important area for HPV infection and its sequelae. Also, in a study that compared the incidence of cancers in HIV-infected individuals referred to an HIV cancer clinic in Mumbai both cervical cancer and anal cancer had increased proportional incidence ratios compared to the general Indian population, indicating that HIV-infected Indian women are at increased risk of developing an HPV-related cancer [90]. There are only two studies of HPV infection in HIV infected women in India [115, 116]. Many Western studies have shown that HPV-associated disease is more prevalent among HIV-infected women compared to HIV-uninfected women[6]. It would be an important addition to the field to evaluate cervical HPV infection and HPV associated disease among HIV-infected Indian women as well as anal HPV infection and disease.

Figure 2:

Reported Prevalences of high-risk HPV (or 16/18) in Indian Women



Only studies with HPV DNA detection included

Table 1: Epidemiological studies of the prevalence of anal HPV infection in men

Author	Date of Publication	Population	N	Prevalence of Any anal HPV	HPV DNA Detection Technique	Risk Factors for infection
Heterosexual Men						
van der Doornum[36]	1994	Heterosexual men with multiple partners attending STI clinics (Netherlands)	85	1.2%	PCR (Types 6/11/16/18/31)	-
Nicolau[52]	2005	Partners of Women with cervical HPV DNA (Brazil)	50	8.0%	HC 2	-
Nyitray[53]	2010	Self Identified Heterosexual Men (Brazil, Mexico, US)	902	12.0%	PCR (PGMY 09/11 primers)	-duration of relationship with primary partner -oral/anal sex with man -lifetime number female partners
Men who have Sex with Men						
Nyitray[58]	2011	Self Identified Heterosexual Men (Brazil, Mexico, US)	1305	12.2%	PCR (PGMY 09/11 primers)	-≥10 female partners - relationship <1 year - HBV diagnosis-
Critchlow[57]	1998	HIV-negative MSM (US)	262	66%	PCR (PGMY 09/11 primers)	- receptive anal intercourse - any sex since last visit
Palefsky[55]	1998	HIV-negative MSM (San Francisco)	262	61%	PCR (PGMY 09/11 primers)	- rectal drug use - rectal discharge - receptive anal intercourse
Van der snoek[40]	2003	HIV-negative MSM attending STI clinic (Netherlands)	241	33%	PCR (Types 6/11/16/18/ 31/33)	- STI infection
Ching-Hong[59]	2004	HIV-Negative MSM (US)	1218	57%	PCR (Types 6/11/16/18/ 31/33)	- receptive anal intercourse -> 5 partners past 6M
Vajdic[76]	2009	HIV-Negative MSM (Australia)	193	79%	HC 2	-

Table 1: Epidemiological studies of HPV infection in women with cervical cancer in India.

Author	Date Of Publication	Population	Age (years) Mean (range)	Number of participants	Prevalence of Any HPV*	Prevalence of Oncogenic HPV*	HPV DNA PCR Primer or Detection Technique	HPV-associated Disease	Risk Factors
Gnanamony[94]	2010	CMC Vellore (Tamil Nadu)	49	150	93%	67% - 16 14 % - 18 5% - 45 4% - 31 3% - 52	PGMY 09/11	CIN 3-ICC	-
Basu[117]	2009	Cervical Cancer Cases (4 states)	51	278	92%	66% - 16 17% - 18 6% - 33	MY09/11 PreTect HPV-Proofer assay	SCC AC	-
Gheit[87]	2009	Pathology Department (Maharashtra)	51.4 (28-86)	180	93%	82% - 16 17% - 45 10% - 18	Multiplex PCR/APEX assay	SCC	-
Travasso[118]	2008	Clinical Samples (Maharashtra)	-	63	-	97% -HR 74% - 16 11% - 18 3% - 33 2% - 31 2% - 45	GP5+/6+	Cervical Cancer	-
Bhatla[119]	2006	Cancer Clinic (Delhi)	48 (25-70)	106	-	98% - HR 74% - 16 14% - 18 11% - 45	PGMY 09/11	ICC	-
Peedicayil[120]	2006	Cervical Cancer treatment (Vellore)	38 CIN 48 ICC	11 119	95% ICC 90% CIN		PGMY 09/11	CIN ICC	-
Rughooputh[96]	2006	Slum dwellers (Ahmedabad)	49.3	17		33% - HR	MY09/11 GP5+/6+	-	-
Sowjanya[93]	2005	Cancer Hospital (Andhra Pradesh)	55 (30-63)	41	-	87% - HR 67% - 16 19% - 18 6% - 33 6% - 35 6% - 45	HC 2 – HR DNA PCR PGMY 09/11		-
Sathish[121]	2004	ICC Surgery Patients (Vellore)	47	58	94.8%		MY09/11	ICC	
Franceschi[109]	2003	Hospital-based (Tamil Nadu)	<41->=56	179	99.4% SSC 100% AC	99.4% SSC 100% AC	GP5+/6+	ICC	-Illiterate> 4 births ,<45 yrs menopause ,toilet in home, husband extramarital affairs

Duttagupta[122]	2002	Cancer referral hospital (West Bengal)	47.29 (24-80)	50	-	82% - 16/18	PGMY 09/11	SSC	-
Saranath[123]	2002	Radiotherapy referrals (Maharashtra)		337	-	77% - 16/18	MY09/MY11	ICC	-
Munirajan[124]	1998	Government Cancer Hospital (Tamil Nadu)	52 (30-70)	43	70%	53% - 16 13% -18	PCR Primers to E6 & E7	ICC	-

* Women may be infected with more than one HPV type at the time of testing

AC=Adenocarcinoma
CIN 1-3=Cervical intraepithelial neoplasia grades 1/2/3
GP5+/6+= HPV DNA PCR consensus primer set to HPV L1 protein
HC2=Hybrid Capture 2
HR=High risk (oncogenic) HPV
ICC=Invasive Cervical Cancer
LSIL= low grade squamous intraepithelial lesion
MY09/11= HPV DNA PCR primer set to L1 protein
NE= Not Evaluated
PGMY 09/11= HPV DNA PCR consensus primer set to HPV L1 protein (based on MY09/11)
SCC=Squamous cell carcinoma

Table 3: Epidemiological studies of the Prevalence of HPV infection in Indian women.

Author	Date	Population	Age Mean (range) Age Curve	N	Prevalence of Any HPV	Prevalence Oncogenic HPV	HPV Detection Technique	HPV-associated Disease	Risk Factors for infection
Datta[106]	2010	Slum dwellers Married (Delhi)	(16-24)	1300	8.4%	7.2% - HR	DNA PGMY 09/11 HC2		
Gupta[88]	2009	Attending GYN No CIN (Delhi)	29 Flat	769	-	16.6% - 16/18	DNA MY09/MY11	-	-Older age -≥ 3 births -Unhealthy cervix -Low SES -Rural
Bhatla[107]	2008	Women with vaginal symptoms (Delhi)	36 (30-74) NE	524	15.5%	13.2% - HR	DNA PGMY 09/11	5% CIN1 8% CIN2+	
Sarkar[99]	2008	Sex Workers (West Bengal)	30 (10 - ≥40) Decrease	229	-	25% - All HR 10% - 16 7% - 18	HC2 - HR	1% LSIL	-<1 year of sex work -Higher # of daily clients -Daily income >= 101 INR
Laikangbam[105]	2007	Community cervical screening (Manipur, Sikkim, West Bengal)	22 (11-55) Flat 20 (10-30) Increases 16 (9-33) Decreases	692 415 1112	7.4% 12.5% 13%	3.5% -16/18 5.3% - 16/18 9.17% - 16/18	DNA MY09/MY11 AND Real-time PCR 16/18 E6	6.7% 11% 11%	- Age curve flat - Prevalence increases with age - Prevalence decreases with age
Aggarwal[103]	2006	Attending GYN Normal cervical cytology (Chandigarh)	38 (19-75) Flat	472	36.8%	8.2% - HR	DNA PCR (E1 primers consensus E6 type specific)	-	-Low SES, Low Edu, -rural
Arora[125]	2005	Attending GYN Neg pap with inflammation (Delhi)	36 (20-60) Decrease	160	-	10% - 16/18	DNA PCR (ORF and E6)	-	
Clifford[104]	2005	Healthy Women	(15-64) NE		14.2%		DNA MY09/MY11		-
Franceschi[102]	2005	Rural Women (Tamil Nadu)	(<25 -≥45) Flat	1943	16.9%	12.5 - HR 3.8% - 16 1.5% - 56 1.2% - 31,32	GP5+/6+	4.9% Ab.Pap	- 0 v. 1-2 pregnancies - Condoms use increased risk
Sankaranarayana[126]	2005	Low-SES rural (Maharashtra)	(30-59) NE	36,938	-	10.3% - HR	HC II	2.3 %CIN1 0.9% CIN2/3	

								0.2% ICC	
Sowjanya[93]	2005	(Andhra Pradesh)	(30-55+) Flat	185	-	10.3% – HR	HC II – HR DNA PCR PGMY 09/11		
Duttagupta[108]	2004	Women attending child health clinic (West Bengal)	30 Muslim-Flat Hindu - Decrease	1044	-	8.5% - 16/18	DNA PCR E6	-	No relationship with Muslim religion
Sankaranarayana[127]	2004	Rural screening camps (Kolkata, Mumbai, Trivandrum)	(25-65) NE	10,123 3,474 4,488	-	6.1-9.0% 7.6% 6.4%	HC II	0.02% CIN 1 0.003 ICC	
Duttagupta[122]	2002	Low SES married rural women (West Bengal)	29 (14-80) Decrease	850	-	8.82% - 16/18	DNA PCR E6	6.8% abnormal cytology	
Saranath[123]	2002	Radiotherapy referrals (Maharashtra)	-	164	-	15.2% - 16/18	MY09/MY11		
Chatterjee[98]	2001	Sex Workers (Calcutta)	28 (19-41) NE	27	-	63% - 16/18	In-situ Hybrid	42% CIN1-3 16% CIN3	-Cervical HSV infection
Gopalkrishna [128]	2000	STI Clinic Patients (New Delhi)	27 (15-44)	50	-	30% - 16	PCR with primer for LCR of HPV 16		

* All studies used cross-sectional study designs or the baseline visit of a longitudinal study.

AC=Adenocarcinoma
CIN 1-3=Cervical intraepithelial neoplasia grades 1/2/3
GP5+/6+= HPV DNA PCR consensus primer set to HPV L1 protein
HC2=Hybrid Capture 2
HR=High risk (oncogenic) HPV
ICC=Invasive Cervical Cancer
LSIL= low grade squamous intraepithelial lesion
MY09/11= HPV DNA PCR primer set to L1 protein
NE= Not Evaluated
PCR=polymerase chain reaction
PGMY 09/11= HPV DNA PCR consensus primer set to HPV L1 protein (based on MY09/11)
SCC=Squamous cell carcinoma

CHAPTER 2: Risk factors for prevalent anal Human Papillomavirus infection among HIV-infected men who have sex with men in San Francisco

Abstract

Objective: Human immunodeficiency virus (HIV)-infected men who have sex with men (MSM) are at high risk of anal cancer compared with the general population. Human papillomavirus (HPV) infection, particularly HPV 16, causes anal cancer. Our aim was to determine the prevalence and risk factors for anal HPV infection in a population of HIV-infected MSM, most of whom were receiving highly active antiretroviral therapy.

Design: We examined data from the baseline visit of a four-year cohort study.

Methods: 318 HIV-infected MSM from San Francisco completed a detailed sexual risk behavior questionnaire. An anal swab was used to collect a sample for HPV DNA testing using L1 HPV DNA PCR. We used log-linear multivariable models to determine risk factors for oncogenic anal HPV infection and anal HPV 16 infection specifically.

Results: Ninety-two percent of HIV-infected MSM had at least one type of anal HPV present; 80% had an oncogenic HPV type and 42% had HPV 16. A higher total number of male partners was associated with anal HPV 16 infection (RR= 1.81 (95% CI: 1.24 - 2.63), p=0.0034)) for 201-1000 lifetime partners compared with 1-200 partners. A higher number of partners (200+) with whom the participant is the insertive partner was also associated with anal HPV 16 infection (RR=1.72 (1.24-2.38)) compared to 0-50 partners. None of the sexual risk factors investigated were associated with oncogenic anal HPV infection in our adjusted models.

Conclusions: The prevalence of oncogenic anal HPV infection, including HPV 16, is high in HIV-infected MSM consistent with previous findings from this and other populations. HIV-infected MSM should be considered for anal cancer screening and counseled about the elevated risk of anal HPV infection associated with increased partners and possible encouraged to btain the HPV vaccine.

Key Words: Human papillomavirus, HPV, HIV, AIDS, men who have sex with men, MSM, anal cancer

Introduction

The prevalence and incidence of anal cancer have been shown to be substantially higher among men who have sex with men (MSM) than among men in the general population, with an incidence of up to 37 per 100,000 [54]. The incidence of anal cancer among HIV-infected MSM is even higher than that among HIV-uninfected MSM; HIV-infected MSM are 59 times more likely to develop anal cancer compared with men in the general population and are twice as likely as HIV-negative MSM [74]. The high rates of anal cancer in this population have not been reduced by antiretroviral therapy (ART), and several papers have documented that the incidence of anal cancer has continued to rise, even after the introduction of ART [129-133].

Like cervical cancer, which is preceded by high-grade cervical intraepithelial neoplasia (CIN 2 or 3), anal cancer is preceded by high-grade anal intraepithelial neoplasia (HGAIN 2 or 3). HIV-infected MSM also have higher rates of HGAIN than HIV-uninfected men [134-139]. Further, like cervical cancer, anal cancer and AIN are associated with human papillomavirus (HPV) infection. Approximately 90% of anal cancers have detectable HPV DNA; two-thirds of those are type 16 and another 9 percent are type 18 [140]. The US Food and Drug Administration (FDA) recently approved an HPV vaccine (Gardasil) that has been shown to be effective in preventing HGAIN and anal HPV infection caused by HPV types 16 and 18 [18], and thus a large proportion of anal cancers are potentially preventable through vaccination.

Consistent with the increased risk of anal cancer and HGAIN, multiple studies have shown that MSM are at high risk for anal HPV infection and that HIV-infected MSM are at even greater risk than HIV-uninfected MSM [55, 57, 137, 139, 141-144]. The prevalence of anal HPV infection in HIV-uninfected MSM has been reported to be between 32% and 60% [30, 40, 55, 56, 145], and almost all HIV-infected MSM have detectable anal HPV infection [55, 75, 76]. It is well established that cervical HPV infection in women is sexually transmitted [146] and there is good evidence that anogenital HPV infection is sexually transmitted in men. Younger age of first sexual experience [41], anal intercourse [33, 55, 56], and number of sexual partners [34, 56, 147, 148] have all been shown to elevate the risk of penile HPV infection in both heterosexual men and MSM. There are fewer data available on risk factors for anal HPV infection among men, but sexual practices, such as receptive anal intercourse, have been associated with an increased risk of infection [55, 57, 59].

Most previous studies investigating the relationship between sexual behaviors and anal HPV infection have been limited in the number and types of sexual behaviors assessed, and have often not distinguished between insertive and receptive anal intercourse. Additionally, many studies have not controlled for potential confounding factors when assessing the relationship between sex and anal HPV infection. We previously performed a cross-sectional study prior to the introduction of ART to begin to understand HPV infection among HIV-infected MSM [55]. This study found that 93% of HIV-infected MSM had anal HPV infection and 38% were infected with HPV 16. Although a thorough history of sexual risk and lifestyle behaviors was collected in this

earlier study, because almost all HIV-infected men had at least one type of anal HPV infection, the study was unable to evaluate risk factors for anal HPV infection.

Here we report the results from the baseline visit of a prospective study designed to assess the natural history of anal HPV infection among MSM. The goal of this analysis is to identify sexual risk behaviors associated with prevalent anal HPV infection in HIV-infected MSM, many of whom were receiving ART. We focus our analysis on the HPV type most associated with anal cancer, HPV 16, and also on all oncogenic HPV types combined.

Methods

HIV-infected MSM were recruited to participate in a four-year prospective follow-up study conducted by the University of California, San Francisco (UCSF), through newspaper advertisements and other community outreach. At the baseline visit, participants completed an interviewer-administered questionnaire; a clinical examination, including collection of an anal swab for HPV testing; and collection of a second anal swab for cytology. All participants also had high-resolution anoscopy (HRA) for detection of anal lesions. Blood was collected for CD4+ lymphocyte counts, which were measured using standardized two- or three-color fluorescence methods. Plasma HIV viral load (HIV VL) was measured using the branched-chain Chiron assay (Chiron, Emeryville, California, USA). All procedures were performed after obtaining written informed consent. The study was approved by the Committee on Human Research of UCSF.

Testing for anal HPV infection was performed as described previously using the polymerase chain reaction (PCR) with L1 consensus primers and probes specific for 29 individual HPV types and a mixture of ten additional types [55]. Beta-globin-negative samples were excluded from analysis. We report the prevalence of each type separately, the prevalence of infection with an oncogenic HPV type, and the prevalence of having any HPV infection (any HPV). The latter is defined by a sample positive with the consensus probe mixture. We define infection with an oncogenic HPV as a positive test for at least one of the following HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 [3]. For our evaluation of risk factors for infection, we consider two outcomes; HPV 16 alone compared to all others, because it is the most common type found in anal cancers; and all oncogenic HPV types combined.

Assessment of potential risk factors

Demographic factors, lifestyle characteristics (including smoking, alcohol and recreational drug use), medical history, prescription medication use, and a history of sexual behavior were collected through an interviewer-administered questionnaire. A medical history of ‘rectal problems’ (i.e. sexually transmitted infections (STIs) as well as hemorrhoids, fissures or fistulas, abscesses, rectal discharge and blood in stool) was also included. We asked about sexual behaviors in three time periods: lifetime, past 12 months and past 30 days. Ever/never practicing a particular behavior was collected as

well as number of partners for each type or behavior. Men were queried about multiple types of sexual behaviors, including sex with men and women, "insertive" anal intercourse (participant inserts his penis into partner's anus) and "receptive" anal intercourse (participant receives his partner's penis into his anus), oral-anal contact (participant's anus receives oral contact), and use of objects in the anus.

Statistical Analysis

Baseline characteristics thought to be related to prevalent HPV infection were examined for association with each of our two outcomes, HPV 16 and oncogenic HPV infection (compared to all others). Categorical variables were assessed for bivariable association using the chi-square test for independence and continuous variables were assessed using analysis of variance (ANOVA) or ranked ANOVAs. Variables were considered significantly associated with prevalent HPV infection if the p-value was <0.05.

To derive adjusted relative risks in order to assess the independent effects of sexual risk behaviors on our outcomes we constructed log-linear multivariable models specifying a binomial distribution (as opposed to the more standard logistic regression in this context). In this analysis, we selected potential sexual risk factors to analyze, as well as potential confounders *a priori,* based on a review of the literature of existing risk factors for HPV infection in men and women. The following sexual risk factors were selected for analysis: number of lifetime female partners; number of lifetime male partners; number of lifetime receptive partners (number of partners with whom the participant was the receptive partner); number of lifetime insertive partners (number of partners with whom the participant was the insertive partner); 12 month total male partners, receptive male partners and insertive male partners, number of 30 day total, receptive and insertive partners, and lifetime number of oral-anal contact partners. Because the sexual risk behaviors were correlated (r>=0.5) and thought to be on similar causal pathways, they were each evaluated in separate multivariable models. Characteristics previously identified as potential confounders of the relationship between a sexual risk behavior and HPV infection were included in these multivariable models. These were: age, race, education, smoking status, alcohol use in the past 12 months, recreational drug use in the past 12 months and current CD4+ level. *A priori* multivariable models were constructed for the association of each of the selected sexual risk factors with HPV 16 and oncogenic HPV infection.

Results

348 HIV-infected men were evaluated for anal HPV infection and of these, samples from 30 (8.6%) were found to have insufficient DNA as determined by inability to amplify the housekeeping gene beta-globin and were excluded from further analyses. Table 1 summarizes the demographic and lifestyle characteristics of the study population. The mean age of the 318 men included in the analysis was 43 years ; 88% were non-Hispanic white, 2% were black, 7% were Hispanic, and 1.6% were Asian (Table 1). More than half (66%) of the men had completed college. Fifty-two percent reported smoking more than 100 cigarettes in their lifetime and 87% reported drinking alcohol in

the past 12 months. Recreational drug use was very high in this population, with 98% of men indicating that they used one or more recreational drugs at least once in their lifetime. The three drugs used most commonly were marijuana (95%), cocaine (78%), and speed (70%). Recreational drug use in the past 12 months was also common, with 78% reporting any use and 30% of men using a non-prescription drug six or more times in the past 12 months.

Nine percent of our study population had been diagnosed with HIV infection in the past 12 months. The mean CD4+ cell count was 446 cells/μL. Seventeen percent had a CD4+ level less than 200 cells/μL, 47% had between 200-500 cells/μL, and 36% had above 500 cells/μL. Most (54%) of our participants had HIV viral load levels <500 copies/μL. Eighty-three percent of participants were taking antiretroviral medications.

These men also self-reported a high number of lifetime sexual partners. Seventy-one percent said that they had more than 200 lifetime male partners and 29% had more than 200 lifetime receptive male partners (Table 2). The mean number of partners with whom the participant was the receptive partner was 7.5 and the mean number of partners with whom the participant was the insertive partner was 6.9. These self-identified MSM often had a history of also having had sex with women. Two-thirds had had at least one female sexual partner in their lifetime and 22% reported 5 or more lifetime female partners. Almost all men (93%) reported a history of oral-anal contact and 61% said that they had had objects inserted in the anus in the past five years.

Prevalence of anal HPV

Almost all men (92%) were positive for at least one type of HPV (Figure 1). The five most common types in descending order were HPV 6 (45%), HPV 16 (42%), HPV 11 (31%), HPV 33 (30%) and HPV 18 (20%). Oncogenic HPV infection was also very common with 80% of men positive for at least one oncogenic HPV type. Among participants positive for at least one identifiable HPV type the mean number of types of HPV found was five.

Bivariable associations of baseline characteristics and anal HPV infection

HPV 16

The only demographic factor that was associated with prevalent HPV 16 infection was education. Fewer men with HPV 16 infection had completed college compared with men without HPV infection (47% v. 62%, p=0.03). Of the recreational drugs evaluated, injection drug use (IDU) was associated with HPV 16. Thirty percent of HPV 16-infected men reported IDU in their lifetime compared with only 15% of the non-HPV 16 infected men (p<0.001). Men with a history of genital or anal gonorrhea infection were more likely to be HPV 16-positive (81% v. 64%, p=0.001) compared with those without such a history.

Of the sexual risk behaviors evaluated, having a higher number of total lifetime male partners (>200), and having a higher number of lifetime insertive partners (>51) were

both significantly associated with anal HPV 16 infection. History of receptive anal sex was not associated with HPV 16 in any of the time periods evaluated at the <0.05 significance level. CD4+ level, HIV VL, and use of ART were also not associated with HPV 16 infection.

Oncogenic HPV

As with HPV 16, oncogenic anal HPV infection was associated with fewer years of education. In contrast to HPV 16, no drug use variable was associated with oncogenic HPV infection at the p≤0.05 significance level. However, oncogenic HPV was associated with history of an anorectal abscess (9% vs. 2%, p=0.05) and history of anorectal discharge (21% vs. 8%, p=0.01).

Only one of the sexual risk behaviors evaluated was significantly associated with oncogenic HPV infection. Having a high (>51) number of lifetime partners with whom the participant was the receptive partner was associated with oncogenic HPV infection (68% vs 44%, p=0.03). CD4+ cell count, HIV VL, and use of ART were not associated with oncogenic HPV infection.

Risk factors for anal HPV infection – multivariable models

HPV 16

Of the 12 sexual risk behaviors evaluated in adjusted analyses, three were significantly associated with HPV 16 after adjustment for age, race, education, smoking, 12-month alcohol use, 12-month drug use, and CD4+ level (Table 3). A higher number of female partners was associated with decreased risk of anal HPV 16 infection among HIV-infected men. Men with 1-4 female partners had an RR of 0.79 (95% CI 0.6-1.05) compared with men reporting no female partners and men with 5+ female partners had an RR was 0.6 (0.41-0.88) when compared with no female partners. Conversely, a higher number lifetime male partners increased the risk of infection, with RRs of 1.81 for 200-1000 partners compared with <200 partners, and 1.68 for 1000+ partners compared with <200 partners. Lastly, having 201+ lifetime insertive partners was also associated with an increase in risk of anal HPV 16 infection (RR: 1.57 (1.57-2.11) compared to 0-50 partners. The number of partners with whom the participant was the receptive partner was not associated with HPV 16 infection in any of the time periods evaluated (lifetime, 12 months, or 30 days).

Oncogenic HPV

None of the 12 sexual risk behaviors evaluated were significantly associated with anal oncogenic HPV in this multivariable analysis (Table 4).

Discussion

The results of this study confirm the very high prevalence of anal HPV infection among HIV-infected MSM in San Francisco we observed in the pre-ART era. Almost all of the men had prevalent anal HPV infection and 80 had a type considered to be oncogenic, consistent with other recent studies that found a high prevalence of anal HPV in HIV-infected MSM in the ART era. De Pokomandy et al (2009) found an even higher prevalence (98%) of anal HPV among Canadian MSM positive for HIV [75]. A study from Australia using Hybrid Capture 2 to detect anal HPV infection found that 95% of HIV-infected MSM had anal HPV infection [76] and a recent French study of found that 75% of MSM were positive for at least one type of HPV and 65% had an oncogenic HPV type [149]. The lower prevalences of HPV found in that study may have been due to differences in the HPV DNA detection method used. Damay *et al.* used a PCR-based method that detected only 24 HPV types (primer targeted E1 gene), whereas our method detects 29 individual types and 10 mixed types (primer targeted to L1 gene). These very high prevalences of HPV infection are of great concern in this population given the strong correlation between anal HPV and anal cancer and the observation that antiretroviral therapy has not been shown to reduce the risk of anal cancer in HIV-infected populations.

We chose to examine risk factors for HPV 16 both because it has been shown to have the strongest association with anal cancer and because it is biologically more aggressive than the other HPV types. HPV 16 is more likely to become a persistent infection and is more likely to be associated with HGAIN [1]. In our analysis we found an increased risk of anal HPV 16 infection associated with an increased number of male partners, consistent with previously published studies [55, 57]. A history of more female sexual partners was associated with a decreased risk of anal HPV 16 infection. This association remained significant even after controlling for total number of male partners and there was not a strong negative correlation between number of female partners, number of male partners or number of receptive partners (data not shown). It may be that men with more female partners engage in different sexual behaviors with their male partners, resulting in decreased exposure to anal HPV. For example, men who have female partners may be more likely to use condoms with their male partners. We did not collect information on condom use and cannot evaluate this relationship in this analysis, but it should be investigated in future studies.

The number of receptive partners was not significantly associated with anal HPV 16 infection. This finding is counterintuitive, given that anal infection should be associated with receptive rather than insertive behavior. However, in our population only three participants had never had receptive anal sex and most (93%) had more than ten lifetime receptive partners. Our study sample therefore did not include a comparison group of men ‘unexposed’ to receptive anal sex.

Similarly, although the lifetime number of receptive partners was associated with having an oncogenic HPV in bivariate analyses, that association did not remain significant in adjusted models. None of the other sexual risk factors evaluated was associated with

having an oncogenic HPV infection. As was the case for analysis of the relationship between receptive anal intercourse and anal HPV 16 infection, a high proportion of participants were positive for oncogenic HPV and we may have had limited power to detect significant associations in adjusted the models.

As described above, one limitation of this study is that we did not collect information on condom use, because at the time the study was designed condom use was not an established protective factor. As a result we do not know what proportion of our participants were having protected sex. This is of particular importance given our population of HIV-infected MSM, who may have received counseling on safe-sex as part of their post-HIV test counseling and potentially as part of their on-going medical care. This possible confounder could have masked an association with receptive intercourse if, for example, men who had more receptive partners were also more likely to use condoms.

Another limitation of this study is that in our cross-sectional analyses we do not know when the anal HPV infection occurred or if a sexual behavior happened before or after the infection. This is of particular importance to with respect to HPV 16 and oncogenic HPV infection which are more likely to persist than infections with non-oncogenic types. It is possible that the risk factors we identified are not associated with acquisition of infection, but with persistence of infection. This is less likely to be true with sexual risk behaviors, which are unlikely to influence persistence of infection.

Our data are consistent with previous studies showing that anal HPV infection is very common among HIV-infected MSM. The high prevalence of infection with HPV 16, the most common HPV type associated with anal cancer, is of particular concern. Our risk factor analysis confirmed that anal HPV infection is likely to be sexually transmitted. The high prevalence of anal HPV infection found in this study, along with the established association of HPV infection with pre-malignant lesions and anal cancer, indicate that HIV -infected MSM are at a high risk for HPV-associated anal disease. Based on our findings and other recent publications, we recommend that HIV-infected MSM be counseled on anal cancer prevention strategies emphasizing safe sex behaviors with all partners, and include information on the increase in risk of anal HPV infection associated with increasing number of male partners.

Table 1: Socio-demographic and lifestyle characteristics of participants (N=318)

Characteristic	N (%)
Demographic factors	
Age (years), mean (± SD)	42.5 (±7.7)
Race/ethnicity	
Non-Hispanic White	281 (88.4)
Black	7 (2.2)
Asian	5 (1.6)
Hispanic	22 (6.9)
Other	3 (0.9)
Education	
Some college or less	140 (44)
Completed college	91 (28.6)
Graduate school	87 (27.4)
Substance use	
Smoked >100 cigarettes	164 (51.6)
How many days per week drank alcohol	
<1 day/week	173 (54.4)
1-2 days/week	81 (25.5)
3-7 days/week	64 (20)
Ever use of cocaine	247 (77.9)
Ever use of LSD/acid	202 (63.7)
Ever use of speed	222 (70)
Ever us of injection drugs	67 (21.1)
Medical history	
Ever anal or genital warts	237 (74.5)
Ever gonorrhea	226 (71.3)
Ever positive syphilis blood test	61 (19.2)
Ever chlamydia	84 (26.4)
Ever infection or abscess in rectum/anus	24 (7.6)
Ever rectal discharge or pus	60 (18.9)
Diagnosed with HIV in past 12 months	9 (2.9)
CD4+ (cells/mL)	
<200	53 (16.9)
200-500	147 (46.8)

Characteristic	N (%)
>500	114 (36.3)
HIV viral load (copies/mL)	
<500	169 (53.8)
500-4000	54 (17.2)
4001-20000	49 (15.6)
>20000	42 (13.4)
Currently taking antiretroviral therapy	
No	53 (16.9)
Yes	260 (83.1)

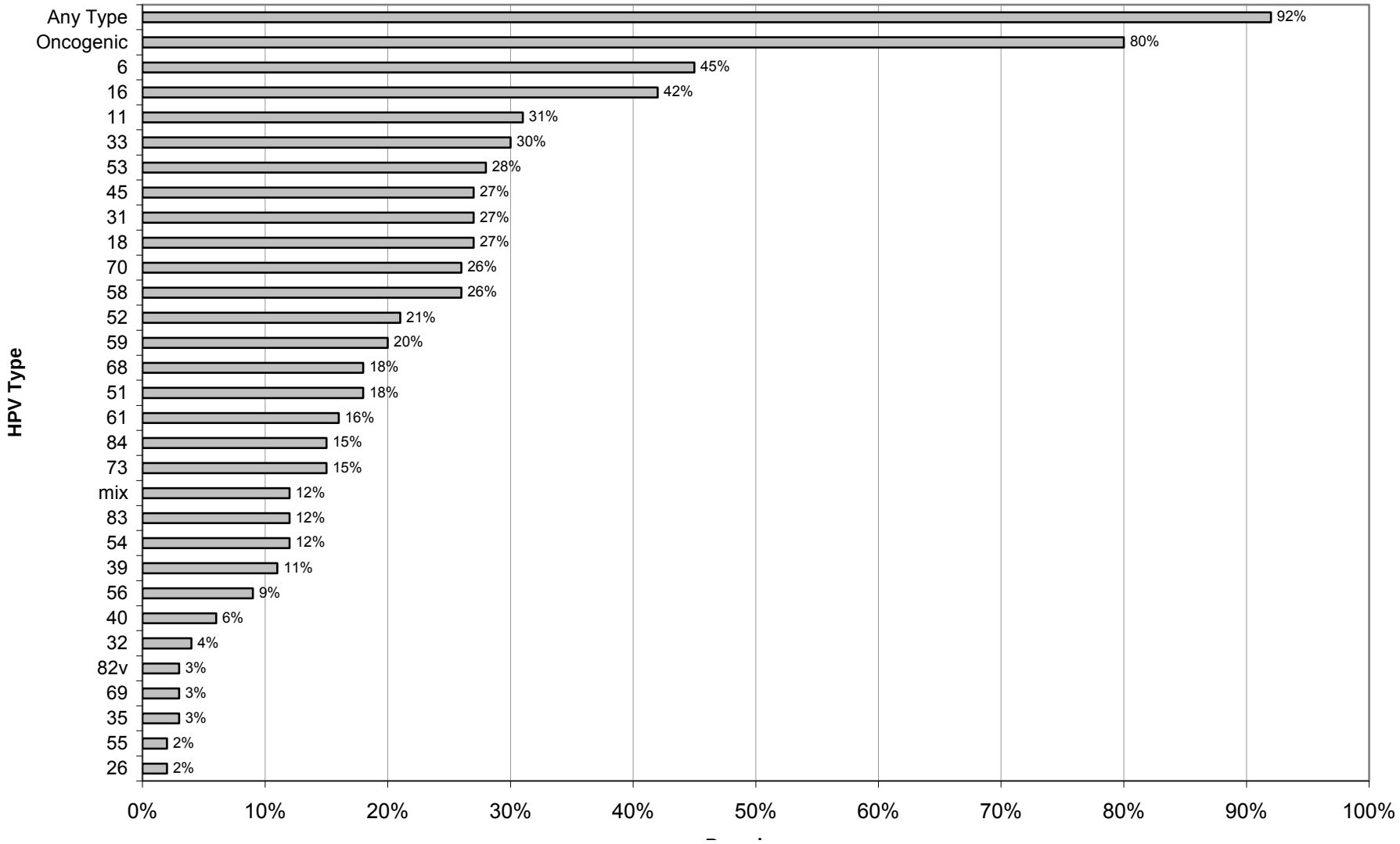
When totals do not reach 318, data were missing; SD, Standard deviation

Table 2: Sexual behaviors of participants (N=318)

Characteristic	N (%)
Lifetime behaviors	
Number female partners	
0	113 (35.5)
1-4	135 (42.5)
5+	70 (22)
Number total male partners	
1-200	92 (28.9)
201-1000	126 (39.6)
1000+	100 (31.4)
Number receptive male partners	
0-50	142 (45.1)
51-200	80 (25.4)
201+	93 (29.5)
Number insertive male partners	
0-50	136 (42.9)
51-200	95 (30)
201+	86 (27.1)
Ever had an oral-anal contact partner	296 (93.1)
Number oral-anal contact partners	
0-10	128 (40.3)
11-50	103 (32.4)
51+	87 (27.4)
Past 5 year behaviors	
Any objects inserted in anus	194 (61)
Past 12 month behaviors	
Number receptive partners, mean (±SD)	7.5 (±17.3)
Number insertive partners, mean (±SD)	6.9 (±15.4)
Past 30 day behaviors	
Number receptive partners, mean (±SD)	1.2 (±3.3)
Number insertive partners, mean (±SD)	1.2 (±2.9)

When totals do not reach 318, data were missing; SD, Standard deviation; insertive, participant is insertive partner during anal intercourse; receptive, participant is the receptive partner during anal intercourse; oral-anal contact, participant recipient of oral-anal contact

Figure 1:Prevalence of HPV infection



* ß-globin-negative specimens were excluded from analysis

Table 3: Associations of sexual risk factors and prevalent HPV 16 infection

	Unadjusted		Adjusted*	
Sexual Risk Factor	**RR (95% CI)**	**p-value**	**RR (95% CI)**	**p-value**
Lifetime behaviors				
Number female partners				
0:0	1.0	0.3	1.0	0.0255
1-4	0.87 (0.65 - 1.15)		0.79 (0.60 - 1.05)	
5+	0.76 (0.53 - 1.10)		0.60 (0.41 - 0.88)	
Number total male partners				
1-200	1.0	0.0125	1.0	0.0034
201-1000	1.65 (1.15 - 2.38)		1.81 (1.24 - 2.63)	
1000+	1.53 (1.04 - 2.25)		1.68 (1.11 - 2.57)	
Number receptive male partners				
0-50	1.0	0.2	1.0	0.06
51-200	0.97 (0.68 - 1.37)		0.82 (0.57 - 1.19)	
201+	1.28 (0.95 - 1.71)		1.27 (0.90 - 1.79)	
Number insertive male partners				
0-50	1.0	0.0207	1.0	0.0046
51-200	1.19 (0.85 - 1.66)		1.15 (0.81 - 1.62)	
201+	1.55 (1.14 - 2.10)		1.72 (1.24 - 2.38)	
Number oral-anal contact partners				
0-10	1.0	0.7	1.0	0.5
11-50	0.98 (0.71 - 1.34)		0.98 (0.71 - 1.35)	
51+	1.13 (0.83 - 1.54)		1.17 (0.84 - 1.63)	
Past 12 month behaviors				
Number male partners				
0-10	1.0	0.3	1.0	0.5
11-50	1.06 (0.78 - 1.46)		1.06 (0.77 - 1.44)	
51+	1.29 (0.94 - 1.78)		1.24 (0.86 - 1.79)	
Number receptive partners	1.00 (1.00 - 1.01)	0.4	1.00 (1.00 - 1.01)	0.5
Number insertive partners	1.01 (1.00 - 1.01)	0.1	1.00 (1.00 - 1.01)	1.0
Past 30 day behaviors				
Number receptive partners	1.02 (0.98 - 1.07)	0.2	1.01 (0.96 - 1.06)	1.0
Number insertive partners	1.03 (1.00 - 1.06)	0.1	1.02 (0.99 - 1.05)	1.0

*Adjusted for age, race, education, smoking, 12 month alcohol use, 12 month drug use, and CD4+ level
Insertive, participant is insertive partner during anal intercourse; receptive, participant is the receptive partner during anal intercourse; oral-anal contact, participant recipient of oral-anal contact

Table 4: Associations of sexual risk factors and prevalent oncogenic HPV

	Unadjusted		Adjusted*	
Sexual Risk Factor	**RR (95% CI)**	**Overall p-value**	**RR (95% CI)**	**Overall p-value**
Lifetime behaviors				
Number female partners				
0	1.0	0.8	1.0	0.5
1-4	1.00 (0.88 - 1.13)		0.99 (0.86 - 1.13)	
5+	1.04 (0.90 - 1.20)		0.98 (0.82 - 1.17)	
Number total male partners				
1-200	1.0	0.09	1.0	0.09
201-1000	1.16 (1.01 - 1.33)		1.10 (0.96 - 1.27)	
1000+	1.07 (0.91 - 1.25)		1.05 (0.90 - 1.22)	
Number receptive male partners				
0-50	1.0	0.02	1.0	0.9
51-200	1.19 (1.06 - 1.35)		1.08 (0.96 - 1.23)	
201+	1.04 (0.90 - 1.20)		1.01 (0.88 - 1.16)	
Number insertive male partners				
0-50	1.0	0.1	1.0	0.5
51-200	1.14 (1.00 - 1.29)		1.07 (0.95 - 1.21)	
201+	1.10 (0.96 - 1.26)		1.06 (0.91 - 1.24)	
Number oral-anal contact partners				
0-10	1.0	0.4	1.0	0.7
11-50	1.08 (0.95 - 1.23)		1.04 (0.92 - 1.18)	
51+	1.08 (0.94 - 1.24)		1.05 (0.89 - 1.23)	
Past 12 month behaviors				
Number total male partners				
0-10	1.0	0.3	1.0	1.0
11-50	1.10 (0.97 - 1.25)		1.06 (0.94 - 1.19)	
51+	1.05 (0.91 - 1.22)		1.05 (0.90 - 1.23)	
Number receptive partners	1.00 (1.00 - 1.00)	0.9773	1.00 (1.00 - 1.00)	1.0
Number insertive partners	1.00 (1.00 - 1.00)	0.8140	1.00 (1.00 - 1.00)	1.0
Past 30 day behaviors				
Number receptive partners	1.01 (0.98 - 1.03)	0.6363	1.00 (0.98 - 1.03)	1.0
Number insertive partners	1.00 (0.98 - 1.02)	0.7975	1.01 (0.98 - 1.03)	1.0

*Adjusted for age, race, education, smoking, 12 month alcohol use, 12 month drug use, and CD4+ level
insertive, participant is insertive partner during anal intercourse; receptive, participant is the receptive partner during anal intercourse; oral-anal contact, participant recipient of oral-anal contact

CHAPTER 3: Incident Infection with and Risk Factors for Anal Human Papillomavirus Infection among HIV-infected Men who have Sex with Men.

Abstract

***Background*.** HIV-infected men who have sex with men (MSM) are at higher risk of anal human papillomavirus (HPV) infection than men in the general population. Little is known about the incidence of and risk factors for type-specific anal HPV infection in this population.

***Methods*.** HIV-infected MSM were enrolled into a prospective cohort study and evaluated for anal HPV DNA, lifestyle factors, and sexual risk behaviors every six months for at least two years.

***Results*.** The overall incidence rate of detectable type-specific anal HPV infection was 21.3 per 100 person-years (PY) (95% CI: 17.7-25.4) and was 13.3/100 PY (10.5-16.6) for oncogenic HPV types. The most commonly detected incident infections were HPV 18 (3.7/100 PY) and HPV 16 (3.5/100 PY). A higher number of recent partners with whom the participant was the receptive partner (OR 2.88 (1.64-5.07) 8+ partners vs. 0-1), a higher number of new partnerships in which the participant was the receptive partner (OR 1.03 (1.01-1.05) per partner), a higher number of new oral-anal contact partnerships in which the participant was receptive partner (OR 1.06 (1.03-1.09)) per partner), and the frequency of receptive anal intercourse (OR 1.08 (1.03-1.13) per act) all significantly increased the odds of detectable incident HPV infection ($p \leq 0.05$).

***Conclusions*.** HIV-infected MSM have a high incidence of oncogenic anal HPV infection. Recent receptive anal sexual behaviors, including receptive anal intercourse and receptive oral-anal contact, are the most important risk factors for incident anal HPV infection.

Key Words: HPV, human papillomavirus, HIV, men who have sex with men, MSM, anus, incidence, receptive anal intercourse

Introduction

The incidence of anal cancer is low among men in the general population, but is considerably higher among men who have sex with men (MSM). Prior to the HIV epidemic, the incidence of anal cancer among MSM was estimated to be as high as 37 per 100,000 [54], which is similar to the incidence rate of cervical cancer among women in Western countries before routine screening was introduced [1]. HIV-infected MSM have an even higher risk of developing anal cancer. They are 59 times more likely to develop anal cancer compared with the general male population and are twice as likely to develop anal cancer as HIV-uninfected MSM [74]. Unlike other virus-associated cancers, the risk of anal cancer has not declined among HIV-infected MSM since the introduction of highly active antiretroviral therapy (HAART) [82, 83, 150, 151]. Instead, the incidence of anal cancer has continued to increase [152], and it is possible that this trend may continue as the HIV-infected population continues to age.

As with cervical cancer, anal cancer is preceded by precursor lesions. Anal intraepithelial neoplasias are classified as either low-grade intraepithelial neoplasia (LGAIN), which are less likely to develop into anal cancer, and high-grade intraepithelial neoplasia (HGAIN), which are more likely to develop into cancer [1]. The prevalence and incidence of these precursor lesions are highest among HIV-infected MSM [31]. Also similar to cervical cancer, HGAIN is strongly associated with human papillomavirus (HPV) infection, particularly with HPV type 16, and approximately 90% of all anal cancers are attributed to HPV infection [1].

In prevalence studies, anal HPV infection is almost universal among HIV-infected MSM, with reported prevalence estimates between 87 and 98% [55, 57, 75, 76]. HPV 16 is among the most common types detected in this population, with prevalences >30% [55, 57, 75, 76]. Risk factors for prevalent anal HPV infection include receptive anal intercourse [57], a greater number of male partners [55, 57], indicators of anal inflammation (for example hemorrhoids) [55, 76], and a lower CD4+ cell count [55, 57, 153]. However, cross-sectional studies are limited in their ability to examine risk factors, given that the prevalent infections are a mix of new and persistent infections. Risk factors for prevalent infection may be factors associated with persistence and not acquisition of new infection.

Few natural history studies have reported on the incidence of type-specific anal HPV infection among HIV-infected [57, 75] men. There is also limited information available about risk factors for incident anal HPV infection. Identifying modifiable risk factors for anal HPV infection is an important step in understanding and potentially preventing anal cancer in HIV-infected MSM.

We conducted a prospective cohort study to assess the natural history of anal HPV infection in MSM in the antiretroviral therapy era. The goal of this analysis is to report the incidence of detection of type-specific anal HPV infection and evaluate risk factors for infection.

Methods

HIV-infected MSM were recruited to a prospective cohort study conducted by the University of California, San Francisco (UCSF), through newspaper advertisements and other community outreach. Enrollment was completed between February 1998 and January 2000, and participants were followed every six months for at least two years. At each visit participants completed an interviewer-administered questionnaire; a clinical examination, including collection of an anal swab for HPV testing; and collection of a second anal swab for cytology. Blood was collected for CD4+ lymphocyte cell counts, which were measured using standardized two- or three-color fluorescence methods. HIV plasma viral load (HIV VL) was measured using the branched-chain Chiron assay (Chiron, Emeryville, California, USA). Written informed consent from participants and the study was approved by the Committee on Human Research of UCSF.

HPV testing

Testing for anal HPV infection was performed as described previously using the polymerase chain reaction (PCR) with L1 consensus primers and probes specific for 29 individual HPV types and a mixture of ten other types [55]. Beta-globin-negative (indicating insufficient DNA) samples were excluded from analysis. Detection of HPV incident infection was calculated for each HPV type individually. Detection of HPV incident infection was defined as a negative test result at baseline for an individual HPV type followed by at least two consecutive positive tests for that type during the follow-up period. Two consecutive positive tests were required because detection on a single occasion is less likely to represent true infection than two consecutive positive tests six months apart, and this is the definition of infection commonly used in vaccine studies [154, 155]. Requiring two positive tests reduces potential outcome misclassification and provides a conservative estimate of incidence. Because of this requirement, participants were included in this analysis only if they had at least two follow-up visits or one year of follow-up.

Assessment of potential risk factors

Demographic factors, lifestyle characteristics; medical history, including antiretroviral medication use; and history of sexual behavior were collected as part of the baseline questionnaire. At each follow-up visit, the interviewer collected information on behaviors and medical history that may have changed since the last interview. Participants were queried about recent smoking, alcohol and drug use; new medical diagnoses and medications used; and recent sexual behaviors. At each visit we asked about sexual behaviors in two time periods: ‘since the last interview’ (SLI), which was approximately six months before the interview, and in the past 30 days.

We asked if the participant had engaged in each sexual behavior (yes/no); the number of partners with whom he had engaged in the behavior; the number of *new* partners with

whom he engaged in the behavior; and the frequency of the behavior. Men were queried about sex with men and with women, “insertive” anal intercourse (participant inserts his penis into partner’s anus) and “receptive” anal intercourse (participant receives his partner’s penis into his anus); oral-anal contact (participant’s anus receives oral contact), and use of objects in the anus.

Statistical analysis

The rate of detection of incident anal HPV infection was calculated as number of participants with incident infection detected divided by person-years of follow-up. An incident infection was assumed to have taken place halfway between the previous visit and the current visit, and person- time was assigned as (current visit date-previous visit date)/2. Exact Poisson confidence intervals were calculated for each incidence rate [156].

Participants who had an incident type-specific HPV infection were censored from that type-specific analysis at future time points, but remained in the analyses for other HPV types. Participants could have ‘any incident HPV’ at multiple visits if they had different type-specific infections at different visits.

We evaluated the bi-variable association of baseline characteristics with any incident infection in participants (n=376 participants) using the chi-square test for independence for categorical variables, and analysis of variance (ANOVA) or ranked ANOVA for continuous variables that were relatively symmetrically distributed or had skewed distributions, respectively. We evaluated bi-variable associations of incident HPV infection with time-dependent characteristics by participant visit (n=926 participant visits). P-values were generated from generalized linear models estimated through repeated measures with generalized estimating equations (GEE) [157], adjusting for potential correlation from taking repeated measures on participants, as well as reporting robust standard errors. We note that the measure of association obtained from this procedure is the odds ratio related to the hazard of new infection.

To evaluate the effect of risk factors on incident anal HPV infection, we constructed multivariable models. Logistic regression models were estimated through repeated measures GEE [157]. Four different model sets were created for each risk factor. Model 1 is the unadjusted model and includes only the risk factor. Model 2 additionally includes the baseline value of the risk factor. Model 3 adds six baseline factors identified as potential confounders (baseline age, race, education, smoking 100+ lifetime cigarettes, baseline CD4+ cell count, and HPV 6 status at baseline). Model 4 adds the potential time-dependent confounder of CD4+ cell count measured at the previous visit.

We selected 11 risk factors *a priori* for analysis and other factors identified in the literature as potentially important risk factors. Seven of these factors were from our ‘since our last interview’ recall period: Injection drug use, number of receptive partners, number of new receptive partners, frequency of receptive intercourse, number of

insertive partners, and number of oral-anal contact partners. Four factors were in the 'past 30 days' recall period: number of new oral-anal contact partners, number of male partners, number of receptive partners, and frequency of receptive intercourse. Additionally, because 'rectal drug use SLI' was associated with incidence of HPV infection in bi-variable analysis, we evaluated recent rectal drug use as an additional risk factor.

All analyses were conducted using SAS 9.2 (SAS, Cary,NC).

Results

Description of study population

Four hundred ninety-three HIV-infected MSM enrolled in the study at baseline. Of those, 41 (8%) were found to have insufficient DNA as determined by inability to amplify the housekeeping gene beta-globin at baseline and were excluded from further analyses and were excluded from further analyses. Of the remaining 451 considered eligible for our follow-up analysis, 83 (18%) did not return for at least two follow-up visits. The participants who did not return for at least two visits did not differ significantly from those who did, in terms of race/ethnicity, education, lifestyle factors, sexual risk behaviors, or HPV prevalence at baseline. Those who did not return were younger in age (41 vs. 45 years, p=0.001). There was also an important difference between the indicators of HIV disease status between the two groups. Those who did not return had a lower mean CD4+ cell count (365 cells/μL vs. 459 cells/μL, p=0.001), a higher mean HIV VL (40,242 copies/μL vs. 10,772 copies/μL, p<0.001), and were less likely to be taking any antiretroviral medications (67% v. 86%, p=0.001).

Three hundred sixty-nine HIV-infected MSM with at least two follow-up visits were included in the analysis. Their mean age was 44.6 years, most (91%) were non-Hispanic white, and 62% had completed college (Table 1 first column). Almost all of the men had been diagnosed with HIV infection more than one year prior to the baseline interview. The mean CD4+ cell count was 459 cells/μL and only 13% had a CD4+ cell count less than 200 cells/μL. Most participants (63%) had an HIV VL of less than 500 copies/μL, 17% had levels between 500 copies/μL and 4000 copies/μL, 12% had between 4001 copies/μL and 20,000 copies/μL, and 8% had greater than 20,000 copies/μL. Eighty-six percent of men were currently taking antiretroviral medication.

Participants reported a high level of sexual activity at the baseline interview. Sixty-four percent of these MSM reported ever having had sex with a woman and 22% had had five or more female partners. Almost all of the men reported both insertive and receptive anal intercourse with men and the number of lifetime male partners was high; 31% had more than 1000 lifetime male partners.

Almost all participants (91%) had a prevalent HPV infection at baseline. Seventy-five percent self-reported having had either anal or genital warts and among these participants the mean number of times they had anal warts was nine.

Incidence of HPV infection

The total number of years of follow-up for the 369 men was 573 years. One hundred and twenty-two men had a detectable type-specific incident anal HPV infection over the two year follow-up period, and the overall incident detection rate was 21.3 per 100 person-years (PY) (95% CI: 17.7-25.4) (Table 2). The most common HPV type detected was HPV type 18, with an incidence rate of 3.7 per 100 PY. The next five most frequent types of incident HPV were HPV 16 (3.5/100 PY), HPV 61 (2.7/100 PY), HPV 33 (2.6/100 PY), HPV 58 (2.3/100 PY), and HPV 31 (2.2/100 PY). The incidence of any oncogenic HPV infection was 13.1/100 PY (95% CI: 10.5-16.6).

Of the 122 men with one or more incident HPV type specific infections, 98 (72%) had an incident infection with only one HPV type, 19 (16%) had two different HPV type infections, three (2%) had three HPV type infections, one (0%) participant had four HPV type infections, and one (0%) participant had six different HPV type infections. Of the 24 men who had more than one HPV type infection, ten had different HPV types detected at different visits and 14 men had incident infections with different HPV types at the same visit.

Men who had an incident HPV infection were less likely to have had a prevalent HPV infection of any type at baseline (87.7% vs. 93.9%, (p=0.04)) and were also less likely to have had a prevalent HPV 6 infection (32% vs. 43%, p=0.04), the most prevalent HPV type at baseline.

Bi-variable associations between risk factors and incident HPV infection

Of the demographic, lifestyle, sexual risk, and medical history factors measured over a participant's lifetime, only two were significantly associated with having an incident HPV infection during the follow-up period (Table 1). Having smoked at least 100 lifetime cigarettes was more common among those without an incident HPV detection (58.7% vs. 46.7%, (p=0.03)). The only other factor associated with incident HPV infection in bi-variable analysis was having been diagnosed with HIV in the past 12 months. Of the men with an incident HPV infection, 4% had a recent diagnosis of HIV infection compared with only 0.8% in those who had been diagnosed with HIV more than 12 months ago (p=0.03).

Recent behaviors showed more significance in association with incident HPV in bi-variable analysis (Table 3). The following recent behaviors SLI were associated with incident anal HPV infection in bi-variable analysis: rectal drug use; number of partners with whom the participant was the receptive partner (receptive); number of new receptive partners; frequency of receptive intercourse; number of new oral-anal contact partners (participant received anal contact); objects in the anus; number of total male partners in the last 30 days; number of receptive partners; and frequency of receptive intercourse in the last 30 days. More men who injected drugs SLI had an incident HPV

infection (3.5% v 1.9%) but this difference was not statistically significant (p=0.4). Other recreational drug use was also not associated with incident infection of anal HPV.

Effect of recent behaviors on incidence of anal HPV infection

Of the two drug use factors evaluated in our multivariable repeated measures analysis, only rectal drug use was significantly associated with incident anal HPV infection. Using rectal drugs SLI increased the odds of detecting an incident HPV infection (OR 2.40 (1.3-4.45), p=0.033).

Of the sexual behaviors reported SLI, many remained significantly associated with anal HPV infection after adjusting for the baseline value of the factor, potential baseline confounders and time-dependent confounders. Number of receptive partners (partners with whom the participant was the receptive partner) (8+ vs. 0-1) increased the odds of anal HPV infection by 2.88 ((1.85-5.45), p=0.002) in the fully adjusted model. The odds of incident HPV infection were also higher for men reporting a greater number of new receptive partners (OR: 1.03 per partner, (1.01-1.05), p=0.006). The frequency of receptive intercourse was also significantly associated with an OR of 2.60 (1.61-4.58, p=0.004) when 1+ times per week was compared to no acts. Having a higher number of new oral-anal contact partners also increased the odds of HPV infection by a small but significant amount per new partner (OR: 1.06 per partner [1.03-1.09], p=0.01). The number of insertive partners (partners with whom the participant was the insertive partner) was not associated with incident HPV infection in the fully adjusted model.

The only behavior reported in the past 30 day recall period that remained significantly associated with incident HPV infection in the fully adjusted model was frequency of receptive intercourse in the past 30 days, which increased the odds of incident HPV infection (OR: 1.08 (1.03-1.13) per partner, p=0.0012).

Discussion

Our study represents one of the few natural history studies of a cohort of HIV-infected MSM, followed prospectively for anal HPV infection together with a detailed evaluation of risk factors for infection. We had a high rate of follow-up with 82% of our participants returning for at least two visits or a total of one year of follow-up.

Overall, the incidence of any anal HPV infection (21.3/100 PY) and of oncogenic anal HPV infection (13.3/100 PY) were high compared with the reported incidences of other sexually transmitted infections (STIs) among HIV-uninfected MSM [40, 158-161]. For example, in a study of MSM from San Francisco, repeat testing for HIV found the incidence rate of new *Herpes simplex* type 2 (HSV-2) infections, to be 3.1/100 PY, [160] much lower than the incidence of HPV we found in our study. Studies elsewhere have reported higher incidences of infection for other STIs including syphilis (16.9/100 PY [158]), *Neisseria gonorrhoeae* (13.7%) and *Chlamydia trachomatis* (20.5%[161], but

even these rates are lower than the rate of anal HPV infection we detected. These high rates of incident HPV infection are of great concern in this population.

Rates of infection with individual anal HPV types were as high as the rates of other STIs commonly seen among MSM. The most common anal HPV type detected was HPV 18, followed closely by HPV 16. These two HPV types are the types most commonly associated with anal cancer and the two types usually found with the highest prevalences in cross-sectional studies of MSM [55, 77]. Three other HPV types (31,33, and 58) with high type-specific incidence rates (>2.0/100 PY) are also oncogenic types. Although these types have rarely been detected in anal cancer specimens, they are known to be associated with cervical cancer and may be important in anal disease in HIV-infected populations. The high incidence rates of oncogenic HPV types imply that the prevalences of these types found in cross-sectional studies are not only the result of persistence of infection, but also represent a high rate of acquisition of new infections. Also important to note is that in our study, 20% of the men with detection of an incident HPV infection had more than one new HPV type detected. A higher number of HPV types is associated with an increased likelihood of HPV-associated disease [59]. The high incidence of infection with oncogenic HPV types and the high proportion of men with multiple HPV types are consistent with the high prevalence and incidence of HGAIN, and of anal cancer.

Few published studies are available for comparison with the rates of incident anal HPV infection. Critchlow (1998) followed HIV-seropositive MSM for two years and found that 38% of men positive at baseline for anal HPV had anal HPV DNA of a new type detected during the follow-up period [57]. Our cumulative incidence of any anal HPV (33%) is comparable to this finding. A recent study of HIV-infected MSM in Canada presented type-specific HPV incidence rates [75]. Their type-specific incidence rates were much higher than the rates we found; however, they defined an incident infection as HPV DNA detected at any single visit after a negative test at baseline. In that study, the incidence rate of HPV 16 was 13.0/100 person-years, compared with our finding of 3.5/100 PY. When we re-calculated our incidence rates using their definition (data not shown) our rates of incident detection were comparably high (e.g., the rate of HPV 16 infection was 14.8/100 PY). Because HPV DNA detected from an anal swab sample could be either from an infection in the participant or the result of HPV virus deposited in the anus during anal intercourse, we believe that our stricter definition of an incident infection we used in our analyses minimizes potential misclassification of the outcome, incident anal HPV.

In our bi-variable analysis of factors associated with any incident anal HPV infection and few demographic, lifestyle, or sexual risk factors measured over the life of the participant, were predictive of incident HPV infection. The one exception was smoking more than 100 lifetime cigarettes, which was significantly associated with *not* having an incident anal HPV infection. However, the association did not remain significant in the multivariable analyses and therefore should be interpreted with caution. Recent behaviors (collected over either the previous 6 months or 30 days) were much more predictive of incident detection of anal HPV infection, implying that recent behaviors are

more important for HPV acquisition than factors measured over a longer recall period. This may be both because accuracy of recall diminishes over time and, more likely, because incident infections reflect recent sexual exposure and personal health more than past history of these factors.

In our multivariable repeated measures analyses, recent sex was an important predictor of incident anal HPV infection. Almost all indicators of receptive anal intercourse were significantly associated with incident anal HPV infection. This is to be expected, as receptive anal intercourse is a plausible means of exposure to HPV. However, the association has proved difficult to demonstrate in cross-sectional studies of HIV-infected MSM [55] both because most MSM have a history of receptive anal behaviors and there was little variation in the variable. In studies of prevalence, risk factors for prevalent HPV infection may be factors associated with persistence of HPV infection. The findings of this analysis offer strong evidence that receptive anal intercourse increases the risk of acquiring anal HPV infection among HIV-infected MSM. The number of new oral-anal contact partnerships, in which the participant's anus receives oral contact, was also associated with incident anal HPV infection infection. This is an important finding suggesting another potential route of exposure to anal HPV infection. Oral-anal contact could also help explain anal HPV infection among men (and women) who deny receptive anal intercourse.

Our study has a number of limitations. We required at least two follow-up visits to be included in our analysis. This produced an important difference between participants included in our analysis and those not included. Men who were excluded had worse measures of HIV disease status than men who were included. It could be that this inclusion criterion induced some selection bias in our study, and may help explain the diminished association between CD4+ cell count seen in our study in comparison to other studies of HIV-infected populations [62-64] given that men with lower CD4+ levels were excluded from the study. Another limitation of our study is that we did not collect information on condom use, which is an important protective factor not accounted for in our analysis. However, the likely effect of condom use would be to reduce the effect size (by preventing infection in those who have receptive sex) and our results represent a conservative measure of the true association. Also, this study was conducted in the early ART era and treatment of HIV infection has improved since that time. Therefore our results may not be generalizable to a population of HIV-infected MSM taking improved ART, who may have better health than our participants, and therefore lower incidences of anal HPV infection.

Our current analysis adds to the existing evidence from prevalence studies that anal HPV infection is common among HIV-infected MSM, and that the type distribution of incident infection is similar to that seen in prevalence studies. Recent receptive anal intercourse is an important predictor of anal HPV infection and oral-anal contact is also a risk factor for incident infection. HIV-infected MSM should be counseled about anal cancer and risk factors for HPV infection. They should also be counseled about potential primary prevention measures such as condom use and the HPV vaccine which

was recently approved for prevention of anal HPV infection with HPV 6,11,16 and 18, and disease associated with these HPV types in men aged 9 to 26 years [17].

Table 1: Baseline socio-demographic and behavioral characteristics by incident HPV infection

	All Participants		No Incident Infection		Incident HPV Infection		
Characteristic	**N**	**(%)**	**N**	**(%)**	**N**	**(%)**	**P-value***
Total N	**369**	**(100)**	**247**	**(66 9)**	**122**	**(33.1)**	
Mean Age (years) (±SD)	44.5	(±8)	44.5	(±7.9)	44.4	(±7.6)	0.7460
Race/Ethnicity							0.5744
Non-Hispanic White	335	(90.8)	226	(91.5)	109	(89.1)	
Black	5	(1.4)	2	(0.8)	3	(21.5)	
Asian/Other	8	(2.1)	6	(2.4)	2	(1.6)	
Hispanic	21	(5.7)	13	(5.3)	8	(6.6)	
Education							0.3238
Some College or Less	144	(39)	103	(41.7)	41	(33.6)	
Completed College	115	(31.2)	74	(30)	41	(33.6)	
Graduate School	110	(29.8)	70	(28.3)	40	(32.8)	
Smoked >100 lifetime cigarettes	202	(54.7)	145	(58.7)	57	(46.7)	0.0298
History of injection drug use	77	(20.9)	55	(22.3)	22	(18)	0.3470
Ever anal or genital warts	279	(75.6)	183	(74.1)	96	(78.7)	0.3338
Number of episodes of anal warts	9	(±24)	10 4	(±27)	6 4	(±20)	0.4180
Prevalent HPV infection at baseline	339	(91.9)	232	(93.9)	107	(87.7)	0.0399
Number female partners							0.9658
0	134	(36.3)	90	(36.4)	44	(36.1)	
1-4	151	(40.9)	100	(40.5)	51	(41.8)	
5+	84	(22.8)	57	(23.1)	27	(22.1)	
Number receptive male partners							0.7547
0-50	147	(40.2)	102	(41.5)	45	(37.5)	
51-200	109	(29.8)	71	(28.9)	38	(31.7)	
201+	110	(30.1)	73	(29.7)	37	(30.8)	
Number insertive male partners							0.7107
0-50	164	(44.6)	111	(44.9)	53	(43.8)	
51-200	115	(31.3)	74	(30)	41	(33.9)	
201+	89	(24.2)	62	(25.1)	27	(22.3)	
Number oral-anal contact partners							0.2215
0-10	142	(38.5)	88	(35.6)	54	(44.3)	
11-50	129	(35)	88	(35.6)	41	(33.6)	
51+	98	(26.6)	71	(28.7)	27	(22.1)	
Currently taking antiretroviral medications	319	(86.4)	214	(86.6)	105	(86.1)	0.9255
HIV diagnosis in past 12 months	7	(1.9)	2	(0.8)	5	(4.2)	0.0316
Mean CD4 T cell count (±SD)	462 6	(±261)	459 7	(±255)	468 7	(±274)	0.7690

	All Participants		No Incident Infection		Incident HPV Infection		
Characteristic	**N**	**(%)**	**N**	**(%)**	**N**	**(%)**	**P-value***
<200	50	(13.7)	30	(12.2)	20	(16.9)	0.4642
200-500	178	(48.9)	123	(50)	55	(46.6)	
>500	136	(37.4)	93	(37.8)	43	(36.4)	
Mean HIV Viral Load (±SD)	10619	(±47159)	10217	(±49557)	11483	(±41732)	0.1270
<500	219	(61.5)	155	(63.8)	64	(56.6)	0.6381
500-4000	61	(17.1)	39	(16)	22	(19.5)	
4001-20000	46	(12.9)	30	(12.3)	16	(14.2)	
>20000	30	(8.4)	19	(7.8)	11	(9.7)	

SD, Standard deviation

*p-value for categorical variable from chi-square, and from ANOVA or ranked ANOVA for normally and non-normally distributed continuous variables

Table 2: Incident Detection of HPV

HPV Type	N[1]	Number Incident Infections	2 yr Cumulative Proportion[2]	Total Person-years	Incidence Rate (Incident Infection/100 PY)[3]	(95% CI)[4]
6	221	5	2.3	348.25	1.4	(0.5-3.4)
11	268	4	1.5	410	1	(0.3-2.5)
16	222	12	5.4	341.5	3.5	(1.8-6.1)
18	268	15	5.6	406.5	3.7	(2.1-6.1)
26	360	1	0.3	555	0.2	(0-1)
31	275	9	3.3	417	2.2	(1-4.1)
32	349	4	1.1	537.75	0.7	(0.2-1.9)
33	278	11	4	422.75	2.6	(1.3-4.7)
35	354	0	0	551	0	(.-0.7)
39	333	4	1.2	511	0.8	(0.2-2)
40	351	5	1.4	539.75	0.9	(0.3-2.2)
45	283	8	2.8	432.5	1.8	(0.8-3.6)
51	305	2	0.7	469.5	0.4	(0.1-1.5)
52	291	5	1.7	449.75	1.1	(0.4-2.6)
53	278	8	2.9	427.75	1.9	(0.8-3.7)
54	333	4	1.2	512.5	0.8	(0.2-2)
55	357	1	0.3	549.5	0.2	(0-1)
56	335	8	2.4	511.75	1.6	(0.7-3.1)
58	283	10	3.5	434	2.3	(1.1-4.2)
59	302	7	2.3	464.5	1.5	(0.6-3.1)
61	308	13	4.2	473.75	2.7	(1.5-4.7)
68	312	1	0.3	485.75	0.2	(0-1.1)
69	358	0	0	555	0	(.-0.7)
70	280	8	2.9	428.5	1.9	(0.8-3.7)
73	326	3	0.9	506.25	0.6	(0.1-1.7)
82	359	1	0.3	557.75	0.2	(0-1)
83	321	2	0.6	496	0.4	(0-1.5)
84	317	8	2.5	488.5	1.6	(0.7-3.2)
Any Type	**369**	**122**	**33.1**	**573**	**21.3**	**(17.7-25.4)**
Oncogenic Types[5]	369	76	20.6	573	13.3	(10.5-16.6)
Non-Oncogenic Types	369	37	10	573	6.5	(4.5-8.9)

Incident Detection Infection, negative for type-specific HPV DNA at baseline followed by 2 consecutive detections of a specific type of type-specific HPV

[1] Participants ß-globin negative at baseline excluded from analysis

[2] # incident infections/participants negative for type-specific HPV DNA at baseline

[3] # incident infections/Person-years at risk

[4] 95% Exact Poisson confidence intervals

[5] HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 [3].

Table 3: Recent lifestyle and behavioral characteristics by incident HPV infection (N=888 participant visits).

Characteristic	Overall N (%)	No HPV N (%)	Incident HPV N (%)	P-value[1]
Recall period: since last interview				
Mean days since last interview	227.3 (±59.7)	227.2 (±57.9)	227.6 (±68.9)	0.9441
Smoked any cigarettes	202 (22.7)	172 (22.9)	30 (22.1)	0.8447
Drank any alcoholic beverages	753 (84.8)	634 (84.3)	119 (87.5)	0.2741
Injected any recreational drugs	19 (2.1)	14 (1.9)	5 (3.7)	0.3850
Used rectal drugs	43 (4.8)	30 (4)	13 (9.6)	0.0281
Developed any new anal/genital warts	103 (11.6)	83 (11)	20 (14.7)	0.2153
Vaginal intercourse	4 (0.5)	3 (0.4)	1 (0.7)	0.6600
Sex with man	768 (86.5)	647 (86)	121 (89)	0.2773
Receptive anal intercourse	509 (57.3)	425 (56.5)	84 (61.8)	0.2660
Number receptive male partners				0.0324
0-1	566 (63.7)	491 (65.3)	75 (55.1)	
2-7	227 (25.6)	190 (25.3)	37 (27.2)	
8+	95 (10.7)	71 (9.4)	24 (17.6)	
Number of new receptive male partners	2.9 (±8.7)	2.4 (±7.5)	5.3 (±13.6)	0.0102
Frequency of receptive intercourse				0.0326
No receptive intercourse	379 (42.7)	327 (43.5)	52 (38.2)	
1-3 times/Mo	399 (44.9)	342 (45.5)	57 (41.9)	
1+ times/week	110 (12.4)	83 (11)	27 (19.9)	
Insertive anal intercourse	496 (55.9)	413 (54.9)	83 (61)	0.1607
Number of insertive male partners				0.0865
0-1	589 (66.3)	509 (67.7)	80 (58.8)	
2-7	210 (23.6)	175 (23.3)	35 (25.7)	
8+	89 (10)	68 (9)	21 (15.4)	
Number of new insertive male partners	2.6 (±7.4)	2.4 (±7.3)	3.4 (±8)	0.1783
Frequency insertive intercourse				0.4087
No insertive intercourse	392 (44.2)	339 (45.1)	53 (39.3)	
1-3 times/Mo	391 (44.1)	326 (43.4)	65 (48.1)	
1+ times/week	104 (11.7)	87 (11.6)	17 (12.6)	
Number oral-anal contact partners				0.0368
0-1	678 (76.4)	578 (76.9)	100 (73.5)	
2-7	169 (19)	148 (19.7)	21 (15.4)	
8+	41 (4.6)	26 (3.5)	15 (11)	
Number new male oral-anal contact partners	1.7 (±5)	1.5 (±4.3)	2.9 (±7.6)	0.0438
Objects in anus	243 (27.4)	197 (26.2)	46 (33.8)	0.0806
Frequency of object use				0.0233

Characteristic	Overall N (%)	No HPV N (%)	Incident HPV N (%)	P-value[1]
0 times	645 (72.6)	555 (73.8)	90 (66.2)	
1-6 times	150 (16.9)	129 (17.2)	21 (15.4)	
7+ times	93 (10.5)	68 (9)	25 (18.4)	
Recall period: Past 30 days				
Number male partners				0.0712
0	194 (21.8)	164 (21.8)	30 (22.1)	
1	303 (34.1)	267 (35.5)	36 (26.5)	
2+	391 (44)	321 (42.7)	70 (51.5)	
Number receptive male partners	0.9 (±1.6)	0.8 (±1.5)	1.2 (±2.1)	0.0780
Frequency receptive intercourse	3.1 (±4.4)	2.9 (±4.2)	4.5 (±5.2)	0.0048
Number insertive male partners	0.9 (±2.2)	0.9 (±2.3)	0.9 (±1.8)	0.9238
Frequency insertive intercourse	3.3 (±5.6)	3.2 (±5)	4.1 (±8.1)	0.3936
Objects in anus	164 (67.5)	133 (67.5)	31 (67.4)	0.9872
Frequency objects used	3.3 (±3.5)	3.3 (±3.8)	3.5 (±2.4)	0.6620
HIV Disease status variables				
Mean CD4+ cell count (Current Visit)	509.5 (±287.6)	513.2 (±289.3)	489.1 (±278.1)	0.3557
<200	98 (11.2)	78 (10.5)	20 (15)	
200-500	382 (43.7)	329 (44.4)	53 (39.8)	
>500	394 (45.1)	334 (45.1)	60 (45.1)	
Mean CD4+ cell count (Previous Visit)	495.2 (±279.8)	494.2 (±279.7)	500.7 (±281.1)	0.8128
HIV Viral load (Current Visit)				0.8451
<500	583 (66.8)	499 (67.3)	84 (64.1)	
500-4000	83 (9.5)	71 (9.6)	12 (9.2)	
4001-20000	112 (12.8)	93 (12.5)	19 (14.5)	
>20000	95 (10.9)	79 (10.6)	16 (12.2)	

receptive partners, participant is the receptive partner
insertive partners, participant is the insertive partner
oral-anal contact, participant is the recipient of anal contact
[1]p-value from generalized estimating equations (GEE) accounting for correlation from taking repeated measures of participants

Table 4: Association of time-varying covariates and Incident HPV, unadjusted and adjusted for potential confounders

	Model 1 Unadjusted		Model 2 Adjusted BL Value		Model 3 Adjusted BL Value BL Confounders**		Model 4 Adjusted BL Value BL Confounders TD Confounders	
Characteristic	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Recall period: since last interview								
Injection drug use	2.01 (0.60 - 6.72)	0.3850	2.19 (0.62 - 7.72)	0.3467	2.05 (0.61 - 6.91)	0.3582	2.07 (0.61 - 6.98)	0.3545
Rectal drug use	2.54 (1.39 - 4.67)	0.0281	2.29 (1.25 - 4.22)	0.0411	2.33 (1.25 - 4.35)	0.0389	2.40 (1.30 - 4.45)	0.0332
Number of receptive male partners								
0-1	1.0		1.0		1.0		1.0	
2-7	1.27 (0.84 - 1.95)	0.0324	1.37 (0.90 - 2.09)	0.0087	1.40 (0.91 - 2.17)	0.0054	1.41 (0.90 - 2.20)	0.0060
8+	2.21 (1.34 - 3.64)		2.59 (1.53 - 4.37)		2.90 (1.65 - 5.10)		2.88 (1.64 - 5.07)	
Number new receptive male partners	1.03 (1.01 - 1.04)	0.0102	1.03 (1.01 - 1.05)	0.0063	1.03 (1.01 - 1.05)	0.0070	1.03 (1.01 - 1.05)	0.0064
Frequency of receptive intercourse								
None	1.0		1.0		1.0		1.0	
1-3 times per month	1.05 (0.69 - 1.60)	0.0326	1.12 (0.73 - 1.71)	0.0216	1.25 (0.79 - 1.97)	0.0107	1.26 (0.80 - 2.01)	0.0121
1+ times per week	2.05 (1.26 - 3.31)		2.23 (1.35 - 3.68)		2.60 (1.49 - 4.54)		2.60 (1.48 - 4.55)	
Number of insertive male partners								
0-1	1.0		1.0		1.0		1.0	
2-7	1.27 (0.81 - 1.99)	0.0865	1.32 (0.84 - 2.07)	0.0477	1.30 (0.81 - 2.07)	0.0600	1.25 (0.77 - 2.02)	0.1201
8+	1.96 (1.15 - 3.35)		2.33 (1.28 - 4.25)		2.26 (1.23 - 4.15)		2.03 (1.09 - 3.78)	
Number new insertive partners	1.01 (0.99 - 1.04)	0.1783	1.02 (0.99 - 1.04)	0.1828	1.02 (1.00 - 1.04)	0.1752	1.01 (0.99 - 1.03)	0.2214
Number of oral-anal contact partners								
0-1	1.0		1.0		1.0		1.0	
2-7	1.14 (0.69 - 1.90)	0.6643	1.28 (0.76 - 2.14)	0.2126	1.37 (0.81 - 2.31)	0.2192	1.44 (0.85 - 2.43)	0.1462
8+	1.23 (0.77 - 1.97)		1.58 (0.95 - 2.64)		1.54 (0.91 - 2.62)		1.64 (0.96 - 2.81)	

	Model 1 Unadjusted		Model 2 Adjusted BL Value		Model 3 Adjusted BL Value BL Confounders**		Model 4 Adjusted BL Value BL Confounders TD Confounders	
Characteristic	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Number new oral-anal contact partners	1.04 (1.01 - 1.07)	0.0438	1.07 (1.04 - 1.10)	0.0109	1.06 (1.03 - 1.09)	0.0145	1.06 (1.03 - 1.09)	0.0127
Times objects inserted into anus								
0-1	1.0		1.0		1.0		1.0	
2-7	1.00 (0.61 - 1.66)	0.0233	1.05 (0.59 - 1.87)	0.0226	1.06 (0.58 - 1.93)	0.0438	1.08 (0.59 - 1.97)	0.1139
8+	2.27 (1.41 - 3.64)		2.38 (1.37 - 4.15)		2.23 (1.24 - 3.99)		2.06 (1.11 - 3.82)	
Recall period: Past 30 days								
Number male partners								
0	1.0		1.0		1.0		1.0	
1	0.74 (0.44 - 1.24)	0.0712	0.74 (0.44 - 1.24)	0.0448	0.75 (0.45 - 1.25)	0.0740	0.76 (0.46 - 1.28)	0.0969
2+	1.19 (0.73 - 1.93)		1.26 (0.77 - 2.05)		1.23 (0.75 - 2.02)		1.22 (0.74 - 2.02)	
Number receptive partners	1.12 (1.01 - 1.23)	0.0780	1.13 (1.02 - 1.25)	0.0555	1.13 (1.01 - 1.27)	0.0640	1.13 (1.01 - 1.27)	0.0655
Frequency receptive intercourse	1.07 (1.03 - 1.11)	0.0048	1.07 (1.03 - 1.12)	0.0058	1.07 (1.03 - 1.13)	0.0054	1.08 (1.03 - 1.13)	0.0049

receptive partners, participant is the receptive partner
insertive partners, participant is the insertive partner
oral-anal contact, participant is the recipient of anal contact

*Generalized linear models estimated with generalized estimating equations (GEE) with a logit link accounting for the correlation between subjects

** Adjusted for age at baseline, race, education, smoking 100+ lifetime cigarettes, and baseline CD4+ level, and HPV 6 status at baseline

CHAPTER 4: Prevalence and risk factors for prevalent anal HPV infection among HIV-infected men who have sex with men in India

Abstract

Background: Little is known about anal HPV infection among men who have sex with men (MSM) in India and even less is known about HPV infection among Indian MSM who are also HIV-infected. The background incidence of HPV-related cancers in both men and women is high in India and Indian HIV-infected MSM may be at especially high risk for anal HPV infection and HPV-associated disease.

Objectives: To determine the prevalence and risk factors for anal HPV infection among Indian HIV-infected MSM.

Methods: We evaluated 298 HIV-infected MSM from two cities in India. Men were tested for anal HPV infection using L1 HPV DNA PCR with probes specific for 29 types and a mixture of 10 additional types. CD4+ level and plasma HIV viral load (VL) were measured. Participants completed an interviewer-administered questionnaire including a sexual history.

Results: Samples from 39 (13%) participants were beta-globin negative and excluded from further analyses. The mean age was 35 years, 84% had <10 years of education, and 48% were married. The mean CD4+ level was 459 cells/μl, 35% had undetectable HIV VLs, and 43% reported currently taking antiretrovirals. The prevalence of anal HPV was 71% (95%CI 65%-77%). In separate multivariable models, ever having had receptive anal intercourse (RR:1.7, 95%CI 1.1-2.5, p=0.01) and higher number of receptive sex partners (partners with whom the participant is the receptive partner) (RR:1.3, 95%CI 1.4-1.7, p=0.02) significantly increased the risk of anal HPV infection. A higher number of female vaginal sex partners was associated with a lower risk of infection (p_{trend}=0.003). Current antiretroviral use also significantly lowered risk of HPV infection after controlling for number of lifetime receptive male partners, age, and CD4+ level (RR:0.8 (0.7-1.0), p=<0.01). "Almost always" condom use by male partner during receptive anal intercourse also significantly reduced risk of infection in adjusted analyses (RR: 0.83 (0.70 - 0.98), p=0.03). CD4+ cell count and HIV VL were not associated with HPV infection in multivariable models.

Conclusions: This is the first report of anal HPV among Indian HIV-infected MSM. Our results show a high prevalence of HPV infection, with sexual behaviors being the most important risk factors.

Introduction

It has become well established that men who have sex with men (MSM) are at greater risk for anal cancer and its precursor lesion, anal intraepithelial neoplasia (AIN), than men in the general population [74, 84, 162, 163]. The incidence of anal cancer among HIV-uninfected MSM in the US is estimated to be 37/100,000, which is close to the incidence of cervical cancer among women in developing countries, including India [84]. HIV- infected MSM in the US are at greater risk of developing anal cancer than HIV-uninfected MSM, with an estimated incidence rate of >100/100,000 [74]. As with cervical cancer, the etiological agent that causes most anal cancers is human papillomavirus (HPV), and HIV-infected MSM are also at greater risk of anal HPV infection.

There is little information available on HPV infection and HPV-associated disease in Indian men. There are some data indicating that HIV-infected men in India may have higher incidence rates of anal cancer than HIV-uninfected men. In a 2008 study conducted at the Tata Memorial Hospital (TMH) in Mumbai among patients registered in the HIV Cancer Clinic, the proportional incidence ratio (PIR) in males for anal cancer was 10.3 (95%CI 4.3-24.8) [164]; in other words, the observed number of anal cancers in HIV-infected males was 10 times greater than the number expected, based on the TMH hospital cancer registry. This study did not include information on the sexual behaviors of the participants, so we do not know if the PIR differed between MSM and heterosexual men.

There is evidence, however, that the incidence of other HPV-related cancers among both Indian men and women is high compared with the incidence in the US and other western countries. Cervical cancer is the most common cancer among Indian women, with an estimated 132,000 new cases of cervical cancer diagnosed each year and 74,000 deaths [91]. The prevalence of cervical HPV infection among Indian women without cervical cancer varies from 7 to 37% [98, 99, 102, 105]. As for HPV-related cancers among Indian men, penile cancer is more common among Indian men than among men in the US. Data from the Indian Council for Medical Research show that the incidence of penile cancer varies from 0.7 to 3.0 /100,000 [86]. Penile cancer represents more than 6% of all cancers in Indian men [86]. It is unknown what percentage of these cases of penile cancer in India can be attributed to HPV infection, although in the US and other western countries 40-50% of men with penile cancer have detectable HPV DNA [84]. Given that HPV is a sexually transmitted infection, the high incidence rates of HPV infection and associated diseases of the cervix and penis in India imply that HPV infection of the anus may also be of concern.

HIV-infected Indian MSM may be particularly vulnerable to anal HPV infection and associated disease. Many Indian MSM are behaviorally bisexual and therefore may serve as a bridge population from their male to their female partners, and from their female to their male partners. Given that the prevalence of HIV is very high (7-63%) among Indian MSM [114, 165-168], there may be a large population of Indian men at high risk of developing anal cancer. Understanding the prevalence of anal HPV infection among HIV-infected MSM, along with potentially modifiable risk factors, is a critical first step in addressing this public health issue.

We conducted a cross-sectional study in two cities in India to determine the prevalence and risk factors for anogenital HPV infection among Indian HIV-infected MSM. In this analysis we report the results for anal HPV infection.

Methods

HIV-infected MSM were recruited from two study sites, Christian Medical College (CMC), Vellore, Tamil Nadu (a large research and teaching institution) and Humsafar Trust (HT), Mumbai (a male sexual health non-governmental organization (NGO)). Men were recruited through outreach workers, and local HIV/AIDS support groups and were also referred from other NGOs. Enrollment occurred from September 2009 to August 2010. Men were eligible for the study if they had had sexual contact with another man in the preceding six months, were HIV-infected, and spoke the local language. Participants completed a questionnaire in the local language (Hindi or Tamil) administered by a male interviewer. They also had a clinical examination, including collection of an anal swab for HPV testing. Blood was collected for a CD4+ lymphocyte count measured by standardized two- or three-color fluorescence methods. Plasma HIV viral load (HIV VL) was measured using the branched-chain Chiron assay (Chiron, Emeryville, California, USA). All procedures were performed after obtaining written informed consent from participant. The consent process included a full description of the study in the local language including potential risks and benefits. All procedures instituted to protect against disclosure of HIV,MSM, or HPV status were described and the benefits to the HIV-infected Indian MSM population of studying this important topic were discussed. The study was approved by the Committee on Human Research of UCSF, and the Institutional Review Boards of both CMC and HT.

Testing for anal HPV infection was performed as described previously using the polymerase chain reaction (PCR) with L1 consensus primers and probes specific for 29 individual HPV types and a mixture of 10 additional types (using a combined probe) for a total of 39 HPV types [55]. Beta-globin-negative samples (indicating insufficient good quality DNA) were excluded from analysis. In the current analyses we report the prevalence of anal HPV as defined by a sample positive with the consensus probe mixture. HPV testing results are preliminary individual results may change in the final analysis. HPV type-specific data are not yet available for inclusion in this analysis, but will be included in the final analysis once these data become available.

Assessment of potential risk factors

Demographic, lifestyle characteristics (including smoking, alcohol, and recreational drug use), medical history, prescription medication use (including use of antiretroviral medications (ARV)), and history of sexual behavior were collected. We asked about sexual behaviors over the participant's lifetime and also in the past 30 days. Ever/never having a particular behavior or partner type was collected, as well as number of partners for each type of behavior. Men were queried about multiple types of sexual behaviors, including sex with men and women, "insertive" anal intercourse (participant inserts his penis into partner's anus) and "receptive" anal intercourse (participant receives his partner's penis into his anus), and oral-anal contact (participant's anus receives oral contact), as well as commercial sex work.

Statistical analysis

Characteristics thought to be related to prevalent anal HPV infection were examined for association with anal HPV infection. Categorical variables were assessed for bi-variable association using the chi-square test for independence. Continuous variables were assessed using analysis of variance (ANOVA) or ranked ANOVAs. Variables were considered significantly associated with prevalent HPV infection if the p-value was <0.05 (no adjustment

was made for multiple comparisons, so one should avoid global inferences). We also assessed differences between men taking ARV medications and those not taking ARV medication, using the method described above.

To derive adjusted relative risks in order to assess the independent effects of sexual risk behaviors on our outcome, we constructed log linear multivariable models specifying a binomial distribution (as opposed to the more standard logistic regression in this context). In this analysis, we selected potential sexual risk factors to analyze, as well as potential confounders *a priori* based on a review of the literature of existing risk factors for HPV infection in men and from our own previous investigations with similar populations in the US. The following sexual risk factors were selected for analysis: lifetime number of women partners, lifetime number total male partners, lifetime number of receptive male partners (number of partners with whom the participant was the receptive partner), condom use by the lifetime receptive partners (partner wears condom) , number of receptive partners in the past 30 days, condom use by receptive partner(s) in the past 30 days, number of lifetime insertive partners (number of partners with whom the participant was the insertive partner), and number of oral-anal contact partners (participant's anus receives oral contact).

Characteristics previously identified as potential confounders of the relationship between a sexual risk behavior and HPV infection were included in these multivariable models. These were: age, education, CD4+ level, and current use of antiretroviral medication. *A priori* multivariable models were constructed for the association of each of the selected risk factors with anal HPV infection.

Results

Two hundred ninety-eight HIV-infected MSM were enrolled into the study; of these, 39 (13%) had insufficient DNA as determined by inability to amplify the housekeeping gene beta-globin and these results were excluded from further analyses. The mean age of the remaining 259 participants was 35 years, 18% had >10 years of education, 65% had 1-10 years and 17% said that they had no education (Table 1). The median monthly income was 3250 rupees (interquartile range (IQR) 1750-6000) or approximately $72. Seventy-seven percent were Hindu, 14% were Muslim, and another 8% reported other religions, including Christian, Buddhist, and Jain. Forty-eight percent of participants were married. Only thirty percent of men had smoked at least 100 lifetime cigarettes, 34% chewed tobacco regularly, and most (60%) had consumed alcoholic beverages. Recreational drug use was uncommon and only 3% had ever used drugs for pleasure. Participants had a median CD4+ level of 431 cells/μL (IQR 270-581), their median HIV viral load was 10,049 copies/mL (IQR 400-74,700), 35% had an undetectable HIV viral load, and 43% were currently taking antiretroviral medications.

Although all men enrolled in the study reported having had sex with men, two thirds also reported had having sexual contact with a woman, 28% reported >5 lifetime vaginal sex partners, and 33% reported vaginal sex in the past six months. The mean age of first sexual contact with a man was 17 years and most men (75%) had more than 100 lifetime male partners. Eighty-seven percent of men reported ever having had receptive anal intercourse, and 44% said they had more than 1000 partners with whom the participant was the receptive partner. Most men had also had insertive anal intercourse (63%) and had had oral-anal contact with a partner (54%). Thirty percent of men reported sex with a female commercial sex worker (FSW), 40% with a male sex worker (MSW), and 64% said that they had received money for having sex with another man.

One hundred and eighty-four men (71%; 95% CI: 65%-77%) were positive for the consensus probe for anal HPV infection. The prevalence of anal HPV infection did not differ by clinical site.

Associations of demographic, lifestyle, and medical characteristics and anal HPV infection

In bi-variable analyses, younger age was associated with anal HPV infection the prevalence of anal HPV infection was 61% among participants aged <35 years compared with 44% in those aged ≥35 years (p=0.03). The only other demographic factor associated with HPV infection was religion. More men who reported their religion as Muslim had anal HPV infection than non-Muslims (17% vs. 7%, p=0.03). Smoking, chewing tobacco, and consuming alcohol were not associated with anal HPV infection.

Associations of markers of HIV disease status and an HPV infection

Having been recently diagnosed with HIV disease (<1 year ago) was more common among those with anal HPV infection (32%) compared with those without anal HPV infection (17%), p=0.04 (Table 2). Currently taking ARV medication was less common among those with anal HPV infection compared to those without anal HPV (37% vs. 57%, p=0.003), and among those taking ARVs, men with anal HPV infection had a lower mean number of years taking ARVs (1.8 vs. 2.7, p=0.04). CD4+ count, HIV VL level, months on current ARV regime, location where they receive their ARV medication, and knowledge of names of ARV medication were not associated with anal HPV infection.

Predictors of ARV medication use in participants

Men who were taking ARV medication differed from men who were not taking ARV medications in a number of respects (Table 3). Men taking ARVs were more likely to have been enrolled at CMC, were older, and had a lower monthly median income. They were also more likely to be married, had a lower number of vaginal sex partners, and had a higher number of receptive male partners. As expected, more of the men taking ARVs had undetectable HIV VLs; however, there was no difference in CD4+ cell counts between the two groups.

Associations of sexual risk factors and anal HPV infection- Multivariable Models

Age was not significantly associated with anal HPV infection after controlling for education, CD4+ level, current ARV use, and number of male receptive partners (Table 4). However, current ARV use remained significantly associated with anal HPV infection in this model and was associated with a reduction in prevalence of anal HPV, RR=0.81 (95% CI: 0.67-0.96, p=0.02).

Almost all models that included measures of receptive anal sex showed a statistically significant association between receptive anal sex and anal HPV infection. Ever having had receptive anal intercourse was associated with an increased prevalence of anal HPV infection (RR:1.67, 1.12-2.48). Having a higher number of partners was also associated with an increased risk (RR: 1.32, 0-100 compared to 101-1000 partners; and RR: 1.30, 0-100 partners compared to 1000+). A higher number of receptive anal intercourse partners in the past 30 days was also associated with increased risk of infection (RR=1.24 and RR=1.34 for 0 partners vs. 1-4 and 5+, respectively). Having male partners that almost always use a condom during receptive anal

intercourse in the past 30 days was associated with a significantly reduced the risk of anal HPV infection (RR: 0.83 (0.70-0.98)).

Number of lifetime vaginal sex partners was also associated with anal HPV infection and having a higher number of female partners was associated with a lowered risk of anal HPV infection. Models including a measure of lifetime vaginal sex partners and either total male partners or total receptive partners could not be run (the model prediction algorithm failed to converge), but these variables were negatively correlated with lifetime number of vaginal sex partners (r=-0.34 and r=-0.46, respectively).

The number of total male partners, number of insertive male partners (partners with whom the participant is the insertive partner), ever having had an oral-anal contact partner, and ever having had oral-anal contact partners were not associated with anal HPV infection in adjusted analyses at the $p<0.05$ cut-off. Neither sex with a commercial sex worker nor accepting money for sex with a man was associated with anal HPV infection in bi-variable or multivariable analyses.

Discussion

This is the first report of the prevalence of anal HPV among HIV-infected Indian MSM. Seventy-one percent of our participants were positive for anal HPV infection. This high prevalence is within the range reported for other populations of HIV-seropositve men worldwide, although at the lower end of the range. Studies conducted by our own research group investigating HIV-infected MSM in San Francisco found a prevalence of >90% [55]. Other studies, from Canada and Australia, that used similar HPV L1 PCR consensus probes also found prevalence levels of >95% [75, 76]. One study, from France, found a lower prevalence (75%) [77] than that found in the US, Canadian, and Australian studies. However, the French investigators used a less sensitive method of identifying HPV DNA (Hybrid Capture 2) that included fewer types and no consensus probe, which together may account for the lower prevalence seen in the French population.

Of the four sociodemographic/medical factors that we included in our multivariable models, only currently taking ARVs remained significantly associated with anal HPV infection after adjusting for the three other demographic factors and one sexual risk behavior (number of receptive partners). Currently taking ARVs was associated with a reduced prevalence of anal HPV infection. This finding is consistent with findings of a reduction in other opportunistic infections following initiation of ARVs [4, 6]. However, the relationship between ARVs and HPV infection and HPV-associated disease is not as clear as the relationship with other opportunistic infections. Neither cervical cancer nor anal cancer incidence have declined after the introduction of ARV therapy [74, 84] and in studies of both cervical and anal cancer among HIV-infected individuals, ARVs have not been shown to lead to regression of high grade intraepithelial lesions or to prevent the progression of high grade lesion to cancer [6]. However, there is some evidence that ARV therapy may help clear a new HPV infection [6]. New HPV infections may be cleared by the more robust immune response in HIV-infected participants taking ARVs. This may help explain our findings of an association between ARV use and prevalent infection. Participants taking ARVs who develop new infection are able to clear them before enrollment in our study.

Another potential explanation for our findings is that men in India taking ARVs are different from men not taking ARVs and that these differences confound the ARV-HPV association. There

were important differences between HIV-infected men taking ARVs and those not taking ARVs, including differences in sexual behavior. Although our multivariable models adjusted for several of these factors, it was not possible to include all of these factors in our multivariable models because of the high prevalence of our outcome (i.e. the models were unstable when more than 4-5 explanatory variables were included). It is also possible that there are unmeasured confounders that explain the relationship seen between anal HPV infection and ARVs. Lastly, because only 30% of participants who reported taking ARV medications knew the names of the medications they were taking, it is possible that some of these men were not taking ARVs, but some other type of medication and this could also have misclassification participants as ARV users and could have inflated the estimate. Future studies of anal HPV infection in this population should include a better measure of ARV use, perhaps collecting information from medical records, or pill identification.

Of the sexual risk factors examined, receptive anal intercourse was the most common behavior associated with anal HPV infection. Measures of this behavior over the lifetime and in the past 30 days both were associated with anal HPV infection. Ever having had receptive anal intercourse and a higher number of receptive male partners both increased the prevalence of anal HPV infection. This finding is consistent with other studies of men that have shown that receptive anal intercourse is a risk factor for anal HPV infection [53, 55, 57, 58, 169], although it has not always been possible to demonstrate this relationship in cross-sectional studies where few men report no receptive anal intercourse. An important protective factor documented in this study was “almost always or always” condom use by the partner during receptive anal intercourse. Condom use has been shown in some studies to be effective in reducing the risk of penile HPV infection ([9, 33, 34, 45] and of genital warts [40], but the relationship between condom use (by the insertive partner during receptive anal intercourse) and anal HPV infection has not been evaluated in other studies.

Another key finding was that a higher number of female vaginal sex partners was associated with a lower prevalence of anal HPV infection. This relationship could not be evaluated controlling for number of either total male partners or number of receptive male partners because the two behaviors are negatively correlated, and therefore having a higher number of vaginal sex partners may be a marker for fewer male receptive partners. Another potential explanation is that participants with more lifetime female partners engage in less risky behaviors with their male partners, for example they are more likely to use condoms with their male parnters if they also have a female partner, or are married. This finding should be evaluated as part of a longitudinal study among Indian MSM in which the prevelance of sex with both men and women is high.

This study had several limitations. As it is the first study of anal HPV in this population, it was designed as a cross-sectional study and therefore has the drawbacks associated with a cross-sectional study. We do not know if the sexual behavior occurred before the anal HPV infection. Another potential limitation to the study is that we did not have a random sample of Indian HIV-infected MSM. It is possible the associations seen cannot be not generalized to all HIV-infected Indian MSM. However, being an MSM and being HIV-infected are both highly stigmatized in India, and it is unlikely that other sampling strategies would have yielded a more representative sample, while ensuring participant confidentiality.

Our study has confirmed the hypothesis that anal HPV infection is common in HIV-infected Indian MSM. Given the large number of Indian MSM living with HIV, this high prevalence of anal HPV infection indicates that many Indian men are at risk of developing anal cancer, which

is potentially preventable through prevention of HPV infection and early treatment of AIN; therefore HIV-infected Indian MSM should be considered for anal cancer screening programs as well as the HPV vaccine.

Receptive anal intercourse was associated with increased risk of anal HPV infection, as was a higher number of partners with whom the participant is the receptive partner. Condom use by the male partner in the recent past reduced the risk of HPV infection. Given that condom use protects against other STIs, as well as protects against acquiring a new strain of HIV, condom use should be recommended to all HIV-infected Indian MSM. Counseling could also include information that condom use by their receptive partners may help prevent anal HPV infection and thereby reduce the risk of anal cancer.

Table 1- Socio-demographic and lifestyle characteristics of participants (N=259)

Characteristic	N (%)
Demographic factors	
Age (years)	
18-25	42 (16.8)
26-35	100 (40)
35+	108 (43.2)
Highest year of school completed	
None	43 (16.6)
1-10	168 (64.9)
10+	48 (18.5)
Median monthly income (INR) (±IQR)	3250 (1750-6000)
Married	123 (47.5)
Religion	
Hindu	201 (77.6)
Muslim	36 (13.9)
Other	22 (8.5)
Self-reported MSM community identification	
Kothi (behaviorally receptive partner)	109 (42.1)
Panthi (behaviorally insertive partner)	16 (6.2)
Bisexual	8 (3.1)
Gay	12 (4.6)
Hijra /Aravani /Transgender (TG)	44 (17)
Double Dick (behaviorally both insertive and receptive partner)	64 (24.7)
Other	6 (2.3)
Substance use	
Smoked more than 100 lifetime cigarettes	80 (30.9)
Chew tobacco regularly	87 (33.6)
Ever consume alcoholic beverage	153 (59.1)
HIV disease status	
CD4+ level (cells/uL)	
<200	35 (13.6)
200-500	121 (47.1)
500+	101 (39.3)
Undetectable HIV viral load level	90 (35)
Currently taking antiretroviral therapy	111 (42.9)

Characteristic	N (%)
Sexual behavior	
Lifetime Number Women Vaginal Sex Partners	
0	99 (38.4)
1-4	86 (33.3)
5-39	36 (14)
40+	37 (14.3)
Mean age first had sex with another male (±SD)	16.8 (±6.3)
Lifetime Total Male Partners	
1-100	65 (25.2)
101-1000	100 (38.8)
1000+	93 (36)
Lifetime Total Receptive Male Partners	
0	34 (13)
1-100	42 (16)
101-1000	67 (26)
1000+	115 (35)

Table 2-Association of HIV disease status and anal HPV Infection

Characteristic	Overall		No anal HPV infection		Anal HPV Infection		P-value*
	N	(%)	N	(%)	N	(%)	
Total N	**259**	**(100)**	**75**	**(29)**	**184**	**(71)**	
Years since first positive HIV test							0.0410
<1	72	(27.8)	13	(17.3)	59	(32.1)	
1-4	95	(36.7)	34	(45.3)	61	(33.2)	
4+	92	(35.5)	28	(37.3)	64	(34.8)	
Mean CD4+ level	458.9	(±254)	475.3	(±268)	452.1	(±248)	0.5610
<200	35	(13.6)	11	(14.7)	24	(13.2)	0.9200
200-500	121	(47.1)	34	(45.3)	87	(47.8)	
500+	101	(39.3)	30	(40.0)	71	(39.0)	
HIV viral load level							0.0646
Undetectable	90	(35)	33	(44)	57	(31.3)	
>400-37,400	80	(31.1)	24	(32)	56	(30.8)	
>37,400	87	(33.9)	18	(24)	69	(37.9)	
Currently taking ARV	111	(42.9)	43	(57.3)	68	(37)	0.0027
Years since first initiating ARV	2.2	(±1.8)	2.7	(±2)	1.8	(±1.6)	0.0400
Months taking current ARV regime	26	(±21.7)	31	(±24.3)	22.8	(±19.3)	0.1080
Location receiving ARV							0.8490
Government Hospital	102	(91.9)	40	(93)	62	(91.2)	
MSF (Medecins Sans Frontieres)	5	(4.5)	2	(4.7)	3	(4.4)	
NGO / Private Charity	1	(0.9)	0	(0)	1	(1.5)	
Private Doctor / Clinic / Hospital	3	(2.7)	1	(2.3)	2	(3.0)	
Names of ARV medications known by participant	30	(27)	10	(23.3)	20	(29.4)	0.4788

*p-value for categorical variable from chi-square, and from ANOVA or ranked ANOVA for normally and non-normally distributed continuous variables

Table 3-Predictors of ARV use

Characteristic	No ARV Use N	(%)	ARV Use N	(%)	P-value*
Clinical Site					0.0001
CMC, Vellore	55	(37.2)	68	(61.3)	
HT, Mumbai	93	(62.8)	43	(38.7)	
Age Categories (years)					<.0001
18-25	38	(27)	4	(3.7)	
26-35	62	(44)	38	(34.9)	
35+	41	(29.1)	67	(61.5)	
Highest year of school completed (years)					0.7089
None	24	(16.2)	19	(17.1)	
1-10	94	(63.5)	74	(66.7)	
10+	30	(20.3)	18	(16.2)	
Mean monthly income (rupees)	5400	(±4722)	3583	(±3764)	<.0001
Religion					0.4353
Hindu	111	(75)	90	(81.1)	
Muslim	24	(16.2)	12	(10.8)	
Other	13	(8.8)	9	(8.1)	
Married	46	(31.1)	77	(69.4)	<.0001
Years since first positive HIV test					<.0001
<1	58	(39.2)	14	(12.6)	
1-4	51	(34.5)	44	(39.6)	
4+	39	(26.4)	53	(47.7)	
CD4+ level					0.1572
<200	15	(10.3)	20	(18)	
200-500	74	(50.7)	47	(42.3)	
500+	57	(39)	44	(39.6)	
HIV VL Level					<.0001
Undetectable	9	(6.1)	81	(73.6)	
>400-37,400	63	(42.9)	17	(15.5)	
>37,400	75	(51)	12	(10.9)	
Lifetime Number Women Vaginal Sex Partners					0.0007
0	19	(25.7)	80	(43.5)	
1-4	21	(28.4)	65	(35.3)	
5-39	18	(24.3)	18	(9.8)	
4+	16	(21.6)	21	(11.4)	
Lifetime Receptive Male Partners					0.0042
1-100	33	(44)	43	(23.5)	
101-1000	14	(18.7)	53	(29)	
1000+	28	(37.3)	87	(47.5)	

*p-value for categorical variable from chi-square, and from ANOVA or ranked ANOVA for normally and non-normally distributed continuous variables.

Table 4-Unadjusted and adjusted associations with anal HPV infection (N=259)

	Unadjusted		Adjusted *	
Characteristic	**RR**	**P-value**	**RR**	**P-value**
Demographics				
Age (years)				
18-25	1.25 (1.01 - 1.55)	0.0427	1.10 (0.88 - 1.37)	0.4156
26-35	1.24 (1.04 - 1.48)	0.0185	1.13 (0.93 - 1.37)	0.2109
35+	1.0		1.0	
Education (years)				
None	1.0		1.0	
0-10	0.97 (0.78 - 1.19)	0.7481	1.02 (0.83 - 1.25)	0.8577
10+	1.04 (0.81 - 1.33)	0.7543	1.02 (0.80 - 1.29)	0.9001
Currently taking ARVs	0.78 (0.66 - 0.93)	0.0046	0.81 (0.67 - 0.96)	0.0184
CD4 level	0.99 (0.96 - 1.02)	0.5250	1.00 (0.97 - 1.04)	0.9080
Sexual Risk Factors				
Number of vaginal sex partners, lifetime				
0	1.0		1.0	
1-4	0.94 (0.80 - 1.09)	0.3941	0.95 (0.80 - 1.12)	0.5452
5-40	0.62 (0.44 - 0.87)	0.0057	0.62 (0.43 - 0.88)	0.0083
40+	0.70 (0.52 - 0.95)	0.0198	0.74 (0.54 - 1.02)	0.0658
Number of total male sexual partners, lifetime				
1-100	1.0		1.0	
101-1000	1.30 (1.03 - 1.64)	0.0271	1.21 (0.96 - 1.52)	0.1117
1000+	1.27 (1.00 - 1.61)	0.0491	1.21 (0.95 - 1.53)	0.1175
Ever had receptive anal intercourse, lifetime	1.70 (1.16 - 2.50)	0.0069	1.67 (1.12 - 2.48)	0.0111

	Unadjusted		Adjusted *	
Characteristic	RR	P-value	RR	P-value
Number of receptive anal sex partners, lifetime				
0-100	1.0		1.0	
101-1000	1.40 (1.11 - 1.76)	0.0047	1.32 (1.04 - 1.69)	0.0236
1000+	1.34 (1.07 - 1.67)	0.0105	1.30 (1.04 - 1.63)	0.0213
Partner almost always used condoms during receptive anal intercourse, lifetime				
No receptive sex	1.0		1.0	
Used condoms less than almost always	1.76 (1.20 - 2.59)	0.0041	1.71 (1.15 - 2.54)	0.0082
Used condoms almost always	1.48 (0.96 - 2.28)	0.0770	1.45 (0.93 - 2.28)	0.1044
Not always compared with always	0.84 (0.67 - 1.05)	0.1288	0.85 (0.67 - 1.08)	0.1778
Number of receptive anal sex partners, Past 30 days				
0	1.0		1.0	
1-4	1.31 (1.04 - 1.66)	0.0218	1.24 (0.98 - 1.57)	0.0722
5+	1.36 (1.08 - 1.71)	0.0098	1.34 (1.06 - 1.68)	0.0133
Partner almost always used condoms during receptive anal intercourse, Past 30 days				
No receptive sex	1.0		1.0	
Used condoms less than almost always	1.48 (1.17 - 1.86)	0.0009	1.48 (1.17 - 1.88)	0.0012
Used condoms almost always	1.27 (1.01 - 1.60)	0.0411	1.23 (0.97 - 1.55)	0.0837
Less than almost always compared with always	0.86 (0.74 - 1.00)	0.0498	0.83 (0.70 - 0.98)	0.0280
Number of insertive anal sex partners, lifetime				
0-100	1.0		1.0	
101-1000	1.21 (0.97 - 1.52)	0.0902	1.11 (0.87 - 1.42)	0.3860

	Unadjusted		Adjusted *	
Characteristic	RR	P-value	RR	P-value
1000+	1.05 (0.73 - 1.52)	0.7812	1.27 (0.83 - 1.94)	0.2714
Ever oral-anal contact	1.14 (0.97 - 1.34)	0.1157	1.08 (0.93 - 1.26)	0.3124
Number of oral-anal contact partners, lifetime				
0	1.0		1.0	
1-10	1.15 (0.96 - 1.38)	0.1412	1.04 (0.88 - 1.24)	0.6493
11+	1.10 (0.92 - 1.33)	0.2974	1.16 (0.96 - 1.40)	0.1295

*Demographic factors adjusted for other demographics in table and number of lifetime receptive male partners. Sexual risk factors adjusted for all demographics included in table (except “Partner almost always used condoms during receptive anal intercourse, Past 30 days”). “Partner almost always used condoms during receptive anal intercourse, Past 30 days” adjusted for demographic factors included in table except current ARV use

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CHAPTER 5: Discussion, Public Health Significance, and Recommendations for Future Research

Summary of Main Findings

The three analyses presented in this dissertation (Chapters 2-4) include important new information on the prevalence and incidence of and risk factors for infection with anal HPV among HIV-infected men who have sex with men (MSM). The prevalence of anal HPV infection was very high in both the San Francisco population and the Indian population, with prevalences between 70-92%. These prevalences are within the range of estimates determined in other US and European populations of MSM [1-4]. Incidence was evaluated only in the US population and was also high compared with the incidence rates of other sexually transmitted infections among MSM [5-7]. Given the well-established link between anal HPV infection and HPV-associated anal disease, including anal cancer, these high prevalence and incidence rates are of great concern. Additionally, because HIV-infected individuals are at greater risk of developing anal cancer than those not infected with HIV, the two populations studied are at high risk for developing anal cancer.

The prevalence, incidence, and main risk factors for prevalent and incident HPV infection in the three studies are summarized in Table 1. MSM who have higher numbers of female partners compared with male partners have a lower prevalence of infection. Conversely, MSM who have a higher total number of male partners, a history of receptive anal intercourse, and more receptive anal intercourse partners (partners with whom the participant is the receptive partner), more frequent receptive anal intercourse, have increased prevalence and incidence of HPV infection. Although the findings are not completely uniform across the three analyses, they do present a consistent picture. Many of the risk factors for HPV infection identified in this dissertation were either not evaluated in past studies or the previous studies were not able to demonstrate an association between the risk factor and anal HPV infection [1, 8]. This dissertation provides good evidence that anal HPV is sexually transmitted among MSM, as is the case with cervical HPV infection among women.

Similarities and differences between the San Francisco and Indian populations and study results

Overall, the results from the studies of the prevalence of HPV infection among MSM in San Francisco and India were quite similar. Very high prevalences of anal HPV infection were found in both populations, and both prevalences were well within the range of what other studies have found (Chapters 2 and 4, respectively). However, the prevalence of anal HPV infection among HIV-infected Indian MSM was lower than that among MSM in San Francisco (71% vs. 93%, respectively). Similar HPV DNA detection techniques were used in both studies, so the differences seen are not likely to be due to a difference in detection of infection.

A possible explanation for the difference in prevalence between the two populations is that the populations themselves are different in many respects. Differences in behaviors between MSM in San Francisco and MSM in India may account for the lower prevalence of anal HPV infection among Indian HIV-seropositve MSM. For example, the participants in the Indian study reported more female partners than participants in the San Francisco study (Chapter 2). Twenty-two percent of Indian men reported more than 10 female sex partners, compared with only 7% of San Francisco men. Indian men also reported a lower frequency of receptive anal intercourse. Thirteen percent of Indian men said that they had never had receptive anal intercourse compared with only 0.8% of San Francisco men. Participants from India were also younger, of different cultural/ethnic backgrounds, reported less substance use, and fewer were taking ARV than participants in San Francisco (Chapter 2). All of these factors could be contributing to the difference in the prevalence of anal HPV between these two populations.

Another important difference between the two studies was the ability to evaluate risk factors for overall anal HPV infection. Because almost all of the men in San Francisco had anal HPV, in order to evaluate risk factors for infection, we chose a modified outcome (anal HPV 16) that may have introduced some bias into the results. The multivariable models compared those with anal HPV 16 to those without HPV 16 infection, but this does not take into consideration those who may have had another type of anal HPV (i.e. HPV 18). Factors that increase or decrease risk of infection are likely similar for different HPV types, and including men with other anal HPV types in our "no disease" group may have introduced error into our results. If, for example, a high number of partners was common to all men who had anal HPV, regardless of type, then our study would not be able to detect a difference in number of partners between those with HPV 16 and those with no HPV 16 (including those with anal HPV of other types). Indeed, we did not the expected association between number of receptive anal intercourse partners and this may be the reason. However, in this study there was no other option because of the high HPV prevalence and the low number of men with no anal HPV infection of any type. Because the overall prevalence of anal HPV was lower among the Indian men, we were able to evaluate any anal HPV infection compared with no HPV infection (Chapter 4). This analysis did show many more significant associations between sexual practices and anal HPV infection. There was also some consistency between these two studies; both identified a higher number of female partners as a protective factor and total number of male partners as a risk factor with similar relative risk estimates. This consistency increases confidence in the HPV infection results found in the San Francisco study. However, given the limitations of the cross-sectional design of both studies, it is important to evaluate these results in comparison to the findings of the incidence study (Chapter 3).

Similarities and differences between the prevalence and incidence studies among MSM in San Francisco

The incidence of anal HPV infection was also very high among the MSM in San Francisco when compared to the incidences of other sexually transmitted infections [5-7]. The distribution of HPV types found was also similar between the two studies, with HPV types 16, 18, and 33 among the most common oncogenic HPV types detected in both the prevalence and incidence studies (Chapters 2 and 3).

There were differences between the risk factors associated with HPV infection in the study risk factors for prevalent HPV 16 and the study that evaluated risk factors for the incidence of any type-specific incident anal HPV infection. However, the significant association of insertive sex (participant is the insertive partner) with prevalent anal HPV infection seen in the prevalence study was not present in the incidence study. It may be that in our study of prevalent infection 'insertive partners' was capturing total number of male partners, including partners with whom the participant had multiple sexual behaviors that could have exposed him to anal HPV (for example, oral-anal contact). The design of the incidence study allowed for shorter recall periods and perhaps more accurate reporting of behaviors, and thus we saw no association with this behavior. A second difference between the two studies was the association seen in the prevalence study between higher numbers of female partners and a decreased prevalence of anal HPV infection (even when controlling for number of male partners). This relationship was also seen in the prevalence study among Indian MSM (Chapter 4). In the incidence study among San Francisco MSM (Chapter 3), no association between recent female partners and incident anal HPV infection was seen. There may be a connection between behaviors with female sex partners and a decrease in risk of anal HPV infection; for example, MSM who have female partners may be more likely to use condoms with their male partners. Controling for condom use with male partners, in the study may have resulted in the association disapearing in the prevalence study, but we did not collect information on condom use. However, the logitudinal analysis that adjusted for baseline anal HPV infection in theory adjusts for unmeasured confounding and thus no association between incident infection and number of female partners was found. The association between female partners and anal HPV infection among MSM should be studied further in a future longitudinal study among Indian men where an association was also noted. The study should ensure that information on both condom use with male partners and number and frequency of female partners are collected.

Ethical considerations

Both of the populations under study were vulnerable populations. Anal HPV infection is a health issue very important to HIV-infected MSM. Although anal HPV infection occurs in heterosexual men, and in HIV-negative MSM, HIV-infected MSM are 59 times more likely to develop anal cancer than men in the general population [9]. Thus, to better understand anal HPV infection and HPV-associated disease in this population, it was important to focus our study on the HIV-seropositive MSM population. The investigators and research staff were all very experienced working with both MSM

and HIV-infected individuals, and received training throughout the study on working with the special needs of this vulnerable population.

The San Francisco study population was recruited through community outreach, newspaper advertisements, and referrals from the treatment and research UCSF HIV/AIDS community. In general, the men in the study were highly educated and very knowledgeable about their health. They were not offered a monetary incentive to participant in the study. A trained HIV counselor was available for counseling on HIV or HPV infection and disease matters and made referrals for medical care or social services as needed. At enrollment the counselor explained the study to the participant in full, answered any questions that the participant had, gave the participant the informed consent document, and observed the participant reading and signing the document. The informed consent document described the study in full including all risks and benefits. For this study, the most important risks were loss of confidentiality, and because all participants had high-resolution anoscopy (HRA), there was a risk of mild pain and a very small chance of persistent bleeding after the biopsy. The investigators protected against the risk of loss of confidentiality by conducting all data collection procedures in a private room, all records were coded and kept in locked files to which only the study investigator had access, and individual identities were not used in any reports or publications. All staff had on-going training and confidentiality was a priority in all interactions with participants and in the handling of study materials or samples. The investigators protected against risk of bleeding after the biopsy by utilizing highly trained anoscopists and using a very gentle technique. No serious bleeds took place over the study period, but a protocol was set up to treat the patient in case of excessive bleeding. Benefits to the participant included the free HRA, close monitoring of any anal lesions found, free treatment of any high grade anal disease found, and the opportunity to contribute new knowledge about HPV infection and disease in HIV-infected MSM. The San Francisco study was approved by the UCSF Committee on Human Research (CHR).

The study in India had similar procedures and risks and benefits to the participants. In India, participants were also recruited through referral community groups and non-governmental organizations and HIV support groups. Because participating in this study often took an entire day (including travel to the study site) participants were reimbursed for a day's lost wages and funds for travel (about $10). Trained HIV-counselor/social workers were available to participants at each visit and conducted all enrollment procedures. Procedures were described to participants in full in their local language (Hindi or Tamil) and the informed consent document was read out loud in full to participants. Although the informed consent was translated (and back translated) into both Hindi and Tamil using the appropriate script, many participants were not able to read, and therefore the study procedures required reading the document aloud to all participants. After answering any questions, counselors collected a signature on the informed consent, and an unsigned version was given to the participant. The most important risk in this study was loss of confidentiality. Again, all staff were highly trained to ensure participant confidentiality and it was a top study priority. All study materials were similarly safeguarded as described for the San

Francisco study. The Indian study participants did not receive HRA, so the most important physical risk was some discomfort in taking the HPV samples (anal swab). The risk of discomfort was reduced by using highly trained clinicians and using a very gentle technique. The study in India was approved by the UCSF CHR, and by the Institutional Review Boards of both Humsafar Trust (the study site in Mumbai) and Christian Medical College (the study site in Vellore).

The results of both studies will be communicated to the participants. A lay summary of the study results will be prepared in the form of a newsletter. For the San Francisco population, this will include the results of both the prevalence and incidence studies, and will include some basic information on HPV, and websites where former participants can obtain more information on HPV infection, disease, treatment, and prevention through HPV vaccination, as well as an invitation to join a future study. The newsletter will be mailed to all participants who consented to be contacted for future studies. We will also include these results in the newsletters of other UCSF studies that target MSM or HIV-infected individuals.

A similar lay summary will be created of the Indian study results in both local languages. Because we did not collect contact information from the participating individuals, we will format this summary as a small poster similar to a conference poster, and it will be displayed at study sites, as well as at any NGOs or support groups which referred patients. The posters will include graphical displays for those who cannot read and clinicians at the sites will be asked to review the main points with any men who cannot read.

Public health significance and recommendations for future research

The results from the three analyses included in this dissertation are important to the health of HIV-infected MSM in the US and India. The results highlight the high prevalence and incidence of anal HPV infection in this population and suggest that they be considered for regular and periodic screening for HPV-associated anal disease. Prevention of anal HPV infection should also be recommended with the quadrivalent HPV vaccine (Gardasil) once it has been proven to be safe and effective in this population. Gardasil was recently approved for use to prevent anal intraepithelial neoplasia (AIN) and anal cancer in both men and women [10]. The risk factors and protective factors for HPV infection identified in the analyses presented in this dissertation have highlighted behavioral factors that could be targeted for modification, for example, use of condoms by the insertive partner during anal intercourse. Additionally, the risk factors could be used to help identify the highest risk groups to target for public health interventions. For example, HIV-infected MSM with over 1000+ lifetime partners could be triaged for HPV-disease screening. This approach would be especially helpful in the Indian MSM population, where high-resolution anoscopy has only recently been introduced and the service will not be widely available for some time.

Future research concerning HPV infection among MSM in San Francisco should include analysis of the data already collected on HPV-associated anal disease,

including risk factors for progression of HPV-related disease and factors associated with regression of HPV-related disease. Research in India should focus on the initiation of a longitudinal study similar to the San Francisco study, which including an evaluation of the role of female partners and condom use. Lastly, and perhaps most importantly, studies should be initiated to evaluate the safety and efficacy of the quadravalent HPV vaccine among HIV-infected MSM in both San Francisco and India. A recent study found that the vaccine is safe and 73% effective in the per protocol analysis among healthy MSM against HGAIN [11]. If it is safe and found also to be effective among HIV-infected MSM vaccinating HIV-infected MSM could reduce the high (and increasing) rates of anal cancer in this population and prevent HPV-related morbidity and mortality.

Table 1: Summary of findings from three studies.

	Prevalence of HPV infection, SF Chapter 2	**Incidence of HPV infection, SF Chapter 3**	**Prevalence of HPV infection, India Chapter 4**
Number of Participants	**318**	**369**	**259**
Prevalence of any HPV infection	92%	NE	71%
Most common prevalent HPV type	HPV6 (45%) HPV16 (42%)	NE	NE
Incidence of any HPV infection (1 year)	NE	21%	NE
Most common prevalent HPV type	NE	HPV18 (3.7%) HPV16 (3.7%)	NE
Mean age of population (years)	43	45	35
CD4+ level			
<200	17%	14%	14%
200-500	47%	49%	47%
>500	36%	38%	39%
Currently taking antiretroviral therapy	83%	86%	43%
≥5 lifetime female sex partners	22%	23%	28%
≥1000 lifetime male sex partners	31%	31%	36%
Never had male receptive partner	0.8%	0.8%	13%
Risk factor for anal HPV infection	**RR**	**OR**	**RR**
5+ lifetime female partners	0.60*	0.96	0.62***
1000+ lifetime male partners	1.68*	NE	1.21
201+ lifetime insertive partners	1.72*	1.0	1.05
8+ receptive partners in past 6 months	NE	2.88**	NE
Frequency of receptive anal intercourse ≥ 1x/week	NE	2.60**	NE
Ever receptive anal intercourse	NE	NE	1.67***
Almost always used condoms, past 30 days	NE	NE	0.83***

***** Statistically significant association with prevalent anal HPV 16 infection
** Statistically significant association with incident anal HPV infection of any type
*** Statistically significant association with prevalent anal HPV infection of any type
NE=Not Evaluated

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