Human papilloma virus types in the oral and cervical mucosa of HIV-positive South African women prior to antiretroviral therapy

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Background: To evaluate the prevalence of human papilloma virus (HPV) infection and types in the oral and cervix mucosa of treatment-naïve HIV-1-positive women with CD4 counts less than 300 cells per ml with no HPV-associated oral lesions.

Methods: Oral epithelium was harvested from the buccal mucosa and lateral borders of the tongue and cervical samples were collected from the endocervical area of 30 women, 22–64 years old. Cytobrush Plus cell collectors were used for sampling both anatomical areas. Genital pathology, obstetric and gynaecological history, co-morbid disease, hormone therapy, sexual behavior and smoking history were assessed via physical examination and clinical interviews. Special investigations included cervical Papanicolau smears, CD4 counts and HIV-1 viral loads. The linear array HPV test was used to determine HPV genotypes present in the specimens.

Results: Oral HPV were identified in 20% (n = 6) of the patients, of which two had infection with two HPV types. Genital HPV was found in 96.7% (n = 29) of the women, of which only 14 had cytological abnormalities on Papanicolau smear. Infection with multiple HPV types were present in 93.1% (n = 27) of the patients, with an average of four HPV types per individual.

Conclusions: South African HIV-positive women with CD4 counts less than 300 cells per ml have a significant risk of cervical HPV strains and multiple strain infection of the cervix. The prevalence of HPV in normal oral mucosa was low but high-risk types were present. Limited correlation between oral HPV types and those identified in the cervical mucosa was found.

Introduction

South Africa is the country with the highest number of people living with human immunodeficiency virus 1 (HIV-1) in the world. By mid-2006, it was estimated that more than half a million adults in South Africa were sick with AIDS of which 51.6% were female (1). A high incidence of HIV-1 infection is seen in the 14- to 24-year-old age group, particularly in young women, with the adult female HIV-1 prevalence peaking at 25–29 years with 32.5% estimated to be infected (1). The majority of HIV-positive patients in South Africa present to the public health care sector only when they reach end-stage HIV disease.

The association of human papilloma virus (HPV) infection of the cervix in women infected with HIV-1 has been well documented in the literature (2–4). It was shown that HIV-1-positive women with $CD4^+$ cell counts of less than 200 copies per ml are at a higher risk of HPV infection than HIV-1-negative women, irrespective of their HIV-1 viral load (2). Recently, a

Brazilian group reported that patients with genital HPV infection have a greater frequency of HPV in their oral mucosa (5). The authors did not state the presence or absence of oral lesions in these patients nor did they specify the strains found in each anatomical area tested (5). Fakhry et al. analyzed oral rinse and cervical–vaginal lavage samples from HIV-positive and HIV-negative women and found oral HPV infections less common than cervical infections in both groups. No reference was made to the presence or absence of oral lesions on clinical examination (6). An increase in HPV-related oral warts associated with antiretroviral therapy (ART) has also been described (7, 8). Most HPV studies were conducted in patients with oropharyngeal papillomas and other neoplasias with very few studies on patients with clinically healthy oral mucosa (8).

No study comparing HPV strains found in the oral mucosa with that found in the cervical mucosa of HIV-1-infected women has been carried out in South Africa. The aim of this study was to evaluate the prevalence of HPV infection and types in clinically normal oral and cervical mucosa of a well-defined ART-naïve female population with end-stage HIV-1 disease using sensitive sampling and detection methods. With molecular testing for HPV becoming more available, the indications for use in a resource-limited country with a high incidence of HIV-1 need further study. The potential role of molecular HPV screening in clinically normal mucosal sites other than the cervix in high-risk populations is not yet clear.

Materials and methods

Patients, clinical examination and sample collection

The study included 30 women attending the Anti-retroviral Clinic at the Pretoria Academic Hospital, for initiation of antiretroviral treatment. The oral cavity of every patient was screened by a senior oral pathologist. Only patients without clinically detectable oral HPV disease were asked to participate in the study and informed consent was obtained. Clinical interviews were carried out and information regarding the patients' sexual practices, contraception use, smoking and medical history was recorded. All the women were antiretroviral naïve with CD4 cell counts of less than 300 cells per ml. Blood for HIV-1 viral load was collected as part of routine standard of care.

Oral epithelial cells were harvested from the buccal mucosa and lateral borders of the tongue using a Cytobrush Plus cell collector (Medscand Medical, Malmö, Sweden). These anatomical sites were brushed with a rolling motion of the brush for at least 10 s to obtain cells from deeper layers of the mucosa without pain or discomfort to the patient (9).

All the patients underwent a thorough clinical examination, including visualization of the cervix and a Papanicolau (PAP) smear. The presence of clinical genital pathology was recorded. Cervical specimens for HPV testing were collected from the endocervical area, including the transformation zone using a Cytobrush Plus cell collector (Medscand Medical) by gently turning the brush a few times in the area.

The cells on the Cytobrush bristles were transferred into a labeled collection tube containing 2 ml of sterile phosphate-buffered saline (PBS), pH 7. The specimens were immediately transported to the laboratory where it was vortexed to remove the cells from the bristles and were centrifuged at 3000 g for 10 min. The cell pellets were then resuspended in 200 µl of sterile PBS and frozen at −70°C until such time that DNA extraction and analysis were performed. This study was approved by the Ethics Committee of the University of Pretoria (Ethics number 25/2005).

Molecular analysis

DNA extraction was performed in batches using the Total Nucleic Acid Isolation Kit (Roche Molecular Systems®, Branchburg, NJ, USA) according to the manufacturer's instructions. The pool of primers is designed to amplify HPV DNA from 15 high-risk genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82), three probable high-risk genotypes (26, 53 and 66) and 19 low-risk types (6, 11, 40, 42, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 83, 84, IS39 and CP6108). The β-globin gene was amplified concurrently to assess cellular adequacy, extraction and amplification for each individually processed specimen. Strict procedures were followed to avoid contamination with negative and positive controls included in each run.

The linear array HPV test (Roche Molecular Systems®), a PCR-based genotyping assay was used to detect and type the specimens in this study according to the manufacturer's instructions.

Results

The women ranged in age from 22 to 64 years with 50% (n = 15) of the patients in the 30- to 39-year-old age group. Due to the immunosuppressed state of the patients, oral and vaginal pseudomembranous candidiasis as confirmed by smear biopsies, gingivitis and cervical inflammation with or without a vaginal discharge were common clinical findings. Two patients had genital warts. Only one patient reported smoking tobacco and two patients were using injectable depot medroxyprogesterone acetate (Depo Provera®, Pfizer Inc., New York, NY, USA). The patients who were sexually active all reported condom use due to their HIV-positive status.

Concurrent oral HPV were identified in 20% (n = 6) of the women (Table 1). Two of them had infections with two HPV types. Two high-risk types (HPV 45 and 59) were identified in two different patients. In 50% of the patients, the oral HPV type(s) were not detected in the patient's cervix.

Table 1 Clinical histories, cervical and Oral HPV types of HIV-1 positive female population sample

Age (years)	CD4 count (cells/ml)	HIV viral load (IU/ml)	Oral pathology	Cervix pathology	Papsmear	Cervical HPV types	Oral HPV
22	266	550 000	None	Inflammation, Candida	Neg	6, 42, 67, 73	81
23	29	822 372	Candida	Candida, Genital warts	Koilocytes, CIN II	18, 45, 53, 54, 56, 58, 70, 73, CP6108	CP6108
24	200	32 000	None	Inflammation	HPV	16, 39, 45, 54, 58, 72	45, 72
26	46	161 264	None	Inflammation	Neg	33	None
26	4	1 000 000	None	Inflammation	Koilocytes, CIN1	16, 39, 56	None
27	147	110 000	None	Suspicious, PVD, Candida	ASCUS	35, 42, 45, 53, 54, 58, 70,	62, 84

						CP6108	
28	137	7700	None	Candida	Suggestive of HPV	16, 52, 62, 67, 73	None
29	139	28 000	Candida	Inflammation, Candida	Neg	35, 42, 55, 81, 83	None
29	20	1 000 000	None	Inflammation, Candida	Koilocytes, CIN1	31, 56, 83	None
29	36	1 200 000	None	Inflammation	Neg	40, 73, 84	None
30	35	220 000	None	Inflammation, PVD	Neg	6, 51, 56, 68, 81, 83	None
30	103	72 000	None	Normal	Neg	35, 70, 72, 84	None
31	106	6600	None	Candida	Koilocytes, CIN II	33, 55 70	None
32	155	39 000	None	Inflammation	Koilocytes, ASCUS	6, 16, 73, 82, CP6108	CP6108
32	20	2772	None	Inflammation, Candida	Neg	35, 45, 66	None
32	162	5800	None	Candida	Neg	70, 84	None
32	7	340 000	None	Inflammation	HSIL/CINIII	59	None
33	153	31 000	None	Inflammation, ectopy	Koilocytes	52, 62, 83	None
33	200	17 000	Candida	Genital herpes	Koilocytes, CIN II	33, 35	None
34	94	710 000	None	Candida	Neg	31, 33, 35, 39, 51, 53, 55, 58, 71, 81	None
35	33	84 000	Candida	Inflammation, PVD, Candida, genital warts	Neg	35, 55, 58, 61, 68, 72, CP6108	None
35	59	152 231	None		Neg	45, 81	None
35	27	130 000	None	Inflammation, ?polyp	Koilocytes, CIN1	35, 71, 81	59
38	105	130 000	None	Inflammation, suspicious	Koilocytes	33, 42, 53	None
39	147	1 500 000	None	Inflammation	Neg	54, 68, 81	None
41	138	68 000	Candida	Inflammation	Neg	55, 61, 81, CP6108	None
42	92	54 735	None	Inflammation	Koilocytes	6, 84	None
46	131	380 000	None	Inflammation	Neg	None	None
53	20	690 000	Candida, Aphthous ulcer	Inflammation, Candida, Erosion	Neg	55, 73, 82, IS39	None
64	59	910 000	None	Inflammation, atrophic	Neg	16, 33	None

Genital HPV was found in 96.7% (n = 29) of the women. The presence of multiple HPV types was common (93.1%; n = 27), with an average of four types per individual (Table 1). Type 35 was most common (26.7% of patients), followed by type 81 (23% of patients). Fourteen of the patients showed cytological abnormalities on Pap smear, all positive for HPV. High-risk HPV types were identified in the cervical smears of 13 of 16 patients with normal cytology on routine cytological examination.

Discussion

The relationship between HPV and oral lesions in HIV-1-infected women and between those HPV strains encountered in the oral cavity versus that in the cervix of the same individual is still uncertain (5, 8, 10, 11).

The impact of HPV infection of the cervix in HIV-1-infected women is well described (2, 3, 12). Current data suggest that concurrent HIV-1 infection increases the risk of HPV persistence and cervical squamous intraepithelial lesions (CSIL), probably through a weakened immune response. Due to the morphological similarities between the cervical and oral mucosa (13) and the well-known epitheliotropic nature of HPV, a link between certain oral epithelial lesions and HPV has been the focus of a meta-analysis study (14). Results from various studies showed that HPV DNA could be found in normal oro-pharyngeal mucosa of healthy individuals (10, 15, 16) as well as in tumor tissue, especially oro-pharyngeal and squamous cell carcinomas (14, 17–19).

Estimates on the prevalence of oral HPV and the genotypes found in normal oral mucosa, premalignant lesions and oral squamous cell carcinoma (OSCC) is highly variable ranging from 0% to 100% and seems to depend on the sampling method, patient profiles, detection method, the types of tissue and populations studied (20, 21).

A shortcoming of many oral HPV studies is the fact that the specific anatomical areas tested for the virus is undefined and usually includes tissue from both the anterior and posterior or oropharyngeal aspects of the oral cavity. There is, however, evidence that tonsillar carcinomas have a stronger association with HPV and this may be in line with data suggesting HPV to infect the normal epithelium in this anatomical location (16, 22, 23).

Non-invasive brush biopsies were used for sampling because cells could be retrieved from specified anatomical locations in the oral cavity rather than oral rinses which will also include cells from undefined locations from the posterior oropharynx. Superficial scraping has been shown to provide more accurate information about the spectrum of HPV genotypes than biopsies, another reason for the choice of sampling in this study (21, 24). The patient population was clearly defined for inclusion in this study as described in Materials and methods.

A greater frequency of HPV in the oral mucosa of patients with genital HPV infection was reported compared with patients without genital HPV infection (5). In our cohort, genital HPV was found in 96.7% (n = 29) of the 30 women tested, 14 with cytological abnormalities on Pap smear and 16 with normal cytology. Seventy-seven percent of all patients had high-risk HPV in their cervical mucosa. The most prevalent HPV types found in this study (HPV 35 and 81) were also significantly different to those described in studies from various regions in Africa (25–30) supporting the fact that the prevalence and distribution of HPV genotypes vary from one geographic region to another. The type-specific concordance between oral and cervical HPV types was low, with only three patients having the corresponding oral strain present in their

cervical mucosa. In all cases, more HPV strains were demonstrated in the cervical than in the oral mucosa.

It has been shown that the prevalence of cervical HPV infection is the highest among young women and appears to drop with increasing age (11, 31). No age-related decrease in HPV prevalence was, however, found in this study, most probably an indication of HPV persistence due to the debilitated or almost absent immune response, a finding supported by other studies (32, 33). The influence of the immune response on the ability to clear HPV infection is demonstrated by studies showing that HIV-1-seropositive women are more likely to shed genital HPV over a longer period of time than HIV-1-seronegative women (34). This permits a high HPV viral load and persistent HPV infection (35). The influence of other risk factors identified in previous studies, i.e. contraceptive use and smoking (36) could not be evaluated in this study due to the small number of participants who used these substances. Detection of cervical HPV DNA has also been associated with high HIV-1 viral loads (2, 35), but no pattern of association between CD4 cell count and the number of HPV genotypes could be demonstrated.

The presence of multiple HPV types in cervical samples from HIV-1-positive compared with HIV-1-negative women patients has been described previously and the results varies from 36% (2) to 45% (35) of HPV-infected HIV-1-positive women. In this study, 90% of the women presented with multiple HPV types with an average of four HPV strains per individual. This could be explained by the inability of the severely impaired cell-mediated immune response to clear the HPV infection in this specific patient population. The clinical impact of multiple strain infection on viral persistence and disease progression cannot be clarified by this study. Future research is needed in this regard.

The impact of this study may be limited due to the small cohort tested, but does serve as a pilot study to direct further research in the HIV-1-positive population of South Africa. Furthermore, only 37 of the most prevalent HPV types were tested for in this study and the presence of other or novel HPV types cannot be excluded. It can, however, be concluded from this study that HIV-1-positive South African women have a significant risk of the presence of high-risk HPV types in their cervical mucosa and that infection with multiple HPV types is extremely common. By contrast, the prevalence of HPV in normal oral mucosa seems to be low, but high-risk types may be present. Oral HPV types in most cases did not correlate with those HPV strains identified in the cervical mucosa. It is clear that clinical examination alone cannot exclude the presence of high-risk HPV types in oral or cervical mucosa and that molecular testing is necessary to diagnose these. Opportunities for further research in this population group are numerous and follow-up testing of patients on therapy needs to be conducted to determine the effect of HAART on the persistence or clearance of these lesions.

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