CASE REPORT

Detection and typing of human papilloma virus in the oral mucosa of patients infected with human immunodeficiency virus

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Summary Recent studies have shown an increased risk of oral warts in HIV+ individuals despite treatment with highly active antiretroviral therapy (HAART). Human papilloma virus (HPV) infection has attracted a great deal of attention, not just because of the difficulty of managing oral warts but also because of the oncogenic potential of certain strains, in particular HPV-16 and -18, which have been detected in 20–30% of oral squamous cell carcinomas. Between 1999 and 2004, DNA extraction was performed using a multiplex PCR reaction to detect and type HPV in 12 HIV-positive adult with a clinicopathologic diagnosis of Oral Warts. HPV was detected in 11 of the 12 oral warts. HPV-32 was present in all subjects, whereas only one subject had a co-infection of HPV-32 and -7. Future studies should examine the specific roles of these specific HPV types and whether a potential link exists for oral premalignant lesions.

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KEYWORDS
HIV; HPV; Oral; Mucosal lesion; Oral warts

Introduction

Papilloma viruses are small (55 nm, 8 kb), double-stranded DNA viruses. Over 200 genotypes of papilloma viruses infect the skin and mucosal surfaces. The human papilloma virus (HPV) is a double-helix virus with about 8000 base pairs in its genome.1–4 These viruses have never been cultured in vitro but have been characterized by molecular methods. They are classified by the molecular similarity of their genetic material and are assigned a genotype number.

Papilloma virus infects the basal keratinocyte of the epidermis, presumably through disruptions of the skin or mucosal surface. At this location, the virus remains latent in the cell as a circular
episome. As the epidermal cells differentiate and migrate to the surface, the virus is triggered to undergo replication and maturation.\textsuperscript{3,4} The process of virus replication alters the character of the epidermis, resulting in cutaneous or mucosal excrescences known as warts.

HPVs are grouped broadly into cutaneous and mucosal types, based on the clinical location of the resulting lesion. Although some overlap exists, most papilloma viruses have distinct anatomic predilections, infecting only certain epidermal sites, such as the skin or genital mucosa. A number of genotypes of these viruses have the potential to transform host cells and are associated with epidermal malignancies. The mechanism for transformation is not known, but viral DNA apparently integrates into the genome of the host cell.

HPV has been found in cervical cancer, tonsillar cancer, and certain types of head and neck cancers.\textsuperscript{5} Furthermore, at least 30 of these have been detected in the oral cavity.\textsuperscript{6,7} The gross appearance of oral warts varies greatly and often reflects the specific HPV genotype causing the lesion.\textsuperscript{6,7} Recent studies have shown an increased risk of oral warts in HIV-infected individuals despite treatment with highly active antiretroviral therapy (HAART). The possibility of increases in both oral and anogenital pathologic conditions due to the human papilloma virus in patients infected with human immunodeficiency virus (HIV) is of concern and is the focus of numerous current research studies. Oral HPV-infection occurrences have not declined since the initiation of HAART but may have increased in white HIV-infected males.\textsuperscript{8} Urban HIV-negative homosexual men in the USA have a high prevalence of anal infection with human papilloma virus (HPV).\textsuperscript{9}

HAART has been responsible for decreasing HIV plasma viral loads, increasing CD4\textsuperscript{+} T lymphocyte counts, prolonging the progression to AIDS, and decreasing the mortality from HIV.\textsuperscript{5} HAART has also decreased the incidence of opportunistic infections, including those of the oral cavity. For example, the incidences of oropharyngeal candidiasis and oral hairy leukoplakia in HIV individuals have significantly decreased, but, in stark contrast, incidence of oral warts in this population has significantly increased. Greenspan et al. have found a
rise in the incidence of oral warts in HIV+ patients in San Francisco in the 1990s. A recent study from Miyako Island in Japan suggested that among healthy individuals, oral HPV infection is uncommon. In this Japanese cohort, HPV-17 and HPV-12 were persistent, while HPV-16 and HPV-53 were transient in normal oral mucosa. With the success of HAART, and possible an increase in HPV occurrence, studied the possibility of different HPV genotypes in oral warts of HIV+ persons. Our objective for this study was to determine and describe the HPV types present in oral warts of HIV+ patients and compare those to non-HIV+ persons.

Materials and methods

Between 1999 and 2004, three hundred and seventy-nine HIV+ adult volunteers were recruited and evaluated at the HIV Outpatient Program (HOP) of the Medical Center of Louisiana at New Orleans for an ongoing longitudinal cohort study on oral mucosal lesions. Of the 379 patients infected with HIV type 1 (HIV-1) in this study, we examined samples from 12 who had a clinicopathologic diagnosis of oral warts. Lesions were categorized as oral warts on the clinical presentation of a solitary, raised cauliflower-like lesion or multiple soft lesions on the tongue, lips, buccal mucosa, or labial mucosa. After being clinically and histologically diagnosed, these lesions were further characterized as having one of three distinct levels of viral activity based on the presence of parakeratosis, koilocytosis, and gross granules in the granular layer and the top one-third of the spinous layer.

Tissue samples were collected by elliptical biopsy after local anesthesia with 2% lidocaine with/without vasoconstrictor, and were divided into three longitudinal fragments: one for histopathological analysis, another for HPV detection and typing, and the third was maintained in liquid nitrogen.

**HPV DNA detection:** DNA extraction was performed using a multiplex PCR reaction containing HPV L1-consensus primers (PGMY09/11) and β-globin primers (GH20/PC04) (supplied by Roche Molecular Systems) to test for sample adequacy according to Roche protocol as described in another study. Quality assurance and contamination avoidance were performed by using separate areas for DNA extraction, PCR set-up, and gel electrophoresis to avoid PCR-contamination. For each PCR run, a master PCR mix was prepared, and water blanks were inserted after every fourth sample. After amplification, products were analyzed by electrophoresis, and the size of the band obtained was compared with the molecular weight standard. DNA extracted from cultured SiHa cells was used as an HPV+ control. PCR products after 40 amplifications were considered positive for HPV if the products demonstrated the 254 base-pair β-globin band and the ~450 base-pair HPV band (Fig. 1).

Results

Of the 12 patients in the experimental group, one was an African American woman; 11 were Caucasian men; 9 received HAART. The mean CD4 cell count was 281 (±210) cells/µL. Clinical, physiological, and virological characteristics of the volunteers who were HPV+ in oral wart lesions are shown in Table 1. Overall, HPV was detected in 11 of the 12 oral warts. Two different HPV types were found (HPV-32; HPV-7). HPV-32 was present

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>HPV type</th>
<th>Sex</th>
<th>Race</th>
<th>Age (years)</th>
<th>CD4 (cells/µL)</th>
<th>HIV-1 viral load (cp/mL)</th>
<th>Anti-HIV therapy</th>
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<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>M</td>
<td>W</td>
<td>32</td>
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<td>HAART</td>
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<tr>
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<tr>
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<td>M</td>
<td>W</td>
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<td>419</td>
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<td>B</td>
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<td>B</td>
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<td>W</td>
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<td>759</td>
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<td>M</td>
<td>W</td>
<td>43</td>
<td>432</td>
<td>&lt;400</td>
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</table>
in all subjects, whereas only one subject had a co-infection of HPV-32 and -7.

Discussion

Increased occurrences of oral warts in HIV+ persons, especially after the institution of HAART have been documented. HPV is associated with several oral lesions grouped clinically as oral warts (verruca vulgaris, squamous cell papilloma, condyloma acuminata, focal epithelial hyperplasia and also with oral leukoplakia with dysplastic change. Oral warts can present in almost any location in the mouth as nodular or raised lesions that appear pink or white depending on the degree of keratinization. Squamous papillomas occur predominantly as solitary cauliflower-like lesions unlike condylomas or FEH, which present as multiple soft lesions that frequently coalesce into nodular tissue masses. The histology of HPV+ mucosal epithelium shows epithelial hyperplasia where HPV is restricted mostly to the spinous layer of the epithelium. The connective tissue layer is usually well vascularized without any inflammatory changes.

HPV oral lesions have been reported in 0.4% of the general population. All persons infected with HPV do not develop oral lesions. HPV DNA detection rate in individuals without any obvious oral lesion (whether HIV+ or not) has varied widely depending on the study population, type of sample collected, method of DNA detection (hybridization versus polymerase chain reaction PCR), and the primers and specific probes utilized. In a recent study, HPV DNA was detected in approximately 36% of HIV+ individuals compared to 6% of HIV—individuals.

Although many strains of HPV have been detected in the oral cavity, the strain most often associated with oral warts is HPV 32. However, these reports were before the advent of HAART. All of our cases were HPV-32, with the exception of one co-infection with HPV-7, a hitherto unreported genotype in the oral cavity. HPV-7, usually associated with benign warts in butchers, has been reported to have a high degree of homology with HPV-40, a rare type, which has been reported in papilloma of the hard palate. It is possible that HIV-1-infected patients present lesions caused by rare or even by new HPV types, which the primer pairs used in the present study did not detect. It is likely that the combination with other primer pairs would not only increase the rate of HPV detection but might also detect other distinct HPV types. However, the omnipresence of HPV-32 in our samples suggests that this genotype is the major force in oral warts, and its prevalence has not changed in the post-HAART era.

HPV infection has attracted a great deal of attention, not just because of the difficulty of managing oral warts but also because of the oncogenic potential of certain strains, in particular HPV-16 and -18, which have been detected in 20–30% of oral squamous cell carcinomas. The increase in oral warts is of particular interest in light of documented increases in cervical and anal HPV infection in HIV-positive individuals causing genital warts and anal dysplasia. Future studies should examine the specific roles of these specific HPV types and whether a potential link exists for oral premalignant lesions.

References


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