ORIGINAL RESEARCH

Human Papilloma Virus Detection in the Oral Mucosa of Human Immunodeficiency Virus Patients: A Study of 24 Subjects

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ABSTRACT

Despite treatment with highly active antiretroviral therapy (HAART) there has been an increased risk of oral warts in HIV+ individuals. So a great deal of attention and interest has been attracted by papilloma virus (HPV) infection , not only because of the difficulty of managing oral warts but also because of the oncogenic potential of certain strains of HPV, specially to HPV type 16 and type 18, which have been detected in 20–30% of oral squamous cell carcinomas. Between 2011 and 2014, DNA extraction was performed using a multiplex PCR reaction to detect and type HPV in 24 HIV positive adult with a clinico-pathologic diagnosis of Oral Warts. HPV was detected in 22 of the 24 orals warts. HPV-32 was present in all subjects, whereas only two patients had a co-infection of HPV type 32 and type 7. Future studies should examine the specific roles of these specific HPV types and whether a potential link exists for oral premalignant lesions.

KEYWORDS: HIV; Human Papilloma Virus; Oral Warts; Mucosal Lesion;

INTRODUCTION

Papilloma viruses are very small (55 nm, 8 kb), double stranded DNA viruses. As much as more than two hundred genotypes of papilloma viruses infect both the skin as well as 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 been characterized by molecular methods but have never been cultured in vitro. They are classified by the molecular similarity of their genetic material and are assigned a genotype number. Generally Papilloma virus first infects the basal keratinocyte of the epidermis, and the virus remains latent for sometime as a circular episome. As the epidermal cells differentiate and migrate to the outer surface, the virus is triggered to undergo replication and maturation.[3,4] The process of virus replication alters the character of the epidermis, resulting in cutaneous or mucosal excrescences known as cutaneous and oral warts respectively. Ocular warts have also been linked with HPV as reported by some ophthalmologists

HPV has been found in certain types of head and neck cancers as well other than cervical cancer, tonsillar cancer, [5] Furthermore, at least 30% of these have been detected in the oral cavity.[5–8] The macroscopic appearance of oral warts varies greatly and they often reflects the specific HPV strain causing the lesion.[6,7] Despite treating these lesions with highly active antiretroviral therapy (HAART), recent studies have shown an increased risk of oral warts in immune-compromised patients especially HIV-infected individuals. So possibility of increase in oral lesions due to the human papilloma virus in patients infected with human immunodeficiency virus (HIV) is of great concern and is under the focus of numerous current research studies.

HAART has been responsible for increasing CD4+ T lymphocyte counts, decreasing HIV plasma viral loads, and thereby decrease the progression of AIDS, and so decreasing the mortality from HIV.[5,8,9] HAART also resulted in decrease 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 due to HAART but, in contrast, the 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.[10] A recent study from Miyako Island in Japan suggested that among healthy individuals, oral HPV infection is uncommon. In this Japanese cohort study, HPV-17 and HPV-12 were persistent, while HPV-16 and HPV-53 were transient in normal oral mucosa.[11] So our aim for this study was to detect human papilloma virus in the oral mucosa of human immunodeficiency virus patients.

MATERIALS AND METHODS

Between 2011 and 2014, seventy HIV+ adult volunteers

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were recruited and evaluated at the ART centre of Ram Manohar Lohia Hospital Lucknow for clinical presentation of oral mucosal lesions. Of the 70 patients infected with HIV type 1 (HIV-1) in this study, we examined samples from 24 who had a final 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 1) parakeratosis, 2) koilocytosis, and 3) gross granules in the granular layer.

After administration of local anesthesia with 2% lidocaine with/without vasoconstrictor, tissue samples were collected by biopsy and were divided into three longitudinal bits: one for histopathological analysis, another for HPV detection, and the third was maintained in liquid nitrogen. DNA extraction was performed using a multiplex PCR reaction containing HPV L1-consensus primers (PGMY09/11) and b-globin primers (GH20/PC04) (supplied by Roche Molecular Systems) to test for sample adequacy according to Roche protocol as described in another study.[12] Contamination avoidance were performed by using separate areas for DNA extraction, PCR set-up, and gel electrophoresis to avoid PCR-contamination. 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 basepair b-globin band and the _450 base-pair HPV band (Fig. 1).

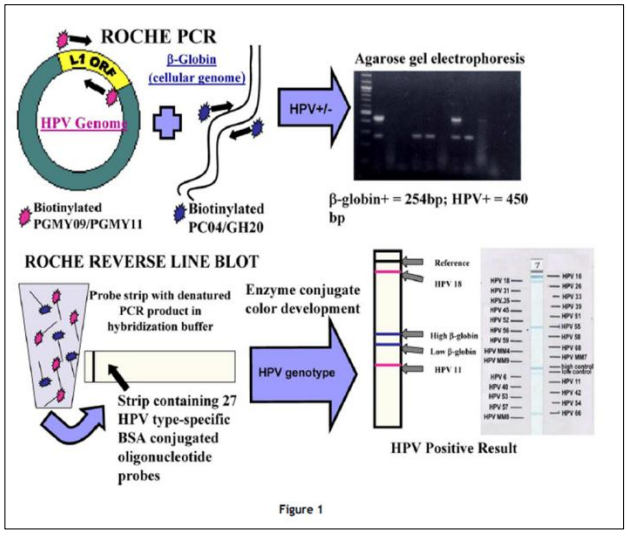

Figure 1

RESULTS

Of the 24 patients in the study group, two were south Indian women; 22 were north indian men; and out of 24 subjects 18 received HAART. The mean CD4 cell count was 278(±209) cells / l ltr. 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 22 of the 24 orals warts. Two different HPV types were found (HPV-32; HPV-7). HPV-32 was present in all subjects, whereas only two subjects had a co-infection of HPV-32 and -7.(Figure 1)

Clinical, physiological, and virological characteristics of the HIV 1 and oral-wart HPV positive patients

Patient No.	HPV type	Sex	Age (years)	CD4 (cells/l L)	HIV-1 viral load (cp/mL)	Anti-HIV therapy
1	32	M	30	401	<400	HAART
2	32,7	M	42	325	89000	NONE
3	32	M	40	412	674	HAART
4	32	M	35	345	<400	HAART
5	32	M	35	45	1,12,567	HAART
6	32	M	40	156	<400	NONE
7	32	M	39	256	52156	HAART
8	32	F	43	102	<400	HAART
9	32	M	33	234	1,00,436	HAART
10	32	M	48	45	<400	HAART
11	32	M	44	678	<400	HAART
12	32	M	44	432	<400	HAART
13	32	M	44	432	<400	HAART
14	32	M	43	345	45367	NONE
15	32	M	30	324	<400	HAART
16	32	F	35	123	678	HAART
17	32,7	M	40	21	1,12,876	HAART
18	32	M	41	234	<400	NONE
19	32	M	40	123	56345	HAART
20	32	M	32	103	<400	HAART
21	32	M	35	246	<400	HAART
22	32	M	46	44	<400	HAART
23	32	M	43	645	<400	HAART
24	32	M	40	456	<400	HAART

Table: 1

DISCUSSION

It has been seen that there are increased chances of oral warts in immune-compromised patients especially in HIV+ persons,.[10,13,14] HPV is associated with several oral lesions grouped clinically as oral warts which may include verruca vulgaris, verrucous carcinoma, squamous cell papillomas, condyloma acuminata, focal epithelial hyperplasia and also with oral leukoplakia with dysplastic change.[15–17] 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 Focal epithelial hyperplasia, which present as multiple soft lesions that frequently coalesce into nodular tissue masses.[6–15] 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 associated oral mucosal lesions have been reported in 0.4% of the general population.[18,19] 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. It was seen in a recent study, that HPV was detected in approximately 36% of HIV+ individuals[20]. Although many strains of HPV have been detected in the oral cavity,[6,21] the strain most often associated with oral warts is HPV genotype 32.[6] However, these reports were before the advent of HAART. In all of our 24 cases, HPV-32 was present in each case, with the exception of two subjects, which were co-infected with HPV-7. HPV-7, usually associated with benign warts in butchers,[22] 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 as well.[23] 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 predominance of HPV-32 in our samples suggests that this genotype is the major type of strain in oral warts, and its prevalence has not changed in the era of post-HAART. HPV infection has attracted a great deal of interest and attention, not just because of the difficulty of managing oral warts but also because of the oncogenic potential of certain types/strains, in particular HPV-16 and -18, which have been detected in 20–30% of oral squamous cell carcinomas.[15,24] 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.[25,26] Future studies should examine the specific roles of these specific HPV types and whether a potential link exists for oral potentially malignant disorders and malignant lesions.

DISCUSSION

1. Cobb MW. Human papilloma virus infection. J Am Acad Dermatol 1990;22:547–66.
2. Swygart C. Human papillomavirus: disease and laboratory diagnosis. Br J Biomed Sci 1997;54:299–303.
3. Fazel N, Wilczynski S, Lowe L, Su LD. Clinical, histopathologic, and molecular aspects of cutaneous human papilloma virus infections. Dermatol Clin 1999;17:521–36.
4. Bonnez W, Reichman RC. Papilloma viruses. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. 5th ed. Philadelphia: Churchill Livingstone; 2000. p. 1630–44.
5. Oh TJ, Kim CJ, Woo SK, Kim TS, Jeong DJ, Kim MS, et al. Development and clinical evaluation of a highly sensitive DNA microarray for detection and genotyping of human papilloma viruses. J Clin Microbiol 2004;42:3272–80.
6. Greenspan D, D' Villiers EM, Greenspan JS, D'Souza YG, Zur HH. Unusual HPV types in oral warts in association with HIV infection. J Oral Pathol 1988;17:482–8.
7. Chang F, Syrjanen S, Kellokoski J, Syrjanen K. Human papilloma virus (HPV) infections and their associations with oral disease. J Oral Pathol Med 1991;20:305–17.
8. Hagensee ME, Cameron JE, Leigh JE, Clark RA. Human papilloma virus infection and disease in HIV-infected individuals. Am J Med Sci 2004;328(1):57–63., Jul.
9. Chin-Hong PV et al. Age-specific prevalence of anal human papilloma virus infection in HIV-negative sexually active men who have sex with men: the EXPLORE study. J Infect Dis 2004;190(on-line edition)
10. Greenspan D, Canchola AJ, MacPhail LA, Cheikh B, Greenspan JS. Effect of highly active antiretroviral therapy on frequency of oral warts. Lancet 2001;357:1411–2.
11. Kazuyo K, Masanori T, Nurtami S, Doralina R, Yumiko N, Burk RD, et al. Low prevalence of HPV infection and its natural history in normal oral mucosa among volunteers on Miyako Island, Japan. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;98:91–6.
12. Lilly EA, Cameron JE, Shetty KV, Lehig JE, Hager S, McNulty KM, et al. Lack of evidence for local immune activity in oral hairy leukoplakia and oral wart lesions. Oral Microbiol Immunol 2005;20:1–8.
13. Leigh J. Oral warts rise dramatically with use of new agents in HIV. HIV Clin 2000;12:7. 314 K. Shetty et al.
14. King MD, Reznik DA, O'Daniels CM, et al. Human papilloma virus-associated oral warts among human immunodeficiency virus-seropositive patients in the era of highly active antiretroviral therapy: an emerging infection. Clin Infect Dis 2002;34:641–8.
15. Chang F, Syrjanen S, Kelloskoski J, Syrjanen K. Human papilloma virus (HPV) infection and their associations with oral disease. J Oral Pathol Med 1991;20:305–17.
16. Miller CS, White DK. Human papilloma virus expression in oral mucosa, premalignant conditions, and squamous cell carcinoma: a retrospective review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;82: 57–68.
17. Leigh JE, Shetty K, Fidel PL. Oral opportunistic infections in HIV-positive individuals: review and role of mucosal immunity. AIDS Patient Care STDs 2004;18:443–56.
18. Bouquet JE. Common oral lesions found during a mass screening examination. J Am Dent Assoc 1986;112:50–7.
19. Knapp MJ. Oral disease in 181,338 consecutive oral examinations. J Am Dent Assoc 1971;83:1288–93.
20. Coutlee F, Trottier AM, Ghattas G, et al. Risk factors for oral human papilloma virus in adults infected and not infected with human immunodeficiency virus. Sex Transm Dis 1997;24:23–31.
21. Volter C, He Y, Delius H, et al. Novel HPV types present in oral papillomatous lesions from patients with HIV infection. Int J Cancer 2003;66:453–6.
22. Oltersdorf T, Campo MS, Favre M, Dartmann K, Gissmann L. Molecular cloning and characterization of human papilloma virus type 7 DNA. Virology 1986;149:247–50.
23. Anderson KM, Allen CM, Nuovo GJ. Human papilloma virus, type 40-associated papilloma, and concurrent Kaposi's sarcoma involving the anterior hard palate of an HIVpositive man. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:80–4.
24. Paz IB, Cook N, Odom-Maryon T, Xie Y, Wilczynski SP. Human papilloma virus (HPV) in head and neck cancer.

Anassociation of HPV 16 with squamous cell carcinoma ofWaldeyer's tonsillar ring. Cancer 1997;79:595–604.
25. Frisch M, Biggar R, Goedert J. Human papilloma virusassociated cancers in patients with human immunodeficiency virus infection and acquired immunodeficiency syndrome. J Natl Cancer Inst 2000;92:1500–10.
26. Silverberg M, Ahdieh L, Munoz A, et al. The impact of HIV infection and immunodeficiency on human papilloma virus type 6 or 11 and on genital warts. Sex Transm Dis 2002; 29: 427–35.

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