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Human papillomavirus frequency in oral epithelial lesions

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BACKGROUND: Oral human papillomavirus (HPV) prevalence varies according to geographical occurrence, the type of lesion, and the method of diagnosis. The polymerase chain reaction method (PCR) appears to be more sensitive and can be easily applicable to epidemiologic studies.

OBJECTIVES: To determine the frequency of HPV and its genotypes in oral lesions among patients attending a reference clinic of a university hospital.

METHODS: PCR was performed to identify HPV DNA from samples of oral epithelial lesions in 80 patients. For HPV DNA amplification, MY09/MY11 consensus primers were used and specific genotypes were identified through restriction fragment of length polymorphism (RFLP) pattern.

RESULTS: HPV DNA was present in 11.3% of patients, and the identified genotypes were 6b, MM4 (W13B), and MM9 (PAP238A).

CONCLUSIONS: HPV DNA frequency in patients with oral epithelial lesions was 11.3%. The genotypes MM4 and MM9 are uncommon in oral lesions, and they are characterized as high-risk HPV types in those types of lesions.
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Keywords: epidemiology; epithelial lesion; human papillomavirus; oral lesion; polymerase chain reaction

Introduction

The human papillomaviruses (HPV) are a group of DNA oncogenic viruses that have been associated with the etiology of several human tumors, particularly squamous tumors of the cervix, anogenital region, skin, and the upper digestive and respiratory tracts (1). These viruses are epitheliotropic; there are more than 120 types, and just over 80 of them have been clearly identified (2). These viruses are associated with papillomatous, hyperplastic, and verrucous lesions in the skin and mucous membrane of various sites (3).

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The oral cavity is covered by an epithelium that has characteristics similar to those of the genital region, and both are exposed to strange bodies and microorganisms, some of which could be potentially carcinogenic (1). Considering that in the genital region this virus is implicated in the development of cancer in the uterine cervix, it is possible that this link would also be present in relation to oral epithelial cancer. Therefore, it has become important to know the frequency of this virus in the oral cavity and also its probable relationship with the presence of the malignant neoplasm in the region.

The frequency of HPV in oral lesions varies with the geographic occurrence, type of lesions and the diagnosis methodology. Nowadays, the polymerase chain reaction (PCR) method seems to be the most sensitive, rapid and less expensive method that can be easily applied in epidemiologic studies (4). The aim of this study was to identify the frequency of HPV in oral epithelial lesions in individuals that seek treatment in a referral service of a university hospital.

Materials and methods

A cross-sectional study was carried out in outpatients attending the stomatology unit of a general university hospital in southern Brazil, between March 1, 2000 and May 31, 2001. All patients with clinical diagnosis for oral epithelial lesion and who had agreed to participate in the study were investigated. A total of 112 patients fulfilled the entry criteria and were allocated in this study. Patients with the following clinically diagnosed epithelial lesions were included in the study: exophytic, ulcerated, hyperplastic for trauma of prothesys (or not), lichen planus, and leukoplakias. Biopsies were taken from the lesions when there was an indication of total excision or the need of a diagnostic definition.

Oral specimens were collected from lesion smears with a cytobrush and immersed in phosphate saline solution. The samples were frozen at $-80^{\circ}C$ for further DNA extraction. QIAamp DNA kit (Qiagen, USA) was used for the DNA extraction according to the protocol indicated for body fluid. To verify the quality of DNA extraction, all samples were submitted to the PCR technique for amplification of β-globin (286 bp) (5) using the PCO4 and GH2O primers, and the samples positives for β-globin were later processed to identify HPV DNA.

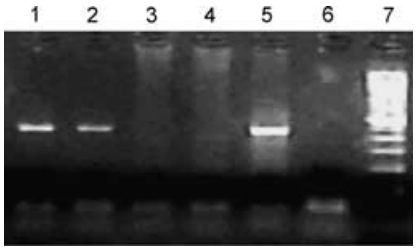

Figure 1 Agarose gel electrophoresis 2.0% ethidium bromide stained of the L1 amplified products obtained with MY09/MY11 consensus primers. Lanes 1 and 2, PCR positive HPV DNA clinical sample; lanes 3 and 4, PCR negative HPV DNA clinical sample; lane 5, PCR positive HPV DNA from HeLa cells; lane 6, reaction negative control (no DNA); lane 7, 100-bp ladder (Invitrogen, Life Technologies, Carlsbad, CA, USA).

PCR with the consensus MY09/MY11 primers was performed to detect the L1 region of the DNA from clinical samples. The method used amplified a 450-bp fragment as described in Manos et al. (6). The clinical samples were processed in duplicate where each PCR was performed, including a negative control (PCR mixture without DNA) and DNA extracted from HeLa cell line, known to be positive for HPV (Fig. 1).

The genotyping was performed through the polymorphic analysis method for enzymatic restriction of amplified PCR product (restriction fragment length polymorphisms (RFLPs)) according to Bernard et al. (7). Bernard's study (7) included the identification and characterization of 41 mucosal HPV types. The enzymes used in the present study for identifying genotypes were *Bam*HI, *Dde*I, *Hae*III, *Hin*fI, *Pst*I, and *Rsa*I. All procedure steps were conducted observing the basic care rules to avoid contamination with DNA from another source as another microorganisms.

Statistical analysis

The frequency of HPV-DNA in oral lesions is described. The characteristics of the samples are compared among patients with positive and negative HPV lesions using the Mann–Whitney and Chi-squared tests. The findings with $P \leq 0.05$ were considered statistically significant.

Results

Samples were collected from 112 individuals. Of these, 32 (28.6%) were excluded (8 for non-histological confirmation of epithelial lesion and 24 negative for β-globin, or that is to say without DNA or with DNA unsuitable for amplification). Eighty individuals remained in the study. The lesions identified in the study population are described in Table 1. Of the studied population, 11.3% (9/80) were HPV positive.

Table 2 describes some social and demographic characteristics of the studied samples according to HPV status. Even though HPV positives were, on average, older than the negative ones, there was no statistically significant difference between them. Also, none of other studied characteristics were significantly different.

Of the nine samples HPV positive, five were from biopsied lesions and four were from not biopsied lesions (Table 1). The identified RFLP genotypes in the lesions were HPV 6b (two lesions), HPV MM4 (W13B) (one lesion), and HPV MM9 (PAP238A) (one lesion). In five lesions, it was not possible to identify the genotype because the previously obtained DNA was degraded.

Table 1 Oral epithelial lesions identified the studied population

Type of lesion	*Biopsied, n (HPV+)*	*Non-biopsied, n (HPV+)*	*Total HPV+*	*Total lesions, n (%)*
Squamous cell carcinoma	2	–	–	2 (2.5)
Papilloma	4 (1)	–	1	4 (5.0)
Hyperplastic epithelial	41 (4)	18 (1)	5	59 (73.7)
Lichen planus	–	5 (1)	1	5 (6.2)
Ulcerated lesion	–	3 (1)	1	3 (3.8)
Leukoplakia	–	7 (1)	1	7 (8.8)
Total	47 (5)	33 (4)	9	80 (100)

Discussion

The aim of this study was to investigate the presence of HPV DNA in oral epithelial lesions of patients attending a stomatology referral service at a general university hospital.

The frequency of 11.3% of HPV positivity observed in this study is among the enormous range described in the literature (5–80%) (8–10). One suggested explanation for the wide variation of this frequency would be the differences of sensitivity and specificity of the detection methodologies used for HPV in oral lesions (8–10). Even within this range, our data suggests a low prevalence of oral HPV infection in the studied population. The main clinical aspects observed in oral lesion did not allow a clear definition of the lesion type. Despite this, the biopsies occurred only when the total removal of the lesion was necessary. Our results indicate that 78.5% (88/112) of the samples were positive for the constitutive β-globin gene, and this is in agreement with the study by Kellokoski et al. (11) who found a detection rate of 85% in DNA from biopsy samples, by PCR.

Table 2 Distribution of social and demographic characteristics according to oral HPV status

Variable	*HPV positives*	*HPV negatives*	*P-value**
Age (years)			
Mean ± SD	53.1 ± 15.2	47.8 ± 16.3	0.27**
Skin color			
White	100.0%	78.9%	0.19***
Non-white	0.0%	21.1%	
Schooling (years)			
≤8	66.7%	70.4%	0.54***
>8	33.3%	29.6%	
Occupation			
Non-qualified	77.8%	69.6%	0.47***
Qualified	22.2%	30.4%	
Family income (US$)			
Up to 217	55.6%	55.9%	0.41****
218–650	11.1%	26.5%	
≥650	33.3%	17.6%	

**P*-value ≤ 0.05 was considered statistically significant.
**Mann–Whitney test; ***Fisher's exact test; ****Chi-squared test.

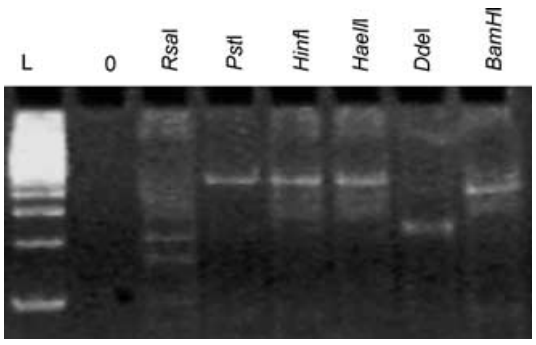

Figure 2 RFLP patterns of MY09/MY11 L1 PCR products used to identify oral HPV types. Silver-stained gels (12.5% acrylamide) are shown. L, 100-bp ladder (Invitrogen, Life Technologies, Carlsbad, CA, USA). 0, PCR negative control (no DNA).

Among the lesions that were part of our samples, we observed 5.0% squamous papilloma and 2.5% of epidermoid carcinoma, but the majority were hyperplastic lesions (51.3%). According to the literature, papillomatous lesions are uncommon, and in our results, the rate is within the expected results, that is 1–4.6% (11). Although the findings lead us to believe in a viral cause for the majority of squamous papillomas, it is impossible to confirm an etiologic role for HPV (12). It is important to mention that of the four papilloma lesions observed in this study and confirmed for histologic tests, only one was positive for HPV (6b) (7).

The genotypes 6b, MM4, and MM9 were identified in the lesions. The HPV 6b has been described by many authors (8, 12–16), and seems to be a common type observed in oral lesions. It is considered of low risk or non-cancer associated. The HPV MM4 is considered a high-risk type (16–18), and it has not been described in buccal cavity lesions. On the other hand, the HPV MM9, currently designated HPV 73, was first identified and sequenced in oral warts with atypia (8), which lead us to believe that it is a low-risk HPV, although this type also has been found in lesions with malignant progression (16, 17) and in immunocompromised patients (15).

In this study, the patient who showed the genotype MM9 (Fig. 2) was a renal transplant patient, using immunosuppressor and corticoid. The HPV seems to be related with the immunosuppression state of the host. There is an increase in the number of diagnostics of HPV in patients with acquired immune deficiency syndrome, renal transplants, pregnancy, or even diseases that require prolonged corticotherapy. It was observed that in these patients, the evolutionary behavior is more aggressive (19). Patients that receive immunosuppressor therapy after organ transplant have an incidence of neoplasia 100 times greater than that in non-transplant patients, controlled by age effect. The cause for this increase is still unclear, but perhaps it is because of a combination of a decrease of the detection of carcinogenic cells by the immune system, chronic antigenic stimulation for the transplanted organ, direct carcinogenic effects of immunosuppressive drugs, or proliferation of the oncogenic virus (20).

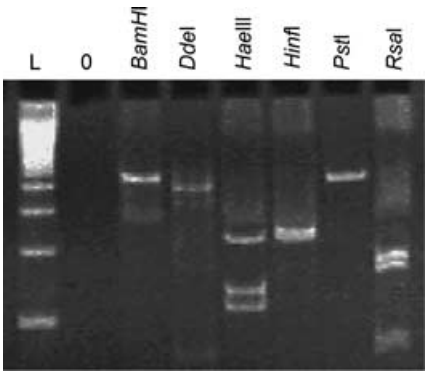

Figure 3 RFLP patterns of MY09/MY11 L1 PCR products used to identify oral HPV types. Silver-stained gels (12.5% acrylamide) are shown. L, 100-bp ladder (Invitrogen, Life Technologies, Carlsbad, CA, USA). 0, PCR negative control (no DNA).

Immunocompromised individuals develop lesions with unusual clinical appearance, which may include HPV types not usually seen in oral lesions. Immunosuppression is a predisposing factor for HPV-associated lesions, which suggests that immune regulation may play a role in the pathogenesis of these lesions (21).

HPV MM4 was identified in one lesion of lichen planus. Zhang et al. (22) showed that lichenoid mucositis, a feature consistent with chronic graft vs. host disease, preceded the development of the post-transplant oral squamous cell carcinoma.

The other genotype identified were HPV 6b (Fig. 3), and it was found in a patient carrying sublingual squamous papilloma. Genotypes 6, 11, and 16 are among the more frequent types reported from papilloma lesions (11, 23). The same genotype was identified in another patient with leukoplakia in the anterior region of the mandible, with past history of a malignant tumor removed from the mouth floor. In leukoplakias and mouth floor lesions, Chang et al. (24) reported that the most frequent genotype is HPV 16; however, genotype HPV 6b, usually associated with benign lesions, was also found in squamous cell carcinoma. This last case leads us to reflect about viral latency and the possibility of there being a reservoir for this virus in the oral cavity (25).

We should mention that in spite of not being possible to genotype all HPV-positive samples, we believe that these findings are quite relevant. That is, our results add to the concept of infection for uncommon HPV types in immunocompromised patients and also increase the possibility of viral latency or, the oral cavity to be a reservoir for this virus, providing that it manifests itself before immunity alteration.

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