

Oncogenic high-risk human papillomavirus in patients with full denture

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Abstract: Human Papillomavirus (HPV) has considerable tropism for epithelial and mucosal tissues and can therefore be found in several anatomical sites, including the oral cavity. This study aimed to investigate the presence of HPV-DNA and the most frequent viral types in patients using full dentures, compare to patients not using full dentures and to associate its presence with socio-epidemiological and behavioral factors. The study consisted of 90 patients with or without full dentures at the time of collection, treated at a public dental clinic. The samples were obtained by exfoliating the oral cavity, and analyzed for HPV-DNA using the nested PCR with PGM09/11 (450-bp), and general primers GP5+/GP6+ (150-bp). Genotyping was performed by specific-type PCR to HPV 6, 11, 16, 18, 31, 33, and 45; and Restriction Fragment Length Polymorphism (RFLP). Pearson's Chi-square test (χ^2) or Fisher's exact test were applied and significant variables in these tests were analyzed by multinomial logistic regression analysis to estimate odds ratio (OR). HPV-DNA was detected in 27.7% of samples and, among those obtained from patients using full dentures, positivity for HPV-DNA was 41.9% ($p = 0.025$). The most frequent viral types were low-risk HPV 6 and 11, and high-risk HPV 31 and 45. Patients who used full dentures had an odds ratio of 2.1 to be positive for HPV DNA. Our results indicate the need for periodic dental follow-up of patients with full dentures in order to preserve the basic conditions of oral health, and also to monitor the appearance of lesions with malignant potential.

Keywords: Oral Health; Papillomaviridae; Polymerase Chain Reaction.

Introduction

Human papillomavirus (HPV), particularly the genus alpha papillomavirus, has considerable tropism for epithelial and mucosal tissues. Therefore, it can be found in several anatomical sites, including the anogenital tract, larynx, esophagus, oral cavity, and others, causing disease ranging from benign lesions, such as epithelial warts, to malignant neoplasms.^{1,2} In recent decades, there has been a marked increase in cases of HPV infection among men and women.^{3,4} HPV infects the basal cell layer of the epithelium, leading to micro trauma at most instances, which causes the basal cells to be exposed and susceptible to virus entry.⁵



The methods of transmission of the virus into the oral cavity have not yet been fully elucidated; some theories point to the importance of vertical transmission, self-inoculation, common use of fomites, and oral sex. The latter has been considered the main form of infection acquisition in the oropharyngeal region.⁶

Although HPV infection is mainly considered to be sexually transmitted, it is also known to be multifactorial because risky behaviors such as high number of sexual partners, smoking, alcoholism, and exposure of the oral mucosa to frequent traumas (like those caused by the use of full dentures) can facilitate infection.^{5,7,8,9,10}

To date, more than 200 types of HPV have been identified, which can be classified according to their oncogenic potential as high oncogenic risk (HR-HPV), including HPV 16, 18, 31, 33, 35, 39, 45, 51, 56, and 58, and low risk (LR-HPV), including HPV 6, 11, 42, 43, and 44.^{9,11} More than 30 types have been isolated in oral lesions or in oral mucosal infections, among which the most frequent are HPV 1, 2, 3, 4, 6, 7, 10, 11, 13, 16, 18, 20, 28, 30, 31, 32, 35, 38, 40, 45, 52, 54, 55, 57, 58, 59, 68, 69, 72, 73, 77, and 85.¹² Seven of these types (HPV 6, 11, 16, 18, 31, 33, and 35) have also been isolated in healthy oral mucosae.^{13,14,15}

Epidemiological studies have found clinical correlation with secondary factors such as poor oral hygiene, excessive exposure to sunlight, injuries caused by lacerated and pointed teeth, and poorly adapted dentures, thus increasing the susceptibility for HPV infection.^{16,17,18,19}

Because of the importance of this virus during the carcinogenesis process, the objective of this study was to investigate the presence of HPV-DNA and the viral types most frequently found in patients with full dentures, and to make an association with the socio-epidemiological aspects and behavioral factors that expose them to infection, which defines the risk of this group for developing HPV infection.

Methodology

Population study and design

This was a cross-sectional, quantitative study with non-probability sampling – a convenience sample. The

subjects involved in the study were patients treated at the Faculty of Dentistry of the Federal University of Mato Grosso do Sul, in Campo Grande city, the Center-Western Region of Brazil, from October 2014 through October 2016. The inclusion criteria were patients of both genders aged 30 years or greater, with or without full dentures. The sample size was obtained considering the case series of the Faculty of Dentistry, corresponding to 15% of the patients attended during the period.

Ethical approval was granted by the Ethics Committee in Research of the Federal University of Mato Grosso do Sul, protocol no. 36389814.2.0000.0021 on 30/09/2014. All participants filled in a questionnaire containing information regarding risk behaviors that may predispose them to HPV infection.

Collection of specimens and detection of HPV-DNA

Samples were collected through 5–10 brushings from pre-established regions of the oral mucosa including the right buccal mucosa (position from top to bottom); left buccal mucosa (position from top to bottom); right, left, and dorsal side of the tongue; and inner regions of upper and lower lips. After collection, DNA was extracted from the samples using the phenol chloroform technique, and quantified using a NanoDrop (Thermo Scientific, Waltham, MA, USA) (180–260 nm). HPV-DNA detection was performed by nested PCR with a pool of consensus primers that amplify PGMY09/11 450-bp DNA sequences within the L1 region of HPV, and general primers GP5+/GP6+ (150-bp) as described previously.²⁰ An endogenous control was used to verify DNA integrity using primers for the human β -globin gene, PC04 and GH20, which amplify a 286-bp sequence. Negative controls for background contamination were added to the DNA template. PCR products were analyzed using 1.5% agarose gel electrophoresis with ethidium bromide staining to visualize the DNA under ultraviolet (UV) light. Molecular weights were determined by comparison with a 100-bp DNA ladder.

Genotyping

HPV-DNA-positive samples were genotyped by PCR using type specific-PCR (TS-PCR) for the

DNA sequences of the E6 and E7 genes of LR-HPV 6 and 11, and HR-HPV 16, 18, 31, 33, and 45.²¹ PCR products were analyzed in a 2.5% agarose gel followed by ethidium bromide staining to visualize the DNA under UV light. Molecular weights were determined by comparison with 100-bp and 50-bp DNA ladders. Restriction fragment length polymorphism (RFLP) was used for samples that were positive for HPV-DNA by PGMY09/11 primers. The PGMY 09/11 PCR product of these samples was purified from the agarose gel using a QIAE X II Gel Purification kit (Qiagen, Dusseldorf, Germany), according to the manufacturer's protocol. The concentration of the extracted material was measured using a NanoDrop, and samples with sufficient material (4.0 ng/ml) were subjected to enzymatic digestion for 1 hr at 37°C. The enzymes used for the reaction were DdeI, HaeIII, RsaI, and PstI. The digestion pattern obtained was analyzed under UV light and interpreted using an algorithm previously described in the literature.²²

Statistical analysis

SPSS 10.0 (SPSS Inc., Chicago, IL) software was used for statistical analysis. For data comparison, Pearson's Chi-square test (χ^2) or Fisher's exact test were applied; for analysis of the variables 'use of full denture' and 'oral sex practice', the test of binary logistic regression was applied.

Results

Prevalence of infection

The study involved 90 patients, mean age of 49.2 years (30–80 years), 36.7% (33–90) males and 63.3% (57–90) females. The patients were divided into two groups: 1) patients using full dentures (FD) (n = 37) mean of 53.10 years (38 to 80 years), among which 70.3% (26–37) were females; and 2) patients not using full dentures (NFD) (n = 53) mean of 49.26 years (30 to 64 years), of which 60.3% (32–53) were females.

After DNA extraction, 92.2% (83/90) of the total samples showed amplification of the human β -globin gene and were considered valid for analysis. HPV-DNA was detected in 27.7% (23–83) of all samples. Among the FD group, 41.9% (13–31, $p = 0.025$) were positive

for HPV-DNA whereas, among the NFD group, the positivity was 19.2% (10–52) (Figure).

Genotyping

Considering all patients involved in the study, the most frequently detected viral types were LR-HPV 6 and 11 (39.1%, 9/23) and HR-HPV 31 (13.0 %, 3/23).

In the FD group, 38.5% (5–13) of the HPV-DNA positive samples showed the presence of LR-HPV, and 15.4% (2–13) showed the presence of HR-HPV. In 7.7% (1–13) of the samples of this group, multiple infection with LR- and HR-HPV was observed, and in 30.8% (4–13) the viral types were not identified by the techniques used. These samples were negative by TS-PCR and did not amplify with PGMY09 / 11 primers that did not allow RFLP analysis. The viral types detected in both of the groups were described in Table.

Socio-epidemiological characteristics

There was no significant difference between the association of age and the presence of HPV DNA among both groups (FD and NFD). Regarding the behavioral characteristics associated with HPV-DNA presence, the variable 'oral sex practice' was significant for all HPV-DNA positive patients and for FD HPV-

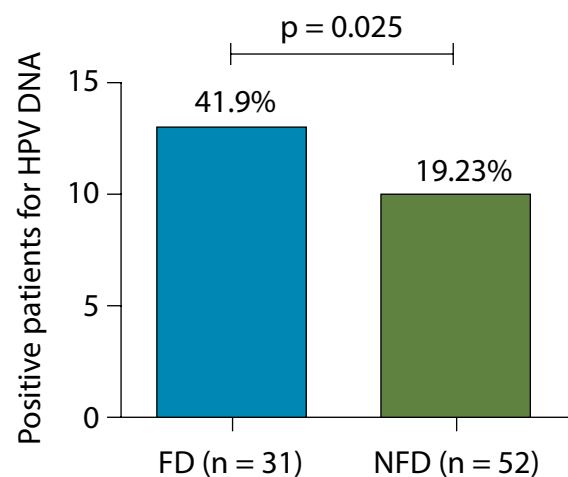


Figure. Distribution of patients positive for HPV-DNA. FD: patients who use full denture and are positive for HPV-DNA. NFD: patients who do not use full denture and are positive for HPV-DNA. * $p = 0.025$. Pearson's Chi-square test (χ^2).

Table. Distribution of the viral types according to oncogenic risk.

Viral types	Groups	
	FD	NFD
	n(%)	n(%)
LR-HPV6	3(23.1)	4(40.0)
LR-HPV11	1(7.7)	-
LR-HPV40	1(7.7)	-
HR-HPV31	2(15.4)	-
HR-HPV91	-	1(10.0)
HR-HPV45	-	1(10.0)
ND	4(30.8)	5(50.0)

LR: Low oncogenic risk; HR: High oncogenic risk; ND: viral types were not genotyped; (-): not found; FD: patients using full dentures that were genotyped (n = 13), and NFD: patients not using full dentures that were genotyped (n = 10). Some patients had two or more viral types (multiple infection).

DNA positive patients (4–23, $p = 0.01$; 1–13; $p = 0.01$ - Fisher’s exact test, respectively).

Considering the significant variables ‘full denture use’ ($p = 0.025$) and ‘oral sex practice’ ($p = 0.01$), for the group of HPV positive patients, we investigated the variables that would be determinant for HPV-DNA presence. Analysis of binary logistic regression revealed that the use of full dentures had an odds ratio of 2.1 to be positive for HPV DNA. ($\chi^2 = 9.037$, $DF = 2$, $p = 0.0011$).

Discussion

The results indicate that patients having full dentures showed a higher frequency of HPV-DNA, the presence of HR-HPV types, and had an odds ratio of 2.1 to be positive for HPV DNA.

Research has shown a great variation in the incidence of HPV infection when detected in the healthy oral mucosa of individuals (0% to 81.1%), by means of different methods of collection and detection in various population groups.^{7,17,23} In the present study, nested PCR revealed a high general prevalence (27.7%) of HPV-DNA in the healthy oral cavity of adults with or without full dentures, suggesting that this observation included subclinical and/or latent infections, with a low number of viral copies, which is common in the oral cavity.¹⁴ Most of these

infections are likely to regress spontaneously to the point of being undetectable, even by use of the most sensitive methods.⁹ In other anatomical sites, our group found an HPV DNA frequency of about 30% in cervicovaginal samples of young women (mean age 35 years) and 83.6% in samples from the glans and coronary sulcus of the penis of heterosexual men (18–65 years).^{24,25,26}

Genotyping of the HPV types detected is highly relevant, considering the oral cavity and the potential risk of developing malignant lesions induced by the highly oncogenic types of the virus. The TS-PCR applied in our study aimed to identify the most prevalent types in the asymptomatic oral mucosa and in benign and malignant lesions.¹ The combination of two methods of genotyping (TS-PCR and RFLP), although still scarce in epidemiological studies, is quite advantageous in the detection of multiple infections.

HPV 6, 11, 16, 18, 31, and 33 were identified in the oral cavity of subjects with and without lesions, and HPV 6 and HPV11 were most commonly found in healthy oral mucosae (23.3% and 6.7%, respectively).²⁷ In our study genotypes described as α -papillomaviruses were observed, unlike other authors.^{28,29} A prevalence study conducted in the USA, with samples of normal oral mucosa, detected the presence of HR-HPV 16, 66, and 51 and LR-HPV 62, 55, and 89,³ whereas in another study, with formalin-fixed paraffin-embedded samples of normal oral mucosa, squamous carcinoma and oral leukoplakias by nested PCR, was detected the presence of types HPV 6, 11, and 16.³⁰

In the oral cavity, a broad spectrum of HPV genotypes were previously isolated, and HPV 6, 11, 16, 18, 31, 33, and 35 were also isolated in the healthy oral mucosa.^{12,13,29} HPV 6, 11, 16, and 18 have mucosal affinity and are responsible for most of the lesions both in the oral cavity and cervical region.^{7,13,31} Among the patients who use full dentures, the most frequently observed types were HPV 6, 11, 31, 40, 45, and 91. LR-HPV presented a higher frequency (16.1%) compared to that of HR-HPV (6.5%). These results contradict the findings in the literature, which describe HPV 16 and 18 as the most common types found in the oral cavity, which, because of their highly oncogenic potential, are considered possible

risk factors for the development of carcinoma in the oral cavity.^{2,4,6,9,10,13,32}

Several authors report that HPV can infect cells of the basal layer of the epithelium provided that there is a rupture, for example, micro traumas in the epithelium.^{1,10,18} In this study we found that 41.9% of patients with full dentures were positive for HPV-DNA, an index higher than the patients who did not use full dentures ($p = 0.025$). These results confirm the expectancy of potentiation of infection by micro traumas caused in the mucosa by the dentures and the consequent facilitation of viral infection.

Transmission of HPV can occur through contact between the injured and healthy mucosa, and for oral infection, several forms of contamination are considered relevant, such as oral sex, autoinoculation, and others. However, abrasion of the mucosa with exposure of the epithelial basal layer cells is necessary for the virus to enter the host cell.^{4,9,13,14} The use of dentures for a long time, often ill adapted, poor hygiene conditions, and other co-factors increase the risk of infection by this virus.^{4,7,10,32} Although in our study age and other factors were not significant, we cannot rule out its possible influence.

Indicators related to sexual activity, such as the number of recent sexual partners, the number of lifetime partners, and the patient's age at the time of the first sexual intercourse have been consistently reported as being the most relevant risk factors for HPV infection. Sexual behavior is crucially associated with HPV infection, because direct contact with the epithelium without the use of adequate protection is

a key factor.^{8,14,33} In this study, the practice of oral sex was associated with HPV infection in both FD and NFD groups, corroborating the importance of 'oral sex practice' as cofactor in transmitting the HPV to this anatomical site.

When the variables 'use of full dentures' and 'oral sex practice' were analyzed together, the former represented an odds ratio of 2.1 to be positive for HPV DNA. The literature shows that people with poor oral health and people with oral disease are significantly more susceptible to oral infections, including those caused by HPV.^{4,7,10,32} Studies have reported that in patients with poor oral health, the prevalence of HPV in oral infection is high.¹⁰ Patients with periodontal disease or other tooth-related problems are also frequently affected by HPV infection.⁷ In addition, the relationship between the number of teeth lost and oral HPV infection has also been studied.^{6,19,20,34} These findings together suggest that poor oral health predisposes to HPV infection.

In conclusion, this study showed the highest HPV-DNA presence in patients with full dentures, including the presence of HR - viral types and infection by more than one viral type. In addition to the use of full dentures, the practice of oral sex was also considered associated to HPV-DNA presence, emphasizing the importance of sexual contact as a mode of transmission. Our results indicate the need for periodic follow-up and orientation to the patients with full dentures in order to preserve basic oral health conditions and also to monitor the appearance of lesions with malignant potential.

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