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Oral Lesions Associated with Human Papillomavirus (HPV)

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Abstract: ***Objective: The aim of this study is to determine the risk of HPV infection in stomatological lesions in the oral cavity. Study design: 53 patients over 21 years, who attended the Department of Oral Medicine of the School of Dentistry of the University of Buenos Aires, Argentina, who presented lesions in the oral cavity clinically compatible with HPV infection. Biopsies were performed in the affected areas and were divided in two parts, one for its histopathological diagnosis, and the other for its HPV determination. Statistical associations were analyzed using IBM Spssstatistics V23 to obtain the Odds Ratio. Results: Of the total of the sample, HPV was positive in 60% of the cases (32/53). The identified genotypes were of low risk (47%) and high risk (53%).HPV16 was the most common of the high risk group (35%) and HPV11 was the most frequent type of the low risk group (53%).Conclusion: We found an association between high-risk HPV and elevated white lesions present in oral mucosa. It is important to identify the type of HPV (high / low risk) present in stomatological lesions since its evolution will depend on this.***

Keywords: Human Papillomavirus, Oral lesions, oral mucosa, Oral pathology, PCR

1. Introduction

HPVs are small non-enveloped double-stranded DNA viruses that belong to the *Papillomaviridae* family. They form a large and diverse group of viruses, with new HPV types being continuously found (1). Human papillomaviruses have a tropism for the squamous epithelium (2). Viral particles infect the basal cells of the epithelium, which are exposed through micro-abrasions or epithelial wounding. The receptors of HPV and the mode of viral entry into the cell are still partly unknown (3).

HPVs cause a wide range of diseases from benign lesions to invasive tumors (1,4). Human papillomavirus (HPV) is an agent responsible for verrucae, condylomas and papillomas at various sites of the body, the oral cavity included (5).Evidence is emerging which suggests that some oral HPV infections might persist**.**

Persistent HPV infection is mandatory for HPV-associated malignant transformation. However, progression of HPV-induced lesions to malignancy requires additional cofactors (2). Micro abrasions or mucosal wounding and the consequent epithelial proliferation mediated by microorganisms and inflammatory cytokines, create an ideal microenvironment for the initial HPV infection and its subsequent persistence, which increases the risk of transmission and its carcinogenic potential (6).

HPV cannot infect us through an intact squamous epithelium. In vivo, it infects the basal layer of mitotically active skin or mucosa through abrasions or wounds inflicted upon the epithelium (7). It is disseminated by direct cell-to-cell contact without the classical signs of viremia (8). After it penetrates the cell, the viral genome is transported to the cell nucleus where it is translated and transcribed. Viral genome is replicated in the following stages: first, early proteins (E1 and E2 are synthesized. As a result of that, some 10 to 200 genome copies are replicated per cell. In the second stage, during the cell cycle, replication occurs in offspring cells at an equal rate. Expression of genes E6 and E7 leads to cell transformation or differentiation. Cells start presenting a faster life cycle and begin to divide more frequently, leading to the formation of benign tumors. At this point, the virus proliferates in the tissue without destroying the cell hosting it. On the third stage, also referred to as the productive stage, large amounts of proteins E1 and E2 start producing thousands of copies of viral deoxyribonucleic acid (vDNA). On the other hand, late proteins (L1 and L2) - fundamental for new virus assembly - are also produced. Viruses are then released from the keratocyte located more superficially (9).

Previous evaluations of HPVs have classified types 16 and 18 as carcinogenic to humans (group 1), types 31 and 33 as probably carcinogenic to humans (Group 2A) and some types other than 16, 18, 31 and 33 as possibly carcinogenic to humans (Group 2B) (10).

Oral HPV infections have been linked to sexual behavior, but recent evidence supports their horizontal, mouth-to-mouth, transmission. Most HPV infections in infants are acquired vertically from the mother during the intrauterine period, during delivery, or later via saliva (2).\

2. Materials and Methods

This study was a part of Program of Support for Clinical Investigation of the School of Dentistry of the University of Buenos Aires, Argentina. It was carried out evaluating healthy patients with oral lesions compatible with HPV infection that were analyzed by nested polymerase chain reaction and subsequent sequencing (PCR). This project was approved by the Institutional Ethics Committee at the School of Dentistry of the University of Buenos Aires in 2017 Resolution (CD) N°628.

A cross sectional descriptive study was performed including 53 adult patients of both sexes, 34 women and 19 men who attended the Department of Oral Medicine of the School of Dentistry of the University of Buenos Aires in the period between February 2017 and February 2018

who presented lesions in the oral cavity clinically compatible with HPV infection.

We selected patients who were over 21 years old and who signed the informed consent and excluded patients who were diagnosed with systemic diseases or were taking retroviral or immunosuppressive medication.

Data Collection

All the data was collected in a protocolled clinical history including age, sex, lesion characteristics and its localization. All patients were asked for laboratory tests (hemogram, coagulogram, hepatogram, glycemia, uremia, uricemia, erythrosedimentation)

Lesion evaluation

The evaluation of the lesions was carried out by stomatologists and regular means of inspection and the operating clinical microscope OPMI PICO S100 CARL ZEISS were used. The lesions were observed in a 1:6 magnification in a sequence of increases.

The clinical lesions suspected of an HPV infection were classified in two groups: flat and elevated. Between the flat lesions we find the white translucent spot, and among the elevated lesions we can find keratosis, vegetations and verrucosities. (Figure 1)

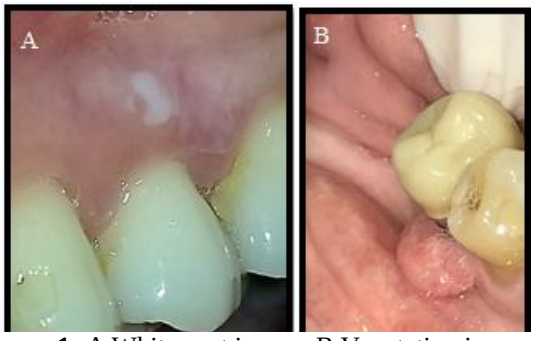

Figure 1: A White spot in gum B Vegetation in gum

Sample collection

Biopsies were performed in the affected areas and were divided in two parts, one was fixed in formalin buffer, included in paraffin and colored with Hematoxylin Eosin for its histopathological diagnosis, and the other was stored in saline solution for its HPV determination, which was analyzed by nested polymerase chain reaction and subsequent sequencing using MY09/11 and GP05+/06+ primers.

Sample processing

The samples were incubated with 200 ul of lysis buffer (Lysis tissue buffer, Roche) and 20 ul of proteinase K in agitation at 650 RPM in a thermostatic bath for 12 hours.

Subsequently, the extraction of DNA from this lysate was carried out in an automated manner with the MagnaPure 96 equipment (Roche) according to the manufacturer's instructions.

To estimate the integrity of the DNA obtained and the absence of PCR inhibitors, a gene fragment of the constitutive gene of β-globin was amplified in all the samples studied, with the universal oligonucleotides BG1 / BG2 (11) by the real-time PCR technique in the LightCycler ® device 2.0 Real-Time PCR System (Roche).

The samples where amplification of β-globin was obtained were subjected to viral DNA detection by PCR (primary PCR), using the universal oligonucleotides MY09 / MY11 which amplify a fragment of 450 bp in the conserved region of the L1 gene of HPV (12).

In order to increase sensitivity, all samples that were negative for amplification with MY09 / MY11 were subjected to a second amplification (secondary PCR) with universal oligonucleotides (GP5 / GP6), located within the sequence recognized by the oligonucleotides MY09 / MY11 and that amplify a fragment of 150 bp. (13,14).

Both PCR reactions were performed on a Veriti® Thermal Cycler (Thermo Fisher Scientific).

As a positive control, a detectable sample of HPV type 16 was used and as a negative control we used a negative sample for HPV. The amplification productswere visualized on a 2% agarose gel with transilluminator.

Capillary electrophoresis of the amplification products resulting from the first and second reaction of PCR was performed, with a previous sequence reaction with the GP5 + / GP6 + primers, in the ABI® sequencer 3500 Genetic Analyzer (Applied Biosystems).

Sequence alignments were performed against the reference HPV L1 gene sequences stored in the GenBank database, by BLAST analysis for the final validation of the HPV genotype.

Statistical analysis

Odds ratio (OR) was used to measure the association between HPV-positive and negative and elevated and flat lesions, using SPSS Statitics V22.

3. Results

A total of 53 patients participated in the study; their mean age was 57 years old with a range of 24–85 and 64% were females. The type of lesions compatible with HPV infection were: verrucosity 49%, keratosis 19%, tumor 10%, white spot 10% and vegetation 9%. Our results showed that 60% (n=32) of the subjects were HPV positive, 21 females and 11 men with a mean age of 56 years. Of the total of the HPV positive cases, 53% (n= 19) was of low risk, being HPV type 11 the most prevalent in our sample and 47% (n=17) corresponded to high risk HPV, where type 16 was the most prevalent of this group (Table 1). We found a coinfection with two types of HPV

in the same lesion in four of our patients 6/35, 16/18, 6/11, 11/16. The detected genotypes of HPV in our sample of the high risk group were 16, 18, 31, 33, 35, 51 and 58 and those that belong to the low risk group were 6, 11, 13, 32, 64 and 72.

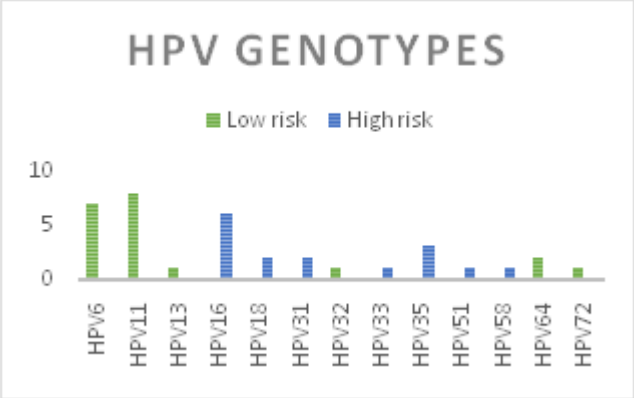

Table 1: High and low risk HPV genotypes. Four patients presented two types of HPV in the same lesion

The most frequent location of the lesions was the gum (29%) followed by the buccal mucosa (21%), tongue (20%) and palate (20%).

The histopathological diagnosis associated with high risk HPV were epithelial hyperplasia 60%, oral carcinoma 27% and oral lichen 13%, meanwhile the diagnosis associated to low risk HPV corresponded to epithelial hyperplasia 78% and oral lichen 22%. (Table 2).

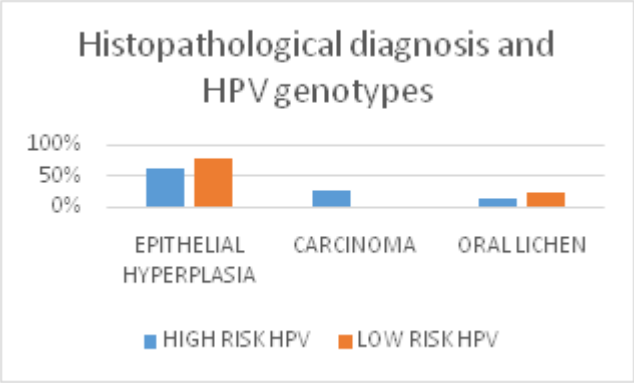

Table 2: Relationship between high/low risk HPV and Histopathological diagnosis

We estimated the risk between HPV positive/negative and white elevated or flat lesions OR: 2,25 (IC95% 0,34-14,83) and the risk between high and low grade HPV and white elevated and flat lesions OR: 1,88 (IC95% 0,27-13,09). (Tables 3 and 4)

Table 3: Estimated risk between HPV positive/negative and white elevated or flat lesions

	White Elevated Lesions	White Flat Lesions	Total
HPV +	27	5	32
HPV -	18	3	21
Total	45	8	53

OR: 2,25 (IC95% 0,34-14,83)

Table 4: Estimated risk between high and low grade HPV and white elevated and flat lesions

	White Elevated Lesions	White Flat Lesions	Total
High Risk HPV	15	2	17
Low Risk HPV	12	3	15
Total	27	5	32

OR: 1,88 (IC95% 0,27-13,09)

4. Discussion

This is the first study addressing the prevalence of HPV in stomatologic lesions in Argentina.

The first clinical presumption of the presence of HPV in oral mucosa is performed by the stomatologist through the identification of certain elementary lesions that were described in this paper and that have been linked for a long time to this kind of infection. However, the confirmation of this clinical diagnosis by anatomopathological study and the detection of the type of HPV present in the lesion are necessary. (10, 15, 16)

In regard to the different types of lesions observed in the stomatological examination, authors such as C. Allen and Fornatora describe a subtype of oral epithelial dysplasia with unique clinical and histological features that would probably have a predictive value for the presence of HPV. These are white, flat, slightly elevated or papillary lesions that histologically present characteristics similar to common dysplasia, such as proliferation and maturation of basal cells, nuclear pleomorphism, increase in the number of mitosis, nuclear prominence, increase in nucleoplasm relation and atypical mitotic figures. (17)

Koss and Durfee introduced the term "koilocytotic atypia" to describe lesions of the cervix that were characterized microscopically by large epithelial cells, with a relatively small and irregular nucleus that was surrounded by a clear glycogen negative halo. It is now known that koilocytes are pathognomonic of HPV infection and that these cellular abnormalities represent the lethal cellular effect of viral reproduction. (18)

On this paper we make a detailed description of the elementary stomatological lesions with clinical inspection and magnification. Where we correlated the high and low risk types with the observable clinical lesion.

Furrer et al. reported that the clinical features considered as signs of putative HPV infection in oral mucosa were: white bright flat lesions with slightly elevated patches or plaques and frankly verrucous lesions. (19)We took this information to initiate the clinical presumption of HPV infection. We found no other authors who took these oral lesions as a reference as suspicious of HPV infection.

In concordance to Ribeiro M et al., in order to enhance HPV detection we used the nested-PCR technique that uses more than one pair of primers. Thus, we were able to

detect the virus even in very low concentrations. High quality DNA is required for this technique to reach its optimal conditions. (20)

In this study we found the presence of multitype (2 types) oral HPV infection in 12,5% of the patients (n=4) and most of them where high risk HPV genotypes. Bui et al. reported the prevalence of multitype (2–6 types) oral HPV infection was 1.5% (2.5% for men, 0.4% for women). Most multitype oral HPV cases (83.8%) harbored one or more oncogenic types. (21)

In this study the prevalence of HPV was 60% and 19% of this corresponded to HPV 16. Gillison et al. said that the prevalence of oral HPV infection among men and women aged 14 to 69 years in the USA was 6.9% and of HPV type 16 was 1%. (22)

Sonawane K et al. postulated that the overall prevalence of oral HPV infection was 11.5%. (23)There are many studies addressing the presence of HPV in the oral cavity of healthy patients without lesions. Our study considered the clinical suspicion of HPV infection in stomatologic lesions. This is the reason why the prevalence of this study was higher than the ones in the previously cited studies.

5. Conclusions

The overall prevalence of HPV oral in our sample was 60% and we found both high and low risk HPV genotypes. The lesions with a suspicion of HPV infection were white translucent spots, among the flat lesions. We found keratosis, vegetations and verrucosities among the elevated ones.

PCR is a very important technique to determine the genotype of HPV present in these lesions because of its high sensitivity.

We found an association between high risk HPV and white elevated lesions, being these oncogenic HPV a risk factor for the development of these lesions.

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