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HPV frequency, p16 expression and risk factors for oral leukoplakia from Córdoba, Argentina

Gerardo Gilligan[1,*], René Panico[2], Cecilia Di Tada[3], Andrea Lucca[4], Mabel Brunotto[5], Eduardo Piemonte[6]

Abstract

Background: Non-homogeneous results about the frequency of HPV in leukoplakia are presented in current literature, possibly linked to different factors. Among them, a diverse HPV geographical distribution was suggested. This study aimed to describe the frequency of HPV in patients diagnosed with leukoplakia.

Patients & Methods: A cross-sectional study was carried out at the Facultad de Odontología, Universidad Nacional de Córdoba. HPV status was studied by polymerase-chain reaction (HPV-PCR) and p16 by immunohistochemistry. HPV status was analyzed concerning the clinical-demographic features using the Fisher test and the McNemar test.

Results: There were included 33 patients diagnosed with different subtypes of oral leukoplakia. HPV genome was detected by PCR in 48.5% (n=16). The most common viral genotype was HPV16. p16 was positive in 27% (n=9) of the cases. The concordance between HPV detection techniques showed poor or weak agreement (Mc Nemar 0.1185). Except for chronic mechanical irritation with HPV-PCR + (p=0.0049), and tongue location with HPV-PCR + (p=0.0366), no significant statistical association between the other included variables was found.

Conclusions: The low frequency of HPV in this study agrees with previous studies of our region. Chronic mechanical irritation could play a role in the development of HPV-associated leukoplakias, mainly located on the ventrolateral tongue.

Keywords: human papilloma virus; oral leukoplakia; risk factors, immunhistochemistry

Frecuencia de VPH, expression de p16 y factores de riesgo de leucoplasia oral de pacientes de Córdoba, Argentina

Resumen

La literatura actual indica que existen resultados heterogéneos en relación a la frecuencia de infección por VPH en leucoplasias, posiblemente debido a diferentes factores. Entre ellos, la una diversa distribución geográfica del virus. El objetivo de este estudio es describir la frecuencia de VPH en pacientes diagnosticados con leucoplasia.

Materiales y métodos: Se realizó un estudio de corte transversal en la Facultad de Odontología de la Universidad Nacional de Córdoba. La infección por VPH fue analizada mediante técnica de PCR e inmunomarcación para p16 mediante inmunohistoquímica. Estos resultados fueron analizados a partir de características clínico-demográficas utilizando el test de Fisher y de McNemar.

Resultados: se incluyeron 33 pacientes diagnosticados con diferentes tipos de leucoplasia bucal. El genoma de VPH fue detectado mediante PCR en el 48.5% (n=16) de los casos. El genotipo viral de mayor frecuencia fue el VPH16. P16 fue positiva en el 27% (n=9) de los casos. La concordancia entre las técnicas de detección de VPH evidenció pobre o débil concordancia (McNemar test 0.1185). A excepción de la relación entre irritación mecánica crónica y PCR-HPV+ (p=0.00949) y la localización en lengua y PCR-VPH+ (p=0.0366), no se evidenciaron asociaciones estadísticamente significativas entre las variables estudiadas.

Conclusiones: La baja frecuencia de VPH en este estudio va en concordancia con otros estudios de nuestra región. La irritación mecánica crónica podría jugar un rol importante en el desarrollo de leucoplasias asociadas al VPH, preferentemente localizadas en el borde de lengua.

Palabras claves: virus de papiloma humano, leucoplasia bucal, factores de riesgo, inmunohistoquímica.

Introduction

The effective and persistent infection of the oral mucosa by the Human Papilloma Virus (HPV) plays a role in the development of several oral conditions. HPV-associated oral lesions can result in different clinical presentations, ranging from hyperplastic to carcinomatous changes[1]. A causal association between HPV and oral leukoplakia (OL), proliferative verrucous leukoplakia (PVL), or oral squamous cell carcinoma (OSCC) has been suggested, although no conclusive evidence for such association has been presented[2].

1 Facultad de Odontología, Universidad Nacional de Córdoba, Argentina. https://orcid.org/0000-0002-5201-1444
2 Facultad de Odontología, Universidad Nacional de Córdoba, Argentina. https://orcid.org/0000-0002-5833-5546
3 Immunohistochemistry Laboratory, Fundación para el Progreso de la Medicina, Córdoba, Argentina. https://orcid.org/0000-0003-2723-2154
4 Molecular Biology Laboratory, Fundación para el Progreso de la Medicina, Córdoba, Argentina.
5 Oral Biology Department, Facultad de Odontología, Universidad Nacional de Córdoba, Argentina. https://orcid.org/0000-0001-8010-1079
6 Oral Medicine Department, Facultad de Odontología, Universidad Nacional de Córdoba, Argentina. https://orcid.org/0000-0001-5955-1139
* Autor para correspondencia:
Correo electrónico: ggilligan@unc.edu.ar

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HPV penetrates the deeper oral epithelial strata, facilitated by superficial microcracks[3,4]. Interestingly, the epithelium of the gingival sulcus is the only oral subsite where basal keratinocytes are physiologically exposed to the oral environment. This observation leads to considering periodontium as a reservoir for this virus[5]. However, in other subsites of the oral mucosa, acute or chronic trauma is necessary to facilitate penetration into the epithelium[6].

A subset of oral potentially malignant disorders (OPMDs) harboured dysplastic changes associated with HPV, and the term HPV-associated oral dysplasia was used to categorize them[7,8]. HPV16 was the most prevalent genotype found in dysplastic OL and erythroleukoplakia mainly located on the tongue and/or floor of the mouth[8].

The analysis of HPV infection in oral conditions could represent a challenge due to dissimilar rates of sensitivity and specificity provided by the available viral diagnostic tests. HPV-DNA detection by PCR cannot distinguish whether HPV is involved in a transient or active infection. The expression of E6/E7 mRNA is considered the gold standard for the diagnosis of oral HPV infection with high concordance and consistency with p16 by immunohsistocmistry[9,10]. HPV oncogenes E6 and E7 are highly associated with carcinogenetic activity and are responsible for inhibition of the tumour suppressors p53 and retinoblastoma protein (pRb). E7-driven inactivation of pRb leads to p16 overexpression. This interferes with cell cycle control and promotes genetic instability and cancer progression[11,12]. However, diffuse p16 positivity by immunohistochemistry is an accurate and reliable method for predicting HPV infection in both high and low-grade cases of OED and OL[13]. Nevertheless, the combination between p16 and genotyping obtained by polymerase-chain-reaction (PCR), could accurately determine HPV infection[14].

In a recent systematic review, South America was the geographical area with the least research on this topic but with the highest rates of HPV[15]. There are heterogeneous results about the frequency of HPV in OL and OSCC. This diversity could be explained by different conditioning factors. The geographical variation in HPV distribution may be due to sexual and cultural habits of the population, ethnicity, or the prevalence of other etiologic factors for OED, such as alcohol and tobacco consumption[15]. Due to a limited number of South American studies addressing this issue, it is important to achieve new original studies. The aim of this study is to describe the frequency of HPV genome and p16 immunoexpression in a cohort of patients with OL and PVL in Argentina. Secondarily, it is proposed to analyze the association between these oral conditions, clinical variables, and associated risk factors.

Material and methods

A cross-sectional study was carried out at the Oral Medicine Department, Facultad de Odontología, Universidad Nacional de Córdoba. There were included 33 patients diagnosed with OL and PVL (according to the criteria of WHO consensus, 2020[16]) between 2017-2019.

The inclusion criteria were patients with clinically and microscopically confirmed diagnosis of OL or PVL, availability of Formalin-fixed and paraffin-embedded (FFPE) and oral exfoliated cells obtained by smears from the lesions, and patients who agreed to give their authorization and sign the informed consent. The exclusion criteria were patients who received previous treatment for OL, PVL, or any type of cancer or prior diagnosis of other OPMD.

Clinical Exam and record of clinicopathological variables

The patients were examined by two calibrated specialists in Oral Medicine. The following variables were recorded in the medical history: age, gender, location of the lesion, tobacco consumption and cumulative exposure to tobacco, alcohol consumption, mate consumption and infusion temperature, and the presence of traumatic sources of chronic mechanical irritation (CMI) (CMI was clinically recorded considering Piemonte et al criteria[17]). In all cases, the biopsy site was selected by three clinicians combining visual inspection, palpation, and Toluidine Blue vital staining. Biopsies from PVL specimens were taken from malignancy-suspicious areas, verrucous lesions, or erythrospatic foci.

For all cases, histopathological diagnosis was performed using conventional hematoxylin-eosin techniques, and recording OED grade[18]. Histopathological findings suggestive of HPV- associated OED were also recorded (karyorrhexis, mitosoid bodies, hyperchromatic nucleus and bright cytoplasmic eosinophilia, dyskeratosis "apoptotic cells"[7,19]).

HPV DNA analysis

The detection of the viral genome was carried out by PCR technique from oral exfoliated cells obtained by smears of the lesions. Smear was performed at the same sites selected to diagnostic biopsies (from exactly the same anatomic subsite).

Viral DNA was isolated from samples obtained with sterile endocervical brushes (Cytobrush K-Kaution Yangzhou Jiangsu, China and Medibrush Plus, Medical Engineering Corporation S.A., Argentina) which were placed in sterile Eppendorf tubes. Oral brush samples were centrifuged for 10´ at 1200 x g. The pellet was resuspended in 200 ul of PBS and the QIAamp DNA Mini Kit (QIAGEN) column purification protocol was followed. Viral genome detection was made by PCR (primers MY09/11 y Gp5+/6+)[20]. As a control, a region of the human Beta globin gene was amplified. The amplification protocol was 3 min at 94°C, 1 min at 94°C, 1 min at 55°C and 1 min at 72°C for 35 cycles and finally at 72°C for 5 min. In positive cases, genotyping was carried out by PCR-RFLP and electrophoresis in 2% agarose gels. The following enzymes were used for digestion: Rsa I- Dde I- Hinf I- Hae III- Pst I - Bam H1 - Sau 3AI. The primers used and the genotypes detected with this technique were those described by Bernard et al, 1994[21].

Immunohistochemistry (IHC) with p16

Sections from paraffin-embedded biopsies were prepared, and immunohistochemistry was performed. FFPE blocks were used to prepare 4-µm-thick sections that were placed

onto positively charged slides. Subsequently, Mouse monoclonal antibody CINtec p16 Histology, Ventana brand, code 705-4713 and UltraView Universal DAB detection kit, from Ventana, code 760-500, counterstaining with Hematoxylin and Ventana bluing, was used and processed in Benchmark GX equipment following the equipment protocols and reagent manufacturer's instructions.

The expression pattern of p16 was studied in the entire length of the epithelium in all specimens. The German semiquantitative scoring system[22] was adopted for the scoring of p16 immunostaining and was graded as Grade 0 (negative staining or < 5% positivity), Grade I (sporadic, 5–10% of nuclear & cytoplasmic positivity), Grade II (focal positivity with > 30% of labelled cells spreading in one tissue area), and Grade III (> 85% of labelled cells spreading in several tissue areas). Grade III was considered as p16 positive. An oropharyngeal-HPV-positive cancer section was taken as a positive control. Semi-quantitative analysis of p16 was evaluated by two blinded investigators and the mean was taken as the final result to prevent interobserver variability.

Subsequently, the cases were subdivided into four subgroups 1) HPV-PCR + / p16 -, 2) HPV-PCR + / p16 +, 3) HPV-PCR - / p16 -, 4) HPV-PCR - / p16 +. The detection of the HPV genome in oral mucosa by PCR could represent an active or a transient infection, that is not linked to host damage or a pathological phenomenon. PCR-HPV+/p16- could be related to a transient infection that is biologically not relevant. HPV-PCR+/p16+ refers to an active infection displaying a biological phenomenon linked to dysplastic or neoplastic changes in the oral mucosa. Consequently, HPV status was considered positive when HPV-PCR and p16 were both positive.

Statistical studies

The qualitative variables were expressed through their absolute and relative frequencies expressed as a percentage. The concordance between HPV-PCR results and p16 was analyzed using the McNemar test. Indicator variables for HPV infection (HPV-PCR & p16) were analyzed concerning the clinical-demographic characteristics using the Fisher test. Infostat software version 2015 was used.

Ethical declarations: All patients signed the informed consent. The present study was approved by the Committee of Ethics in Research in Health Sciences (CIEIS), Facultad de Odontología, Universidad Nacional de Córdoba, Argentina (Protocol 11-T 2016).

Results

In our study, a total number of 33 patients with OL and PVL diagnoses were included. Of these patients, 69.7% (n=23) were women, while the remaining 30.3% were men (n=10). Patients were aged from 24 to 86 years old, with an average age of 61.48 years and a median of 63 years. The average age according to sex was similar (61.65 in women and 61.16 in men). The location on the ventrolateral tongue was the most frequent among the patients (n=19; 58%). The subsequent sites involved were gingiva (n=5, 16%), buccal mucosa (n=6, 16%), and palate (n=3, 10%).

Table 1 shows demographic, clinicopathological features, and HPV tests performed. Of 33 included patients, 70% corresponded to unifocal OL (n=23) and 30% (n=10) to PVL. Regarding the histopathological findings, 24.24% (n=8) corresponded to hyperkeratosis without dysplasia, 36.36% (n=12) to mild dysplasias, 30.30% to moderate dysplasias (n=10) and 9.9% (n=3) corresponded to severe dysplasias. The three cases of severe dysplasias were female patients without classical risk factors (non-smokers-non-drinkers). Histological patterns typical of koilocytic dysplasias or HPV-associated OED were not observed in any case.

Among them, 75.75% (n=25) were smokers and 48.48% were alcohol drinkers (n=16). The average pack/years (p/y) was 24.27 p/y. Regarding the clinical exam, it was noted that 54.54% (n = 18) of OL were associated with CMI sources. The combined origin of trauma (fixed or prosthetic factors added to a parafunctional habit) was the most frequent source of CMI (72.22%; n=13).

Of 33 OL samples, the HPV genome was detected by HPV-PCR in 48.5% (n=16) of the cases. The most common viral genotype was HPV16 (n=6), followed by low-risk HPV6 (n=2), HPV11 (n=2), and HPV84 (n=1). HPV31 was also detected in one case. In four cases of PCR-HPV +, genotypification was not possible. The p16 protein was positive in 27% (n=9) of the cases, being more frequent to find positivity in women than in men (43% vs 11%). The distribution of cases according to HPV-PCR and p16 is shown in Table 2. The concordance between the HPV-PCR results and p16, analyzed by the Mc Nemar test was 0.1185 and by the Cochran-Mantel-Haenszel test was 0.5788 (poor or weak agreement).

In relation to HPV-PCR + cases of OL, 81% of them (n=13) were associated with CMI, 75% (n=12) were smokers and 50% (n=8) were drinkers. Except for CMI with HPV-PCR + (OR=10.4, CI=95% 2.03-53.2, p=0.0049), and tongue location with HPV-PCR + (OR=5.42, CI=95% 1.19-24.5, p=0.0366), it was not observed a significant statistical association between the clinical-demographic variables and the HPV indicator variables (PCR & p16) (Table 3). This scenario is evidenced in the cases represented in Figure 1, Figure 2, and Figure 3.

Discussion

The association of HPV with OL is considered a controversial topic. Some research groups identified high-risk HPV and viral DNA in OPMD[23]. However, HPV was also reported in healthy oral mucosa as well[24]. In a recent meta-analysis of 36 original studies, 24 of them presented data on the frequency of HPV in OL, with scarce or no combination of tests of HPV

Table 1. Demographic, clinical and biomolecular characteristics.

Variable	Category	n	%	p
Age	<63	16	48.48	0.8618
	63 o +	17	51.52	
Sex	Female	23	69.7	**0.0236**
	Male	10	30.3	
Site	Multifocal	6	18.18	**0.0003**
	Unifocal	27	81.82	
Tongue	Tongue-	19	57.58	0.3841
	Tongue+	14	42.42	
Diagnosis	Leukoplakia	23	69.7	**0.0236**
	Proliferative Leukoplakia	10	30,3	
HPV-PCR	PCR-	17	51.52	0.8618
	PCR+	16	48.48	
p16	p16-	24	72.73	**0.009**
	p16+	9	27.27	
HPV	HPV-	28	84.85	**0.0001**
	HPV+	5	15.15	
Dysplasia	Mod/sev dysplasia	13	39.39	0.223
	No/mild dysplasia	20	60.61	
Inheritance	Inheritance-	22	66.67	0.0555
	Inheritance+	11	33.33	
Arsenic	As-	24	72.73	**0.009**
	As+	9	27.27	
Tobacco	Tobacco-	8	24.24	**0.0031**
	Tobacco+	25	75.76	
Cumulative Tobacco	<10 pack/ year	16	48.48	0.8618
	10 pack/year or more	17	51.52	
Alcohol	Alcohol-	16	48.48	0.8618
	Alcohol+	17	51.52	
Mate	Mate hot/ veryhot	15	45.45	0.6015
	Mate no/ warm	18	54.55	
Chronic mechanical irritation (CMI)	CMI-	15	45.45	0.6015
	CMI+	18	54.55	

detection, and with only two studies from South America[25]. A recent study from Sweden, Brazil and Romania showed that the prevalence of HPV in OL is low[26].

It is challenging to assign a causal role to HPV in OPMD since there are different results in terms of its frequency. There were described several determinants of these inconsistencies, including viral detection and material collection techniques, as well as the HPV heterogeneous geographical distribution. Nevertheless, there were found few studies that combined two or more HPV detection techniques and correlated them with clinical and histopathological variables. The single-use of lab test for HPV diagnosis could reveal limited results. It was suggested that other assays such as HPV-PCR for HPV DNA, HPV16 / 18 fluorescent in situ hybridization (FISH), and p16 IHC have suboptimal performance when used as stand-alone tests, with FISH being the gold standard[27]. Hence in this study, a combination of HPV-PCR and p16-IHC was used to ascertain positivity.

Some studies addressing HPV infection in OL by combining HPV-PCR and p16 were found in the current literature. In the study by Sushma et al, it was found that 86% of OL with HPV-PCR + and 10% were positive for p16 by IHC. The study included 50 cases of OL. They also found viral DNA in all cases with positive p16 by immunohistochemistry. The most frequent subsite was the buccal mucosa due to the origin of the patients characterized by specific habits linked to gutkha chewing[28]. Bhosale et al did not detect HPV infection with PCR and p16[29]. Sundberg et al did not find viral DNA in OL, however, they found 17% of cases with p16 overexpression[2]. Yang et al showed that the prevalence of HPV DNA in exfoliated cells from OL was 4.9%. Among them, the patients were predominantly male, without risk habits, located on the tongue, and with a histopathological diagnosis of mild dysplasias. Furthermore, 43 cases (41.8%) were p16 over-expressed. Although p16 expression did not correlate with clinical variables, it was correlated with HPV positivity[14]. In our study, we found 48.4% of OL positive for HPV- PCR and 27.2% positivity for p16, with no correlation between the variables. Only in 5 cases (15.15%), both techniques were positive. In agreement with them, the frequency of HPV infection in our study was low.

Previous studies combining HPV-PCR and p16 show little association between both techniques. Our results are positioned in intermediate values for each of the techniques according to the obtained results of other authors. The variability of HPV-PCR and p16 separately could reflect in these studies the influence of geographical and cultural variability on the prevalence of HPV. However, the absence of association between HPV-PCR and p16 could also be associated with modifications in the techniques used or with factors not yet fully identified.

The differences between HPV-PCR and p16 found in our study (48.4% vs 27.2%) and the poor concordance between both techniques to evaluate HPV infection, lead to consider several aspects of viral infection and its role in oral carcinogenesis.

Table 2. Correlation between HPV-PCR and p16

	PCR +	PCR -	Total
p16 +	5 (15.15%)	4 (12.12%)	9 (27.27%)
p16 -	11 (33.33%)	13(39.39%)	24 (72.73%)
Total	16 (48.48%)	17 (51.51%)	33 (100%)

The detection of viral DNA in 48.4% of our cases, in the absence of p16 positive samples, could indicate a transient infection or the finding of a biologically trivial infection[30]. Thus, HPV-DNA detection by PCR cannot distinguish whether HPV is involved in a transient or an active infection. The expression of E6/E7 mRNA is considered the gold standard for HPV infection and the result shows a high consistency with p16 IHC[9]. Although p16 may serve as a good prognostic indicator, our study demonstrated that it is not satisfactory when used exclusively as the only HPV detecting method. A recent study from Tomo et al found p16 immunoexpression in OL without HPV detection by linear array, suggesting that p16 is not dependent on HPV in OL[31].

In our study, except for lingual location and the presence of CMI, no other variables presented a statistically significant association with HPV detection techniques. The role of CMI in HPV infection was an addressed issue mentioned in previous studies. Nevertheless, this consideration is difficult to prove in humans and consequently, this association is supported by hypothetical evidence. Previous experimental models showed that a previous wound at the site of inoculation improves the infection by Papilloma Virus and CMI could stimulate the reactivation of a latent Papilloma virus[6]. This relationship could be explained by the biological requirement for the presence of a minor wound that facilitates the entry of the virus into immature cells of the basal layer, which express

Table 3. correlation between clinical variables and HPV-PCR, p16, and HPV status

		HPV-PCR			p16			HPV status		
Clinical variables	Category	PCR-	PCR+	p	p16-	p16+	p	HPV-	HPV+	p
Age	<63	6	10	0.1694	10	6	0.2587	13	3	0.6562
	63 o +	11	6		14	3		15	2	
Sex	Female	13	10	0.4646	15	8	0.2166	19	4	0.6642
	Male	4	6		9	1		9	1	
Site	Multifocal	5	1	0.1748	5	1	0.6546	6	0	0.5563
	Unifocal	12	15		19	8		22	5	
Tongue	Tongue-	13	6	0.0366	13	6	0.6982	16	3	0.905
	Tongue+	4	10		11	3		12	2	
Diagnosis	Leukoplakia	10	13	0.2587	17	6	0.9999	19	4	0.6642
	Proliferative leukoplakia	7	3		7	3		9	1	
Dysplasia	Mod/sev dysplasia	4	9	0.0799	8	5	0.4251	10	3	0.36
	No/mild dysplasia	13	7		16	4		18	2	
Inheritance Morbida	Inheritance-	10	12	0.4646	17	5	0.4555	19	3	0.9999
	Inheritance+	7	4		7	4		9	2	
Arsenic (As)	As-	14	10	0.2587	17	7	0.9999	21	3	0.5971
	As+	3	6		7	2		7	2	
Tobacco	Tobacco-	4	4	0.9999	4	4	0.1695	6	2	0.5736
	Tobacco+	13	12		20	5		22	3	
Cumulative Tobacco	<10 pack/year	7	9	0.4935	14	2	0.1175	15	1	0.3353
	10 pack/year or more	10	7		10	7		13	4	
Alcohol	Alcohol-	8	8	0.9999	12	4	0.9999	12	4	0.1748
	Alcohol+	9	8		12	5		16	1	
Mate	Mate hot/veryhot	6	9	0.3028	10	5	0.9999	12	3	0.9999
	Mate no/warm	11	7		13	5		15	3	
Chronic mechanical irritation (CMI)	CMI-	12	3	0.0049	10	5	0.6968	13	2	0.9999
	CMI+	5	13		14	4		15	3	

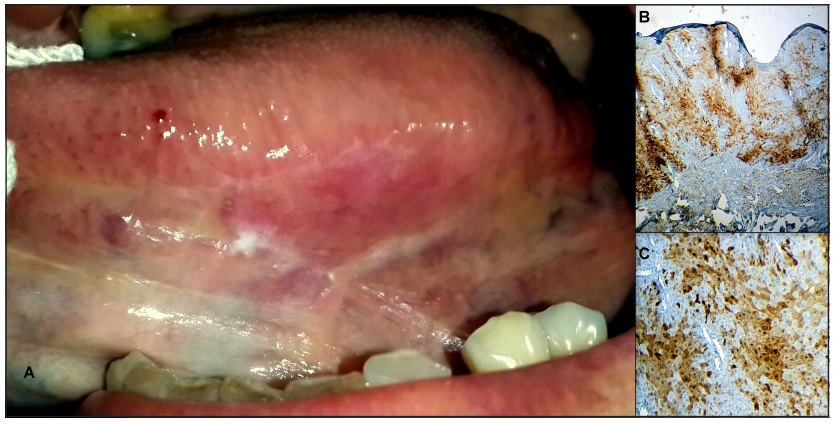

Figure 1. A. A non-smoker, non-drinker, 62-year-old woman with a white plaque mixed with slightly red components with an extended rough surface located on the ventrolateral tongue. PCR-HPV +, genotype 16. **B and C.** Positive immunohistochemistry for p16 involving all epithelial strata.

specific membrane receptors. Receptors from the family of integrins and syndecans are usually overexpressed when the epithelium is exposed to mechanical trauma[32]. CMI generates a hyperproliferative status of the oral epithelium, increasing the persistence, even in suprabasal strata, of immature cells (immunolabelled with cytokeratins 19) with an immunohistochemical pattern similar to basal keratinocytes[33]. This phenomenon linked to CMI could facilitate the persistence and the viral infection of the oral mucosa.

The statistical association between the presence of HPV-DNA, lesions located on the ventrolateral tongue, and CMI sources was also considered by other authors. In this regard, Perry et al showed that OSCCs occur predominantly at sites of potential CMI. These authors highlighted the relationship between HPV infection in CMI-oral subsites (such as the mobile tongue) and the frequency of OSCC[34]. Such clinical variables are associated with HPV-PCR and not with p16. Therefore, CMI when affecting the lateral tongue could increase the risk for viral entry to basal keratinocytes. However, other poorly understood biological events should be studied to understand the persistence of an effective HPV infection relevant to oral carcinogenesis.

PVL is an oral condition originally associated with HPV infection. However, Bagan et al showed a lack of association between this PVL and HPV[35]. Interestingly, a recent metanalysis showed that the pooled HPV prevalence in PVL was 24.7% (95% CI 1.8-62.0) with a wide range of detection[23]. In our study, 3/10 patients diagnosed with PVL showed p16 and PCR-HPV positive results. The weak association between HPV-DNA presence and PVL and OL may be explained by the hyperkeratosis which characterizes these oral conditions, avoiding access to the viral genome[36]. However, if OL is chronically traumatized, ideal conditions for viral entry could be created.

Another possibility is that HPV infection leads to a dysplastic transformation via its oncogenic stimulus. Then, the virus would be removed as a result of the cellular exfoliation or the host immune system action. This mechanism, already proven in some cervical cancers, is called the "hit and run theory" and results in an HPV-induced tumor not harbouring the viral DNA anymore, neither in the episomal nor integrated form[36].

While the frequency of HPV in the healthy population in Córdoba, Argentina is 3%[24], and in other countries of the region, such as Brazil, 6.2%, this percentage increases to 44% in oral conditions such as OL[37]. Our study shows a similar percentage of HPV infection (by PCR-HPV) in OL with 48.5%. This displays that the behaviour of HPV infection within OPMD could be similar in different areas of South America. However, HPV genotyping shows differences. The most frequently detected genotypes in OL in the study of Della Vella et al were HPV6 and 11[36]. In the Brazilian population, the most frequent genotypes of HPV in OL were HPV16 and HPV18 alike, while in our population the most frequent was HPV16, followed by low-risk HPV[6,11,84]. Interestingly, another study conducted in Córdoba, Argentina, detected HPV84[38]. In one patient of our cohort of OL, HPV84 was also detected. This genotype is infrequently found in the region with some studies describing its presence in HIV-positive patients[39]. The most frequent HPV genotypes found in our study agree with the HPV genotypes reported in this same population but obtained from cervical samples[40]. Consequently, the tetravalent and nonavalent HPV vaccines could cover the most frequent spectrum of HPV in our population.

In this series, HPV16 was the most prevalent genotype, followed by low-risk HPV[6,11,84]. The most frequently detected genotypes in OL in the study of Della Vella et al were HPV6 and 11. They are considered not carcinogenic by the International Agency for Research of Cancer (IARC), due to their poor interference with the host cell life cycle. Nevertheless, low-risk genotypes were found in vaginal intraepithelial neoplasia and larynx and nasopharyngeal lesions, with a higher prevalence than high-risk HPV[36]. These findings suggest that in a subset of OL and other OPMDs, low-risk HPV could also play a co-carcinogenic effect within the multifactorial and multistep process of oral carcinogenesis.

Limitations: The follow-up and clinical monitoring of these patients over time was not a variable included in this study. Because of the evolution of OSCC in some patients with high-risk HPV-associated leukoplakia, further studies are needed to evaluate the biological behaviour of these OPMD and OED associated with a viral infection.

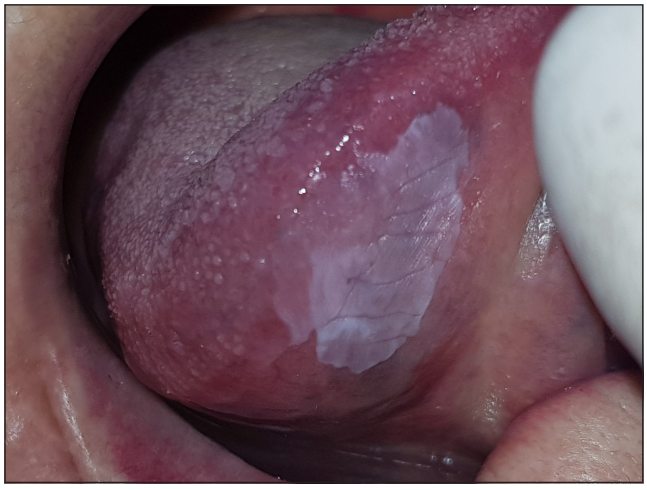

Figure 2. A non-smoker, non-drinker, 80-year-old woman with a white lesion located on the ventrolateral tongue. PCR-HPV + not identified, p16 positive.

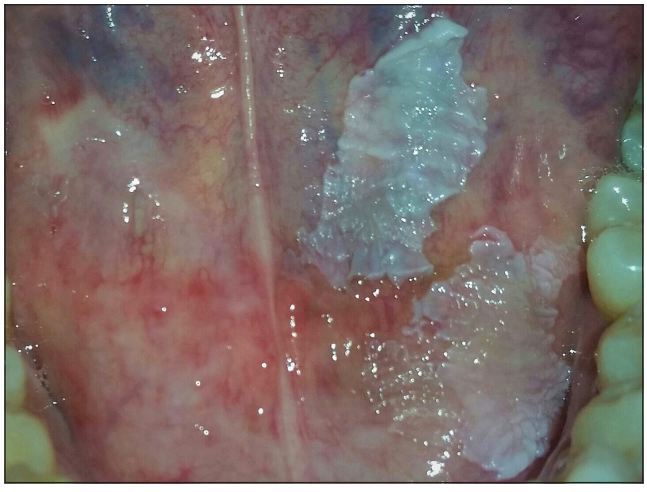

Figure 3. A light smoker, non-drinker, 40-year-old woman with a white plaque with a verrucous surface located on the ventral tongue. PCR-HPV + not identified, p16 positive.

The small number of patients included in the present study could be considered a limitation. Nevertheless, the existing results and the clear absence of statistical significance suggest that even increasing the population under study, would have been unlikely to modify the final results. Due to the evolutionary cycle of HPV, it would have been ideal to follow up on oral lesions by monitoring HPV status. Nevertheless, that would not have ensured that a negative test turns positive either. Although p16 in combination with HPV-PCR has shown adequate sensitivity and specificity to be used together as indicators of HPV infection, greater reliability could have been obtained by performing HPV ISH and detection of E6E7RNAm, which were not performed because of limited resources.

In conclusion, the low frequency of oral HPV infection in OL detected by HPV-PCR and p16 agrees with previous studies, even from those developed in other geographical areas. Further studies are needed to correlate clinical settings with molecular and viral detection variables to establish possible links between this viral infection and other oral conditions. CMI could play a role in HPV infection of OL, mainly those located on the lateral tongue border.

Ethical considerations

All patients signed the informed consent. The present study was approved by the Committee of Ethics in Research in Health Sciences (CIEIS), Facultad de Odontología, Universidad Nacional de Córdoba, Argentina (Protocol 11-T 2016).

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Conflicts of Interests. The authors declare no conflicts of interest for this study.

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