

HUMAN PAPILLOMA VIRUS IN ORAL MUCOSA AND ITS ASSOCIATION WITH PERIODONTAL STATUS OF GYNECOLOGICALLY INFECTED WOMEN

Liliana Fuster-Rossello¹, Estela Ribotta¹, Cecilia Cuffini², Margarita Fuster-Juan³

¹ Department of Periodontics, Independencia Foundation for Advanced Studies in Dentistry, Cordoba, Argentina

² Institute of Virology, National University of Cordoba, Argentina

³ Department of Gynecology, Maternity and Neonatology, National University Hospital of Cordoba, Argentina

ABSTRACT

The aim of this study was to determine whether Human Papillomavirus was present in tongue and periodontium of periodontally healthy and diseased women who had genital lesions caused by the virus. Thirty non-menopausal women, systemically healthy and diagnosed with gynecological HPV lesions, were referred by the Gynecology Service Department of the University Maternal Neonatal Hospital of the City of Córdoba. Anamnesis, oral mucosa examination and periodontal clinical assessment were performed. Three brush samples were taken per patient: two from the same periodontal location (external epithelium of the gum and internal epithelium of the periodontal sulcus/pocket), and the third from the tongue. The 90 samples were submitted to Pap cytology and Polymerase Chain Reaction. The data were statistically analyzed by "Chi Square Test" (χ^2) and "Kappa Index"

(κ). High prevalence of HPV was found in the tongue (30%) and periodontal tissues (15%). High risk (HR) genotype -16 was detected with the highest percentage (67%), and genotypes -52 and -6 were also detected. Whenever HPV was present in periodontal location, it was also identified in the tongue of the same patients, of whom 88.89% reported that they practiced oral sex. It is worth noting the clinical finding of stomatologic lesions compatible with foliate papillitis in patients with positive intraoral HPV. High prevalence of HPV was found in the female population in Córdoba, with genotype -16 being detected at the highest percentage. No positive correlation was found between HPV and higher incidence and severity of periodontal lesions.

Key words: Human Papilloma Virus; oral mucosa; periodontal disease

VIRUS PAPILOMA HUMANO EN MUCOSA BUCAL Y SU RELACIÓN CON EL ESTADO PERIODONTAL DE MUJERES GINECOLÓGICAMENTE INFECTADAS

RESUMEN

El objetivo del estudio fue identificar la presencia del virus Papiloma Humano en lengua y periodonto de mujeres sanas y enfermas periodontales con lesiones genitales del mismo. Se evaluaron treinta mujeres, no menopáusicas, de entre 18 y 50 años de edad, derivadas del Servicio de Ginecología del Hospital Universitario Materno Neonatal de la ciudad de Córdoba, sistémicamente sanas y con diagnóstico ginecológico de lesiones por HPV. Se realizó, anamnesis, inspección de mucosas bucales, examen clínico periodontal y la toma de tres escobillados por paciente, dos de un mismo sitio periodontal (epitelio externo de encía y epitelio interno del surco/bolsa periodontal) y el otro de lengua. Las 90 muestras obtenidas fueron sometidas a estudios citológicos de Papanicolaou y a estudios moleculares de amplificación de ácidos nucleicos por Reacción en Cadena de la Polimerasa. Los datos fueron agrupados y analizados por el "Test Chi Cuadrado" (χ^2) y el "Índice de Kappa" (κ). Fue demostrada la alta prevalencia

de la presencia del virus papiloma tanto en lengua (30%), como en tejidos periodontales (15%). El genotipo -16 de alto riesgo (HR) fue identificado en mayores porcentajes (67%) encontrando, también, el genotipo -52 y el -6. Siempre que el HPV estuvo presente en los sitios periodontales fue detectado, también, en la lengua de las mismas pacientes, de las cuáles el 88,89% practicaba sexo oral. Se destaca el hallazgo clínico de lesiones estomatológicas compatibles con papilitis foliada en las pacientes HPV intrabucal positivas. Se señala en la población femenina de Córdoba, Argentina, la alta prevalencia de la presencia de HPV donde el genotipo -16 fue detectado en mayor porcentaje. En la muestra analizada no fueron positivamente correlacionados la presencia de HPV y la mayor incidencia y severidad de lesiones periodontales.

Palabras clave: Virus papiloma humano; mucosa oral; enfermedad periodontal

INTRODUCTION

Periodontitis results from the complex interaction among infectious agents which, coupled with host susceptibility, promotes the destruction of the supporting tissues of teeth¹⁻³.

Even though there is no doubt that bacteria are responsible for periodontal diseases, they do not

explain the diversity and magnitude of the clinical manifestations found^{4,5}.

Findings and implications in the pathogenesis and severity of Cytomegalovirus (CMV), Epstein-Barr Virus type 1 (EBV-1) and Herpes Simplex Virus (HSV)⁶⁻¹⁰ in some of these diseases encouraged the search for other viral agents capable of residing in

healthy and unhealthy oral tissues, but which have not been linked to periodontal pathologies.

Human papillomaviruses (HPVs) that belong to the Papillomaviridae family consist of a group of epitheliotropic viruses associated with cell proliferative processes and may be benign (warts and condylomas) and/or pre-malign or malign (leukoplakia and carcinomas) in the stratified epithelia of the skin and mucosa. HPV virions are small particles 55nm in diameter composed of a double-stranded circular chain of 8,000 pairs of DNA bases whose amino acid sequences classify them into more than 100 genotypes, inside a naked icosahedral capsid shell.^{11,12} HPVs with mucous tropism infect the anus-genital area and the oral-larynx-trachea-bronchial mucosa¹³⁻¹⁷. Based on their association with cervical cancer, they are classified as: high-risk, including the predominant types -16, -18, -31 and -45; and low-risk viruses such as -6, and -11^{18,19}. Papillomavirus -16 has been considered a single infection in more than 80% of squamous cell carcinomas of uterine cervix and in 30% of the oropharynx, and it also shows a strong association with tonsil cancer.²⁰⁻²² Table 1 shows the detection of the Papillomavirus in oral mucosa and its identification method as reported by several authors^{6,23-26}.

HPV is biologically linked to differentiation program of the host's basal keratinocytes. The basal cells of junctional epithelium directly exfoliate the gingival sulcus and have a high capacity for proliferation and cell renewal. Syndecan-1 and heparan sulfate proteoglycans -specific cell surface receptors in susceptible individuals- have been shown to be present in these marginal periodontium epithelial cells. They represent basic conditions for the initial adhesion, subsequent biology and survival of the HPV^{27,28}.

In addition to these features, this epithelium is non-keratinized, permeable, fairly thin, and immersed in a humid environment which creates ideal conditions for viral development²⁹. Similar circumstances

are observed in the cervical anatomical transition zone, between the mature epithelium of the exocervix and the stratified epithelium of the endocervical canal, the preferred location for the virus^{30,31}. Epidemiological studies have shown that HPV infections of the female genital tract are the most common sexually transmitted diseases around the world¹⁸.

Oral transmission can occur by oral-genital or oral-oral sex, or by autoinoculation^{32,33}.

It is suspected that the tongue facilitates intra-oral contamination from one infected site to another, in addition to enabling horizontal and vertical microbial transmission among humans, which would justify the need to research other mucosal reservoirs.¹⁷ As the presence of HPV modifies the biological reactions of tissues, the aim of this study was to analyze the periodontal status of women with HPV genital lesions and detect whether there was also presence of HPV in the periodontal sulcus and/or pocket and the tongue.

MATERIALS AND METHODS

This cross-sectional observational study was approved by the Ethics and Discipline Committee of the Hospital Universitario Materno Neonatal of Córdoba, HUMN, Argentina, following the principles of the Declaration of Helsinki. It was carried out from March 2006 to February 2008. All subjects signed an informed consent to participate.

The sample consisted of 30 non-menopausal women between the ages of 18 and 50 (32 ± 8 years old), with HPV gynecological diseases, referred by the medical team involved in the Gynecology Service of HUMS, who contributed with systemic, colposcopic, cytological and histopathological clinical studies through biopsies. Only the patients presenting endocervical diseases, such as epithelial cell anomalies (CIN I, CIN II and CIN III) were referred.

Table 1: Detection of the Papilloma Virus in Marginal Periodontium.

Reference	Study Site	Sampling method	Viral diagnosis	HPV%	Type of HPV studied/ HPV found
Parra and Slots 1996	Gingival crevicular fluid	Moistened paper point	PCR	17	-
Saglam et al., 1996	Tissue	Biopsy	PCR	100	-
Bustos et al., 2001	Tissue	Biopsy	PCR	92.31	-
Hormia et al., 2005	Tissue	Biopsy	PCR	26	Pull high risk / 6, 11, 16
Horewicz et al., 2010	Tissue	Biopsy	PCR	0	16 / 0

Inclusion and Exclusion Criteria

Systemically healthy non-menopausal women with gynecological diagnosis of HPV diseases, having at least 12 teeth distributed in both maxillae, were included. Pregnant women and women with severe systemic diseases which might affect the progression of periodontal disease (e.g., diabetes and immunologic disorders), presence of fungal oral infections or oral infections by other viruses were not included. Patients medicated with non-steroidal anti-inflammatory drugs, corticoids, antibiotics and antiseptics within 6 months prior to the beginning of the study were also excluded.

Study Design and Measurement of Clinical Periodontal Condition

Oral and periodontal examinations were performed on all subjects. A single calibrated operator recorded all the indices and parameters. The calibration was done at Independencia Foundation, and inter- and intra-operator agreement kappa (k) was 0.85 to 0.90. Plaque index (PI), periodontal probing depth (PD), clinical attachment level (CAL), and gingival bleeding (BOP) were recorded at 6 places for all the teeth in the mouth, using a Williams probe marked out in millimeters (Hu Friedy, Chicago, IL).

The periodontal diagnosis was based on the current classification of the American Academy of Periodontology, where the patients with chronic periodontitis presented ≥ 6 teeth, each with at least one site with a PD and CAL ≥ 5 mm. Women with gingivitis presented $\geq 20\%$ of sites with plaque, gingival inflammation signs and bleeding upon gentle probing where PD or CAL measurements were not > 4 mm. Periodontally healthy subjects did not have PD or CAL > 4 mm and $< 20\%$ of sites showed bleeding upon gentle probing³⁴.

Sampling Strategy

Ninety samples were taken using oral swabs: 30 from the tongue and 60 from periodontal sites (healthy subjects and subjects with gingivitis or periodontitis). These were collected from the external and internal (sulcus or pocket) gingival epithelium of a single periodontal site, and tongue (three from each subject), to be tested by both cytological Papanicolaou (PAP) studies and Polymerase Chain Reaction (PCR) Technique.

Periodontal samples were taken after removing supragingival plaque with sterile cotton swabs and

isolating the sites with cotton rolls. The internal gingival walls were swabbed with a sterile micro brush (Dental Micro Brushes, small, Microbrush International) which had proved suitable for collecting epithelial cells in a pilot test; and the external walls of the same sites were swabbed in the same way with another identical brush. For the tongue samples, the patients were asked to swallow several times, after which a sterile cytology collector brush (Endocervical collector brush, Medisul) was used to swab ventral portions and borders of the tongue. The brushes were placed in sterile plastic tubes with TE buffer (10ml of Tris-HCL (ph 6.4) in 1ml of EDTA 0.2M (ph 7.5), which were labeled with the sampling sites and patient's name, and kept at 4°C until the nucleic acid was extracted³⁵. Before placing the micro brushes and/or cytology collector brushes in the tube for processing, a smear of each sample was made on a sterile microscope slide, fixed with lacquer and identified for subsequent histological studies.

PCR and PAP Studies

DNA was detected by polymerase chain reaction (PCR) with MY09 and MY11 "primers" directed towards the L1 protein encoding gene, common to all mucosa-tropic papillomaviruses. To confirm the quality of the samples, a PCR targeting the coding region of β -globin was done. Then the genotype was obtained through Restriction Fragment Length Polymorphism (RFLP), with restriction enzymes³⁶⁻³⁸. For the cytological diagnosis, the pathognomonic sign of HPV infection is constituted by the presence of koilocytes, or "empty cells", described as epithelial cells of relatively small and irregular nucleus surrounded by a clear perinuclear halo, which represents the lethal cell effect of the viral reproduction. Furthermore, other morphological changes such as dyskeratosis, macronucleosis and keratinocytes multinucleation have also been associated with this infection³⁹⁻⁴¹.

Data Analysis

The data were grouped in charts, including two or more variables, using the "Chi Square Test" (χ^2) to verify their association. For the concordance analysis between the diagnostic procedures PCR-Tongue and PAP-Tongue, the "Kappa Index" (κ) was used. The statistical significance was established at $p < 0.05$.

RESULTS

Table 2 shows the characteristics of the study population, including age, gynecological diagnosis, periodontal status, and viral identification methods.

Papillomavirus (HPV) DNA was detected in 30% (9/30), 13.33% (4/30), and 16.67% (5/30) of the samples from tongue and internal and external periodontal sites, respectively. It is worth mentioning that whenever HPV was present, both in the internal and/or the external epithelium of the periodontal site, it was also identified in the tongue. HPV genotype -16 was found at the highest percentage (67%), and genotypes -6 and -52 were also detected. The cytological signs were positive in 10% (3/30) of the samples, represented by macronucleosis, hyperchromasia, isolated binucleation, scattered parakeratosis and dyskeratosis. Only one sample presented perinuclear cytoplasmic halos, whose PCR provided negative results for HPV.

A unique finding worth mentioning is the appearance of foliate papillae in the posterior third of the tongue edge, reddish, enlarged, and sometimes separated by deep grooves, as well as acanthosis appearance, as seen in Fig. 1. Similar signs have been described in the literature as Foliate Papillitis, without having been linked to the presence of the virus^{42,43}. This kind of papillitis was detected in 39% of the study cases, where tongue PCRs were positive in 78% of the samples. Ninety-three percent of the patients reported that they practiced genital-oral sex.

The results of this study did not provide categorical data to strongly associate HPV with periodontal disease.

DISCUSSION

In this study, the virus was found in the tongue of 30% (9/30) of the patients, and in the internal epithelium of 13% (4/30) and external epithelium of the periodontal study sites of 16.6% (5/30). The results confirm previous studies that used molecular biology techniques and found the virus in the normal oral mucosa of the adult population in widely varying percentages (10-13% to 81.1%)^{6,14,16,17,24,25}. The variations in the findings may depend on several factors, such as viral identification techniques, sampling site, and method used to obtain the sample. In the oral cav-

Table 2: Clinical, demographic, PCR and PAP data.

Variable	Categories	Number of Patients	Percentage %
Age	19-27	12	40.00
	28-36	8	26.67
	37-45	10	33.33
Gynecological Data	CINI	19	63.33
	CINII	6	20.00
	CINIII	5	16.67
Periodontal Data	Gingivitis	12	40.00
	Periodontitis	11	36.67
	Healthy	7	23.33
Smoking	Smoker	7	23.33
	Non- smoker	23	76.67
Genital - Oral sex	Negative	2	6.67
	Positive	28	93.33
Foliate Papillitis	Negative	14	46.67
	Positive	16	53.33
PCR - Tongue	Negative	21	70.00
	Positive	9	30.00
PCR - Internal	Negative	26	86.67
	Positive	4	13.33
PCR - External	Negative	25	83.33
	Positive	5	16.67
PAP - Tongue	Negative	27	90.00
	Positive	3	10.00
PAP - Internal	Negative	27	90.00
	Positive	3	10.00
PAP - External	Negative	26	86.67
	Positive	4	13.33



Fig. 1: Foliate papillae showing, reddish, enlarged appearance (A) (B), sometimes separated by deep grooves and acanthosis (C) (D).

ity there are highly keratinized mucosas such as the gum, in which there are very few nucleated cells. This may explain the discrepancy between samples from these areas and samples from the tongue in the same patient.

It is also important to highlight that the burden of HPV infection and disease varies in different regions of the world, with higher prevalence and incidence in low-income countries and in the most deprived sectors of society^{12,18}. Finding the virus in 29.6% of the cytological samples taken from the periodontal sites of the internal and external epitheliums – 13% (4/30) and 16.6% (5/30) respectively – matches the findings of Marketta Hormia, Stina Syrjänen et al., who found the virus in 26% (8/31) of the gingival biopsy specimens from the internal and external epithelium. In said study, HPV was present in the internal epithelium and oral epithelium of only one patient, while all the other positive samples belonged exclusively to the external epithelium²⁵. The variations in the frequency of HPV presence may be due to the different sampling methods, which were obtained through biopsies in other studies²⁴⁻²⁶. Recent publications confirm that the presence of the virus was much greater in molecular tests on cytological samples than in biopsies.

It is also interesting to analyze the study by Parra and Slot (1996) on the presence of different kinds of viruses in the gingival fluid of patients with advanced periodontitis, which found HPV in 17% of the samples⁶. These samples were obtained by placing sterile paper points in periodontal pockets for 20 seconds, a technique which might not obtain epithelium cells properly from all the moistened paper points. In our study, in contrast, it is important to note that in addition to using cytological samples for molecular identification of viruses, a novel sampling technique was used: taking the epithelial swab using micro-brushes. This enabled epithelial cells to be collected more predictably and seemed to be a suitable method for obtaining viable epithelial cells from which viruses could be studied.

When the samples were classified, genotype -16 was detected at highest percentage (67%), and genotypes -6 and -52 were found in two of the samples. Both, -16 and -52 are considered high oncogenic risk. These findings coincide with the research done by Syrjänen (2000) and Miller and Johnstone (2001)^{13,44}, where 37 out of more than 100 virus genotypes were positive in the oral cavity; and HPV-16 was also the most frequently found, followed by -6^{17,21,33}. Consequently, and coinciding with Hornia et al., the epithelium of both oral

and sulcar marginal periodontium could represent a latent site for viral persistency²⁵. It is important to note that in all the patients in whom HPV was present in the internal and/or external epithelium of the selected periodontal site, it was also found in the tongue, so it may be suspected that the tongue facilitates intraoral contamination from one infected site to another, and enables the transmission of the virus among humans both horizontally and vertically³², in addition to which most of the women in the study reported that they regularly practiced oral sex.

The preference of the virus for tonsillar epithelium, described in the literature at percentages higher than 51% in tonsillar carcinomas, may be related to the greater presence of the virus in posterior third of the tongue, where the lymphoid tissue has its greatest expression, and may be related to the enlarged, reddened appearance of the foliate papillae. This is a clinical finding that calls for exhaustive oral inspection^{20,21,23}.

The persistent coexistence of both infections -periodontal and viral- in the same host over time, could be checked by means of longitudinal studies, but not in cross-sectional studies such as ours, which limits this work.

Taking into account the natural history of this viral infection, some patients, whether due to immunologic inability to eliminate the virus or because of periodic genito-oral and oral-oral or other reinfections, could perpetuate its presence in the mouth and determine the difference between a transient and a persistent infection⁴⁵, which may be one of the fundamental conditions influencing proliferative lesions and inflammatory lesions in the oral cavity.

CONCLUSIONS

The study sample was unable to link the presence of Papillomavirus in the oral cavity to periodontal status.

The high prevalence of HPV-16 in the Argentine population examined is noteworthy. Oral sex may be a risk factor for the development of this oral infection, and the results of this study increase interest in this field of research.

It would be of interest to study the coexistence of both periodontal and viral persistent infections in a same host over longer periods of time.

CORRESPONDENCE

Dr. Fuster Rossello Liliana
 Juramento 2743, Parque Chacabuco
 CP 5008, Córdoba-Argentina
 E-mail: lilianfuster@arnet.com.ar

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