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ORIGINAL ARTICLE

Prevalence and risk factors for HPV infection in normal oral mucosa of Chilean dentistry students.

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Abstract: Aim: To determine the prevalence and risk factors for HPV infection in normal oral mucosa of Chilean dentistry students. Materials and methods: A cross-sectional study was performed. The study group was comprised of 103 individuals between 18 and 33 years old. A self-administered survey of cancer family history, sexual habits, smoking and alcohol drinking was applied. Oral mucosal samples were taken using a sterile swab. Subsequently, all samples were analyzed by polymerase chain reaction technique (PCR). Results: Results were negative for HPV detection in all analyses. Of the study population, 58% had a family history of cancer, 40.9% had had more than 3 sexual partners, 76.3% had sexual intercourse before the age of 19, 66.3% had engaged in oral sex, 69.9% drank alcohol and 20.6% were smokers. Conclusions: The group studied is exposed to various risk factors for HPV infection, so it is necessary to educate about the relationship between them and the spread of the virus. Despite the presence of risk factors, the detected prevalence of HPV was 0%.

Keywords: *Oral cavity; Buccal mucosa; PCR; HPV 16; HPV 18.*
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INTRODUCTION.

The human papilloma virus (HPV) belongs to the family Papillomaviridae. There are more than 100 varieties known in humans[1] that specifically infect the basal epithelial layer of the skin and mucosa[2].

Nowadays there is no doubt that HPV infection is responsible for cervical cancer. Based on its ability to generate mutagenic cell changes HPV has been classified into high and low risk. HPV 16 and 18[1] are among the most well-known varieties of high-risk oncogenic genotypes.

In the oral mucosa HPV infection has been identified as a potential risk factor in the genesis of oral cancer, 32.5% of them are positive, caused by a malignant strain of HPV[3]. It is transmitted by direct sexual contact through solutions of continuity in the genital and oral epithelium[1]. In general, the presence of HPV in the oral mucosa, associated or not with clinical lesions, has been favored by the presence of predisposing risk factors that facilitate its spread. These are mainly: sexual habits, family history of cancer, use of immunosuppressive drugs, smoking and alcohol consumption[4-8].

At present, prevalence of HPV in normal oral mucosa reported in international literature ranges from 4.5% to 6.9%. The group with a higher risk of HPV ranges between 3.5% and 3.7%; considering only infection by HPV 16, the range goes from 1.0% to 1.3%[9-10].

In Chile there are no epidemiological data on HPV in normal oral mucosa in the following databases: PubMed, SciELO, Latindex and Ministry of Health. There are only published studies on HPV prevalence in relation to cervical cancer. Therefore, the objective of this study was to determine the prevalence and risk factors for infection with human papilloma virus in normal oral mucosa of Chilean dentistry students.

MATERIALS AND METHODS.

This research was approved in March 2014 by the Ethics Committee of the School of Dentistry at Universidad Andres Bello.

A cross-sectional study based on the genomic detection of HPV 16 and 18 in keratinocytes of the oral mucosa of a group of students was conducted. The sample was selected by convenience.

A target population of 1140 students of the School of Dentistry at Universidad Andres Bello, campus Santiago, in March 2014, was included in the study. The students were informed and invited to participate privately and internally within the institution.

Participants attended the dental clinic between March and October 2014. Each of the volunteers was explained verbally about the study and was given an informed consent, which was read and signed.

Participants were assured about the confidentiality and anonymity of the gathered data. Additionally, they were given a self-administered questionnaire.

Then participants were examined by an oral pathologist, who took a sample of their oral mucosa.

Finally, samples were processed by PCR in the Laboratory of Oral Microbiology and Biotechnology at Universidad Andres Bello, Santiago.

Self-administered questionnaire

Participants received a self-administered questionnaire containing 4 topics: family history of cancer; history of sexual habits, information on sexual activity, age of sexual initiation, number of sexual partners, oral sex practice and protection; history of smoking and alcohol consumption; use of immunosuppressive drugs.

Clinical examination

An oral pathologist examined oral tissues of volunteer students in a dental unit (UniK 4T, Kavo, USA) under dental light (A-dec 500, A-dec, USA) and a dental tray. The criterion for inclusion of students was having an oral mucosa without pathology.

The criterion for exclusion of the subjects was that they were immunized against HPV and/or were affected by diabetes.

Collection of the samples

The researcher used sterile cotton swabs (Deltalab, Chile) to collect the samples, performing a vigorous smear for a standardized time of one minute in the areas of the left and right buccal mucosa, upper and lower attached gingiva, and hard and soft palate.

Samples were stored in sterile Eppendorf tubes (Sorenson, USA) and transported immediately at room temperature for processing.

Sample processing

For processing samples DNA extraction was performed. Each sample was suspended in 560uL of TE buffer, 30uL of SDS (Merck, USA) and 10ul of proteinase K (Merck, USA). Then 600uL phenol: chloroform: isoamyl were added: (25:24:1) (Winkler, Chile).

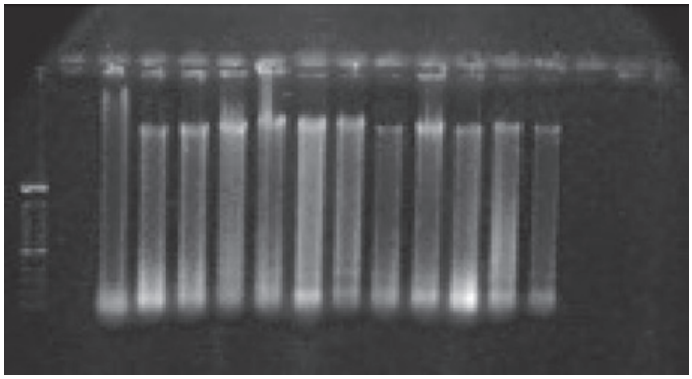
Afterward 400uL of the aqueous phase were removed and put in a new sterile Eppendorf tube. On these 400uL, 40uL of 3M sodium acetate (Winkler, Chile) and 1000uL of absolute ethanol (Merck, USA) at $^{-}$20ºC were added.

Then the obtained sample was kept at $^{-}$4ºC for 24hrs. Subsequently it was centrifuged (MiniSpin, Eppendorf, USA) for 20 minutes, excess liquid was removed and the DNA sample was resuspended in 20ul of sterile distilled water. The success of the technique was verified by 0.8% agarose gel electrophoresis (Lasken, Chile).

After DNA extraction, PCR method was performed; for this, the conventional GP5+/GP6+ primers were used to detect generic HPV and a mix GoTaq® Green Master Mix (Promega, USA). After that, samples were placed in the thermal cycler (PCR System ProFlex, USA) with the following program: 95ºC (5m), 45X (94ºC (30s), 40ºC(30s), 72ºC (30s)), 72ºC (10m).

Once HPV was detected, eight primers were used to specifically detect the presence of HPV 16 and 18, two for the inner chain (inner F and R) and two for the outer chain (outster F and R). Finally, for the presence of HPV 16 and/or 18, electrophoresis with 1.5% agarose was performed. (Fig.1)

Figure 1. Electrophoresis for DNA extraction in 0.8% agarose gel. Bands in each lane indicate the presence of not degraded DNA that can be used for PCR. Microbiology and Biotechnology Laboratory, Universidad Andres Bello.



Data recording

Variables were registered in Microsoft Excel 2014. These were gender, age, sexual habits, alcohol consumption, smoking, history of cancer and use of immunosuppressive drugs, and presence of HPV 16 and 18.

The criteria for microbiological diagnosis of HPV 16 and 18 in the oral mucosa through the PCR technique was based on those reported by Beder *et al.*[5]

Data analysis

Description of the qualitative variables is presented in percentage and frequency distribution tables. The mean and standard deviation was calculated for quantitative variables. Statistical analysis was performed using SPSS 20.0 (IBM, USA).

RESULTS.

A group of 103 students participated in the study. Sample consisted of 74 women (71.8%) and 29 men (28.2%). Average age was 22.8 years ± 3.9. The description of the risk factors studied is shown in Table 1. HPV 16 and 18 were negative in the 103 samples analyzed.

DISCUSSION.

In recent years the scientific community has developed a keen interest in the investigation of HPV and its etiopathogenic role in various cancerous diseases, highlighting varieties 16 and 18 for their high oncogenic potential[12,13]. There are no studies on the prevalence of HPV in normal oral mucosa in the Chilean population, increasing the need for detection.

The results of this study show the absence of HPV 16 and 18 in the oral mucosa of the participants despite the strong presence of risk factors associated with the infection. However, this is not surprising because the technique used for the sampling was carried out on the surface epithelium of the oral mucosa. The virus must be established in the basal layers for a period long enough to allow its replication and relocation to the outer epithelial layers. Therefore, even when the subject is infected with HPV in the basal layers, tests may not show positive results by exfoliating surface cells of the mucous layer.

Our finding is consistent with the findings of Eskenazi *et al.*[14] and Bouda *et al.*[15]. This can be attributed to the PCR technique applied (conventional PCR) and to the fact that the primers used were the same.

On the contrary, the results of this study do not agree with Flake *et al.*[16], Turner *et al.*[17] and Sanchez *et al.*[18], who reported a prevalence of 2.5%, 2.6% and 80% respectively. These differences can be explained by the detection method used[19], the sensitivity of the technique and the types of primers used[18].

Specifically, Sanchez *et al.*[18] obtained samples by scraping with citobrush, obtaining cells from the basal layer. For processing they applied the conventional PCR technique, but with different primers than the ones employed in this study. Flake *et al.*[16] and Turner *et al.*[17] used saliva sample and the processing method was quantitative PCR technique, which has a different sensitivity to conventional PCR technique.

Although we found no presence of HPV in its oncogenic forms 16 and 18 in the oral mucosa of students, they may be considered a population at risk for HPV infection due to the high rate of students who were sexually active before the age of 20, the number of sexual partners, oral sex practice and oral sex without protection.

Sanchez *et al.*[18] and D'Souza *et al.*[7] reported similarly

Table 1. Risk factors for infection with human papilloma virus.

History	Characteristic	n	% validity
Sexual habits			
Sexual activity	Yes	89	87.3
	No	13	12.7
Total		102	100
Type of sexual relationship	Romantic	75	84.3
	Sex worker	3	3.4
	Unknown	0	0
Two of the above categories together	2+	9	10.1
Three of the above categories together	3+	2	2.2
Total		89	100
	0-5	95	95
Number of people kissed with open mouth	More than 6	5	5
Total		100	100
Age of onset of sexual activity	Under 13	1	1.1
	14-16	27	30.3
	17-19	40	44.9
	Over 20	21	23.6
Total		89	100
Number of sex partners	0-2	58	59.2
	03-05	24	24.5
	6-10	13	13.3
	More than 10	3	3.1
Total		98	100
Oral sex practice	Yes	67	66.3
	No	34	33.7
Total		101	100
Number of people you have had oral sex with	1	28	41.8
	2-5	34	50.7
	More than 5	5	7.5
Total		67	100
Use protection when having oral sex	Yes	6	8.8
	No	62	91.2
Total		68	100
Alcohol consumption			
	Drinker	72	69.9
	Non drinker	6	5.8
	Former drinker	25	24.3
Total		103	100
Smoking			
(cigarettes)	Smoker	21	20.6
	Non-smoker	22	21.6
	Ex-smoker	59	57.8
Total		102	
Another type of tobacco	Yes	3	3.3
(tobacco for chewing, snuff, cigars, pipe)	No	87	96.7
Total		90	100
History of cancer	Yes	60	58.3
	No	43	41.7
Total		103	100
Immunosuppressive drugs	Yes	4	4.1
	No	94	95.9
Total		98	100

high rates regarding sexual habits. However, unlike our study, they found a high prevalence of HPV in the oral mucosa in both sexes. This can be attributed to the fact that the patients they studied may have had some type of sexually transmitted disease.

Regarding smoking and alcohol consumption, it can be clearly seen that alcohol is more prevalent than smoking, finding that coincides with those of D'Souza *et al.*[7] The effects of smoking and alcohol on HPV in the oral mucosa remains unclear. On the one hand, stimulation of the harmful elements would cause a thickening of the oral mucosa and eventually increased keratinization of it[14], preventing a potential HPV infection in the basal layer of the oral epithelium. Moreover, smoking could inhibit immune function facilitating the resistance of HPV, in addition to the genetic damage that can cause in keratinocytes.

Only a very small percentage of the students in the sample admitted the use of immunosuppressive drugs, which may be due to the fact that the study population consisted of healthy young people.

The association between immunosuppression and HPV infection is studied mainly in genital tract lesions, where it is classified as the most important predictive risk factor for recurrence[20]. Therefore, promoting behaviors that stimulate immunity may be effective in preventing HPV infection in the oral cavity.

Although it is well known that HPV infection with high oncogenic potential is the main risk factor for developing cervical precursor lesions and cervical carcinoma, this alone is not enough, since it also involves genetic and epigenetic alterations for the development of neoplasia[21]. For this reason, the presence of direct cancer family history in more than 50% of the population studied is a cause of concern, since HPV infection in their oral mucosa could facilitate the development of oral epithelial dysplasia or oral carcinoma.

It would be interesting to conduct a follow-up study on the subjects who participated in this study due to the strong presence of known risk factors for the development of potentially malignant and malignant lesions associated with HPV.

This is why it is urgent to educate people about the risk factors that facilitate the spread of HPV, even more if we consider that young people currently enjoy greater sexual freedom.

This technique can be applied in future studies to more diverse populations in terms of age, educational levels and socio-economic status, in order to confirm if the presence of the risk factors described above is indeed associated with the presence of HPV in the oral mucosa of the Chilean population.

Prevalencia y factores de riesgo de infección por virus papiloma humano en mucosa oral normal de estudiantes de odontología chilenos.

Resumen: Se realizó un estudio de corte transversal. El grupo de estudio fue constituido por 103 individuos entre 18 y 33 años. Se aplicó una encuesta autoadministrada sobre antecedentes familiares de cáncer, hábitos sexuales y consumo de tabaco y alcohol. Se tomaron muestras de la mucosa oral utilizando un hisopo estéril. Posteriormente, todas las muestras fueron analizadas mediante técnica de reacción en cadena de la polimerasa (PCR). Resultados: Los resultados fueron negativos para la detección de VPH en todos los análisis. De la población estudiada el 58% presentó antecedentes familiares de cáncer, el 40,9% ha tenido más de 3 parejas sexuales, el 76,3% se inició sexualmente antes de los 19 años, el 66,3% ha practicado sexo oral, un 69,9% es bebedor de alcohol y el 20,6% es fumador. Conclusiones: El grupo analizado está expuesto a diversos factores de riesgo de infección VPH, por lo que es necesario educar acerca de la relación entre estos y el contagio con el virus. A pesar de la presencia de factores de riesgo en los encuestados, la prevalencia detectada de VPH fue de 0%.

Palabras clave: *Cavidad oral; Mucosa bucal; PCR; VPH16; VPH18.*

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