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Oral squamous papilloma: clinical, histologic and immunohistochemical analyses

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Abstract: Oral squamous papilloma (OSP) is a benign proliferation of the stratified squamous epithelium, which results in a papillary or verrucous exophytic mass. Twelve patients suspected to have oral papilloma underwent excisional biopsy for histopathologic and immunohistochemical analysis. The majority of the patients (75%) were females, and the most prevalent site was the tongue, followed by the palate. The round and whitish form was present in 58.4% of the cases. The lesions were softened/flaccid in 66.7% of cases and a pedunculated attachment was seen in 75% of the lesions. The histopathologic examination revealed hyperparakeratosis, occasional basal hyperplasia, and koilocyte-like cells in 100% of the specimens. Immunohistochemical assays utilizing BP53-12 and Pab240 antibodies for p53 protein showed negative or weak immunostaining (91.6%) for both immunomarkers in all the epithelial layers examined. The findings suggest the benign nature of the lesions and small possibility of becoming malignant. (J Oral Sci 51, 367-372, 2009)

Keywords: oral squamous papilloma; protein p53; immunohistochemical; clinical features; histopathology.

Introduction

Oral squamous papilloma (OSP) is a benign proliferation of the stratified squamous epithelium, which results in a papillary or verrucous exophytic mass (1-3) induced by human papillomavirus (HPV). The sites of predilection for localization of the lesions include the tongue and soft palate, but any surface of the oral cavity can be affected (3).

At least 150 different types of HPV have been identified already (4). The viruses considered to be of high oncogenic potential include HPVs 16, 18, 31, 33, 35, 39, 45, 51, 55, 56, 58, 59, 66 and 68 (5), and these have been associated with the development of various types of human neoplasias, particularly lesions of epithelial cells of the uterine cervix, urethra, anogenital region, skin, digestive tract, tracheal/bronchial and nasal mucosa and oral mucosa (6,7). DNA sequences of HPV 16 and 18 are found in approximately 85% of invasive squamous cell carcinomas and their precursors, such as grave dysplasia and carcinoma in situ. Infection by HPV acts as an initiator, and additional somatic mutations are essential, where the occurrence of these alterations is facilitated by smoking, other co-existent infections, dietary deficiencies and hormonal changes, all considered co-factors in the pathogenesis of cervical tumors. DNA of the HPV has been detected in about 95% of cervical tumors (4,8).

One of the functions of p53 protein is the decisional role in DNA repair or the induction of the cell to enter apoptosis (4,9), thereby being a negative regulator of cell growth (10). p53 protein, when activated, is capable of repressing cell growth and signaling cell death (apoptosis).

Protein E6 of HPVs of high oncogenic risk is capable of being associated with protein p53, where the viral protein E6 recruits the cellular protein E6AP which functions as a ubiquitin ligase for the complex containing p53. This recruitment results in the ubiquitination of p53 followed by its rapid degradation. Without p53 protein, the

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cell loses the capacity of perceiving and repairing possible DNA damage, and cell division thereby continues without repair (4). Protein p53, when modified, is stable and easily detectable in immunohistochemical assays (10).

The aim of the present study was to evaluate 12 patients with oral squamous papilloma, based on clinical and histopathologic characteristics and immunohistochemical expression of protein p53.

Materials and Methods

Sample

Twelve patients at the Universidade Federal Vale do Jequitinhonha and Mucuri (UFVJM) were evaluated; patients were both sexes and aged 4 to 65 years. Oral papilloma were subjected to excisional biopsy and later histopathological and immunohistochemical analysis of the lesions. All the patients underwent detailed clinical examinations, and characteristics were noted. This study was approved by the research committee of UFVJM.

Histopathologic examination

After clinical evaluation, excisional biopsy of the lesion was performed and the specimens were fixed and stained with hematoxylin eosin, for routine histological analysis. The strict histopathologic criteria for oral squamous papilloma (OSP) was as follows: squamous epithelium arrayed in finger-like projections, normal maturation pattern and presence of hyperparakeratosis in the epithelium, koilocytosis as a result of perinuclear cytoplasmic vacuolization of cells of the spinous layer of the epithelium, producing perinuclear pale/clear halos, and pyknosis and the occasional presence of basilar hyperplasia (11).

Immunohistochemistry for p53 protein

Immunohistochemical analysis was carried out using the antibodies Pab-240 and BP53-12, specific for protein p53. BP53-12 recognizes both mutant and wild-type p53 and Pab-240 is mutant-specific. All epithelial layers (basal, spinous, granular and cornified) were examined for staining. From each case 4-µm thick sections were prepared and mounted on silanized glass microscope slides. Tissue sections were deparaffinized with xylene, hydrated using a graded alcohol series and treated with 0.6 % H_2O_2 in methanol for 10 min to eliminate endogenous peroxidase activity. For antigen retrieval, the sections were immersed in 10 mM sodium citrate buffer (pH 6.0) and boiled twice for 12 min in a high-intensity microwave oven. At this point, incubation was carried out with the following primary antibodies: anti-p53 clone PAb-240 and BP53-12 (diluted 1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The LSAB + kit (Dako Corporation, Glostrup, Denmark) was used for application of the biotinylated antibody and peroxidase-labeled streptavidin, according to the manufacturer's instructions. The reactive products were visualized by immersing the sections for 3 min in 0.03% diaminobenzidine solution, containing 2 mM H_2O_2. The sections were then counterstained with Mayer's hematoxylin, dehydrated and mounted. Sections of oral squamous cell carcinoma with known PAb-240 and BP53-12 expression were used as positive control. Negative controls were reactions with the omission of each primary antibody. Cells were considered to be positive for the p53 protein antigens for clones PAb-240 and Bp53-12 if there was any staining of the nucleoplasm or nucleoli, regardless of the staining intensity. The PAb-240-positive and BP53-12-positive cells were quantified by light microscopy at ×200 magnification. Positively stained cells in each epithelial layer, basal, spinous, granular and cornified layers, were counted in 3 fields for each layer.

Results

The majority of the patients (75%) were female, and the age of the subjects ranged from 4 to 65 years (average 27.6 years). All the patients evaluated presented with solitary lesions. The time of development of the lesions varied from 2 months to 20 years and the most prevalent site was the tongue with 5 lesions (41.7%), followed by the palate (4 lesions, 33.3%), lip (2 lesions, 16.7%) and labial commissure (1 lesion, 8.3%). The size of the lesions varied between 0.2 cm and 1.2 cm. The round shape was the most prevalent (7, 58.4%), followed by the cauliflower shape (3, 25%); 58.4% (7) of the lesions were whitish in color. Eight lesions (66.7%) had a softened/flaccid consistency, and pedunculated attachment was evident in 9 lesions (75%) (Table 1).

The epithelium showed a normal maturation pattern and the majority of the lesions were hyperparakeratotic. There was a discrete increase in the number of cells in the basal layer (basilar hyperplasia) of the epithelium in at least one segment of the whole area evaluated, along with the presence of koilocyte-like cells in 100% of the slides examined (Figs. 1-3).

Immunostaining for protein p53 was primarily negative or weakly positive (91.6%, 11 slides) for the immunomarkers BP 53-12 and Pab 240 in all the epithelial layers evaluated (basal, spinous, granular and cornified) (Table 2, Figs. 4 and 5). Only one slide showed a strongly positive staining for the two immunomarkers, and this belonged to a male patient of 44 years, whose lesion was located on the soft palate.

Table 1 Clinical profile of the oral squamous papilloma cases

Cases	Age (years)	Sex	Lesion Localization	Number of Lesions	Size (cm)	Time of evolution (months)	Shape	Color	Consistency	Attachment
1	4	F*	Lower lip	1	0.3	72	Cauliflower	Whitish	Softened/flaccid	Sessile
2	6	F	Labial comissure	1	0.5	3	Cauliflower	Whitish	Softened /flaccid	Sessile
3	8	M**	Lower lip	1	0.2	2	Rounded	Rosy/pink	Softened /flaccid	Pedunculated
4	14	M	Tongue dorsum	1	0.3	36	Rounded	Whitish	Softened /flaccid	Pedunculated
5	20	F	Hard palate	1	1	72	Cauliflower	Whitish	Rubber-like	Pedunculated
6	24	F	Tongue lateral bord	1	1.2	Did not inform	Rounded	Rosy	Fibrous	Sessile
7	27	F	Tongue dorsum	1	0.2	24	Periform	Whitish	Softened /flaccid	Pedunculated
8	33	F	Soft palate	1	0.4	Did not inform	Rounded	Rosy	Softened /flaccid	Pedunculated
9	43	M	Soft palate	1	0.4	4	Lobulated	Reddish/red	Softened /flaccid	Pedunculated
10	44	F	Tongue dorsum	1	0.8	24	Rounded	Whitish	Rubber-like	Pedunculated
11	44	F	Tongue dorsum	1	0.3	24	Rounded	Whitish	Rubber-like	Pedunculated
12	65	F	Soft palate	1	0.5	72	Rounded	Red	Softened /flaccid	Pedunculated

*F: female **M: male

Fig. 1 Histopathological aspect of oral squamous papilloma, with the presence of papillary finger-like projections (asterisks, ×40, H–E staining).

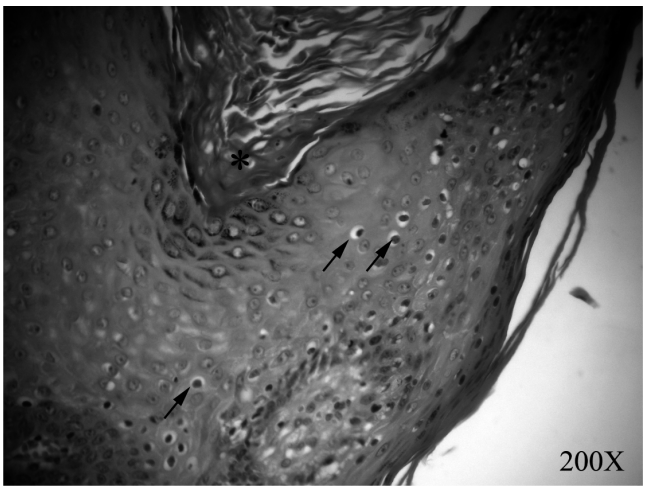

Fig. 2 Histopathological aspect of oral squamous papilloma with the presence of koilocyte-like cells (arrows) and hyperkeratosis (asterisk, ×200, H–E staining).

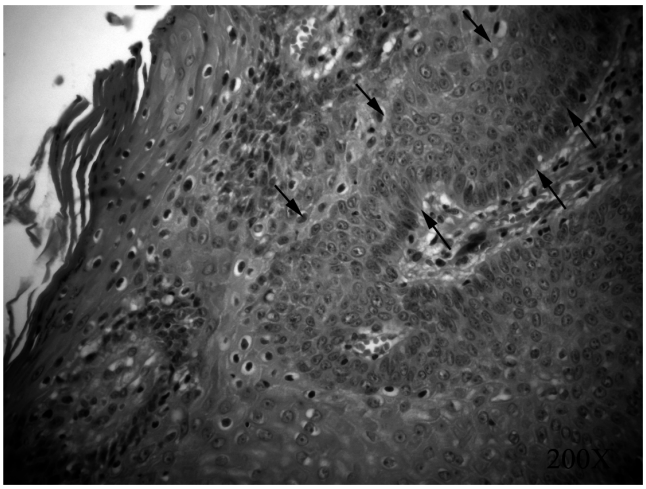

Fig. 3 Histopathological aspect of oral squamous papilloma with the presence of basilar hyperplasia (arrows, ×200, H–E staining).

Discussion

The majority of the lesions in the present study were found to be round in shape, whitish, flaccid in consistency and pedunculated. Such findings are consistent with those in the literature. It is known that the rate of recurrence of solitary lesions is low, compared to multiple lesions which show a clinically different behavior (3,12). In the present study, all of the specimens displayed solitary lesions, and there was no recurrence to date after resection of the

Table 2 Protein p53 immunostaining for the epithelial tissue according to its layers

Epithelial tissue layers (n = 12)	Protein p53 immunomarker			
	PAb-240		BP-53-12	
	Positive	Negative	Positive	Negative
Basal	2	10	6	6
Spinous	3	9	2	10
Granular	1	11	2	10
Cornified	1	11	1	11

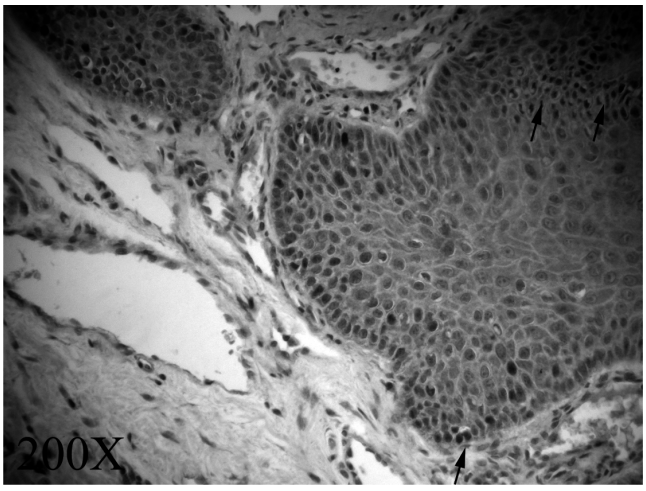

Fig. 4 Immunopositivity to p53 protein (clone BP 53-12) in cells of basal and spinous layers (arrows).

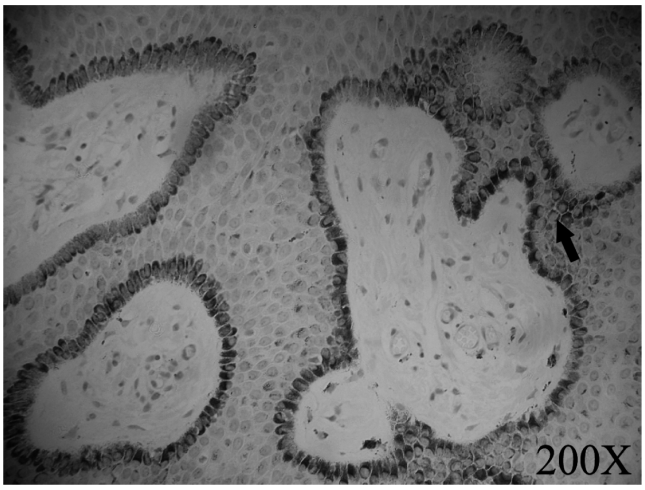

Fig. 5 Immunopositivity to p53 protein (clone Pab-240) in cells of basal layer (arrow).

lesion.

As the papillomavirus completes its replication cycle in differentiated cells of the outer layer of the epithelium and releases virions through shedding of these cells, the exposure of the immune system of the infected individual to viral antigens is relatively small. Infection with HPV thereby tends to be more persistent (13). This was demonstrated in the present study, with the lesions being seen up to 20 years, without any changes.

In the present work, histopathologic examination revealed indication of viral presence in 100% of the specimens. This occurred due to the observation of koilocyte-like cells in the spinous layer of the epithelium (2-4). Basilar hyperplasia was found occasionally in the present study. This characteristic should be considered with caution, since its presence and some mitotic activity can be confused with mild epithelial dysplasia (3). Atypical cells and mitoses, which were not observed in the samples, are very uncommon in papillomas, as they are benign lesions. When they occur, a papillary carcinoma may be suspected (12). The whitish color of the lesions was clinically observed in 7 papillomas in this study, indicating the thickness of the cornified layer (3).

The viral presence should be confirmed with the help of PCR (polymerase chain reaction). DNA of the HPV can also be detected in tissue by *in situ* hybridization using radioisotope-labeled specific probes (3,4). However, hybridization is less sensitive than PCR, which is considered the most suitable method for the detection of HPV (14). In the present work, the diagnosis of papilloma was based only on clinical and histopathologic characteristics of the lesion.

Presumably, the papillomas are induced by HPVs 6 and 11, considered to be of low oncogenic potential (3,4). It has also been suggested that all oral papillomas harbor the DNA sequence of HPV (15). In our study, the presence of koilocyte-like cells in all the slides can be considered highly suggestive of viral infection. The DNA sequences of HPV 6 and 11 have been detected in 50 to 68% of oral papillomas (3,15). Of the 14 papillomas evaluated by Fregonesi et al. (14) using in situ hybridization, 14% (2) were positive for HPV 6/11, 22% (n = 3) were positive for HPV 16/18, and the great majority (64%, 9) were negative for HPV. However, Major et al. (16) found 100% PCR positivity for HPV 6 or HPV 11 DNA in 10 papillomas due to recurrent respiratory papillomatosis. Generally, in children with laryngeal papillomatosis, there can be co-infection by HPVs 6 and 11. However, in adults with this lesion, this co-infection is rare, as it is caused only by one of the viruses (13). This has stirred controversy over the true origin of papillomas, since not all lesions are induced by the virus.

The absence of immunostaining for p53 protein in the great majority of the slides examined in the present work indicates the benign nature of the lesions with slow cell

proliferation, evidenced by their slow development. In only one slide there was intense staining for the 2 immunomarkers in all the epithelial layers examined, which suggests the presence of virus with high oncogenic potential (HPV 16 and 18). Alternatively, it could have been caused by the presence of coexisting external factors. This patient was a 44-year-old male who smoked cigarettes and drank alcohol. He was informed about his condition and counseled for cessation of these habits. The transformation of potentially malignant cells into malignant cells occurs due to inactivation of genes for regulators of cell proliferation, such as p53. The mutation in gene p53 is found in human malignant neoplasms with functional inactivation of protein p53 and over expression of the gene (17,18), resulting in loss of control of the cell cycle and in increased cell proliferation in the tumor (19).

However, oncogenes themselves are not sufficient to give rise to malignant neoplasia in the oral cavity. A variety of mechanisms can lead to the functional inactivation of protein p53, including the interaction with viral or cellular oncoproteins (20). The protein E6 of HPV-16 is capable of binding to protein p53, enhancing its degradation and altering the control of cell growth (21). According to Barzal-Nowosielska et al. (22), infection by HPV and/or alterations in protein p53 can co-exist in papillomas of the oral cavity, as these authors demonstrated over expression of p53 in 55% (n = 36) of the cases of papillomas evaluated. However, the authors also found over expression of p53 in 12 of 23 HPV-negative papillomas (52%), besides over expression of protein in 8 of 13 HPV-positive papillomas (61.5%). This coexistence could not be demonstrated in the present study, since only one (8.4%) of the 12 specimens evaluated showed positive immunostaining for protein p53. This specimen could have been in an active growth phase with possible disruption of cell cycle regulation, and the location could have been at risk for cancer development (23). Copete et al. (24) observed 93% positivity for p53 in the epithelial cells of the basal and parabasal layers of 28 papillomas. According to these authors, this high prevalence of positivity for protein p53 in oral papillomas is in consonance with the concept that the accumulation of mutant or wild-type protein p53 in the nucleus could allow augmented cell proliferation.

We concluded that the papilloma lesions in this study were most prevalent in females, being unique, with localization on the tongue, appearing whitish in color, round in shape, and with flaccid consistency and pedunculated attachment. Histopathologic examination of the specimens was compatible with viral infection. Immunohistochemical assays for p53 protein were negative for the great majority of the specimens evaluated, suggesting a benign character for the lesions and a small risk of becoming malignant. Further studies are warranted to elucidate this relationship.

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