Association of human papilloma virus with atypical and malignant oral papillary lesions

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Objective. This study aimed to examine atypical and malignant papillary oral lesions for low- and high-risk human papillomavirus (HPV) infection and to correlate HPV infection with clinical and pathologic features.
Study Design. Sections of 28 atypical papillary lesions (APLs) and 14 malignant papillary lesions (MPLs) were examined for HPV by in situ hybridization and for p16 and MIB-1 by immunohistochemistry; 24 conventional papillomas were studied for comparison.
Results. Low-risk HPV was found in 10 of 66 cases, including 9 APLs and 1 papilloma. All low-risk HPV−positive cases showed suprabasilar MIB-1 staining, and the agreement was statistically significant ($P < .0001$). Diffuse p16 staining combined with high-risk HPV was not seen in any of the cases. A subset of HPV$^-$ APLs progressed to carcinoma.
Conclusions. Oral papillary lesions are a heterogeneous group. Low-risk HPV infection is associated with a subset of APLs with a benign clinical course. Potentially malignant APLs and MPLs are not associated with low- or high-risk HPV. (Oral Surg Oral Med Oral Pathol Oral Radiol 2014;117:722-732)

Papillary lesions of the oral mucosa present with a range of clinical and histologic appearances, from benign to malignant. Squamous papilloma is a common and benign lesion that is clinically well-demarcated, round or oval in shape, and generally less than 1 cm in diameter. Recurrence is rare after conservative excision. Histologically, the papillary projections are covered by acanthotic stratified squamous epithelium with a normal pattern of maturation and occasionally basilar hyperplasia.[1] Less frequently, there are lesions that architecturally resemble a papilloma, but with atypical clinical or histologic features. Atypical clinical features include larger size, irregular outline, progressive growth, and recurrence. Atypical histologic features include variably shaped rete ridges and dysplasia of the epithelium that covers the papillary projections. Exophytic, papillary epithelial proliferations with dysplasia, referred to as dysplastic oral warts, have been described in HIV$^+$ patients.[2-4] However, atypical papillary lesions (APLs) of the oral mucosa in immunocompetent persons have not been well documented with respect to diagnostic criteria and biologic potential.

The nomenclature of malignant papillary lesions (MPLs) is controversial, and the term may include papillary carcinoma, papillary squamous cell carcinoma, and exophytic squamous cell carcinoma.[5-10] Papillary carcinomas and papillary squamous cell carcinoma have both been defined as exophytic, papillary neoplasms in which the epithelium covering the surface projections shows either carcinoma in situ or pronounced cellular pleomorphism with keratinization at the surface.[5,6,11] Other authors have characterized the epithelium of papillary squamous cell carcinoma as high-grade dysplasia with no surface keratinization.[8] Exophytic squamous cell carcinoma has histologic features that overlap with papillary squamous cell carcinoma, but the surface projections are broad-based and bulbous rather than finger-like.[9] There seems to be

Statement of Clinical Relevance

Oral papillary lesions are a heterogeneous group with variable clinical behavior. Preliminary evidence suggests that low-risk human papillomavirus (HPV)−positive atypical papillary lesions run a benign clinical course. Potentially malignant and malignant papillary lesions are not associated with HPV infection.

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agreement that stromal invasion in this group of neoplasms is usually superficial and may be difficult to confirm unequivocally, and the diagnosis of carcinoma may be made on the basis of the extent of growth of the exophytic mass even in the absence of definitive stromal invasion.[8-10] These lesions are considered distinct on one hand from conventional squamous cell carcinoma with both exophytic and endophytic invasive components, and on the other hand from verrucous carcinoma. They are distinguished from the former by a wholly exophytic papillary architecture of the carcinoma and from the latter by significant dysplasia and infiltrative islands rather than blunt, pushing invasion.[8-10]

Human papillomavirus (HPV) has been shown to cause a variety of papillary lesions of skin and mucosa, including verruca vulgaris, condyloma acuminatum, focal epithelial hyperplasia, and recurrent respiratory papillomatosis.[12] Previous studies have examined benign, atypical, and malignant oral papillary lesions for the presence of HPV using a variety of methods, including immunohistochemical staining for HPV common antigen, in situ hybridization (ISH) with DNA probes, polymerase chain reaction (PCR), and in situ reverse transcriptase PCR.[13-21] The reported prevalence of HPV in oral squamous papilloma varies from 13% to 68%, and all positive cases are low-risk HPV types 6 and 11.[15,18,21] HPV is more commonly detected in oral condyloma acuminatum, but there is considerable overlap in the histopathologic features of condyloma and papilloma of the oral mucosa.[16,22] There is a strong association between HPV and oral exophytic epithelial lesions in immunosuppressed patients.[4,13,19] The prevalence of HPV in malignant oral verrucous or papillary lesions in immunocompetent patients is much lower.[14,17,20]

This study examined a group of atypical and malignant papillary lesions for low- and high-risk HPV and correlated the detection of HPV with clinical and pathologic features, with the aim of improving our understanding of these uncommon oral lesions.

MATERIALS AND METHODS

This was a retrospective study of biopsy specimens of oral mucosal lesions submitted to the Oral Pathology Diagnostic Service (OPDS) at the Faculty of Dentistry, University of Toronto, by general and specialist dentists in the province of Ontario, Canada. This study was approved by the Research Ethics Boards of the University of Toronto (protocol 26571) and the University Health Network (UHN) (protocol 12-5269-TE). A search of the OPDS files from 2005 to 2011 was conducted using the keyword "papillary." A total of 28 APLs and 14 MPLs were identified. For comparison with the APLs and MPLs, 24 cases of squamous papilloma accessioned during the same period were also collected for study. Lesions diagnosed as inflammatory papillary hyperplasia of the palate were excluded from the study, because this is well recognized as a reactive mucosal condition. No patients included in the study had a history of immunosuppression. Fourteen cases of normal oral epithelium from specimens diagnosed as fibroepithelial polyp or amalgam tattoo, from a variety of oral sites, were used as normal controls.

Clinical follow-up information on cases of APL and MPL was obtained from the clinician who submitted the biopsy. Some of the patients with a persistent lesion after biopsy were referred to the Department of Head and Neck Surgery at the Princess Margaret Cancer Centre, UHN, for further treatment. Follow-up information for these patients was obtained by chart review at UHN.

In situ DNA hybridization for high- and low-risk HPV

In situ hybridization (ISH) for HPV DNA was performed on 4-μm-thick sections of formalin-fixed, paraffin-embedded (FFPE) biopsy specimens, using the Inform HPV II Family 6 probe (B) for low-risk HPV and the Inform HPV III Family 16 probe (B) for high-risk HPV on a Ventana Benchmark automated slide stainer according to the manufacturer's instructions (Ventana Medical Systems Inc, Tucson, AZ, USA), as previously described.[23] The Family 6 probe detected low-risk HPV types 6 and 11. The Family 16 probe detected high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 66. A case of condyloma acuminatum was used as the positive control for the Family 6 probe and normal oral epithelium was used as the negative staining control. For the Family 16 probe, high-risk HPV 3-in-1 control slides supplied by the manufacturer were used, including CaSki cells ($HPV16^+$, 200-600 copies per cell), HeLa cells ($HPV18^+$, 10-50 copies per cell), and T24 cells (HPV^-). In addition, a case of $HPV16^+$ oropharyngeal carcinoma was used as positive control for the Family 16 probe. ISH for HPV DNA was scored as positive when blue reaction product was seen in the nucleus of lesional cells, in either a homogeneous or punctate pattern. Staining that was not localized to the nucleus of epithelial cells was interpreted as background staining. Positive and negative controls were included in each run of ISH, and the expected results were obtained. All cases were scored independently by 2 observers (C.M. and G.B.) following the aforementioned criteria, and there was no disagreement between observers.

Immunohistochemical staining

Immunohistochemical staining for p16 protein (cyclin-dependent kinase inhibitor 2A, *CDKN2A*) was performed