Comparison of the prevalence of human papilloma virus infection in histopathologically confirmed premalignant oral lesions and healthy oral mucosa by brush smear detection

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Objective. The role of human papilloma virus (HPV) infections in oral carcinogenesis is an important topic of research in maxillofacial oncology. Nevertheless, the association between such infections in the oral cavity and the development of oral precancerous lesions remains unclear. The aim of this study was to evaluate the association between oral HPV infections and oral leukoplakia or erythroplakia.

Study Design. The case control study included 118 patients with manifest oral leukoplakia or erythroplakia, who underwent surgical biopsy, including a histopathologic grading of the lesion, and 100 control patients without any oral lesions. HPV detection was achieved with a noninvasive brush smear method (Digene Cervical Sampler, Hybrid Capture II-Test). Logistic regression analysis was performed to assess the associations.

Results. A significant association was found between high-risk oral HPV infection and the presence of oral premalignant lesions ($P = .001$). Among all other evaluated parameters, only smoking showed a significant association with the presence of oral lesions.

Conclusions. Oral HPV infections may play a role in the pathogenesis of premalignant oral lesions. (Oral Surg Oral Med Oral Pathol Oral Radiol 2015;119:333-339)

The association between anogenital human papilloma virus (HPV) infections and anal or genital cancer is indisputable.[1,2] During the last decade, HPV has also become a topic of research in maxillofacial oncology, especially with regard to the pathogenesis of malignant oral and oropharyngeal tumors. Meanwhile, the causal role of HPV infections has been proved at least for a subgroup of oropharyngeal squamous cell carcinoma.[3]

Oral HPV infections seem to be present in up to 70% of patients with tonsillar carcinoma.[4,5] Additional evidence for a role of HPV in head and neck oncology is seen in the increased incidence of HPV-positive oral and oropharyngeal squamous cell carcinoma (OSCC) in the last 30 years,[6,7] especially in tonsillar carcinoma.[8] At the same time, a decrease in HPV-negative OSCC has been noted during the same period.[6,8,9] The fact that patients with HPV-positive carcinomas seem to have a lower likelihood of tobacco and alcohol consumption in comparison with HPV-negative patients reinforces the hypothesis of a viral infection as a (co)causal factor in oral carcinogenesis at some sites.[6,10]

In contrast to OSCC, little evidence exists in the literature for an association between oral HPV infections and premalignant oral lesions, such as dysplastic leukoplakia or erythroplakia. The described rates of carcinomatous transformation of oral leukoplakia, regardless of its pathogenetic origin, range from 0.7% to 2.9%.[11,12] Leukoplakia may present as simple hyperkeratosis or show different grades of dysplasia (mild, moderate, severe), indicating a premalignant condition. In contrast to these varying histologic appearances, erythroplakia shows severe epithelial dysplasia, carcinoma in situ, or even invasive carcinoma in more than 90% of the cases.[13] Tobacco and areca nut chewing are two known etiologic factors in the development of oral leukoplakia and erythroplakia, in contrast to the unknown role of HPV.[14]

HPV is strictly epitheliotropic and infects either the cutaneous epithelium or the mucosal squamous epithelium, depending on the genotype.[14-16] Genotypes infecting the mucosal epithelium have been categorized into high-risk types (e.g., HPV 16/18/31/33/35/39/45/

Statement of Clinical Relevance

The study suggests a possible association between oral human papilloma virus (HPV) infection and premalignant oral lesions.

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51/52/56/58/59/68), on the basis of their epidemiologic association with carcinoma of the cervix uteri, and into low-risk types (e.g., HPV 6/11/42/43/44).[17] The reported prevalence of HPV infection in oral precancerous lesions shows an extreme variance from 0% to 100%.[18-23] The cause for this variance may be related to the different HPV detection methods used and different ethnic or geographic origins of the patients examined and the inappropriate grouping of lesions from different anatomic sites of the mucosa of the upper aerodigestive tract, not only from the oral region.[16,20,24-26] Additionally, according to a recent investigation, a higher prevalence rate of high-risk HPV may be found with regard to oral lesions that have a higher degree of epithelial dysplasia, especially in the floor of the mouth.[19]

A decrease of oral HPV-positivity—from normal mucosa, to hyperkeratotic and dysplastic leukoplakia, to malignant lesions—has been described and has been explained by the possible role of HPV only in the early stages of oral carcinogenesis.[8,23]

Thus, the reported results suggest a causal connection between premalignant oral lesions respectively, early stages in oral carcinogenesis and the presence of HPV in the oral cavity. In addition, the pathomechanism of high-risk HPV infections with regard to carcinoma of the cervix uteri supports this hypothesis. During the integration of high-risk HPV-DNA in cervical mucosal cells, damage occurs in the viral genes *E1* and *E2*. These two genes regulate the expression of the viral proteins E6 and E7, which inhibit the cellular tumor suppressor genes (*p53*, *pRB*).[11] The result is the overexpression of these proteins, which may promote malignant transformation of the infected cell.

The aim of this study was to investigate the association between premalignant oral lesions and the presence of oral HPV infection (low-risk or high-risk HPV), the HPV detection method consisted of a simple brush smear test (Digene Hybrid Capture 2).

MATERIAL AND METHODS

To compare the prevalence of oral HPV infection in patients with potentially premalignant oral lesions (leukoplakia or erythroplakia) with patients who were clinically oral-healthy, oral HPV testing was conducted on 218 consecutive patients between 19 and 50 years of age. Of these subjects, 118 ("lesion group") had been referred to the Department of Craniomaxillofacial and Oral Surgery of the Medical University of Innsbruck between March and September 2013 for histopathologic examination of clinically diagnosed leukoplakia or erythroplakia (Figure 1).

The "oral healthy" control group consisted of 100 consecutive patients, who had visited the Department of Craniomaxillofacial and Oral Surgery for other miscellaneous reasons (e.g., control visits after trauma or surgery, implantology) and did not show any lesions of the oral mucosa. The inclusion criteria comprised the following:

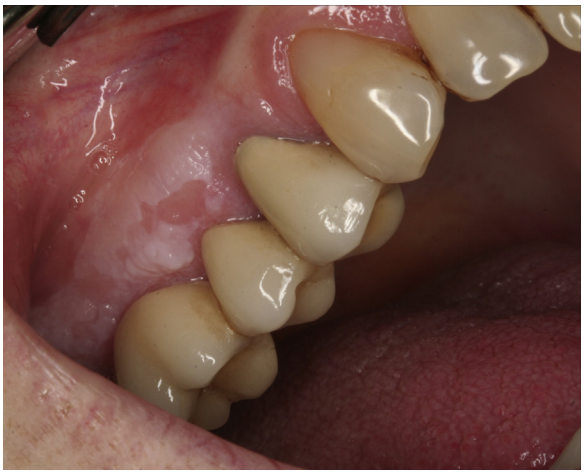

Fig. 1. Leukoplakia in the maxillary gingival region.

- No history of oral or oropharyngeal cancer
- Age: 19 to 50 years
- Capable of understanding and giving informed consent
- For patients in the lesion group: manifestation of leukoplakia or erythroplakia; histopathologic examination performed
- For patients in the control group: absence of any lesion in the oral mucosa

Patients were excluded for the following reasons:

- The study questionnaire (see below) was incomplete or missing
- In the lesion group, the histopathologic examination revealed invasive carcinoma

The Digene Cervical Sampler HC2 Hybrid Capture procedure, a simple brush smear test routinely used in gynecologic HPV detection, was used as the HPV testing method. Its application in the clinical diagnostics of oral HPV infections has already been evaluated in different trials, which had confirmed the reliability, sensitivity, and efficacy of this noninvasive testing method for the detection of oral HPV.[27-29]

All patients were asked to answer a questionnaire regarding tobacco and alcohol consumption, sexual behavior (lifetime number of oral and vaginal sexual partners) and family history of head and neck tumors. Panoramic radiography and a detailed clinical investigation were conducted in every case.

After clinical examination, two oral brush smears from the left and the right buccal mucosa from all