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:

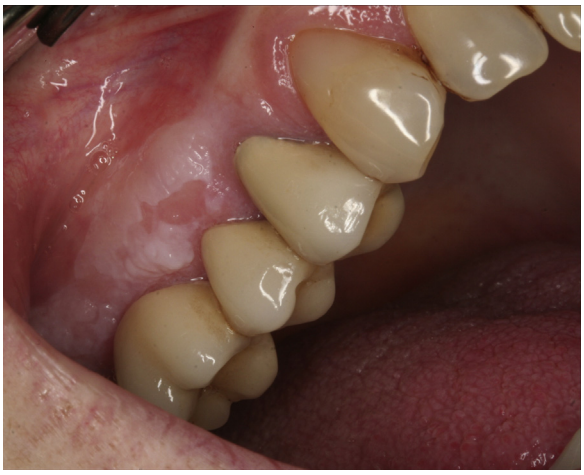

Fig. 1. Leukoplakia in the maxillary gingival region.

- 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

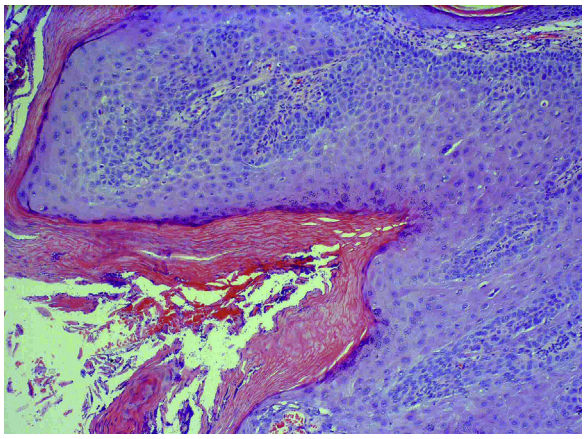

Fig. 2. Histologic aspect of a high-risk HPV−positive, mild dysplastic leukoplakia, presenting cellular pleomorphism and nuclear hyperchromatism, architectural disorders in the basal zone, and inflammatory cell infiltration (hematoxylin and eosin, original magnification ×10).

patients, as well as from the affected region in the case of the lesion group, were taken by the investigators. All 118 patients of the lesion group underwent surgical biopsy followed by histopathologic examination at the Department for Craniomaxillofacial and Oral Surgery. In all patients, the clinical diagnosis of leukoplakia or erythroplakia was confirmed by histopathologic investigation (Figure 2), which focused on possible dysplastic changes and their grade and not on histopathologic HPV detection.

After specimen collection through a brush smear, HPV detection was done with the HC2-HPV-DNA-testing (QIAGEN, NL) using the Hybrid Capture 2 technology, a nucleic acid hybridization assay with signal amplification that utilizes microplate chemiluminescent detection. The analysis was performed by the Laboratory for Clinical Biochemistry of the Department for Gynecology and Obstetrics. This test helps distinguish "no infection," "low-risk HPV-infections," (HPV 6/11/42/43/44), and "high-risk HPV infections" (HPV 16/18/31/33/35/39/45/51/52/56/58/59/68).

The collected data were processed and analyzed anonymously using the statistical package SPSS 19.0. Mean value and standard deviation (SD) were assessed for age, and numbers with percentages were assessed for the other variables. Logistic regression analysis was performed to investigate the associations; the results are presented as odds ratios (ORs) with 95% confidence intervals (CIs). Two-sided *P* values less than .05 were considered statistically significant.

The study has been approved by the local ethical committee of the Medical University of Innsbruck (nr. AN5192 328/4.8). The guidelines of the Helsinki Declaration have been followed in the present study.

RESULTS

The study population consisted of 218 patients. Of these, 118 presented with premalignant oral lesions ("lesion group"), and 100 ("control group") did not show any oral pathologies; 110 patients were female (50%) and 108 male (50%). In the lesion group, there were 55 female (47%) and 63 male patients (53%), whereas the control group consisted of 55 female (55%) and 45 male (45%) patients.

Overall, the mean age was 29.1 (SD 6.2) years, whereas the mean age in the lesion group was 29.7 (SD 6.9) years and in the control group 28.2 (SD 5.1) years.

In the lesion group, 86.4% (n = 102) of patients presented with oral leukoplakia, whereas 13.6% (n = 16) showed erythroplakia. The pathologically affected sites were the buccal mucosa (46.6%, n = 55), the maxillary or mandibular gingiva (40.7%, n = 48), and other miscellaneous oral sites, such as the palate, the tongue, or the floor of the mouth (12.7%, n = 15). With regard to the histologic diagnosis, 17% (n = 20) of the lesions presented as hyperkeratosis, 34.7% (n = 41) showed mild dysplasia, 35.6% (n = 42) showed moderate dysplasia, and 9.3% (n = 11) showed severe dysplasia; 3.4% (n = 4) of the lesions were classified as carcinoma in situ.

History of oral and vaginal sexual partners, as well as positive family history regarding head and neck cancer, as well as data on alcohol and tobacco consumption and HPV infection, are shown in Table I. The smoking habit category was subdivided into nonsmoking, mild smoking (<10 pack years), and heavy smoking (>10 pack years).

With regard to the brush smear testing, 95% (n = 207) of all patients showed concordant test results among two (in the control group) and three (lesion group) brush smears. In 5% (n = 11), a positive HPV test result could be recorded only in one of the brush smears, whereas out of the 11 patients, 6 were found in the control group and 5 in the lesion group.

In the unadjusted analysis, significant results were found with regard to age, smoking, and HPV infection (Table II). The OR was 3.5 (95% CI 1.7-7.2) for mild smokers and even larger among heavy smokers at 5.4 (95% CI 2.4-12.3). With regard to oral HPV infections, an OR of 3.2 (95% CI 1.6-6.2) was found for infections with high-risk HPV infections in consideration of all of the patients in the lesion group (see Table II). If only patients with dysplastic lesions were considered, the OR changed to 3.4 (95% CI 1.7-6.4).

After adjustment for other variables, the results did not change significantly. In the multivariable analysis, a significant relationship (95% CI 1.8-8.8) between high-risk HPV infection and the presence of a leukoplakia or erythroplakia with an OR of 4.0 was found ($P = .001$). If only dysplastic lesions of the lesion group were

Table I. Characteristics of control and lesion group

	Control group (n = 100)	*Lesion group (n = 118)*
Leukoplakia	-	102 (86.4%)
Erythroplakia	-	16 (13.6%)
Histologic diagnosis		
Hyperkeratosis	-	20 (17%)
Mild dysplasia	-	41 (34.7%)
Moderate dysplasia	-	42 (35.6%)
Severe dysplasia	-	11 (9.3%)
Carcinoma in situ	-	4 (3.4%)
Affected anatomic region		
Buccal mucosa	-	55 (46.6%)
Maxillary and mandibular gingiva	-	48 (40.7%)
Other sites (palate, tongue, mouth floor)	-	15 (12.7%)
Mean age (SD)	28.2 (5.1)	29.7 (6.9)
Gender		
Female	55 (55%)	55 (47%)
Male	45 (45%)	63 (53%)
Head and neck cancer in family		
No	93 (93%)	106 (90%)
Yes	7 (7%)	12 (10%)
Smoking status		
Non-smoking	77 (77%)	52 (44%)
Mild smoking*	14 (14%)	33 (28%)
Heavy smoking*	9 (9%)	33 (28%)
Regular consumption of alcohol		
No	38 (38%)	48 (41%)
Yes	62 (62%)	70 (59%)
Number of vaginal sexual partners		
None	6 (6%)	10 (8%)
1 to 5	53 (53%)	59 (50%)
6 to 10	26 (26%)	22 (19%)
>10	15 (15%)	27 (23%)
Number of oral sexual partners		
None	9 (9%)	9 (8%)
1 to 5	61 (61%)	65 (55%)
6 to 10	16 (16%)	23 (19%)
>10	14 (14%)	21 (18%)
Low-risk HPV status		
Negative	80 (80%)	100 (85%)
Positive	20 (20%)	18 (15%)
High-risk HPV status		
Negative	86 (86%)	78 (66%)
Positive	14 (14%)	40 (34%)

SD, standard deviation.
*Mild: <10 pack years; heavy: >10 pack years.

considered, the OR was 4.3 ($P = .001$). The association between low-risk HPV infections and the presence of oral lesions was not significant. For all other determined parameters, only tobacco consumption showed a significant association with the presence of oral lesions (see Table II).

HPV infection rates differed among the groups, depending on the histologic grading of the lesions (Table III). For low-risk HPV infections, the highest infection rate was recorded in hyperkeratotic lesions (n = 10; 50% of all hyperkeratotic lesions), followed by carcinoma in situ (n = 1; 25% of all carcinomas in situ),

Table II. Logistic regression analyses

	Unadjusted odds ratio (95% CI)	*P*	*Adjusted odds ratio (95% CI)*	*P*
Age (per 10 years)	1.5 (1.0-2.4)	.07	1.5 (0.9-2.5)	.13
Gender (female reference)	1.4 (0.8-2.4)	.22	1.2 (0.6-2.2)	.62
Head/neck cancer in family	1.5 (0.6-4.0)	.41	1.4 (0.5-4.1)	.58
Smoking				
Mild	3.5 (1.7-7.2)	<.001	4.4 (2.0-9.5)	<.001
Heavy	5.4 (2.4-12.3)	<.001	6.2 (2.5-15.1)	<.001
Alcohol use	0.9 (0.5-1.5)	.69	0.6 (0.3-1.2)	.14
Many vaginal sexual partners*	1.0 (0.6-1.8)	.94	0.6 (0.3-1.5)	.27
Many oral sexual partners*	1.4 (0.8-2.4)	.26	1.5 (0.6-3.6)	.41
Low-risk HPV infection	0.7 (0.4-1.5)	.36	0.5 (0.2-1.3)	.16
High-risk HPV infection	3.2 (1.6-6.2)	<.001	4.0 (1.8-8.8)	<.001

CI, confidence interval.
*Six or more partners.

Table III. Human papilloma virus presence in different lesions

Lesion grading (n = 118)	*Low-risk HPV-positive (n = 18)*	*High-risk HPV-positive (n = 40)*
Hyperkeratosis (n = 20)	10	4
Mild dysplasia (n = 41)	4	13
Moderate dysplasia (n = 42)	3	15
Severe dysplasia (n = 11)	-	7
Carcinoma in situ (n = 4)	1	2

mild dysplasia (n = 4; 9.8% of all mild dysplasias), and moderate dysplasia (n = 3; 7.1% of all moderate dysplasias). Low-risk HPV presence was not noted in cases with severe dysplasia. For the high-risk HPV infections, the highest rate was seen in patients with severe dysplasia (n = 7; 63.3% of all severe dysplasias) and a single case of carcinoma in situ (n = 2; 50% of all carcinomas in situ), followed by moderate dysplasia (n = 15; 35.7% of all moderate dysplasias), mild dysplasia (n = 12; 29.3% of all mild dysplasias), and hyperkeratotic lesions (n = 4; 20% of all hyperkeratotic lesions).

DISCUSSION

The results of the present study suggest a significant association between the presence of premalignant oral lesions and positive results for presence of oral HPV. The prevalence of HPV infection in oral lesions in the current analysis agrees with the findings of Miller and Johnstone,[30] who described an HPV infection rate two to three times higher in patients with leukoplakia compared with oral-healthy persons. For high-risk

HPV infections, the association was even more significant. Our results reinforce the hypothesis of a possible causal role of HPV infection, especially high-risk HPV infection, in the pathogenesis of some cases of oral leukoplakia and erythroplakia.[31]

McCord et al. found a significant association between oral high-risk HPV infections and cases of severe oral epithelial dysplasia.[19] In a second analysis, the same group did not find any association between high-risk HPV infections and potentially malignant papillary oral lesions but only for low-risk HPV infections and papillary pathologies with a benign clinical course.[22] It may be presumed that HPV infections, especially with regard to the high-risk group, have different infection properties and carcinogenic effects, depending on the underlying tissue pathology. This fact may also account for the inconclusive results of Samman et al., who did not find a significant HPV involvement in oral verrucous carcinoma or hyperplasia.[32] With regard to the remaining variables investigated in this study, the role of tobacco consumption in oral carcinogenesis has already been documented and is well known.[33] The present findings confirm the association between smoking habits and the development of oral leukoplakia and erythroplakia (Table II.)

Additional challenges in comparing different studies with regard to the prevalence of oral HPV infections are the different detection methods used. Numerous testing methods are available, including in situ hybridization (ISH), polymerase chain reaction (PCR)−based assays and immunohistochemical testing for p16 expression. All these techniques are based on the analysis of tissue samples and are, therefore, invasive. However, cytologic methods, including the method used in the present study, can eliminate the need for invasive tissue acquisition. Despite the variety of these testing possibilities, no standard approach for oral HPV testing has been defined until now. Due to the different limitations of each method, a recent publication suggested a multimodal strategy, with no allowance for testing errors, in determining the oral HPV status in special cases.[34] The Digene Cervical Sampler HC2 Hybrid Capture procedure used in this study represents a simple, convenient, and reliable method not only for detection of cervical HPV infection but also for testing for oral HPV infection. Previous investigations have proven the specificity, sensitivity, and reliability of this tool for oral applications as well.[27-29] To evaluate the efficacy of the Digene HC2 Hybrid Capture, Chaudhary et al. determined the oral presence of high-risk HPV in 430 patients with oral submucous fibrosis or OSCC, detecting the viral infection by PCR and the Digene HC2® assay simultaneously.[27] The results of both tests were compared, whereby the PCR represented the control method. In the case of patients with oral submucous fibrosis, sensitivity and specificity were found to be 73.7% and 92.5%, respectively, with concordant results from both tests in 85.4%. In the case of OSCC, sensitivity and specificity were even higher, with 87.14% and 92.76% and a level of agreement (88.3%) of both diagnostic tools.[27] In contrast to PCR, the Digene HC2 is a noninvasive, convenient, and simple option in oral HPV detection. Therefore, it should be considered as a valuable tool in dentistry and oral surgery research, especially for epidemiologic purposes.

According to D'Souza, a high lifetime number of oral-sex partners (≥6) seems to play an additional important role in oral carcinogenesis.[4,10] In the case of premalignant oral lesions, no significant association between the number of oral or vaginal sexual partners and the presence of premalignant oral lesions could be found in the present study. Tendentially, patients of the lesion group stated a higher number of different sexual partners for oral sex practices. Nevertheless, the comparison with the control group was not statistically significant. Of course, oral cancer does not arise from clinically evident premalignant lesions in every patient. Therefore, the present study is not comparable with D'Souza's findings. However, this may explain the lack of association between sexual activity and the development of premalignant lesions, such as leukoplakia or erythroplakia.

The present study has some limitations, the first being the age of the investigated patients. Undoubtedly, the effects of many cancerogenic factors, especially smoking, are time related. Since the age of a considerable portion of the study population was below 40 years, the data with regard to these factors may not completely applicable with regard to older populations.

The second limitation is that the test method used in the study may not distinguish between actively transforming oral HPV infections and cellular DNA integration or inactive infections. However, as the U.S. Food and Drug Administration (FDA) determined a HPV target concentration of 1 picogram per milliliter with regard to the analytical sensitivity of the Hybrid Capture 2 test, accidental contamination leading to a positive HPV test result may be excluded. Nevertheless, the HC2 assay cannot determine HPV as the etiologic cause of a premalignant lesion but only confirm the viral presence in the investigated area. In addition, the Hybrid Capture 2 Test cannot detect the presence of a specific HPV type but only allows a distinction between the two clinically relevant groups—"low risk" and "high risk" HPV.

The third limitation was that in 87.3% of the "lesion group" patients, the affected sites were the buccal mucosa or the gingiva, which are sites with a low risk of

malignant transformation compared with anatomic regions at high risk, such as the floor of the mouth, the lateroventral tongue, or the soft palate. To gain additional information regarding the role of HPV in the genesis of premalignant oral lesions and in oral carcinogenesis, further studies evaluating only sites with a known high malignant potential would be desirable. Additionally, only 83% of the histologically investigated lesions showed a premalignant condition with dysplasia, and the remaining 17% presented only hyperkeratosis. Considering only dysplastic lesions and enlarging the study population could also provide more detailed information regarding the correlation of HPV and premalignancy.

According to the results of the present study a few considerations regarding HPV vaccinations may be made. The effectiveness of vaccination for the prevention of cervical HPV 16 infection has already been demonstrated.[35] In contrast to carcinoma of the cervix, premalignant oral lesions are not gender specific. If HPV is ultimately demonstrated to have a causative role in some cases of oral carcinogenesis, vaccination could considered an option in the prevention of oral cancer, similar to its routine application in gynecologic HPV prevention.

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