CONCISE COMMUNICATION

Human Papillomavirus DNA in Oral Mucosal Lesions

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This study determined the presence of human papillomavirus (HPV) DNA in oral mucosa cells from 121 patients with different types of oral mucosal lesions (13 squamous cell carcinomas, 59 potentially malignant lesions, 49 benign erosive ulcerative lesions) and from 90 control subjects. HPV DNA was detected by nested polymerase chain reaction, and genotype was determined by DNA sequencing. HPV prevalence was 61.5% in carcinomas, 27.1% in potentially malignant lesions, 26.5% in erosive ulcerative lesions, and 5.5% in control subjects. The risk of malignant or potentially malignant lesions was associated with HPV and was statistically significant. HPV-18 was found in 86.5% of HPV-positive lesions but was not associated with a particular type of lesion and was found in 80% of the HPV-positive control subjects. HPV infection was related to older age but not to sex, smoking, or alcohol use; the presence of lesions in the oral cavity increased the risk of HPV infection.

Human papillomavirus (HPV) is a small DNA virus that infects the squamous epithelium of the skin or mucosa. It causes both asymptomatic infections and various benign or malignant lesions. Of the ~25 HPV genotypes that infect the genital mucosa, a subgroup termed “high-risk” HPV (e.g., HPV-16, -18, and -31) has been detected in up to 99% of anogenital malignancies and is considered to be causally linked to cervical cancer [1].

At present, there is less information on the potential role of HPV in oral carcinogenesis, which is suggested by histologic similarities between lesions of the oral and genital mucosa and by studies showing that HPV can immortalize oral keratinocytes in vitro [1]. Although high-risk HPVs have been detected in malignant and potentially malignant oral lesions, they have also been found in normal oral mucosa [2, 3].

Understanding the role of HPV in oral carcinogenesis is complicated by different frequencies (0%–100%) of HPV infection in potentially malignant lesions and in oral cancer [4, 5], probably due to differences in sampling methods (biopsy, washings, brushings), patient profiles, and sensitivity of methods (in situ hybridization, polymerase chain reaction [PCR]). In this study, we investigated the prevalence of HPV infection in oral brushings from patients with different types of mucosal lesions and compared these with samples from a group of control subjects without oral mucosal disease. We also assessed whether HPV status, as assessed by PCR amplification and sequence analysis, was related to age, sex, alcohol use, smoking, or presence of lesions in the oral cavity.

Subjects and Methods

Study subjects. Oral brushings were obtained over 6 months (July–December 2000) from 211 consecutive subjects referred to the Department of Oral Sciences, University of Palermo, Palermo, Italy. Of these subjects, 121 (57.3%) presented with oral mucosal lesions [13 [10.7%] with oral squamous cell carcinoma [OSCC], 59 [48.8%] with potentially malignant lesions—25 with oral leukoplakia and 34 with oral lichen planus, both without epithelial dysplasia—and 49 [40.5%] with benign erosive-ulcerative lesions [EULs], likely of traumatic origin] and were termed the patient group. Ninety persons (42.7%) with hard dental tissue–related diseases and no sign of mucosal lesions were termed the control group. The patients were 57 men (mean age, 52 years; range, 18–89 years) and 64 women (mean age, 58 years; range, 18–94 years). The control subjects were 47 men (mean age, 40 years; range, 20–76 years) and 43 women (mean age, 42 years; range, 21–75 years). Information on age, smoking, and alcohol use was obtained by personal interview.

Sample collection and processing. Oral specimens were obtained by cytobrush (RAM; Mirandola), as described elsewhere [6]. Biopsy samples were taken from OSCCs and from potentially malignant lesions. In patients, samples were brushed from the lesion site; in control subjects, samples were obtained from >1 oral cavity site (buccal mucosa, tongue border, or mouth floor) and were pooled in the same tube.
PCR analysis. DNA extraction was done, as described elsewhere [6]. All clinical samples were checked for DNA by amplification of the human β-globin gene and were tested in duplicate. Three types of control samples were included in each reaction series: blank control, HPV-negative Wi cells (negative control), and HPV-18 DNA-positive HeLa cells in dilutions of 20,000–50,000 down to 2–5 HPV DNA copies (positive control). All control samples were prepared and analyzed in parallel with the clinical specimens, to ensure that proper reaction conditions were maintained. Special care was taken to control contamination. Standard precautions for PCR reactions were observed [7], and the different PCR steps (reaction mix preparation, sample preparation, amplification, and electrophoresis) were done in separate rooms. HPV DNA was amplified in a nested PCR assay (MY09-MY11 primer pair in combination with GP5-GP6 primer pair), as described elsewhere [8]. The MY-PCR detected 200–500 copies of HPV DNA (product size, ~450 bp) and the MY/GP-PCR ~2–10 copies (product size, ~40 bp). Amplifications were done in a DNA thermal cycler (Mastercycler gradient; Eppendorf). PCR products were analyzed in 2% agarose gel.

Sequencing analysis. The HPV genotyping was based on direct sequencing of MY- or MY/GP-PCR fragments [9]. Amplification products were purified by Microcon YM-100 (Amicon; Millipore). The sequence of both DNA strands was determined by the BigDye Ready Reaction kit (Perkin-Elmer Applied Biosystems) in an automatic sequencer (ABI Prism 310 analyzer; Perkin-Elmer Applied Biosystems). Alignments were obtained from the GenBank on-line BLAST server and downloaded HPV sequences from the HPV database (http://hpv-web.lanl.gov).

Data analysis. We analyzed HPV prevalence by using the standardized normal deviate Z test. Associations were investigated by the Fisher exact test, odds ratios (ORs), and corresponding 95% confidence intervals (CIs). We used the logistic regression model (SAS Institute) to examine whether HPV status depended on age, sex, smoking, alcohol use, or presence of lesions.

Results

Overall, we detected HPV DNA in 42 (19.9%) of 211 oral samples—by MY-PCR in 3 (7.1%) of 42 cases and by MY/GP-PCR in 39 cases (92.8%). Figure 1 shows PCR products from HeLa cells and oral samples. HPV infection was diagnosed significantly more often in patients (37 [30.6%] of 121) than in control subjects (5 [5.6%] of 90; P < .0001; OR, 7.49; 95% CI, 2.81–19.98). Of the lesions, 8 (61.5%) of 13 OSCCs, 16 (27.1%) of 59 potentially malignant lesions (7 leukoplakia and 9 lichen planus), and 13 (26.5%) of 49 EULs were HPV positive. HPV detection was significantly related to OSCCs (P = .002) and potentially malignant lesions (P = .009) but not to EULs (P = .079).

The differences in HPV detection were statistically significant between OSCCs and potentially malignant lesions (61.5% vs. 27.1%; P = .0086) or EULs (61.5% vs. 26.5%; P = .0088) but not between potentially malignant lesions and EULs (27.1% vs. 26.5%; P = .3299). The risk of oral lesions associated with HPV infection was higher for OSCCs (OR, 14.4; 95% CI, 1.37–21.98) and for potentially malignant lesions (OR, 7.8; 95% CI, 1.69–36.1) but did not reach a clear statistical significance for EULs (OR, 6.1; 95% CI, 0.98–19.2).

HPV-18 was the most frequent genotype (36 [85.7%] of 42 HPV-infected lesions) and was found alone or in combined in-

![Figure 1](https://example.com/figure1.png)
infection in 7 (87.5%) of 8 OSCCs, 14 (87.5%) of 16 potentially malignant lesions, 11 (84.6%) of 13 EULs, and 4 of 5 control subjects. More rarely detected were HPV-16, -33, -31, and -6; mixed HPV infections (genotypes 6/18 and 33/18) were found in 5 samples.

The risk of HPV detection (table 1, model 1) increased ~3% when age increased by 1 year, but other effects were not significant at the 5% level. When the possible effect of an oral lesion was included in the model (table 1, model 2), the odds of HPV presence for EULs, potentially malignant lesions, and OSCCs were 5.3, 7.8, and 55 times greater, respectively. Of note, the risk associated with age in this model was no longer statistically significant, due to a higher risk associated with presence of lesions.

**Discussion**

In this study, the presence of HPV DNA was detected in ~20% of the oral samples analyzed: in most cases (~93%), by the second step of our PCR system (sensitivity, 2–10 copy numbers) but not by the first (sensitivity, 200–500 copy numbers), This finding indicates that the oral HPV infections were weakly productive and is consistent with prior observations that HPV DNA–positive oral samples generally produce weaker PCR products than HPV-positive cervical specimens [2, 10]. Further investigations are needed to determine whether virus load could be an additional marker of oral HPV infection, as recently proposed for HPV-related cervical lesions [11].

HPV infection was diagnosed significantly more often ($P < .0001$) in patients than in control subjects and was associated with roughly a 7.5-fold increased risk of lesions. This finding is in contrast to studies that detected high percentages (40%–60%) of HPV in normal oral mucosa [3] but in accordance with that of Ostwald et al. [10], who found a very low prevalence of (subclinical) HPV infection in patients with healthy oral mucosa.

At present, the association of HPV with OSCC is not as clear as its association with cervical cancer [1, 11]. We detected HPV in 61.54% of OSCCs, which is within the range of 25%–75% reported in most PCR-based studies [4, 12]. Even though approaches other than HPV DNA detection alone (e.g., integration into cell chromosomal DNA or analysis of E6 and E7 gene expression) are also required to assess the contribution of HPV in OSCC, our findings of HPV association and a >14-fold higher risk of OSCC confirm a possible role for HPV in oral carcinogenesis and should be taken into account.

HPV involvement in the etiology of potentially malignant lesions (e.g., oral leukoplakia and oral lichen planus) has been proposed by some authors [4, 13]. Our finding of a 27.12% HPV detection rate (associated with nearly an 8-fold increased risk of potentially malignant lesions) is consistent with the HPV prevalence reported for such lesions (17%–40.8%) in other PCR-based studies [4, 12]. Because different histopathologic types of potentially malignant oral lesions have been identified (e.g., erythroplakia; verrucous and nodular leukoplakia; and erosive, atrophic, and reticular oral lichen planus) [13], long-term follow-up of the forms of potentially malignant lesions found to be HPV positive could help to clarify the effective role of HPV in the etiology of these lesions.

A further finding in the present study was the detection of HPV DNA in 26.53% of oral mucosal EULs, which, to the best of our knowledge, has not previously been reported. Because HPV cannot be thought to be responsible for this type of lesion, its detection could be due to the oral lesion per se, similarly to findings in the genital mucosa, where mechanical trauma can lead to epithelial basal cell exposure and subsequent HPV infection [1]. Our data on HPV infection in oral EULs, if substantiated by studies with larger numbers of patients, could have both screening and etiologic implications. Thus, long-term follow-up of patients with a history of HPV-positive EULs could be helpful in evaluating any clinically different behavior (tendency to persistence or recurrence or likelihood of progression to premalignant stages), compared with HPV-negative EULs.

Unexpectedly, HPV-18 was the most prevalent (~86%) genotype detected in all positive samples, and it was not associated with increased risk of oral lesions. Few cases (~12%) of mixed type HPV infections were diagnosed; however, because HPV genotyping based on cycle sequencing alone has limited ability for mixed HPV infection analysis [9], our data should be interpreted with caution. Very low percentages (2%–12%) of other HPV types (HPV-33, -31, or -6) were found, with an unexpectedly low prevalence (~5%) of HPV-16, a genotype frequently reported in oral lesions and carcinomas [4, 12]. These findings could indicate a different distribution pattern of HPV

**Table 1.** Logistic regression models of human papillomavirus in oral mucosal cells.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Odds ratio (95% confidence interval)</th>
<th>$P^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.03 (1.00–1.05)</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Smoking$^b$</td>
<td>1.64 (0.64–4.19)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.50 (0.12–2.06)</td>
<td></td>
</tr>
<tr>
<td>Sex$^c$</td>
<td>1.04 (0.45–2.39)</td>
<td></td>
</tr>
<tr>
<td>Alcohol use</td>
<td>0.96 (0.36–2.61)</td>
<td></td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.99 (0.96–1.02)</td>
<td></td>
</tr>
<tr>
<td>Smoking$^b$</td>
<td>1.50 (0.55–4.11)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.38 (0.08–1.84)</td>
<td></td>
</tr>
<tr>
<td>Sex$^c$</td>
<td>0.94 (0.37–2.38)</td>
<td></td>
</tr>
<tr>
<td>Alcohol use</td>
<td>0.66 (0.22–1.99)</td>
<td></td>
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<tr>
<td>Erosive ulcerative lesions</td>
<td>5.27 (1.56–17.82)</td>
<td>&lt;.05</td>
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<tr>
<td>Potentially malignant lesions</td>
<td>7.83 (2.22–27.62)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Oral squamous cell carcinoma</td>
<td>55.02 (9.17–330.12)</td>
<td>&lt;.001</td>
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</table>

$^a$ Statistical significance set at $P < .05$.

$^b$ Compared with nonsmokers.

$^c$ Female vs. male.
genotypes, probably reflecting the geographic origins of samples. Considerable genetic differences between Sicilians and other Italian populations were recently described [13]. Also, HPV-18 is the most frequent genotype in oral HPV infections in the Greek population [14], which is genetically similar to central and southern Italian populations [15].

When we measured demographic factors potentially associated with oral HPV infection, regardless of the presence of oral lesions, we found only older age to be a significant risk determinant for the presence of HPV DNA. Analysis of the possible effects of a lesion in the oral cavity showed that all types of oral mucosal lesions were associated with risk of HPV infection: In particular, the increased (~5-fold) risk associated with oral mucosal EULs is highly suggestive of a causal link and identifies these traumatic benign lesions as a possible predisposing factor for oral HPV infection.

Studies of oral HPV infection are still in the early phase, and aspects such as HPV load and persistence in infected cells of the oral mucosa need to be analyzed in more detail. Further studies to find the prognostic value, if any, of HPV infection as a biomarker for early diagnosis of oral mucosal malignant disease, especially relating to the design of a chemopreventive approach, will also be important.

References