ORIGINAL ARTICLE

Human papillomaviruses in oral carcinoma and oral potentially malignant disorders: a systematic review

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OBJECTIVES: Human papillomavirus (HPV) in oral carcinoma (OSCC) and potentially malignant disorders (OPMD) is controversial. The primary aim was to calculate pooled risk estimates for the association of HPV with OSCC and OPMD when compared with healthy oral mucosa as controls. We also examined the effects of sampling techniques on HPV detection rates.

METHODS: Systematic review was performed using PubMed (January 1966–September 2010) and EMBASE (January 1990–September 2010). Eligible studies included randomized controlled, cohort and cross-sectional studies. Pooled data were analysed by calculating odds ratios, using a random effects model. Risk of bias was based on characteristics of study group, appropriateness of the control group and prospective design.

RESULTS: Of the 1121 publications identified, 39 cross-sectional studies met the inclusion criteria. Collectively, 1885 cases and 2248 controls of OSCC and 956 cases and 675 controls of OPMD were available for analysis. Significant association was found between pooled HPV-DNA detection and OSCC (OR = 3.98; 95% CI: 2.62–6.02) and even for HPV16 only (OR = 3.86; 95% CI: 2.16–6.86). HPV was also associated with OPMD (OR = 3.87; 95% CI: 2.87–5.21). In a subgroup analysis of OPMD, HPV was also associated with oral leukoplakia (OR = 4.03; 95% CI: 2.34–6.92), oral lichen planus (OR = 5.12; 95% CI: 2.40–10.93), and epithelial dysplasia (OR = 5.10; 95% CI: 2.03–12.80).

CONCLUSIONS: The results suggest a potentially important causal association between HPV and OSCC and OPMD.

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Keywords: oral cancer; precancer; premalignancy; meta-analysis; case–control; human; HPV; lichen planus; leukoplakia; erythroplakia; adults; biopsy; cytology

Introduction

The specific role of human papillomaviruses (HPV) in the development of premalignant and oral squamous cell carcinoma (OSCC) continues to be debated topic (Syrjänen and Syrjänen, 2000, Adelstein et al, 2009) despite the well-established fact that the vast majority of cervical squamous cell carcinoma of uteri (CC) is attributable to HPV infection (zur Hausen, 1994, 2002, zur Hausen and de Villiers, 1994). The papillomavirus family (Papillomaviridae) is a highly diverse group of small non-enveloped DNA tumor viruses (de Villiers et al, 2004). HPVs are identified by complete sequence analysis, and classified by type, on the basis of their sequence homology within the capsid protein gene L1, the most conserved gene within the genome (de Villiers et al, 2004; de Villiers and Gunst, 2009). Hence, HPV types are referred to as genotypes. In humans, over 120 HPV genotypes have been fully sequenced (de Villiers et al, 2004; de Villiers and Gunst, 2009). HPVs have also been...
classified as high or low risk types based on the clinical behavior of the virally infected tissues.

The HPV virion is approximately 55 nm in diameter and consists of a closed circular double stranded DNA genome, with a size of size almost 8000 bp. Overall, the HPV genome has the capacity to encode eight proteins: E1, E2, E4-E7, the non-structural proteins involved mainly in replication, transcription and transformation and L1 and L2, the structural proteins that compose the capsid. HPV specifically target the undifferentiated proliferative basal cells of epithelial mucosa that are exposed following tissue trauma. HPV proteins, especially the oncoproteins E6 and E7 of the high risk HPVs (HR-HPVs), interact with different degrees of affinity, with host cell proteins to disturb the normal epithelial differentiation and apoptosis by stimulating cellular proliferation, DNA synthesis and inhibition of cell cycle regulators (Doorbar, 2007). The interactions between E7 and pRB and E6 and p53 have been characterized (Münger et al, 1989; Werness et al, 1990). Continued and aberrant expression of the E6 and E7 genes of the HR-HPVs leads to genomic instability, and mutational events that can result in malignant transformation (Stanley et al, 2007). Proteins of low-risk HPVs (LR-HPVs) have a low affinity for tumor suppressor proteins. Thus, these viruses have low oncogenic potential and the infections are usually self-limited. Persistent infection with HR-HPVs increases the risk of cancer, while the low-risk types may be associated with benign lesions. In women with CC, to date 15 HR-HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82), three probable (HPV 26, 53, and 66) and 12 LR-HPV types (HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and 89) have been identified, based on pooled case-control data. HPV16 is the most potent type to cause cancer at different anatomical sites, causing around 50% of all cervical cancer (Muñoz et al, 2004, IARC, 2007; Smith et al, 2007).

In normal oral mucosa, the following HPV types have been detected: HPV 2, 6, 7, 11, 13, 16, 18, 31, 33, and 35. The significance of HPV in healthy-appearing oral mucosa is not known. To date, in oral benign and malignant lesions so far, 24 types have been detected: HPV 1, 2, 3, 4, 6, 7, 10, 11, 13, 16, 18, 31, 32, 33, 35, 39, 45, 51, 52, 55, 56, 57, 58, 59, 66, 69, 72, and 73 (Syrjänen and Syrjänen, 2000, Kreimer et al, 2010). Of significance these included 13 HR-HPVs and probable HR-HPV that have been associated with CC.

As early as 1983, an original observation and hypothesis was presented, that implicated HPV as a risk factor in a subset of oral cancers (Syrjänen et al, 1983). Since then, several studies have focused on HPV detection in oral cancer but results have been conflicting (Miller and White, 1996, Syrjänen and Syrjänen, 2000, Ragin et al, 2007, Adelstein et al, 2009) and lacked the design rigor of case-controls studies. By contrast, the data on HPV association with oro-pharyngeal cancer is increasingly compelling (Melin et al, 2000, D’Souza et al, 2007; Adelstein et al, 2009; Ang et al, 2010). Miller and Johnstone were the first to present a meta-analysis based on pooled data from non-controlled studies between 1982 and 1997 to estimate HPV prevalence in tissues with precancerous and cancerous features and normal oral mucosa. They found that the frequency of HPV detection in normal oral mucosa [10.0%; 95% confidence interval (CI), 6.1–14.6%] was significantly less than in leukoplakia (22.2%; 95% CI, 15.7–29.9%), intra-epithelial neoplasia (26.2%; 95% CI, 19.6–33.6%), verrucaous carcinoma (29.5%; 95% CI, 23–36.8%), and OSCC (46.5%; 95% CI, 37.6–55.5%). The pooled odds ratio (OR) for the subset of studies directly comparing the prevalence of HPV in normal mucosa and OSCC was 5.4, confirming the trend observed in the overall sample (Miller and Johnstone, 2001), but once again their analyses were not based on case-control studies.

In the review of Kreimer et al (2005), HPV prevalence in OSCC was 23.5% (Kreimer et al, 2005). HPV16 was the most common type present and was detected in 16.0% of OSCC, accounting for almost 70% of HPV-positive cases. HPV18 was the next most common oncogenic HPV type, detected in 8% of OSCC (Kreimer et al, 2005; Adelstein et al, 2009).

The wide variations in HPV detection rates have been explained by differences in sampling (e.g. oral scrapings, cells acquired with mouthwash, or biopsies) and the sensitivity and specificity of HPV testing methods. Kellkokoski et al (1992) showed that the same samples taken from healthy oral mucosa tested HPV-positive in 3.8% and 29.4% with dot blot hybridization and polymerase chain reaction (PCR), respectively.

**Oral potentially malignant disorders**

The common term ‘oral potentially malignant disorders’ (OPMD) has been suggested for oral precancers, including both oral precancerous lesions (e.g. leukoplakia, erythroplakia, and oral proliferative verrucous leukoplakia) and oral precancerous conditions (e.g. lichen planus and submucous fibrosis). All oral mucosal lesions that carry a risk of malignant transformation are included under this term (Warnakulasuriya et al, 2007; van der Waal, 2009). In the present systematic review, the following OPMD were included: oral lichen planus (OLP), leukoplakia, erythroplakia and oral proliferative verrucous leukoplakia (OPVL).

*Oral lichen planus* is a chronic autoimmune disorder of unknown etiology in which predominantly T lymphocytes accumulate beneath the epithelium and increase the rate of differentiation of stratified squamous epithelium, resulting in either epithelial thickening or atrophy with or without ulceration (Epstein et al, 2003). In the literature published prior to 1998, 107 OLP samples were tested for the presence of HPV DNA with either *in situ* hybridization (ISH) or dot blot hybridization and 23% were positive. The most prevalent types detected were HPV 6 and 11, followed by HPV 16. Since that time, 1929 samples (either scrapings or biopsies) from normal oral mucosa, have been tested for HPV DNA, and 11% were positive (Syrjänen and Syrjänen, 2000). In comparison with normal oral mucosa the HPV detection rate in OLP was...
twice as high. Although additional studies (Giovannelli et al, 2002; Campisi et al, 2004a,b) have detected the prevalence of HPV in OLP, there has been no systematic review of the literature evaluating the strength of this association.

Leukoplakia has been defined as white plaques of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer (Warnakulasuriya et al, 2007). In a review evaluating the literature prior to 1998, 890 leukoplakic and keratotic lesions had been tested for HPV DNA, of which 25.4% were HPV-positive (Syrjänen and Syrjänen, 2000). Recently, Szarka et al (2009) detected HPV more frequently in lesions than in controls (P \leq 0.001 in all comparisons). HPV prevalence increased gradually with increasing severity of the lesions; 32.8%, 40.9%, and 47.7% in OLP, oral leukoplakia (OL), and OSCC, respectively. HPV copy number distribution patterns roughly corresponded to prevalence rates, but OLP and OL were comparable. HPV prevalence differed significantly between two OLP groups classified as either higher malignancy risk or lower malignancy risk lesions (42.6 vs 22.4%).

Erythroplakia has long been considered as the oral lesion with the highest potential for malignant transformation. The 1978 WHO definition is still used, and describes erythroplakia as ‘a fiery red patch that cannot be characterized clinically or pathologically as any other definable disease’ (Warnakulasuriya et al, 2007). By 1998, only 11 oral erythroplakia lesions had been tested for HPV DNA, and 54.5% tested HPV16 positive (Syrjänen and Syrjänen, 2000).

Oral proliferative verrucous leukoplakia is a rare, but distinct high-risk clinical form of OL (van der Waal, Reichart, 2008, Mete et al, 2010). Conflicting results exist in the literature concerning the presence of HPV in OPVL (Palefsky et al, 1995; Campisi et al, 2004a; Bagan et al, 2007). By 1998, 215 OPVL lesions had been tested for HPV, with the detection rate of 26.5% (Syrjänen and Syrjänen, 2000).

There are few follow-up studies on HPV and OPMD. In 1996, Nielsen et al found that an overall HPV detection rate in premalignant lesions was 40.8% (n = 49), while no patients in the control group (n = 20) were HPV-positive. All patients who developed oral cancers within 4–12 years were positive for HPV. By contrast, Yang et al (2009) detected HPV DNA in 22.8% of 167 OL lesions, which underwent malignant transformation. The most significant predictor for malignant transformation was found to be the recurrence of OL after treatment (P = 0.03), and not the HPV status.

The primary aim of this systematic review was to calculate the pooled estimates of the odds ratio (OR) for the association of HPV with OSCC and OPMD (cases), when compared with healthy oral mucosa (controls). We tested the null hypothesis that there is no difference in HPV prevalence between cases and controls. The secondary aim was to examine the effect of sampling technique (tissue vs exfoliated cells) on these risk estimates of HPV infection.

### Materials and methods

#### Literature search

A systematic literature search of MEDLINE from 1966 through September 2010 and EMBASE from 1990 through September 2010 was conducted without language restriction entering the following terms: HPV, human papillomavirus, oral squamous cell carcinoma, oral precancer, oral premalignancy, oral cancer, oral verrucous carcinoma, lichenoid, oral lichen planus, oral dysplasia, leukoplakia, erythroplakia, submucous fibrosis, oral verrucous leukoplakia, oral keratoacanthoma, oral Bowen’s disease, oral, mouth, oropharyngeal, risk factor, frequency, prevalence, epidemiology and serology as both medical subject heading (MeSH) terms and text words. Moreover, reference lists of previous meta-analyses and other relevant papers were searched. All abstracts were reviewed independently by two preselected standardized reviewers (AA, GL or PA, IB). When the article was considered relevant by the two reviewers, the full papers were obtained and evaluated.

#### Inclusion criteria

Studies addressing the relationship between HPV infection and OSCC and between HPV infection and OPMD were included in the present systematic review when they met the following criteria:

- Randomized controlled trials (RCTs) testing HPV vaccine efficacy and including OSCC and/or OPMD among outcomes or;
- Cohort studies comparing OSCC and/or OPMD incidence among subjects with and without HPV infection or;
- Case–control or cross-sectional studies comparing HPV infection among subjects with and without OSCC and/or OPMD.

Furthermore, the following criteria had to be met:

- Clinical and histological diagnosis of OSCC and OPMD specified;
- HPV infection based on detection of HPV by DNA detection in the tissue biopsies or exfoliated cytology samples;
- Studies that have healthy individuals as controls.

Studies were excluded if they:

- Included patients with malignancies different from cancer of the mouth (C01-C06, excluding the C01.9, base of the tongue), e.g. oro-pharyngeal cancer, and when it was impossible to extract cancer of the mouth data;
- Included among cases or controls, HIV-positive subjects, transplant patients or defined immunosuppression;
- Investigated pediatric patients (under 17 years) specifically.

#### Critical appraisal

From all eligible reports that met the inclusion criteria, two reviewers (AA and PA) independently extracted
relevant information and HPV DNA data. The follow-
ing quality criteria were adopted:

1. For RCTs, allocation concealment method, masking of the study and loss of participants to follow up;
2. For cohort studies, appropriateness of the control group: subjects belonging to the control group must not differ significantly from those of the study group, except for HPV infection (gender and age must be matched, subjects of the control group must be selected from the study base), length of follow up (at least 5 years) and prospective design (i.e. data and samples collected specifically for the study).
3. For case–control studies, characteristics of the study group (consecutive, unsellected patients with OSCC and/or OPMD), appropriateness of the control group: subjects belonging to the control group must not differ significantly from those of the study group, except for the diagnosis of OSCC and/or OPMD (gender and age must be matched, subjects of the control group must be selected from the study base) and prospective design (i.e. data and samples collected specifically for the study).

Each of the criteria for RCTs, cohort studies and case–control studies were rated as ‘met’, ‘unmet’, or ‘unclear’. The global validity of the study was assessed using three categories:

1. Low risk of bias: all of the criteria met;
2. Moderate risk of bias: one or two criteria unclear;
3. High risk of bias: at least one criterion unmet or three criteria unclear.

**Data extraction**

A standardized data extraction form was prepared and tested for review of three articles independently by five reviewers, which resulted in some changes in the form. Critical appraisal of all the studies that had been selected based on the inclusion criteria was then carried out without masking the name of authors, institutions or journal. The form was used to extract data from the study. The eligibility, validity and design including: HPV DNA detection methods, tissue samples for HPV DNA testing within or immediately adjacent to the actual lesion, intra-individual controls from mucosa not adjacent to the lesion (site specified) or inter-individual controls (site specified), and outcome information (including HPV type) were recorded on the extraction form for each study. When studies included data from different anatomical sites, only data on the cancers of the mouth were extracted. When this was not possible, the study was excluded. Furthermore, detailed information was sought on the methodology of HPV DNA detection from the eligible papers. This included how the samples were collected (rinse, brush, biopsy that provide either exfoliated cells or tissue for HPV-testing), and the type of HPV detection method (ISH, PCR, other DNA techniques), HPV genotyping and HPV quantification with real-time PCR on DNA or mRNA level. Positive and negative controls were always included in the HPV testing methods, but not specifically extracted onto the form.

**Statistical analysis**

The data were analyzed using Review Manager 5, a copyrighted freeware developed by the Cochrane Collaboration, for preparing and maintaining reviews (www.cochrane-net.org/revman). The primary analysis was the prevalence of HPV DNA in lesions and in the samples taken from the controls at any anatomical site of the mouth. The association between oral lesions and HPV prevalence was estimated by calculating OR and the 95% confidence interval (CI). When absence of events in one of the groups caused problems with computation of OR, 0.5 was added to all values for that study, except when absence of events involved both study and control groups, in this case OR was undefined (Yu et al, 2010). As heterogeneity among studies was expected on the basis of large variability in HPV prevalence across different countries, a random effect was used to calculate the summary estimate using Mantel-Haenszel method (Mantel, 1958, Greenland and Robins, 1985).

A sensitivity analysis was planned, excluding studies of lower methodological quality (i.e. studies at high risk of bias). To investigate potential for publication bias, the funnel plot of the OR of the included studies was checked for asymmetry (Sterne and Egger, 2001). Statistical heterogeneity was assessed with the $I^2$ statistic that has been conventionally adopted to indicate low, moderate, and high heterogeneity to values of 25%, 50%, and 75% (Higgins et al, 2003).

**Results**

**Results of the search strategy**

In total, 1121 papers were identified from the database searches and the full texts of 62 papers were acquired for further inspection. Of these, 24 papers were excluded because of inappropriate study design, while 39 studies met the criteria of a case–control design. Among these papers, no RCTs or cohort studies were identified. The papers included in this analysis were published between 1987 and 2009, included the following languages: English, Chinese, German, and Spanish.

**Critical appraisal of the included studies on OPMD and OSCC**

On the basis of the established criteria, the risk of bias in the studies included was quite high. In fact, none of the 39 included studies met all three criteria, three studies met two criteria (Giovannelli et al, 2002; Herrero et al, 2003; DeBanth et al, 2009), 11 met one criterion (Lei et al, 1996; Mao et al, 1996; Nielsen et al, 1996; Bustos et al, 1999; Patiman et al, 2001; Kansky et al, 2003; Koppikar et al, 2005; Hansson et al, 2005; Cianfriglia et al, 2006; Anaya-Saavedra et al, 2008), while the remainder of the studies did not fulfill any of the predefined quality criteria. In particular, only four studies enrolled consecutive patients. In nine studies, cases and controls were matched and four studies had a prospective design. No study was defined low risk of bias and only two were evaluated as a moderate risk of
bias (Herrero et al., 2003; Debanth et al., 2009). For this reason, sensitivity analysis was not performed.

**HPV and OSCC**

The characteristics of the studies included in this review are summarized in Table 1 and the results of the meta-analysis are shown in Figure 1. The 33 studies comprised a total of 1885 OSCC patients and 2248 controls. HPV prevalence across all studies was higher among OSCC samples than in controls with the exception of four investigations (Cox et al., 1993; Herrero et al., 2003; Sugiyama et al., 2003; Tachezy et al., 2009). The risk estimates for HPV association with OSCC varied from 0.32 (95% CI: 0.02–5.70) to 363.00 (95% CI: 13.76–9575.31), with significant heterogeneity between the studies ($I^2 = 71\%$). The pooled OR across all studies was 3.98 (95% CI: 2.62–6.02), indicating a significantly increased risk of HPV among the cases, when compared with the controls.

Figure 1 also shows the secondary analysis of HPV prevalence in cases and controls, stratified by the HPV sampling technique. In studies using biopsies for HPV detection in both cases and controls, the risk for HPV association had an OR = 3.30; 95% CI: 2.08–5.23. In studies that used biopsies for HPV testing of the OSCC cases and exfoliate cells for the controls, the risk for HPV association had an OR = 8.61; 95% CI: 3.52–21.09. In studies using exfoliated cells for HPV testing of both OSCC cases and controls, the risk estimates were not statistically significant. Sub-analysis also disclosed that the heterogeneity was mainly attributed to the two small subgroups, while studies examining tissue only showed an acceptable level of heterogeneity ($I^2 = 47\%$).

The visual examination of the symmetry of the funnel plot (Figure 2) did not suggest a large publication bias.

**HPV and OPMD**

The characteristics of the studies included in the review are summarized in Table 2 and the results of the meta-analysis are shown in Figure 4. Altogether, there were

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<tr>
<th>Study</th>
<th>Sample</th>
<th>Detection method$^a$</th>
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<tr>
<td>Maitland et al, 1987</td>
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<td>Szarka et al, 2009</td>
<td>Tissue Exfoliated cells</td>
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ISH, in situ hybridization.

$^a$At this stage, no distinction was made according to the PCR methods, whether single PCR, nested PCR or quantitative PCR. In most studies, single PCR was used with the primers targeting L1 gene. Thus, also no distinction was made at this stage between the HPV genes amplified for HPV testing nor the primers used for HPV testing were targeting the L1 or E genes.
956 patients with OPMD and 675 controls in these studies. In all of the investigations, the HPV detection rate was higher in the OPMD group than in the controls. The OR of HPV DNA detection in OPMD varied from 1.67 (95% CI: 0.17–16.22) to 363.00 (95% CI: 6.41–20565.48). The pooled estimate across all studies was 3.87 (95% CI: 2.87–5.21), indicating a significantly increased risk of HPV among OPMD patients when compared with controls. Interestingly, despite the great variability in these studies, the heterogeneity of the results of the whole group was close to 0 ($I^2 = 1\%$).

Figure 4 also shows the secondary analysis of HPV prevalence in cases and controls stratified by the method of sampling. Studies using either tissue biopsies or exfoliated cells for HPV testing (both in cases and controls).
controls) showed very similar risk estimates: OR = 3.69 (95% CI: 2.22–6.13) and OR = 4.66 (95% CI: 3.00–7.23), respectively. However, when biopsies were used for HPV testing in the OPMD cases and exfoliated cells in the controls, the difference in HPV prevalence between the cases and controls lost its significance.

As shown in Figure 5, the visual examination of the symmetry of the funnel plot did not suggest a large publication bias.

In a subgroup analysis of different entities of OPMD (Figure 6), the risk estimates for their association with HPV were statistically significant as follows: (1) unspecified OMPD (OR 4.44; 95% CI: 2.64–7.49), (2) lichen planus (OR 5.12; 95% CI: 2.40–10.93), (3) leukoplakia (OR 4.03; 95% CI: 2.34–6.92), and (4) epithelial dysplasia (OR 5.10; 95% CI: 2.03–12.80). In these subgroups, we also calculated the pooled estimates for HPV16 associations as summarized in Figure 7. Difference between cases and controls remained statistically significant for OLP (OR 5.61; 95% CI: 2.42–12.99) and OL (OR 4.47; 95% CI: 2.22–8.98).

**Discussion**

This study showed a strong association between the presence of HPV DNA, and specifically HPV16, and OSCC. This was not the case with OPVL and carcinoma in situ (CIS) because of the small size of the cohorts. This is the first and most comprehensive meta-analysis performed at this point in time that utilized a strict case–
control setting. For the present analysis, the literature was reviewed up to September 2010.

In the emerging era of HPV vaccines, interest in the role of HPV in cancers other than the genital tract has substantially increased. Recently, the association of HPV with a subset of oro-pharyngeal and particularly tonsillar cancer has been confirmed (Mellin et al., 2000; D’Souza et al., 2007). The role HPV in oral cancer (OSCC) has been under debate since the first report suggested this association in 1983 (Syrjanen et al., 1983). In this systematic review, we investigated the prevalence of HPV in OSCC and OPMD in strict case–control settings. Importantly, we only included studies where all lesions were histologically confirmed. In addition, unlike previous reviews, we included only studies detecting HPV in tissue samples or exfoliated cells. As a result of the adherence to these strict inclusion criteria, only 39 studies out of 1121 reports were eligible for the analysis. For OSCC, 33 studies including 1885 cancers and 2248 controls were analyzed, giving this review a substantial statistical power. Importantly, all studies based exclusively on HPV serology were excluded, because HPV seropositivity only signifies a past- or present-HPV exposure of the individual, with no indication of the exact site of infection. Most HPV serology studies have correlated HPV seropositivity with genital HPV infection and the concordance has been poor. Furthermore, no studies exist that demonstrate HPV seroconversion after oral HPV infection.

HPV and OSCC
Several previous reviews exist on HPV prevalence in normal, benign, premalignant, and malignant oral lesions. However, none of the earlier studies fulfill the strict definitions set forth for the present analysis. Miller and Johnstone were the first to publish a meta-analysis on HPV prevalence in precancer lesions, cancer and normal oral mucosa. Pooled data from non-controlled studies published between 1982 and 1997 showed that HPV was 2–3 times more likely to be detected in oral precancer lesions, and 4.7 times more likely to be present in oral carcinomas, when compared with normal mucosa (Miller and Johnstone, 2001). Pooled OR for a subset of studies directly comparing the prevalence of HPV in normal mucosa and OSCC was 5.37. The probability of detecting HR-HPV in OSCC was 2.8 times higher than that of LR-HPV types. Syrjanen reviewed the HPV literature published prior to 1998, and the pooled HPV detection rates in normal oral mucosa, OL and OSCC were 13% (with PCR), 25% (PCR or ISH) and 33% (PCR), respectively (Syrjänen and Syrjänen, 2000).

The present meta-analysis showed that HPV significantly increases the risk for OSCC, as compared with the controls (OR 3.98, 95% CI: 2.62–6.02). The pooled HPV prevalence was 33.7% in the OSCC group and 12.0% in controls, which closely agrees with the findings by Termine et al. (2008). However, the present findings are somewhat lower than those detected by Miller and Johnstone (2001) but higher than reported by Kreimer et al. (2005) and Smith et al. (2004). Kreimer et al. (2010) found any HPV in 4.5% (95% CI: 3.9–5.1) of the oral exfoliate samples from 4070 healthy individuals while HPV16 genotype accounted for 28% of all HPV types detected.

Based on our results, we can also estimate that the increased risk for OSCC is related mostly to HPV16 (pooled OR = 3.86; 95% CI: 2.16–6.87). However, the studies included in our analysis showed moderate heterogeneity. By contrast, Hobbs et al. (2006) reported only a weak association between HPV16 and OSCC.

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**Table 2 Characteristics of studies investigating human papillomavirus infection in oral potentially malignant disorder (OPMD) and controls**

<table>
<thead>
<tr>
<th>Study</th>
<th>Condition</th>
<th>Cases</th>
<th>Controls</th>
<th>Detection method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maitland et al, 1987</td>
<td>Not otherwise specified OPMD</td>
<td>Tissue</td>
<td>Tissue</td>
<td>PCR</td>
</tr>
<tr>
<td>Holladay and Gerald, 1993</td>
<td>Dysplasia; carcinoma in situ</td>
<td>Tissue</td>
<td>Tissue</td>
<td>PCR</td>
</tr>
<tr>
<td>Cox et al, 1993</td>
<td>Lichen planus; leukoplakia</td>
<td>Tissue</td>
<td>Tissue</td>
<td>PCR</td>
</tr>
<tr>
<td>Nielsen et al, 1996</td>
<td>Not otherwise specified OPMD</td>
<td>Tissue</td>
<td>Tissue</td>
<td>PCR and ISH</td>
</tr>
<tr>
<td>Mao et al, 1996</td>
<td>Dysplasia; carcinoma in situ</td>
<td>Tissue</td>
<td>Tissue</td>
<td>PCR</td>
</tr>
<tr>
<td>Gopalakrishnan et al, 1997</td>
<td>Proliferative verrucous leukoplakia</td>
<td>Tissue</td>
<td>Tissue</td>
<td>PCR</td>
</tr>
<tr>
<td>Bouda et al, 2000</td>
<td>Oral hyperplasia; dysplasia</td>
<td>Tissue</td>
<td>Exfoliated cells</td>
<td>PCR and ISH</td>
</tr>
<tr>
<td>Sand et al, 2000</td>
<td>Lichen planus; leukoplakia</td>
<td>Tissue</td>
<td>Tissue</td>
<td>PCR and ISH</td>
</tr>
<tr>
<td>Patiman et al, 2001</td>
<td>Oral hyperplasia; dysplasia</td>
<td>Tissue</td>
<td>Exfoliated cells</td>
<td>PCR</td>
</tr>
<tr>
<td>Giovannelli et al, 2002</td>
<td>Not otherwise specified OPMD</td>
<td>Tissue</td>
<td>Tissue</td>
<td>PCR</td>
</tr>
<tr>
<td>Sugiyama et al, 2003</td>
<td>Dysplasia</td>
<td>Tissue</td>
<td>Exfoliated cells</td>
<td>PCR</td>
</tr>
<tr>
<td>OFlahartara et al, 2003</td>
<td>Lichen planus</td>
<td>Exfoliated cells</td>
<td>Exfoliated cells</td>
<td>PCR</td>
</tr>
<tr>
<td>Campesi et al, 2004a,b</td>
<td>Lichen planus; leukoplakia</td>
<td>Exfoliated cells</td>
<td>Exfoliated cells</td>
<td>PCR</td>
</tr>
<tr>
<td>Cianfriglia et al, 2006</td>
<td>Lichen planus</td>
<td>Exfoliated cells</td>
<td>Exfoliated cells</td>
<td>PCR</td>
</tr>
<tr>
<td>Luo et al, 2007</td>
<td>Not otherwise specified OPMD</td>
<td>Tissue</td>
<td>Tissue</td>
<td>PCR</td>
</tr>
<tr>
<td>Llamas-Martinez et al, 2008</td>
<td>Leukoplakia</td>
<td>Exfoliated cells</td>
<td>Exfoliated cells</td>
<td>PCR</td>
</tr>
<tr>
<td>Debanth 2009</td>
<td>OPMD; dysplasia</td>
<td>Cells</td>
<td>Cells</td>
<td>RNA probe</td>
</tr>
<tr>
<td>Majunder et al, 2009</td>
<td>Leukoplakia</td>
<td>Tissue</td>
<td>Exfoliated cells</td>
<td>PCR</td>
</tr>
<tr>
<td>Szarka et al, 2009</td>
<td>Lichen planus; leukoplakia</td>
<td>Exfoliated cells</td>
<td>Exfoliated cells</td>
<td>PCR</td>
</tr>
</tbody>
</table>

ISH, *in situ* hybridization; OPMD, oral potentially malignant disorder.
Importantly, the association of HPV with OSCC was significant only when HPV was detected in the biopsy samples, being strongest and most consistent when studies used biopsy samples for HPV detection in both cases and controls. However, the HPV association was also significant when biopsy samples of OSCC were compared with cytology of the controls. This is consistent with the view that HPV infection is multi-focal, i.e., exfoliated cytology is positive even if taken outside of the lesion of interest. This finding has also been confirmed in the genital tract (Barzon et al., 2010). The significant association was completely lost when only exfoliated cells were used to analyse HPV in both the cases and the controls. This finding is supported by Herrero et al. (2003) who showed that HPV DNA in exfoliated cells was not associated with HPV DNA detection in OSCC biopsies. Thus, when future studies exploring the relationship between HPV and OSCC are designed, only biopsied tissues should be used for HPV testing, to obtain the most accurate results.

**HPV and OPMD**

To the best of our knowledge, this is the first systematic review showing a strong association between HPV detection and OPMD, when the same sampling technique was used for both cases and controls (HPV DNA detected in biopsy samples: OR = 3.69; 95% CI: 2.22–6.13; HPV detected in exfoliated cells OR = 4.66; 95% CI: 3.00–7.23). This significance was lost, however, when the sampling methods were different for the cases and the controls. Importantly, when the different subgroups of histological entities among OPMD were dissected, the association of HPV with OLP, OL and epithelial dysplasia remained statistically significant, while OPVL, and CIS did not reach statistical significance. This might be related to the limited number of cases and controls in the OPVL-, epithelial dysplasia-, and CIS studies. HPV prevalence in these OPMD lesions was quite similar as reported by Miller and Johnstone (2001) and Syrjänen and Syrjänen (2000), but higher in the controls than reported by Kreimer et al. (2010). However, Kreimer et al., 2010 included in their meta-analysis only the
studies with more than 50 subjects sampled with scrapings or oral rinse only and tested with PCR. The present meta-analysis also showed that HPV16 was significantly increased in OL and OLP compared with controls. The strength of the association between HPV and OLP was somewhat unexpected and especially interesting in view of the acceptance of OLP as a premalignant condition. However, it is not easy to explain why a chronically inflamed epithelium thought to be attacked by T cells should be prone to support HPV infection. One explanation could be that ulceration is frequent in OLP making it more susceptibility for HPV infection. Another potential reason could be the chronic use of steroids which may induce immune suppression that could regulate HPV replication. Follow-up studies are needed to elucidate if the natural histories of HPV-infected OL and OLP lesions differ from those without HPV infection. Until now, few follow-up studies exist on the progression of OPMD toward malignancy and the role of HPV remains contradictory (Nielsen et al, 1996, Yang et al, 2009).

**HPV testing**

Human papillomavirus testing method is critical for the estimation of HPV prevalence in different oral diseases. Sampling techniques together with widely divergent PCR methods in different studies explain most of the variability in HPV prevalence among OSCC and control samples. Most meta-analyses on HPV and genital diseases are based on PCR as the gold standard. General or consensus primers targeting L1 gene are most frequently used for HPV detection because they are able to identify several HPV genotypes at the same time. All studies ISH can be an even more sensitive method than PCR in cases where only a few cells in the sample contain high copy numbers of the virus that are not detectable with PCR (Syrjänen, 1990). In the present meta-analysis, no distinction was made between the PCR primers used in HPV detection. Inadequate sample purification because of PCR inhibition has been shown to result in significant underestimates of oral HPV prevalence (Puranen et al, 1997; D’Souza et al, 2007). Moreover, the quality of the sample (e.g. frozen or fixed) might significantly affect the HPV testing results. Termine et al showed in their meta-analysis (1988–2007) that the detection rate of HPV with ISH was higher in formalin-fixed and paraffin-embedded biopsies derived from OSCC than in other head and neck cancers (38.1% vs 24.1%). When PCR was used, the detection rate increased to 39.9% (Termine et al, 2008). Transcriptional activity of HPV oncogenes E6 and E7 is important in understanding the role of HPV in oral diseases. Until now, however, only a few studies on transcriptional activity of HPV in OSCC or OPMD are available (Koskinen et al, 2003, Badaracco et al, 2007). In all future studies, the sampling, processing of the samples as well as PCR protocols should be standardized to allow for more precise comparison of the results. One should also recognize the limitations of the selected HPV testing method with regard to the samples used. Most of the studies have focused only on HPV16 or a restricted panel of other HR-HPV genotypes. Further studies might reveal other genotypes associated with OPMD and OSCC.

One shortcoming of this meta-analysis is that we were not able to assess the detailed anatomic location or histological variants of the oral lesions associated with HPV because of the lack of the data in original papers. Importantly, because most of these studies have failed to collect the data on smoking, drinking history, sexual habits, age or other potential risk factors, we made no attempt to analyze the confounding risk factors of HPV. One limitation is also that we did not categorize the studies by strength of study design. By the strict selection criteria used here, the majority of the included studies were considered to have an inherent risk of bias. Also very few of the studies that used negative and positive controls stated
another methodological limitation is the efficiency of nucleic acid isolation, which could have been influenced by time and temperature of storage.

In conclusion, the results of this meta-analysis showed a strong association between HPV and OPM-D/OSCC, thus justifying the rejection of the null hypothesis that HPV is equally prevalent in normal oral mucosa.

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oral mucosa and OPMD or OSCC. For HPV testing, biopsy samples are more appropriate than exfoliated cell samples. In the total lack of prospective cohort studies, we were unable to take a position on the temporal relationship between HPV infection and oral malignancies. To formally confirm the role of HPV as an etiological agent of OSCC, additional evidence is required, summarized in the ‘modified Koch’s postu-

Figure 7 Forest plot of human papillomavirus (HPV) 16 prevalence in different subgroup among oral potentially malignant disorder (OPMD) and control samples.
lates’ (Haverkos, 2004): (1) viral infection precedes the development of cancer, which has not yet been formally shown, although the current case–control approach gives the best evidence available to date to support this concept; (2) viral genome present in tumor lesions or in tumor cells, a finding that has been confirmed by this review; (3) epidemiologic association between the presence of the virus and development of cancer, a fact that was not shown in the present review because no randomized case–control studies or follow-up studies have been published; (4) ability of the virus or viral proteins to transform cells in vitro, which has been clearly shown; (5) ability of the virus or viral proteins to promote tumor formation in animals; an experimental model exists where HPV16 E6/E7 expression together with carcinogen 4NQO induces oral cancer in transgenic mice within 9 months (Strati et al., 2006, Jabbar et al. 2010); (6) finally, prophylactic HPV vaccination eliminates OSCC, however, obtaining this ‘final proof’ will require at least 20 years, before the oral cancer prevalence in HPV vaccinated and non-vaccinated subjects can be ascertained epidemiologically.

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Author contributions

All authors of the group 4 have actively participated in the execution of this study.

References


Review.


