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ORIGINAL ARTICLE

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

S Syrjänen[1], G Lodi[2], I von Bültzingslöwen[3], A Aliko[4], P Arduino[5], G Campisi[6], S Challacombe[7], G Ficarra[8], C Flaitz[9], HM Zhou[10], H Maeda[11], C Miller[12], M Jontell[13]

[1]*Department of Oral Pathology, Institute of Dentistry, Faculty of Medicine, University of Turku, Turku, Finland;* [2]*Dipartimento di Medicina, Chirurgia e Odontoiatria, Universita' degli Studi di Milano, Italy;* [3]*Department of Oral Microbiology and Immunology, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg, Sweden;* [4]*Department of Stomatology, Faculty of Medicine, University of Tirana, Albania;* [5]*Department of Biomedical Sciences and Human Oncology, Oral Medicine Section, Lingotto Dental School, University of Turin, Turin, Italy;* [6]*Settore di Medicina Orale "V.Margiotta", Dipartimento di Discipline Chirurgiche ed Oncologiche, Universita' di Palermo, Palermo, Italy;* [7]*Oral Medicine, King's College London Dental Institute, Guys Hospital, London, UK;* [8]*Reference Center for the Study of Oral Diseases, Department of Odonto-Stomatology, Florence, Italy;* [9]*Oral and Maxillofacial Pathology, The University of Texas Health Science Center at Houston Dental Branch, Houston, TX, USA;* [10]*Department of Oral Medicine, West China College of Stomatology, Sichuan University, Chengdu, China;* [11]*Department of Oral Pathology, School of Dentistry, Aichi Gakuin University, Nagoya, Japan;* [12]*Department of Oral Health Practice, College of Dentistry, University of Kentucky, Lexington, KY, USA;* [13]*Department of Oral Medicine, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg, Sweden*

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

Correspondence: Stina Syrjänen, Department of Oral Pathology, Institute of Dentistry, Faculty of Medicine, University of Turku, Lemminkäisenkatu 2, FI-20520 Turku, Finland. Tel: +358-2-3338349, Fax: 358-2-3338399, E-mail: stina.syrjanen@utu.fi
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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. HPVs 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 (Syrjanen *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 (Mellin *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%), verrucous 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. Kellokoski *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 presence 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 is '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,b; 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 following 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, unselected 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

Table 1 Characteristics of studies investigating human papillomavirus infection in oral squamous cell carcinoma and control samples

Study	*Sample*		*Detection method*[a]
	Cases	*Controls*	
Maitland *et al*, 1987	Tissue	Tissue	ISH
Chang *et al*, 1989	Tissue	Tissue	ISH
Yeudall and Campo, 1991	Tissue	Tissue	PCR
Cox *et al*, 1993	Tissue	Tissue	ISH
Holladay and Gerald, 1993	Tissue	Tissue	PCR
Mao, 1995	Exfoliated cells	Exfoliated cells	PCR
Lei *et al*, 1996	Tissue	Tissue	PCR
Mao *et al*, 1996	Tissue	Tissue	PCR
Cruz *et al*, 1996	Tissue	Tissue	PCR
Gopalakrishnan *et al*, 1997	Tissue	Tissue	PCR
Wang *et al*, 1998	Tissue	Tissue	PCR
Bustos *et al*, 1999	Tissue	Exfoliated cells	ISH
Sand *et al*, 2000	Tissue	Tissue	PCR
Bouda *et al*, 2000	Tissue	Exfoliated cells	PCR
Cao *et al*, 2000	Tissue	Tissue	PCR
Patiman 2001	Tissue	Tissue	PCR
Patiman *et al*, 2001	Tissue	Tissue	PCR
Giovannelli *et al*, 2002	Exfoliated cells	Exfoliated cells	PCR
Herrero 2003	Exfoliated cells	Exfoliated cells	PCR
Regezi *et al*, 2002	Tissue	Tissue	PCR
Sugiyama *et al*, 2003	Tissue	Tissue	PCR
Kansky *et al*, 2003	Tissue	Tissue	PCR
Chang *et al*, 2003	Tissue	Tissue	PCR
Zhang *et al*, 2004	Tissue	Tissue	PCR
Koppikar *et al*, 2005	Tissue	Exfoliated cells	PCR
Hansson *et al*, 2005	Exfoliated cells	Exfoliated cells	PCR
Luo *et al*, 2007	Exfoliated cells	Exfoliated cells	PCR
da Silva *et al*, 2007	Tissue	Tissue	PCR
Anaya-Saavedra *et al*, 2008	Tissue and exfoliated cells	Exfoliated cells	PCR
Llamas-Martínez *et al*, 2008	Tissue	Tissue	PCR
Majunder *et al*, 2009	Tissue and exfoliated cells	Exfoliated cells	PCR
Tachezy *et al*, 2009	Exfoliated cells	Exfoliated cells	PCR
Szarka *et al*, 2009	Tissue	Exfoliated cells	PCR

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.

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.

Figure 3 shows the comparison between the presence of HPV16 among OSCC and controls. A total of 725 OSCC cases were compared with 539 controls in 18 included studies. An OR of 3.86 (95% CI: 2.16–6.87) was statistically significant. Heterogeneity was less than that detected in the OPMD analysis (I^2 = 49%).

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

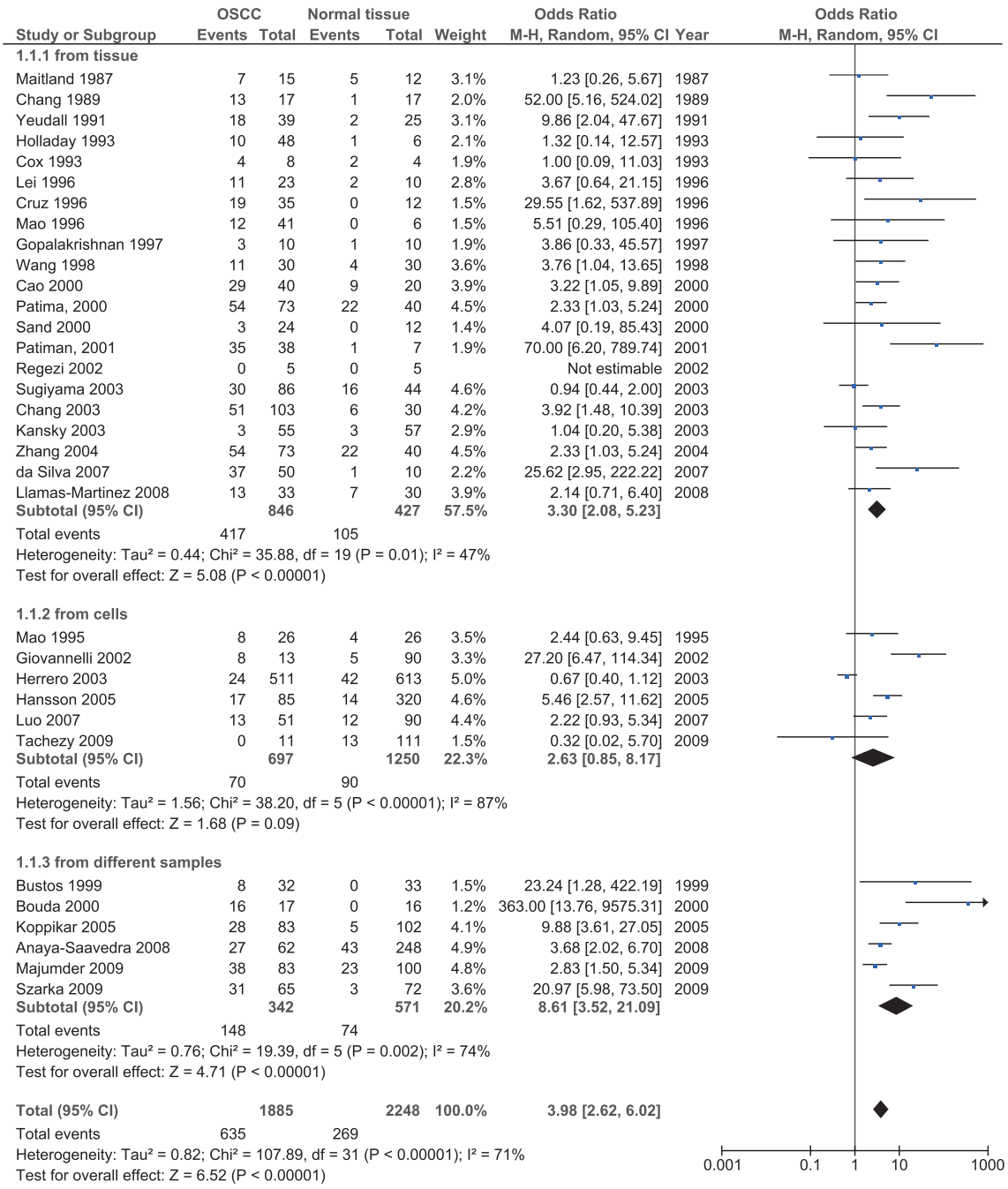

Figure 1 Forest plot of human papillomavirus (HPV) prevalence in oral squamous cell carcinoma (OSCC) and control samples. Biopsied samples and exfoliated cells were used for HPV testing both in cases and controls in 1.1.1 and 1.1.2, respectively. In 1.1.3 the biopsy samples of OSCC were tested for HPV while in the controls exfoliated cells were used for HPV testing. Studies are ordered by year of publication. The square and horizontal line correspond to the study odds ratio and the 95% confidence intervals. The area of the squares reflects the weight each trial contributes in the meta-analysis. The diamond represents the combined odds ratio with its 95% confidence intervals

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

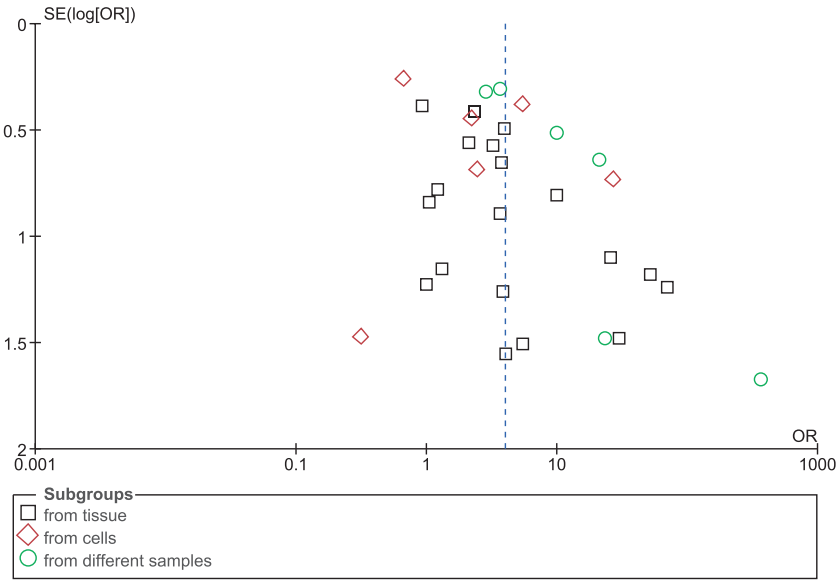

Figure 2 Funnel plot of the studies investigating human papillomavirus (HPV) infection in oral squamous cell carcinoma (OSCC) and control samples

Study or Subgroup	OSCC Events	OSCC Total	Normal tissue Events	Normal tissue Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Chang 1989	13	17	1	17	4.3%	52.00 [5.16, 524.02]	1989
Yeudall 1991	10	39	0	25	3.1%	18.15 [1.01, 325.36]	1991
Holladay 1993	10	48	1	6	4.4%	1.32 [0.14, 12.57]	1993
Cox 1993	4	8	2	4	4.1%	1.00 [0.09, 11.03]	1993
Mao 1995	8	26	4	26	7.9%	2.44 [0.63, 9.45]	1995
Cruz 1996	15	35	0	12	3.1%	18.90 [1.04, 344.45]	1996
Gopalakrishnan 1997	3	10	1	10	3.9%	3.86 [0.33, 45.57]	1997
Wang 1998	11	30	4	30	8.2%	3.76 [1.04, 13.65]	1998
Bustos 1999	1	32	0	33	2.6%	3.19 [0.13, 81.25]	1999
Cao 2000	18	40	6	20	9.0%	1.91 [0.61, 5.98]	2000
Sand 2000	1	24	0	12	2.5%	1.60 [0.06, 42.13]	2000
Bouda 2000	13	19	0	16	3.0%	68.54 [3.53, 1329.30]	2000
Kansky 2003	1	55	1	57	3.3%	1.04 [0.06, 17.00]	2003
Sugiyama 2003	30	86	16	44	11.2%	0.94 [0.44, 2.00]	2003
Zhang 2004	43	73	13	40	10.9%	2.98 [1.33, 6.69]	2004
Hansson 2005	12	85	3	85	8.1%	4.49 [1.22, 16.55]	2005
Llamas-Martinez 2008	11	33	0	30	3.1%	31.18 [1.74, 557.27]	2008
Szarka 2009	18	65	2	72	7.1%	13.40 [2.97, 60.49]	2009
Total (95% CI)		**725**		**539**	**100.0%**	**3.86 [2.16, 6.87]**	
Total events	222		54				

Heterogeneity: Tau² = 0.62; Chi² = 33.22, df = 17 (P = 0.01); I² = 49%
Test for overall effect: Z = 4.58 (P < 0.00001)

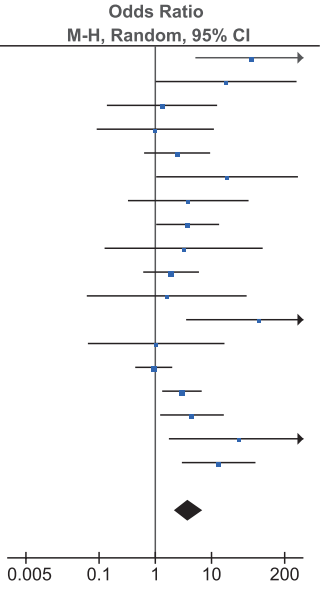

Figure 3 Forest plot of human papillomavirus (HPV) 16 prevalence in oral squamous cell carcinoma (OSCC) and control samples

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–

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

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

ISH, *in situ* hybridization; OPMD, oral potentially malignant disorder.

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; Adelstein *et al*, 2009; Ang *et al*, 2010). 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. Syrjänen 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

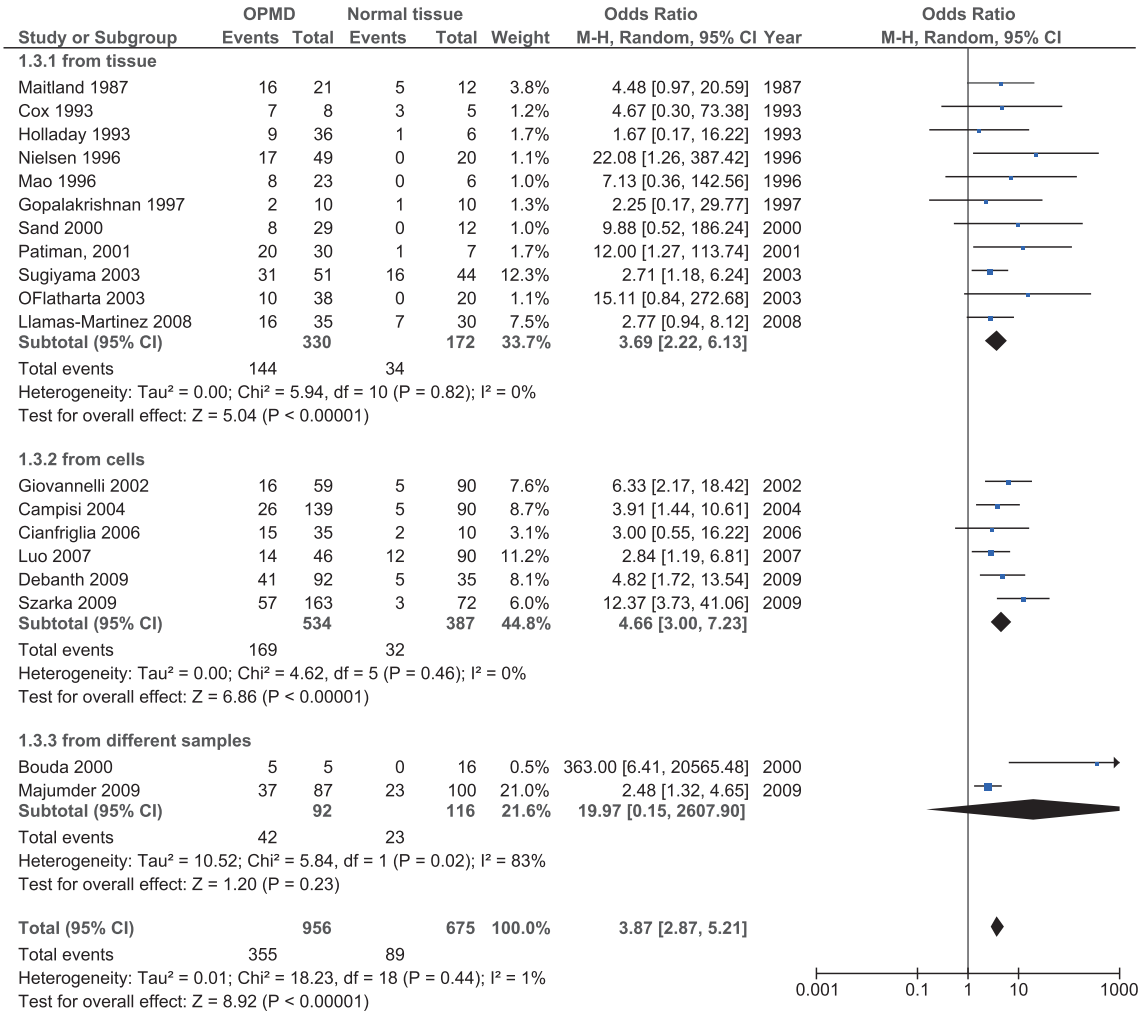

Figure 4 Forest plot of human papillomavirus (HPV) prevalence in oral potentially malignant disorder (OPMD) compared with control samples in the 18 studies included. Biopsied samples and exfoliated cells were used for HPV testing both in cases and controls in 1.3.1 and 1.3.2, respectively. In 1.3.3 the biosy samples of OSCC were tested for HPV while in controls exfoliated cells were used.

(OR = 2.0; 95% CI: 1.2–3.4). 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

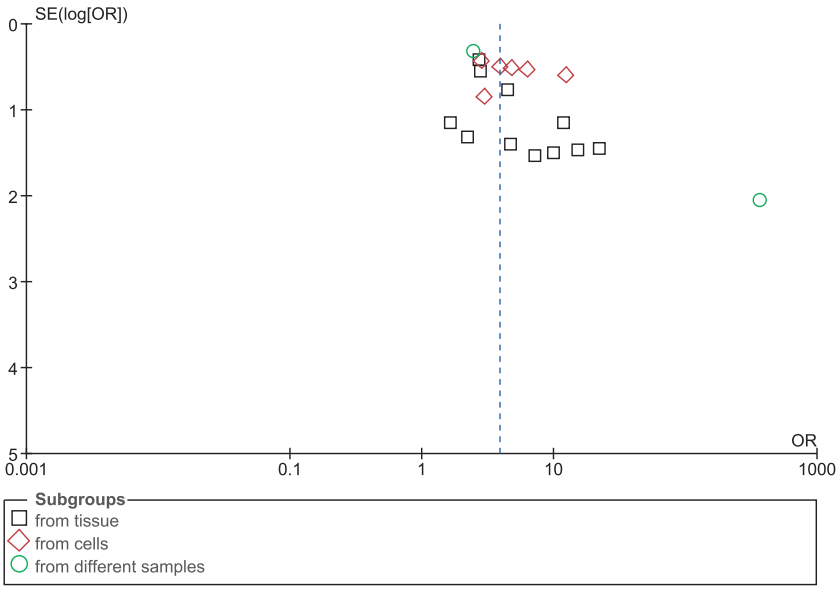

Figure 5 Funnel plot of the studies investigating human papillomavirus (HPV) infection in oral potentially malignant disorder (OPMD) and control samples

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 the 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

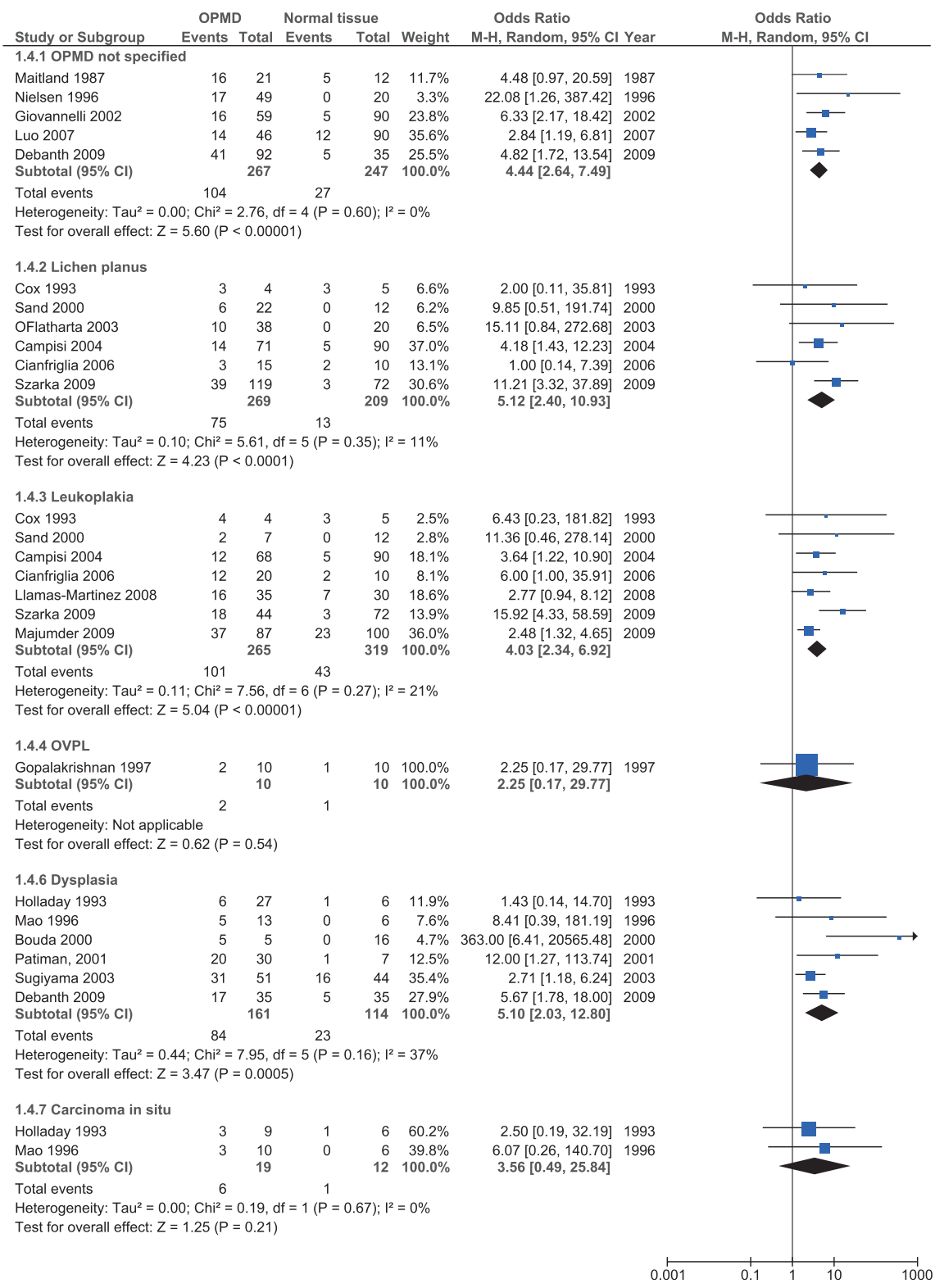

Figure 6 Forest plot of human papillomavirus (HPV) prevalence in different subgroup among oral potentially malignant disorder (OPMD) and control samples

the sensitivity of the PCR method used. 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 OPMD/OSCC, thus justifying the rejection of the null hypothesis that HPV is equally prevalent in normal

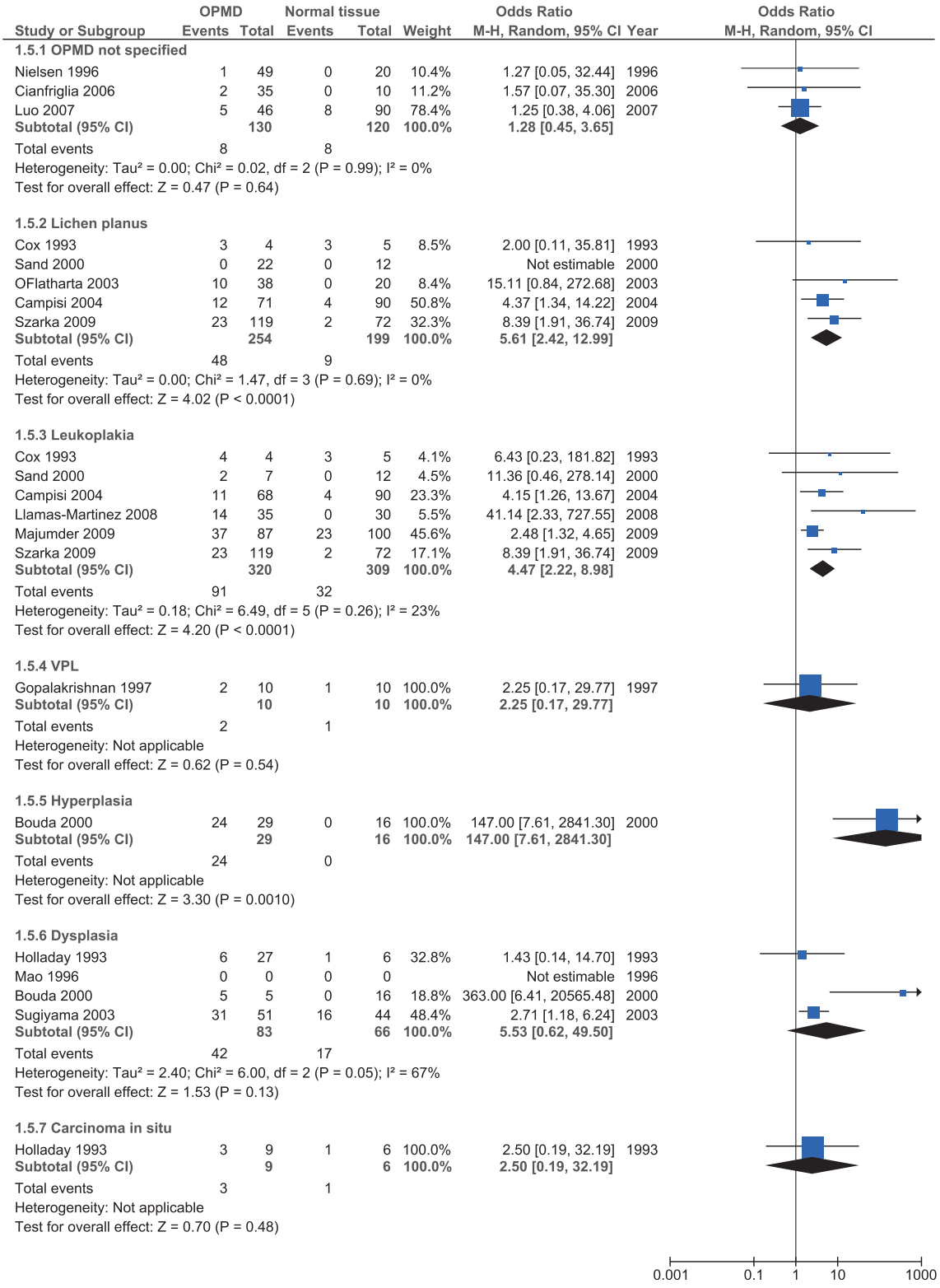

Figure 7 Forest plot of human papillomavirus (HPV) 16 prevalence in different subgroup among oral potentially malignant disorder (OPMD) and control samples

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-

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

Adelstein DJ, Ridge JA, Gillison ML *et al* (2009). Head and neck squamous cell cancer and the human papillomavirus: summary of a National Cancer Institute State of the Science Meeting, November 9–10, 2008, Washington, D.C. *Head Neck* **31:** 1393–1422.

Anaya-Saavedra G, Ramírez-Amador V, Irigoyen-Camacho ME *et al* (2008). High association of human papillomavirus infection with oral cancer: a case–control study. *Arch Med Res* **39:** 189–197.

Ang KK, Harris J, Wheeler R *et al* (2010). Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med* **363:** 24–35.

Badaracco G, Rizzo C, Mafera B *et al* (2007). Molecular analyses and prognostic relevance of HPV in head and neck tumours. *Oncol Rep* **17:** 931–939.

Bagan JV, Jimenez Y, Murillo J *et al* (2007). Lack of association between proliferative verrucous leukoplakia and human papillomavirus infection. *J Oral Maxillofac Surg* **65:** 46–49.

Barzon L, Militello V, Pagni S *et al* (2010). Distribution of human papillomavirus types in the anogenital tract of females and males. *J Med Virol* **82:** 1424–1430.

Bouda M, Gorgoulis VG, Kastrinakis NG *et al* (2000). ''High risk'' HPV types are frequently detected in potentially malignant and malignant oral lesions, but not in normal oral mucosa. *Mod Pathol* **13:** 644–653.

Bustos DA, Pavan JV, Carricart SE *et al* (1999). Human papillomavirus detection in oral cancer lesions in the city of Córdoba. *Rev Fac Cien Med Univ Nac Cordoba* **56:** 65–71 (Spanish).

Campisi G, Giovannelli L, Aricò P *et al* (2004a). HPV DNA in clinically different variants of oral leukoplakia and lichen planus. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **98:** 705–711.

Campisi G, Giovannelli L, Ammatuna P *et al* (2004b). Proliferative verrucous vs conventional leukoplakia: no significantly increased risk of HPV infection. *Oral Oncol* **40:** 835–840.

Cao J, Zhang ZY, Patima, Zhang YX, Chen WT (2000). Human papillomavirus infection and p53 alteration in oral squamous cell carcinoma. *Chin J Dent Res* **3:** 44–49.

Chang KW, Chang CS, Lai KS, Chou MJ, Choo KB (1989). High prevalence of human papillomavirus infection and possible association with betel quid chewing and smoking in oral epidermoid carcinomas in Taiwan. *J Med Virol* **28:** 57–61.

Chang JY, Lin MC, Chiang CP (2003). High-risk human papillomaviruses may have an important role in non-oral habits-associated oral squamous cell carcinomas in Taiwan. *Am J Clin Pathol* **120:** 909–916.

Cianfriglia F, Di Gregorio DA, Cianfriglia C, Marandino F, Perrone Donnorso R, Vocaturo A (2006). Incidence of human papillomavirus infection in oral leukoplakia. Indications for a viral aetiology. *J Exp Clin Cancer Res* **25:** 21–28.

Cox M, Maitland N, Scully C (1993). Human herpes simplex-1 and papillomavirus type 16 homologous DNA sequences in normal, potentially malignant and malignant oral mucosa. *Eur J Cancer B Oral Oncol* **29B:** 215–219.

Cruz IB, Snijders PJ, Steenbergen RD *et al* (1996). Age-dependence of human papillomavirus DNA presence in oral squamous cell carcinomas. *Eur J Cancer B Oral Oncol* **32B:** 55–62.

Debanth S, Singh PA, Mehrotra R, Singh M, Gupta SC, Pandya S, Chowdhury A, Singh M (2009). Human papillomavirus infection and premalignant lesions of the oral cavity: a cross sectional study in Allahabad, North India. *Asia-Pacific Journal of Clinical Oncology* **5:** 111–118.

Doorbar J (2007). Papillomavirus life cycle organization and biomarker selection. *Dis Markers* **23:** 297–313.

D'Souza G, Kreimer AR, Viscidi R *et al* (2007). Case–control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med* **356:** 1944–1956.

Epstein JB, Wan LS, Gorsky M, Zhang L (2003). Oral lichen planus: progress in understanding its malignant potential and implications for clinical management. *Oral Surg Oral Med Oral Pathol* **96:** 32–37.

Giovannelli L, Campisi G, Lama A *et al* (2002). Human papillomavirus DNA in oral mucosal lesions. *J Infect Dis* **185:** 833–836.

Gopalakrishnan R, Weghorst CM, Lehman TA *et al* (1997). Mutated and wild-type p53 expression and HPV integration in proliferative verrucous leukoplakia and oral squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **83:** 471–477.

Greenland S, Robins JM (1985). Estimation of a common effect parameter from sparse follow-up data. *Biometrics* **41:** 55–68.

Hansson BG, Rosenquist K, Antonsson A *et al* (2005). Strong association between infection with human papillomavirus and oral and oropharyngeal squamous cell carcinoma: a population-based case–control study in southern Sweden. *Acta Otolaryngol* **125:** 1337–1344.

zur Hausen H (1994). Molecular pathogenesis of cancer of the cervix and its causation by specific human papillomavirus types. *Curr Top Microbiol Immunol* **186:** 131–156.

zur Hausen H (2002). Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* **2:** 342–350, Review.

zur Hausen H, de Villiers EM (1994). Human papillomaviruses. *Annu Rev Microbiol* **48:** 427–447.

Haverkos HW (2004). Viruses, chemicals and co-carcinogenesis. *Oncogene* **23:** 6492–6499, Review.

Herrero R, Castellsagué X, Pawlita M *et al* (2003). Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. *J Natl Cancer Inst* **95:** 1772–1783.

Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003). Measuring inconsistency in meta-analyses. *BMJ* **327:** 557–560.

Hobbs CG, Sterne JA, Bailey M, Heyderman RS, Birchall MA, Thomas SJ (2006). Human papillomavirus and head and neck cancer: a systematic review and meta-analysis. *Clin Otolaryngol* **31:** 259–266, Review.

Holladay EB, Gerald WL (1993). Viral gene detection in oral neoplasms using the polymerase chain reaction. *Am J Clin Pathol* **100:** 36–40.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans (2007). Human papillomaviruses. *IARC* **90:** 1–670.

Jabbar S, Strati K, Shin MK, Pitot HC, Lambert PF (2010). Human papillomavirus type 16 E6 and E7 oncoproteins act synergistically to cause head and neck cancer in mice. *Virology* **407:** 60–67.

Jalouli J, Ibrahim SO, Mehrotra R *et al* (2010). Prevalence of viral (HPV, EBV, HSV) infections in oral submucous fibrosis and oral cancer from India. *Acta Otolaryngol* **130:** 1306–1311.

Kansky AA, Poljak M, Seme K *et al* (2003). Human papillomavirus DNA in oral squamous cell carcinomas and normal oral mucosa. *Acta Virol* **47:** 11–16.

Kellokoski JK, Syrjänen SM, Chang F, Yliskoski M, Syrjänen KJ (1992). Southern blot hybridization and PCR in detection of oral human papillomavirus (HPV) infections in women with genital HPV infections. *J Oral Pathol Med* **21:** 459–464.

Koppikar P, deVilliers EM, Mulherkar R (2005). Identification of human papillomaviruses in tumors of the oral cavity in an Indian community. *Int J Cancer* **113:** 946–950.

Koskinen WJ, Chen RW, Leivo I, Mäkitie A, Bäck L, Kontio R, Suuronen R, Lindqvist C, Auvinen E, Molijn A, Quint WG, Vaheri A, Aaltonen LM (2003). Prevalence and physical status of human papillomavirus in squamous cell carcinomas of head and neck *Int J Cancer* **107:** 401–6.

Kreimer AR, Clifford GM, Boyle P, Franceschi S (2005). Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev* **14:** 467–475.

Kreimer AR, Bhatia RK, Messeguer AL, González P, Herrero R, Giuliano AR (2010). Oral human papillomavirus in healthy individuals: a systematic review of the literature. *Sex Transm Dis* **37:** 386–391, Review.

Lei L, Li H, Sun Y (1996). Study of HPV in oral squamous cell carcinoma. *Zhonghua Kou Qiang Yi Xue Za Zhi* **31:** 375–377 (Chinese).

Llamas-Martínez S, Esparza-Gómez G, Campo-Trapero J *et al* (2008). Genotypic determination by PCR-RFLP of human papillomavirus in normal oral mucosa, oral leukoplakia and oral squamous cell carcinoma samples in Madrid (Spain). *Anticancer Res* **28:** 3733–3741.

Luo CW, Roan CH, Liu CJ (2007). Human papillomaviruses in oral squamous cell carcinoma and pre-cancerous lesions detected by PCR-based gene-chip array. *Int J Oral Maxillofac Surg* **36:** 153–158.

Maitland NJ, Cox MF, Lynas C, Prime SS, Meanwell CA, Scully C (1987). Detection of human papillomavirus DNA in biopsies of human oral tissue. *Br J Cancer* **56:** 245–250.

Majunder M, Indra D, Roy PD *et al* (2009). Variant haplotypes at XRCC1 and risk of oral leukoplakia in HPV non-infected samples. *J Oral Pathol Med* **38:** 174–180.

Mantel N (1958). An experimental design in combination chemotherapy. *Ann N Y Acad Sci* **76:** 909–931.

Mao EJ (1995). Prevalence of human papillomavirus 16 and nucleolar organizer region counts in oral exfoliated cells from normal and malignant epithelia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **80:** 320–329.

Mao EJ, Schwartz SM, Daling JR, Oda D, Tickman L, Beckmann AM (1996). Human papilloma viruses and p53 mutations in normal pre-malignant and malignant oral epithelia. *Int J Cancer* **69:** 152–158.

Mellin H, Friesland S, Lewensohn R, Dalianis T, Munck-Wikland E (2000). Human papillomavirus (HPV) DNA in tonsillar cancer: clinical correlates, risk of relapse, and survival. *Int J Cancer* **89:** 300–304.

Mete O, Keskin Y, Hafiz G, Kayhan KB, Unur M (2010). Oral proliferative verrucous leukoplakia: underdiagnosed oral precursor lesion that requires retrospective clinicopathological correlation. *Dermatol Online J* **16:** 6.

Miller CS, Johnstone BM (2001). Human papillomavirus as a risk factor for oral squamous cell carcinoma: a meta-analysis, 1982–1997. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **91:** 622–635.

Miller CS, White DK (1996). Human papillomavirus expression in oral mucosa, premalignant conditions, and squamous cell carcinoma: a retrospective review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **82:** 57–68.

Münger K, Werness BA, Dyson N, Phelps WC, Harlow E, Howley PM (1989). Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. *EMBO J* **8:** 4099–4105.

Muñoz N, Bosch FX, Castellsagué X *et al* (2004). Against which human papillomavirus types shall we vaccinate and screen? The international perspective *Int J Cancer* **111:** 278–285.

Nielsen H, Norrild B, Vedtofte P, Praetorius F, Reibel J, Holmstrup P (1996). Human papillomavirus in oral premalignant lesions. *Eur J Cancer B Oral Oncol* **32B:** 264–270.

OFlatharta C, Flint SR, Toner M, Butler D, Mabruk MJ (2003). Investigation into a possible association between oral lichen planus, the human herpesviruses, and the human papillomaviruses. *Mol Diagn* **7:** 73–83.

Palefsky JM, Silverman S Jr, Abdel-Salaam M, Daniels TE, Greenspan JS (1995). Association between proliferative verrucous leukoplakia and infection with human papillomavirus type 16. *J Oral Pathol Med* **24:** 193–197.

Patiman, Zhang Z, Cao J (2001). Research on expression of human papillomavirus type 16 and telomerase in oral lesions. *Zhonghua Kou Qiang Yi Xue Za Zhi* **36:** 119–121 (Chinese).

Puranen MH, Yliskoski MH, Saarikoski SV, Syrjanen KJ, Syrjanen SM (1997). Exposure of an infant to cervical human papillomavirus infection of the mother is common. *Am J Obstet Gynecol* **176:** 1039–1045.

Ragin CCR, Modugno F, Gollin SM (2007). The epidemiology and risk factors of head and neck cancer: a focus on human papillomavirus. *J Dent Res* **86:** 104.

Regezi JA, Dekker NP, Ramos DM, Li X, Macabeo-Ong M, Jordan RC (2002). Proliferation and invasion factors in HIV-associated dysplastic and nondysplastic oral warts and in oral squamous cell carcinoma: an immunohistochemical and RT-PCR evaluation. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **94:** 724–731.

Sand L, Jalouli J, Larsson PA, Hirsch JM (2000). Human papilloma viruses in oral lesions. *Anticancer Res* **20:** 1183–1188.

da Silva CE, da Silva ID, Cerri A, Weckx LL (2007). Prevalence of human papillomavirus in squamous cell carcinoma of the tongue. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **104:** 497–500.

Smith E, Ritchie J, Yankowitz J *et al* (2004). Human papillomvirus prevalence and types in newborns and parents. *Sex Transm Dis* **31:** 57–62.

Smith JS, Lindsay L, Hoots B *et al* (2007). Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. *Int J Cancer* **121:** 621–632.

Stanley MA, Pett MR, Coleman N (2007). HPV: from infection to cancer. *Biochem Soc Trans* **35:** 1456–1460.

Sterne JA, Egger M (2001). Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. *J Clin Epidemiol* **54:** 1046–1055.

Strati K, Pitot HC, Lambert PF (2006). Identification of biomarkers that distinguish human papillomavirus (HPV)-positive versus HPV-negative head and neck cancers in a mouse model. *PNAS* **103:** 14152–14157.

Sugiyama M, Bhawal UK, Dohmen T, Ono S, Miyauchi M, Ishikawa T (2003). Detection of human papillomavirus-16 and HPV-18 DNA in normal, dysplastic, and malignant oral epithelium. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **95:** 594–600.

Syrjänen S (1990). Basic concepts and practical applications of recombinant DNA techniques in detection of human papillomasvirus infection. *APMIS* **98:** 95–110, Review.

Syrjänen S, Syrjänen K (2000). HPV infections of the oral mucosa, Chapter 17. In: Syrjänen K, Syrjänen S, eds. *Papillomavirus infections in human pathology*. J. Wiley & Sons: New York, pp. 379–412.

Syrjanen K, Syrjanen S, Lamberg M, Pyrhonen S, Nuutinen J (1983). Morphological and immunohistochemical evidence suggesting human papillomavirus (HPV) involvement in oral squamous cell carcinogenesis. *Int J Oral Surg* **12:** 418–424.

Szarka K, Tar I, Fehér E *et al* (2009). Progressive increase of human papillomavirus carriage rates in potentially malignant and malignant oral disorders with increasing malignant potential. *Oral Microbiol Immunol* **24:** 314–318.

Tachezy R, Klozar J, Rubenstein L *et al* (2009). Demographic and risk factors in patients with head and neck tumors. *J Med Virol* **81:** 878–887.

Termine N, Panzarella V, Falaschini S *et al* (2008). HPV in oral squamous cell carcinoma vs head and neck squamous cell carcinoma biopsies: a meta-analysis (1988–2007). *Ann Oncol* **19:** 1681–1690, Review.

de Villiers EM, Gunst K (2009). Characterization of seven novel human papillomavirus types isolated from cutaneous tissue, but also present in mucosal lesions. *J Gen Virol* **90:** 1999–2004.

de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H (2004). Classification of papillomaviruses. *Virology* **324:** 17–27.

van der Waal I (2009). Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. *Oral Oncol* **45:** 317–323.

van der Waal I, Reichart PA (2008). Oral proliferative verrucous leukoplakia revisited. *Oral Oncol* **44:** 719–721.

Wang J, Li J, Huang H, Fu Y (1998). Detection of the E7 transform gene of human papilloma virus type 16 in human oral squamous cell carcinoma. *Chin J Dent Res* **1:** 35–37.

Warnakulasuriya S, Johnson NW, van der Waal I (2007). Nomenclature and classification of potentially malignant disorders of the oral mucosa. *J Oral Pathol Med* **36:** 575–580.

Werness BA, Levine AJ, Howley PM (1990). Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science* **248:** 76–79.

Yang SW, Lee YS, Chen TA, Wu CJ, Tsai CN (2009). Human papillomavirus in oral leukoplakia is no prognostic indicator of malignant transformation. *Cancer Epidemiol* **33:** 118–122.

Yeudall WA, Campo MS (1991). Human papillomavirus DNA in biopsies of oral tissues. *J Gen Virol* **72:** 173–176.

Yu LM, Chan AW, Hopewell S, Deeks JJ, Altman DG (2010). Reporting on covariate adjustment in randomised controlled trials before and after revision of the 2001 CONSORT statement: a literature review. *Trials* **11:** 59, Review.

Zhang ZY, Sdek P, Cao J, Chen WT (2004). Human papillomavirus type 16 and 18 DNA in oral squamous cell carcinoma and normal mucosa. *Int J Oral Maxillofac Surg* **33:** 71–74.