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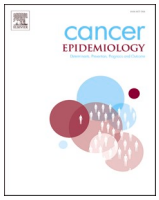

A systematic review and meta-analysis of the association of human papilloma virus infections with ocular surface squamous neoplasia

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ABSTRACT

The incidence of Ocular Squamous Surface Neoplasia (OSSN) is increasing, particularly in populations with high HIV prevalence and higher solar irradiance. Human Papilloma Virus (HPV) is considered a precursor/co-factor to OSSN. We aimed to quantify the association between HPV and OSSN and analyse co-factors in this association, including geographical differences and pathology of the comparator group. We used the DerSimonian and Laird method to compute summary odds risk estimates in a random effects model. The I^2 statistic was used to quantify heterogenicity. Subgroup analyses, sensitivity analyses and meta-regression were used to explore sources of heterogeneity. Twenty-one studies were included. The odds of HPV was significantly higher in OSSN lesions than benign lesions. The pooled odds ratio was 9.2 (95 % CI: 5.0–16.9) ((I^2 = 56.1 % (95 % CI: 26 %-74 %)). In subgroup analysis, the odds ratio was lower in studies from African countries (with high HIV prevalences) and countries closer to the equator. The effect size was lower when ocular surface diseases such as pterygium were used as the comparator group rather than healthy tissues. We report a strong association between HPV and OSSN. The odds of HPV was 9.2 times higher in conjunctival cancers than benign tissues. This association was muted in African countries and countries closer to the equator, highlighting the role of UV radiation and HIV as co-factors in OSSN development. Muting of the association may also signal a role of pterygium as precursor lesions to OSSN, or that HPV may be involved in their development.

1. Introduction

Ocular surface squamous neoplasia (OSSN), the predominant malignancy of the ocular surface, involves abnormal growth of dysplastic squamous epithelial cells on the surface of the eye [1]. OSSN encompasses a spectrum of disease from benign (squamous papilloma), to pre-invasive (conjunctival intraepithelial neoplasia including carcinoma in-situ) and invasive disease (squamous cell carcinoma (SCC) and mucoepidermoid carcinoma) [2]. Benign growths such as pterygia and pinguecula are not included in the spectrum of OSSN.

OSSN is rare globally, but the incidence varies widely by geographic region, with rates as high as 35 per million per year in sub-Saharan Africa [3], a region that accounts for 67 % of the global population of people living with human immunodeficiency virus (HIV) [4]. Individuals with HIV have a three to 30-fold increased risk of developing OSSN [5–8]. This suggests a viral infection- or immunosuppression-related carcinogenic pathway which could extend to other infections such as human papillomavirus (HPV).

HPV, the most common sexually transmitted infection globally, is associated with both benign and malignant conditions, with 4.5 % of all cancers in humans attributed to HPV [9]. Each person, regardless of gender, has a 50 % lifetime infection risk [10]. Over 200 HPV genotypes have been identified and categorised according to whether they target cutaneous or mucosal cells. These are further classified as low- and high-risk based on their potential to cause malignant cell transformation. Low-risk HPV subtypes (e.g., HPV-6, HPV-11) cause benign lesions such as cutaneous and anogenital warts and are generally grouped as cutaneous subtypes. High-risk HPV subtypes (commonly HPV-16, HPV-18) are linked to anogenital and oropharyngeal cancers [11].

The incidence of OSSN is higher in regions with high levels of ultraviolet (UV) solar radiation, particularly those within 30 degrees latitude of the equator [12]. Cumulative UV light exposure is a risk factor in OSSN pathogenesis [12–14].

The role of HPV in OSSN has been previously considered; however, divergent findings have left uncertainty regarding a definitive

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association [15–17]. Given HPV's oncogenic potential on surfaces with a similar histology to the ocular surface, and the involvement of other viral infections in OSSN, it is plausible that HPV, or certain subtypes, are associated with OSSN development. This systematic review and meta-analysis aims to investigate the association between HPV and OSSN.

2. Methods

2.1. Search strategy

A comprehensive search of PubMed and Embase databases was conducted in October 2023 for studies of HPV prevalence in people with OSSN. The search strategy (Supplementary file 1) included MeSH headings and abstract/title keywords with no limitation placed on publication year or language. Title/abstract and full-text screening were performed independently in Covidence [18] by both authors with discrepancies resolved by discussion. Full-text articles in languages other than English were translated at the full-text review stage to determine eligibility. References of included papers and review articles were also searched to identify articles not captured by the initial search.

The study protocol was developed following PRISMA guidelines for systematic reviews [19] and registered with PROSPERO (ID: CRD42024505775).

2.2. Study selection

Cohort, case control and cross-sectional studies were eligible for inclusion if they: included a surgical biopsy of the conjunctiva or cornea, involved a histopathological diagnosis of OSSN (OSSN group), tested for HPV using PCR techniques, included a comparator group of participants with healthy or benign ocular lesions (e.g. pinguecula, pterygia, conjunctivitis, cataracts), and reported the prevalence of HPV in both OSSN and comparator groups.

Literature reviews, case series and case reports were excluded. Studies in which the comparator group consisted solely of participants with conjunctival papillomas (considered part of the OSSN spectrum and associated with mucosal HPV [20]), cutaneous malignancies (e.g., melanoma, basal cell carcinoma) or precursor cutaneous lesions (e.g., actinic keratosis) were also excluded. If the comparator group included participants with the above pathologies but provided sufficient data for their removal, the study was included.

2.3. Data extraction

Data were extracted independently by both authors. Discrepancies were resolved by consensus. Extracted data included: author, publication year, study location, type of tissue sample used for PCR testing and HPV subtypes isolated, number of participants in OSSN and comparator groups including their respective histopathological diagnoses, and number of cases in each group positive for HPV. Information on HIV status was collected if available based on the commonly reported association between HIV and OSSN [21].

2.4. Risk of bias

Both authors independently assessed the risk of bias of included studies using the Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomized Studies in Meta Analysis. Specific quality-related criteria were assigned to three categories: selection (4 points), comparability (2 points), and exposure (3 points) with a maximum of 9 points. Studies that scored greater than 7 were considered high-quality, and those scoring less than 5 were considered low quality. All others were considered moderate quality [22].

2.5. Statistical analysis

For studies with no infected cases in either the OSSN or comparator group, we added 0.5 continuity correct to all cells, then estimated the odds ratio (OR) and respective 95 % confidence intervals (95 % CI) using the metan command of STATA (Stata Corp., College Station, TX, USA) [23]. Studies with no events in both the OSSN and comparator group were excluded from the meta-analysis. Given the high heterogenicity amongst studies, weights were assigned using the Dersimonian-Laird method using a random effects model with the heterogeneity estimate taken from the Mantel Haenszel model. Between study variance was measured using Tau2. The null hypothesis of homogeneity was measured with Cochrane's Q test. The degree of inconsistency between studies was evaluated using I^2 statistic with 95 % CI. We used the ranges of 0–40 % as low, 30–60 % as medium, 50–90 % as substantial and 75–100 % as considerable heterogenicity. A sensitivity analysis, using a one-by-one exclusion method, was conducted to assess whether any studies in the meta-analysis significantly impacted the overall results.

Subgroup analyses were used to explore potential effects of the following:

- African versus non-African countries
- latitude of country of origin - $>$ 30 degrees versus $\leq$ 30 degrees latitude to the equator (proxy for cumulative UV exposure)
- type of comparator tissue - healthy ($\geq$ 70 % of the control group was healthy tissue) versus ocular surface diseases
- HIV positive serology - studies where $>$ 50 % of the cases were HIV positive

Publication year was explored with cumulative meta-analysis. Meta-regression was used to explore sources of heterogenicity, using the specific effect estimate as the dependent variable and study variables as independent variables. Visual inspection of a funnel plot and Egger's regression test were performed to assess for publication bias.

3. Results

The search strategy yielded 403 papers, from which 21 (12 case-control, 8 cross-sectional and 1 cohort study) met the eligibility criteria for inclusion (Fig. 1). Studies from Asia (n = 6), Africa (n = 6), North America (n = 5), Europe (n = 2) and Australia (n = 2) were published between 1995 and 2022 and enrolled 832 OSSN cases and 874 control samples that included healthy conjunctival specimens and a range of non-malignant diagnoses, predominantly pinguecula and pterygium (Table 1). PCR testing for identification of HPV revealed one or more HPV subtypes in 40.9 % of histologically confirmed OSSN cases (n = 336) and 9 % of control samples (n = 79). Assessment of study quality revealed most studies were of moderate quality (n = 16), with four being low quality and only one considered high-quality (Table 1).

3.1. Meta-analysis

The meta-analysis comparing risk of HPV in OSSN with that of benign lesions included 18 studies. Three studies with no HPV positive cases in both OSSN and control groups were excluded [15,24,25]. The meta-analysis showed a strong association between HPV and OSSN (OR = 9.2; 95 % CI: 5.0–16.9) (Fig. 2). There was substantial heterogeneity [(I^2 = 55.5 %; 95 % CI: 24 %-74 %, p = 0.002) (Cochrane's Q = 38.18, df17 p = 0.002) (Tau2 = 0.31)].

3.2. Subgroup analysis and meta-regression

Subgroup analysis and meta-regression were performed to identify potential sources of heterogeneity.

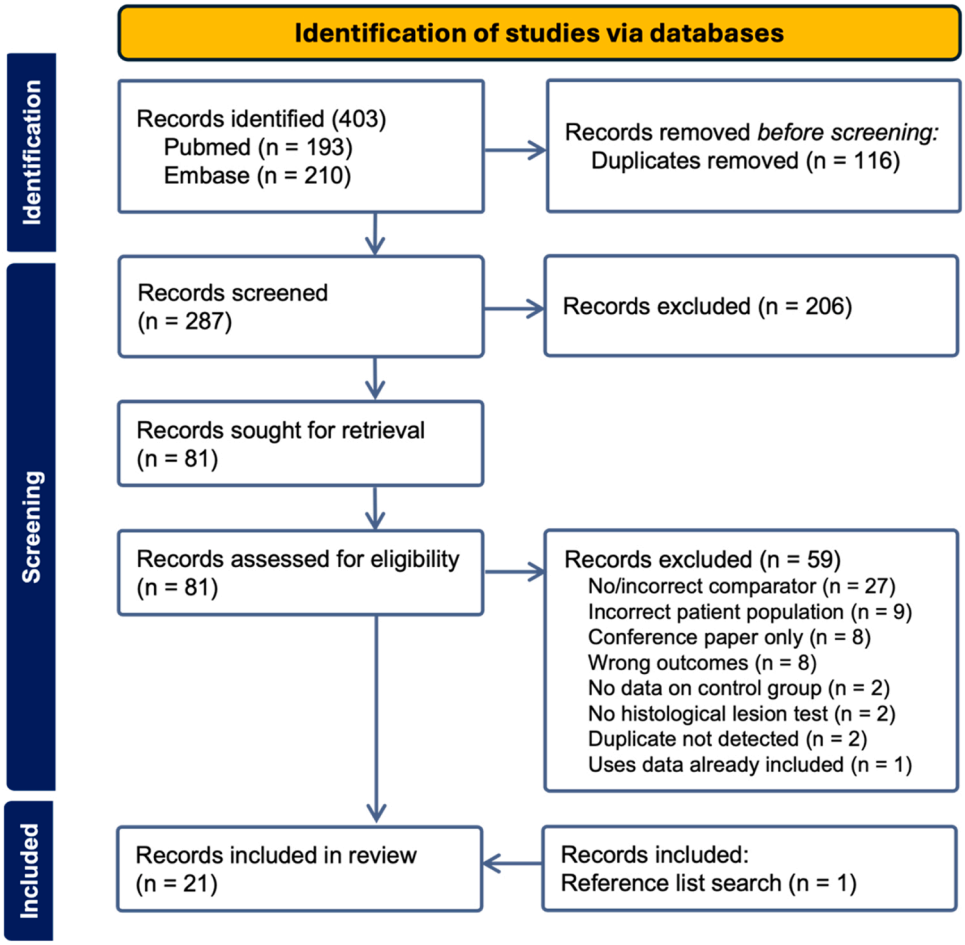

Fig. 1. Study inclusion flow diagram based on the 2020 PRISMA statement.

3.3. African countries

Studies from African countries (n = 7) had a lower effect size (OR = 4.5; 95 % CI: 2.3–8.8) in comparison with studies from other countries (n = 11) (OR = 20.2; 95 % CI: 8.2–49.8), which was significant on meta-regression (p = 0.027). There was less heterogeneity in studies of non-African (39 %, p = 0.089) and African (48.2 %, p = 0.072) countries than in the overall analysis (Fig. 3).

3.4. HIV positive population

HIV status of cases and controls was not mentioned in 12 studies, and positive HIV serology was an exclusion criteria in another [26] (Table 1). Four studies, all from African countries, included cases with HIV positive serology, with HIV positive cases in each accounting for > 50 % of total cases [7,27–29]. Whilst not recorded, Ateenyi-Agaba et al. suggested that a majority of their Ugandan case cohort was likely to be HIV positive [30]. Waddell et al. reported > 50 % HIV positive participants in overall HIV data, but these were not defined in PCR-tested samples [31]. The remaining African study did not measure HIV, however prevalence of HIV in Mozambique was 11.6 %. Since it is likely that in all seven studies from African countries, > 50 % of cases were HIV positive we did not conduct a separate meta-analysis of the four that strictly fulfilled our inclusion criteria.

3.5. Latitude of study population

Studies from countries with an average latitude > 30° from the equator (n = 5) had a higher effect size (OR = 69.0; 95 % CI: 8.9–535.4) compared with studies from countries ≤ 30° of the equator (n = 13) (OR = 6.3; 95 % CI: 3.7–10.6) (Fig. 4). This was significant on meta-regression (p = 0.016). Compared to the overall analysis, heterogeneity was decreased in both groups (> 30° latitude: I^2 = 46.9 %, p = 0.111; ≤ 30° latitude: I^2 = 41.2 %, p = 0.059).

3.6. Healthy comparator tissue

Across all studies meta-analysed, comparator tissue included 27 % healthy conjunctiva, 10.7 % pterygium and 4.2 % pinguecula. Two studies provided overall control numbers and listed comparator tissue type without specific numbers of each, neither of which included healthy, pterygium or pinguecula tissue [7,27]. The effect size of studies in which the control group comprised ≥ 70 % healthy tissue (n = 5) was greater compared to studies in which control groups included < 70 % healthy tissue (≥ 70 % healthy tissue: OR = 46.5; 95 % CI: 5.1–420.7; < 70 % healthy tissue: OR = 6.4; 95 % CI: 3.6–11.4) (Fig. 5A). This was not significant on meta-regression (p = 0.093). Heterogeneity was higher in the ≥ 70 % healthy tissue group (I^2 = 69.9 %, p = 0.01) than both the < 70 % healthy tissue group (I^2 = 42.0 %, p = 0.06) and the overall analysis.

To further investigate the effect of comparator tissue on the effect size of the HPV-OSSN association, studies with pterygium in the control group and allowed pterygium-specific data to be extracted, were pooled. This showed a muted effect size, approximately two-thirds of the overall group (pooled OR = 6.0, 95 % CI 2.4, 15.1) (Fig. 5B), and low heterogeneity (I2 0.0 %, p = 0.52).

Table 1
Characteristics of the 21 studies included in the review.

Author and year	Tissue type	HPV genotypes tested	OSSN group			Comparator group (control)			NOS score
			Number of cases and diagnosis	HPV positive cases (%)	HIV positive cases (%)	Diagnosis	HPV positive controls (%)	HIV positive controls (%)	
Adachi 1995 [47]	FFPE	16, 18	2 SCC	1 (50)	Not mentioned	9 healthy conjunctiva	0	Not mentioned	6
Asadi-Amoli 2011 [16]	FFPE	16, 18, 31, 33, 35, 52, 58	50 SCC	46 (92)	Not mentioned	50 healthy conjunctiva	0	Not mentioned	4
Ateenyi-Agaba 2004 [30]	Fresh frozen	5, 8, 10, 11, 12, 14, 16, 18, 20, 22, 23, 24, 36, 37, 38, 45	21 SCC	18 (85.7)	'likely a majority of our SCC patients were HIV+ '	10 pterygium, 7 pinguecula, 4 solar keratosis, 1 pigmented naevi	9 (40.9)	likely a few controls were HIV+ '	5
Ateenyi-Agaba 2010 [27]	FFPE, fresh frozen	Broad spectrum (mucosal and cutaneous)	94 SCC, 39 CIN	67 (43.6)	113 (85.0 %)*	285 (cataract, chalazion, corneal tears, eye trauma)	40 (10.5)	128 (48.4)*	8
Auw-Haedrich 2008 [54]	FFPE	16	12 CIN	2 (16.7)	Not mentioned	14 healthy postmortem conjunctiva, 1 inflamed conjunctiva	0	Not mentioned	6
Carrilho 2013 [50]	FFPE	Broad spectrum (mucosal and cutaneous); Specific primers 16, 38	8 SCC, 11 CIN	11 (57.9)	Not mentioned	3 pinguecula, 1 melanosis, 1 conjunctivitis	0	Not mentioned	5
Chauhan 2012 [49]	FFPE, fresh frozen	All major	44 SCC, 20 CIN	7 (10.9) (4 SCC; 3 CIN)	Not mentioned	15 limbal stem cell deficiency	0	Not mentioned	5
de Koning 2008 [28]	FFPE	Broad spectrum (mucosal and cutaneous)	24 SCC, 57 CIN (81 overall)	38 (46.9 %) (10 SCC; 28 CIN)	52 (64 %) (22 HIV- and 7 unknown)	29(15 pinguecula, 3 chronic inflammation, 2 pyogenic granuloma, 2 cavernous angioma, 7 other)	11 (37.9)	10 (34) (19 HIV-)	5
de La Parra-Colin 2022 [26]	FFPE, fresh frozen	Broad spectrum (mucosal and cutaneous)	2 SCC, 20 CIN	9 (40.9)(1 SCC; 8 CIN)	Excluded HIV+ cases	22 pterygium	1 (4.5)	Excluded those HIV+	6
Dushku 1999# [24, 54]	FFPE	All known	4 SCC, 4 CIN	0	Not mentioned	13 pterygium, 10 limbal tumours, 1 pinguecula	0	Not mentioned	5
Guthoff 2009# [15]	FFPE	6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	18 (SCC, CIN)*	0	0	11 pterygium, 5 healthy conjunctiva	0	0	5
Karcioglu 1997 [48]	FFPE	16, 18	31 SCC, 14 CIN	25 (55.6) (17 SCC; 8 CIN)	Not mentioned	19 healthy conjunctiva, 31 corneal scar, 20 climatic droplet keratopathy	6 (31.6)	Not mentioned	5
Kuo 2006 [55]	FFPE	Broad spectrum (mucosal and cutaneous)	9 CIN	9 (100)	Not mentioned	4 pterygium, 2 lymphoid proliferation, 2 superior limbic keratoconjunctivitis	0	Not mentioned	5
McDonnell 1989 [33]	FFPE	16, 18	3 SCC, 13 CIN	12 (75) (3 SCC; 9 CIN)	Not mentioned	1 nevus, 1 pterygium, 1 seborrheic keratosis*	0	Not mentioned	4
Scott 2002 [53]	FFPE	16, 18	10 CIN	10 (100)	Not mentioned	10 clinically uninvolved conjunctival from same eyes of cases; 5 healthy controls	0	Not mentioned	7
Simbiri 2010 [29]	FFPE, fresh frozen	6, 11, 16, 18, 31, 33	28 (SCC, CIN)	20 (71.4)	28 (100 %)	8 pterygium	4 (50)	8 (100 %)	5
Tabrizi 1997 [56]	FFPE	6, 11, 16, 18, 31, 33	88 CIN	34 (38.6)	Not mentioned	66 no/minimal dysplasia	5 (7.6)	Not mentioned	6
Tornesello 2006 [7, 55]	FFPE	Broad spectrum (mucosal and cutaneous); Specific primers for 16, 38	29 SCC, 57 CIN	17 (19.8)	56 (65.1 %)	63 (benign eye lesions, eye trauma)	1 (1.6)	15 (23.81) (24 HIV-)	6
Tulvatana 2003# [25]	FFPE	Broad spectrum	16 SCC, 14 CIN	0	Not mentioned	23 healthy conjunctiva	0	Not mentioned	4
Waddell 1996 [31]	FFPE	16	20 SCC*	7 (35)	Not defined in PCR samples	9 pinguecula, 6 inflamed conjunctiva*	2 (13.3)	Not defined in PCR samples	5
Woods 2013 [32]	FFPE, fresh frozen	Broad spectrum	24 SCC, 46 CIN	3 (6.5) (SCC)	0	42 pterygium, 69 healthy conjunctiva	0	0	4

# Excluded from meta-analysis
CIN = Conjunctival intraepithelial neoplasia; FFPE = Formalin-fixed paraffin-embedded; HIV = Human immunodeficiency virus; HPV = Human papilloma virus; NOS = Newcastle-Ottawa Scale; PCR = Polymerase chain reaction; SCC = Squamous cell carcinoma
* Data represents only samples tested using PCR

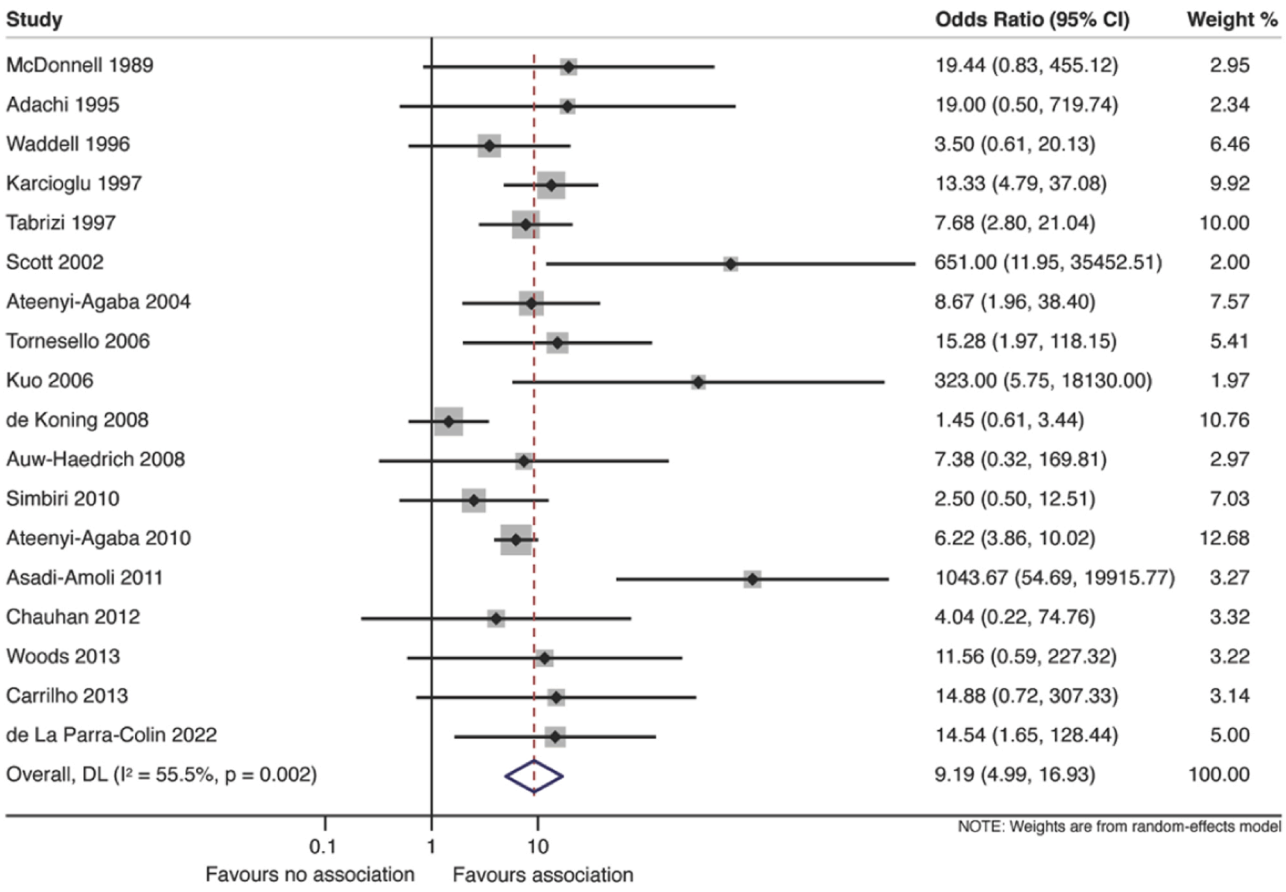

Fig. 2. Forest plot of studies investigating the association between HPV and OSSN. CI = confidence interval.

3.7. Year of publication

Cumulative meta-analysis (used to assess effect of publication year on overall effect size), showed a consistent association between HPV with OSSN from 1989 to 2022 (Fig. 6). Meta-regression confirmed that publication year did not significantly alter the overall effect size ($p = 0.79$).

3.8. Publication bias, sensitivity analysis and heterogeneity

Visual inspection of the funnel plot revealed an asymmetric distribution, indicative of publication bias, with smaller studies with weaker associations being under-represented (Supplementary file 2). This was confirmed by Egger's test for small-study effects that indicated the significant asymmetry ($p = 0.04$).

A sensitivity analysis assessing influence of individual studies on effect size, showed the pooled effect size ranged from 7.2 (95 % CI: 4.3–12.1) to 10.7 (95 % CI: 5.1–22.5) when any single study was removed (Supplementary file 3). Exclusion of Asadi-Amoli et al. [16] decreased the effect estimate from 9.2 (95 % CI: 5.0–16.9) to 7.2 (95 % CI: 4.3–12.1), a 22 % relative decrease, suggesting it exerts most influence on effect size.

A sensitivity analysis of study quality (as determined by quality appraisal) showed that removal of the two low quality studies in the meta-analysis [32,33] reduced the effect estimate to 9.1 (95 % CI 4.7–17.6; I^2 60.1 %), a relative decrease of only 1.1 %. This indicates study quality did not influence the effect size of the HPV- OSSN association.

To explore sources of heterogeneity, meta regression using the effect size as the dependent variable was conducted (Table 2). This showed latitude $> 30^\circ$ ($p = 0.016$), and studies in African countries ($p = 0.027$) as significant covariates. No factors remained significant on multivariate analysis, where there is likely to have been collinearity between variables, although latitude tended toward significance.

4. Discussion

This review and meta-analysis showed a strong association between HPV and OSSN. The odds of HPV in conjunctival cancers was 9.2 times that in healthy/benign tissues. This is similar to Ramberg et al. [34] and Gichuhi et al. [35] that showed HPV infection increased the odds of OSSN by 8.4 and 4.0 times, respectively. Our subgroup analyses revealed geographic and latitudinal variations, suggesting co-factors such as UV exposure and HIV, may interact with HPV in OSSN pathogenesis.

Previous studies that have investigated the HPV- OSSN association have yielded variable results, potentially due to differences in:

- HPV detection methods (PCR, *in-situ* hybridisation, immunohistochemistry) and inclusion/exclusion of secondary HPV result verification.
- PCR sensitivity and specificity, primer designs, DNA templates, use of positive/negative controls and nested PCR.
- Primers used (type-specific, broad spectrum and/or high-risk) and resultant HPV genotype coverage.
- Formalin-fixed paraffin-embedded versus fresh frozen tissue (better preservation of DNA, RNA and proteins) for PCR, and collection/processing procedures

- Classification of OSSN, lack of histological confirmation of OSSN, and inclusion of non-healthy conjunctiva as a comparator.

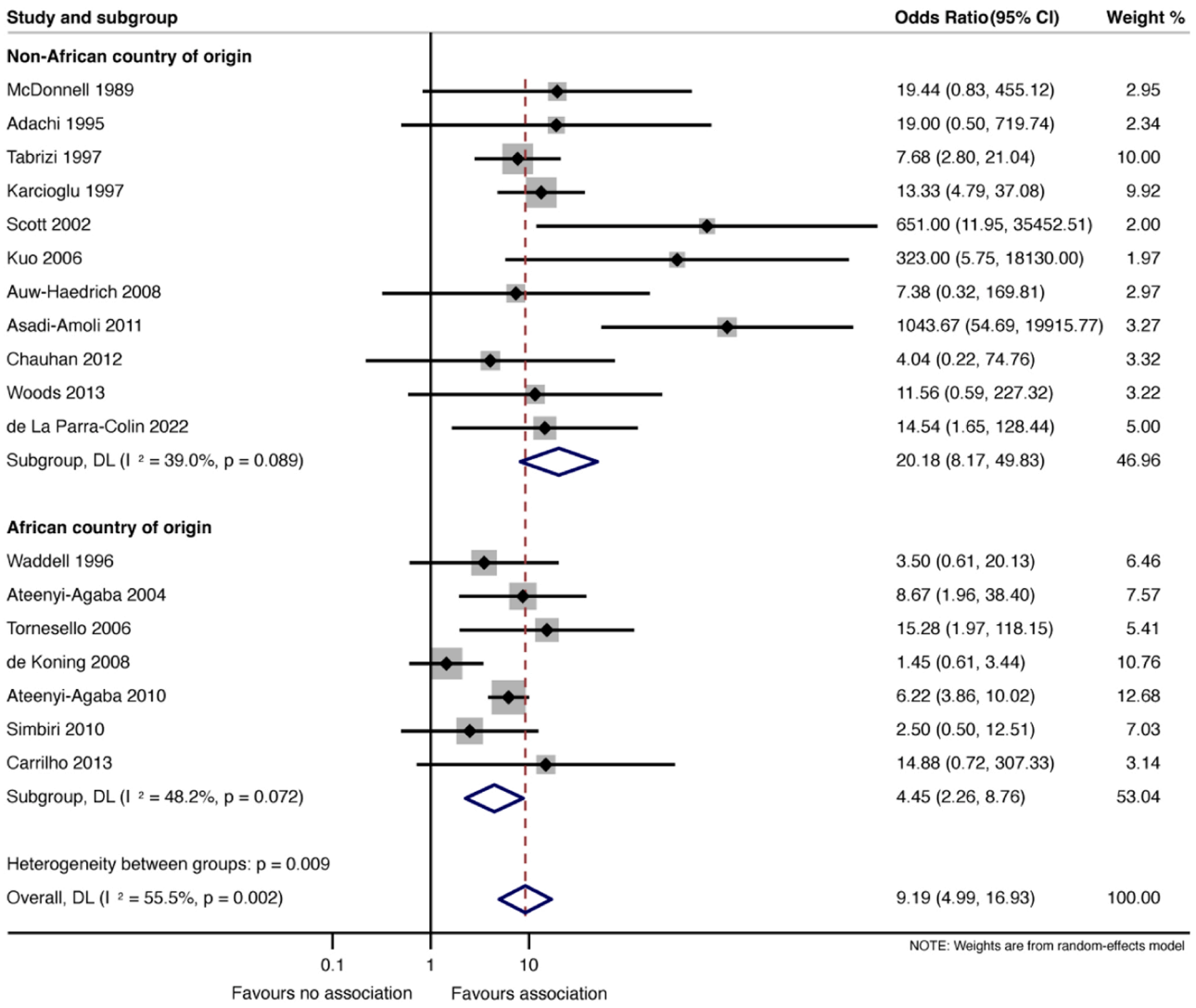

Fig. 3. Association of HPV and OSSN grouped by studies emanating from African and non-African countries. CI = confidence interval.

This review minimised heterogeneity between studies by including only those that used: surgical biopsies with histologically confirmed diagnoses; PCR-based HPV detection and allowed for exclusion of specific diagnoses (papillomas, cutaneous malignancies, precursor lesions) from the comparator group.

4.1. *Latitude, African countries and HIV*

This review is the first to examine a possible interplay between HPV and latitude. Latitude was an independent risk factor for OSSN, with lower HPV association in countries closer to the equator and therefore higher levels of UV radiation. This is consistent with Gichuhi et al. that showed a stronger HPV-OSSN association in North America and Asia (both > 30 degrees latitude of the equator) than in African countries [35].

UV radiation is a known risk factor in OSSN pathogenesis [25,36,37]. OSSN aetiology is complex with a potential multifactorial interplay of factors like viral infection (HPV, HIV), UV radiation, immunosuppression and chemical mutagenesis. This study suggests a role of HPV and UV radiation, the relative importance of which may be geographically dependent. In high UV regions like Africa, UV radiation may be more influential than HPV due to its direct mutagenic effects on ocular cells. Combined UV-induced and HPV-mediated p53 disruptions can promote oncogenesis. UV radiation also causes ocular immunosuppression, facilitating HPV persistence and replication, and reactivating latent virus [38].

In regions with lower UV radiation, other etiological factors, like HPV, may play a more prominent role. The prevalence of different HPV genotypes varies by geographical location. In a global study of cervical cytology results of women, the most carcinogenic genotype, HPV-16 (which all studies included in the current review tested for), was more prevalent in North America (38.9 %) and Europe (35.2 %) than Africa (25.6 %) [11]. Similarly in men, a higher prevalence of HPV-16 was reported for Europe and North America (7 %) than Sub-Saharan Africa (4 %) [39]. The results of the latitude sub analysis suggests that in regions with lower UV radiation and higher prevalence of high-risk HPV genotypes (i.e. North America and Europe), HPV may be more dominant in the development of OSSN. In contrast, high UV radiation and lower prevalence of high-risk HPV genotypes may link UV exposure more strongly to OSSN development, with HPV playing a lesser role. Understanding these regional differences may help tailor preventive strategies and clinical management approaches for OSSN.

Given the limited data on HIV status in individual studies, we did not examine the effect of HIV on the HPV-OSSN association. We used African countries as a surrogate marker for HIV, given all 7 included countries likely fulfilled the inclusion criteria of > 50 % HIV positive cases, despite data available in only four studies. HIV prevalence in African countries in this review (Botswana 16.4 %, Malawi 7.1 %,

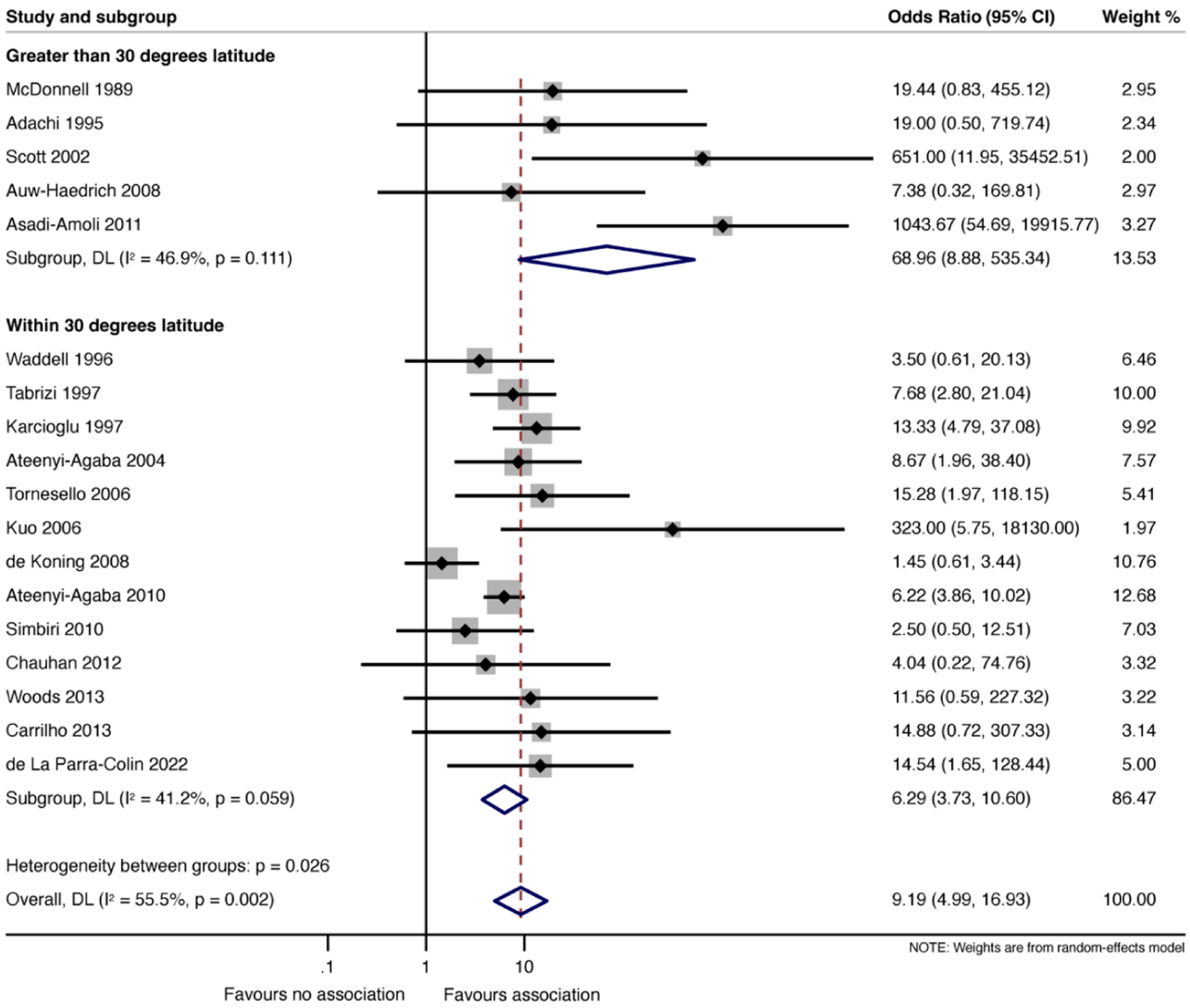

Fig. 4. The relationship between HPV and OSSN grouped by the study population's average latitude from the equator. Both groups, $> 30^{\circ}$ and $\leq 30^{\circ}$ latitude, showed an association between HPV and OSSN, however this was greater in the populations that reside further from the equator. CI = confidence interval.

Mozambique 11.6 %, Uganda 5.1 %) is much higher than the global HIV prevalence of 0.6 % [40]. We are unable to differentiate between the dual risk factors of radiation and HIV in these countries. Previous studies have however shown HIV is a risk factor for OSSN development [35].

4.2. Comparator tissue

The type of comparator tissue in included studies influenced the observed effect size with a higher effect size in studies with predominately healthy tissue compared to those with a higher percentage of diseased/injured conjunctiva. This review excluded studies in which the comparator group included only papilloma tissue or where papilloma data could not be separated from the control data, due to the strong association with OSSN. Previous studies show HPV prevalence in conjunctival papilloma ranges from 58 % to 92 % [20,41–43]. Selective exclusion of papillomas, cutaneous malignancies or precursor lesions from the control group may explain the higher association observed in this study compared to Gichuhi et al. [35]. It also highlights the importance of appropriate control selection, as healthy tissue offers a clearer contrast in HPV prevalence in OSSN than inflamed or diseased tissues.

We did not exclude other inflammatory lesions such as pterygia. Both OSSN and pterygia have uncertain pathogenesis, with UV radiation and oncogenic viral infection as common factors [44]. HPV prevalence in pterygia varies widely, from 0 % to 100 % [45,46]. Our results showed that with at least 70 % healthy conjunctiva in the control group, the odds of HPV in OSSN were more than 46.5 times higher compared to the overall control group. In contrast, if diseased conjunctiva contributed more than 30 % of the comparator sample, the odds were only 6.4 times higher. The reduction in effect size with increased diseased tissue may indicate that benign lesions, like pterygia, may be precursors to OSSN, and more likely to be HPV positive.

4.3. Year of publication

Advancements in PCR sensitivity and specificity have improved HPV genotype detection. Some early studies in our review used HPV-16 and/or HPV-18 specific primers [31,33,47,48] whereas recent studies used advanced primers to detect a broad spectrum of HPV genotypes [26,49,50]. Despite enhanced diagnostic capabilities, publication year did not affect the HPV-OSSN association. Improved HPV detection may have similarly increased detection rates in both OSSN and comparator tissue, including non-healthy conjunctiva like pterygium.

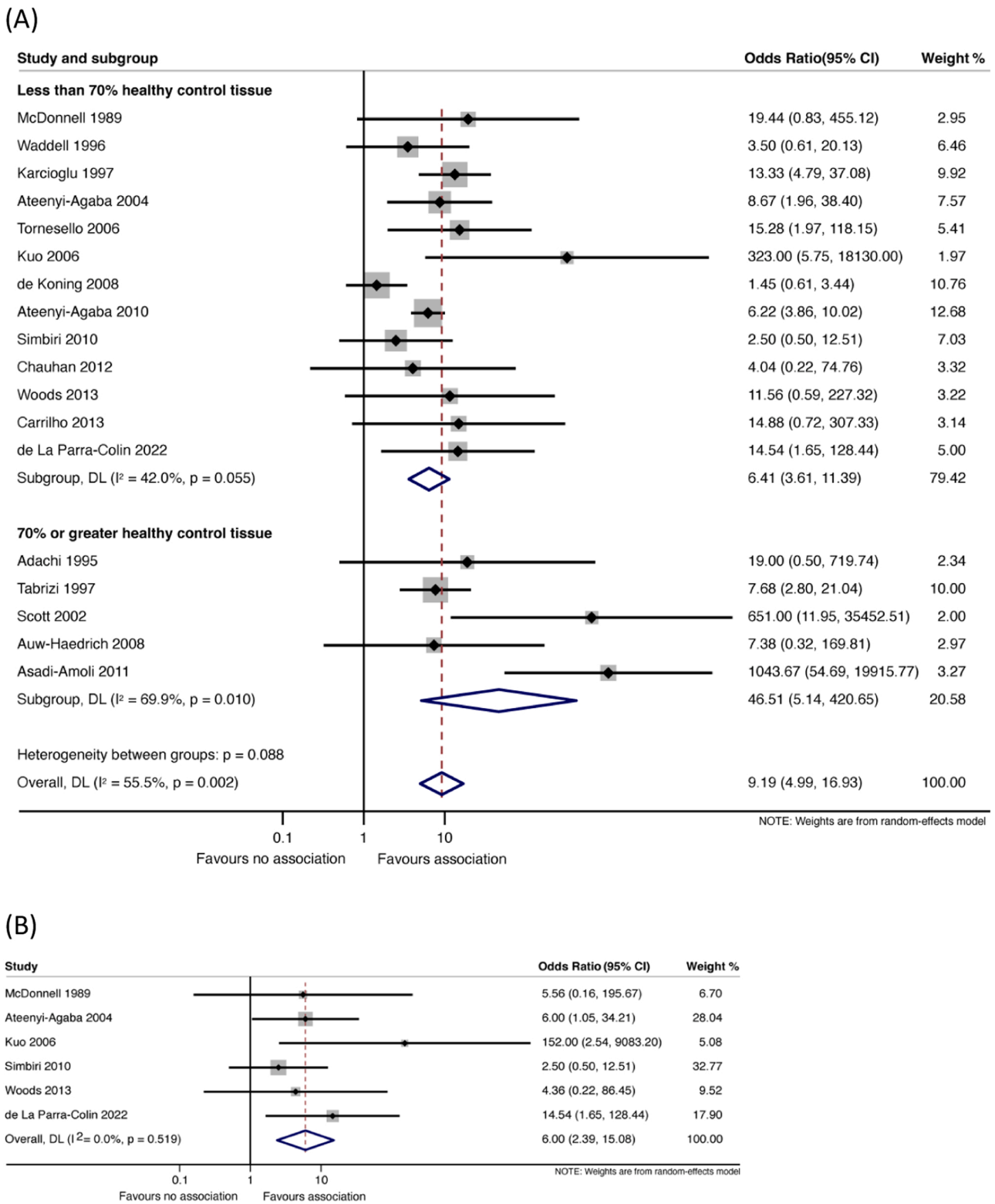

Fig. 5. Healthy comparator tissue. (A) Forest plot of odds ratios of the association between HPV and OSSN grouped by studies in which the comparator tissue comprised < 70 % or ≥ 70 % healthy conjunctival tissue. (B) Meta-analysis of studies in which pterygium and HPV data could be extracted individually. CI = confidence interval.

4.4. Strengths and limitations

The HPV genotypes tested and identified varied across studies. Some distinguished between mucosal and cutaneous subtypes, but our study did not. The International Agency for Research on Cancer classifies 12 mucosal high-risk HPV genotypes (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) as carcinogenic in humans, with HPV-16 being the most oncogenic [51]. Despite the predominance of carcinogenic mucosal HPV genotypes, Carreira et al. [21] reported a stronger association between cutaneous HPV subtypes and OSSN. Given the

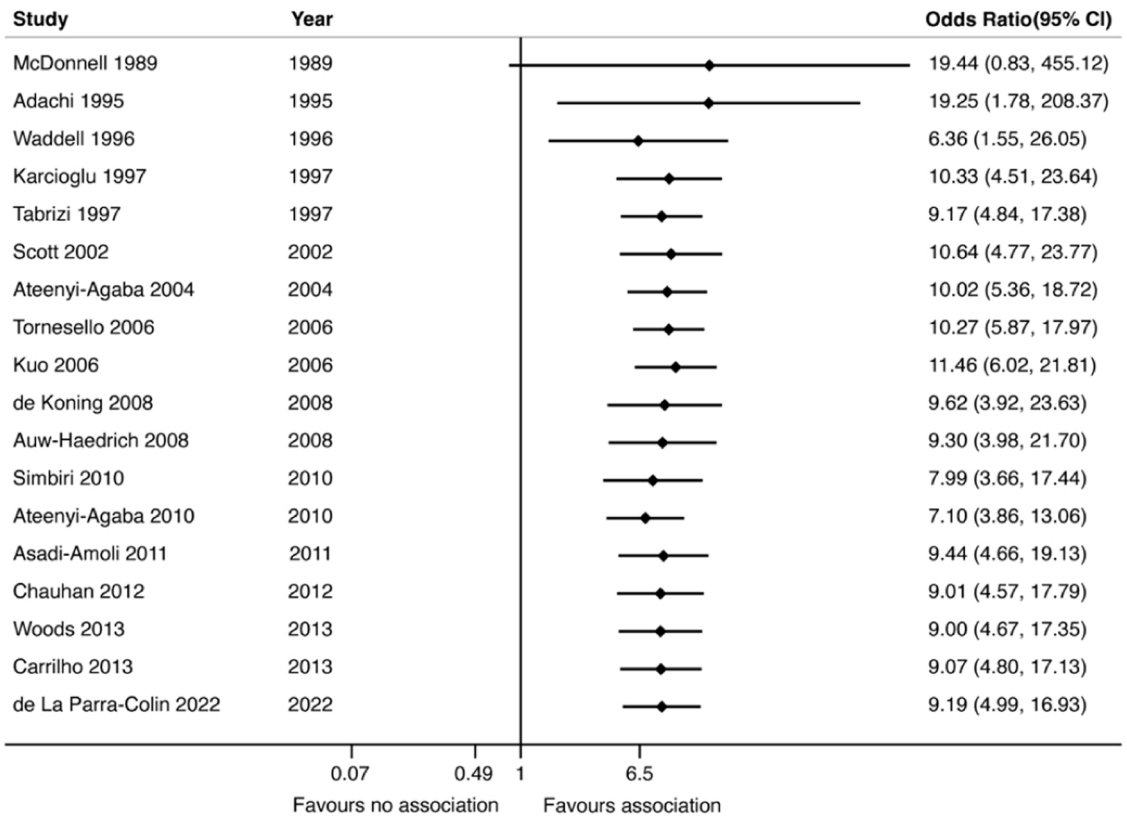

Fig. 6. Cumulative meta-analysis describing the association between HPV and OSSN. This indicates a consistent trend towards an association from the first paper in 1989. CI = confidence interval.

Table 2
Meta-regression analysis with outcome odds ratio of HPV infection in OSSN versus controls.

	Univariate coefficient (95 % CI)	p-value	Adjusted coefficient (95 % CI)	p-value
Publication year	−0.003 (−0.11–0.10)	0.946	0.03 (−0.07–0.13)	0.512
Latitude > 30°	−2.37 (−4.25 - −0.50)	0.016	−1.83 (−4.22–0.54)	0.118
African countries	−1.40 (−2.62 - −0.19	0.027	−0.71 (−3.24–1.82)	0.552
> 70 % healthy control tissue	1.44 (−0.27–3.15)	0.093	−0.026 (−2.16–2.11)	0.980

oncogenic potential of HPV-16 and the large number of high-risk mucosal HPV genotypes, a stronger association with OSSN might be expected. Many smaller studies of mucosal HPV included in the subgroup analysis by Carreira et al. [21] showed individual strong associations with OSSN [33,47,52,53] but this was muted by two larger studies showing no association [27,28].

Despite attempts to homogenise included studies, significant heterogeneity remained. Sensitivity analysis showed removal of low-quality studies did not significantly affect the HPV-OSSN association, however most included studies were of moderate quality, potentially affecting robustness. In addition, inclusion of Asadi-Amoli et al. [16], which had the most influence on effect size, may have contributed to an overestimation of the overall HPV-OSSN association.

The association between HPV and OSSN may be confounded by factors like HIV status, which was inconsistently reported across studies. Given the higher OSSN prevalence in HIV-positive individuals, future studies should systematically control for HIV status to isolate the effect of HPV on OSSN risk.

5. Conclusion

This review and meta-analysis provide evidence supporting an association between HPV infection and OSSN. The findings underscore the multifactorial nature of OSSN pathogenesis, involving HPV alongside UV radiation, HIV co-infection and immunosuppression. Understanding the complex interplay between these factors to develop targeted public health interventions is crucial for mitigating the burden of OSSN globally. Further research in diverse global populations may also help capture regional variations in HPV prevalence and OSSN incidence to better understand the geographic differences in the association between the two.

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CRediT authorship contribution statement

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Declaration of Competing Interest

Neither author has any conflict of interest to declare.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.canep.2025.102799.

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