HPV-associated head and neck cancers in the Asia Pacific: a critical literature review & meta-analysis

Running title: HPV-associated HNSCC in Asia Pacific

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Abstract:

Background: Malignancies of the upper aero-digestive tract are a major public health problem, especially in the Asia Pacific. Certain Human papillomaviruses (HPVs) are well-established risk factors for carcinoma of the uterine cervix and for a subset of head and neck carcinomata: however their true importance in different populations and anatomical subsites remains unclear. The major risk factors in Asia Pacific remain smoked/smokeless tobacco, areca nut, alcohol abuse and poor diet, with evidence for HPVs. We review published evidence of association of HPV with anatomical site-specific Head & Neck Squamous Cell Carcinoma (HNSCC) in these populations and attempt a meta-analysis.

Materials and methods: From MEDLINE/PubMed/WEB-of SCIENCE/EMBASE/Scopus databases we found 66 relevant studies: 15 case-control studies met our inclusion criteria for meta-analysis, totaling 1146 cases & 618 controls. HPV detection rates, sample site and size, and methods of tissue preservation and HPV detection were tabulated for each study.

Results: Studies were heterogeneous in terms of sample selection and method of detection of HPVs. Most were of limited quality. Approximately, 37.55 % of overall HNSCC biopsy specimens of 66 studies reported HPV positivity of any subtype. PCR (polymerase chain reaction) was the mostly used detection method and HPV16 was the most common genotype detected. In meta-analysis, pooled prevalence of high risk types of HPV in fifteen selected studies, irrespective of anatomic site, was 40.92 % (469/1146): pooled odds ratio 4.82; (95% CI: 3.62 – 6.42).

Conclusion: In view of the significant association of HPV with HNSCC, studies with accurate subsite classification and more sensitive detection methods are necessary. Accurate data from this geographical region are essential to inform public health policies and treatment decisions, especially as studies from Europe and North America reveal HPV-driven cancers to be less aggressive, permitting treatment de-intensification.

Keywords: Head and neck cancer, human papillomavirus, Asia pacific populations, polymerase chain reaction (PCR), meta-analysis.

Introduction:

Head and Neck Cancers (HNC) represent a biologically heterogeneous group of neoplasms involving numerous distinct anatomical sites within the head and neck. Use of the term usually excludes the skin, central nervous system, eyes, lymphatic system, and endocrine glands. Most malignancies of the head and neck arise from the mucous membranes lining [International Classification of Disease tenth revision (ICD-10)]; Lips (C00); base of the tongue (C01), oral cavity including the anterior two-third of the tongue (C02 - C06); tonsils (C09); the oropharynx (C10); nasopharynx (C11) and accessory sinuses (C12); the hypopharynx (C13), the vallecula and the larynx (C32) (1). Most malignancies in this region (approx. 85%) specifically excluding nasopharyngeal carcinomas, which are biologically distinct are morphologically characterized as Head & Neck Squamous Cell Carcinomas (HNSCC) and share common risk factors (2).

Nasopharyngeal carcinomas are often recorded separately because they are biologically distinct, having a strong association with Epstein-Barr virus (3, 4). HNSCC is the fifth most common cancer diagnosed in the world (5). It is also one of the most common malignancies throughout Asia Pacific, including the Indian Subcontinent, accounting for 40-50% of all cancers (6). Tobacco, both smoked and unsmoked, areca nut (in these populations usually consumed as a component of betel quid) and alcohol use/abuse, often in the context of diets poor in antioxidants and minerals, are well-established major risk factors, so it is no surprise that HNSCC are most common in those countries where the consumption of tobacco, areca and/or alcohol is high (Australia, Brazil, China, France, India, Japan, Netherlands, Papua New Guinea, South Africa and Switzerland) (7). Recent studies have shown a rising incidence of Squamous Cell Carcinoma (SCC) of the oropharynx, despite the reduction of tobacco use in several of these countries (8-11). This suggests increasing prevalence of another risk factor, now clearly identified as persistent infection with so-called “high risk” types of human papillomavirus (HPV) (12, 13).

HPVs are a large family of viruses (the Papillomaviridae) that are small in diameter (55nm), epitheliotropic, with a genome 7200 – 8000 base pairs in length (14). Depending on their nucleotide sequences, HPVs are classified into more than 100 different genotypes, about 15 of which are regarded as “high risk” due to their oncogenic potential

(15, 16), amongst which, HPV16 and HPV18, are especially closely linked with malignant transformation (17, 18). Approximately 25 HPV types are associated with oral lesions (17), and more than 90% of cases of HPV-related HNSCC are associated with HPV type 16 (19, 20). Although HPV-associated HNSCC is well documented in the western world including Australia, studies on South Asia, South East Asia and East Asia are few and inconsistent. We present here a critical review of published studies. Clearly, further work on large case series with well-defined anatomical subsites and utilizing the sensitive and specific methods available is required.

Epidemiology & Risk Factors:

HNC is a serious problem in much of the world. The burden is approximately 599,637 new cases per annum worldwide: 300,373 for lip plus oral cavity; 156,877 for larynx; 142,387 for other pharynx (oro- & hypo-pharynx) excluding the nasopharynx (International Agency for Research on Cancer, Globocan 2012, http://globocan.iarc.fr/Pages/fact_sheets_population.aspx, accessed on 21 July, 2014) (21). While there is considerable geographical variation, the major burden is in developing countries. This includes South Asia where the annual incidence is 194,742 new cases and mortality 136,199 p.a. (an age-standardized rate of approximately 20.70 per 100,000 persons p.a.), South East Asia with 35,228 incidence rate and East Asia with the incidence of 79,477 per annum (International Agency for Research on Cancer, Globocan 2012, http://globocan.iarc.fr/Pages/fact_sheets_population.aspx, accessed on 21 July, 2014), and this is largely because of extensive use of tobacco and areca nut, poverty and poor oral health (21). The overall incidence of HNSCC in the Asia Pacific is 314,778 new cases per year, which is more than half of the total HNSCC cases worldwide. The mortality rate is also very high, approximately 199,537 p.a.

Although HPV-associated cancers are best described in cancer of the uterine cervix, where >99% of cases harbor HPV (22), a significant association of HPV with HNSCC has shown an overall worldwide proportion of 25-35 %, but with substantial variability (23-25). Since the association of HPV with HNSCC was first reported in the mid 1980s,

rising rates of oropharyngeal cancer (C01, C09, C10 & C12) especially of the tonsil (C09) and base of the tongue (C01) have been shown in Europe and in the USA, (26-29). In the USA, between 1984 and 2004, the incidence rates of HPV-associated oropharyngeal cancer (C01, C09, C10 & C12) increased from 0.8/100,000 to 2.6/100,000 person – year (from 16.3% to 71.7%) (27). In Sweden and Finland the incidence of HPV-associated tonsil (C09) carcinoma rose by 3-fold during 1970 (29%) to 2007 (93%) and by 2-fold between 1956 and 2000, respectively (30, 31). This specific subsite of oropharyngeal cancer (tonsil carcinoma) has also shown 3-fold increase in England between 1995 and 2010 (32). Significant trends have been described in Australia, where HPV-related oropharyngeal cancer (C01, C09, C10 & C12) increased from 20.2% (1987-1995) to 63.5% (2006-2010) (33). A recent meta-analysis of contemporary studies on HPV-associated oropharyngeal cancer (C01, C09, C10 & C12) estimates approximately 69.7% prevalence in North America and 73.1% in Europe (34). Unfortunately, such studies from Asia Pacific region have been reported show-striking differences. Reports only from India have given prevalence rates of the association of HPV with oropharyngeal cancer (C01, C09, C10 & C12) approximately 27.28 % (22.85 - 31.71%) (35, 36).

Head and neck cancer sites with high prevalence have a distinctly different structure, and encompass the lymphoid mucosae of Waldeyer's ring (31, 37). This area of the oropharynx is an embryonic transformation zone, and most HPV-positive oropharyngeal cancers (C01, C09, C10 & C12) arise from this (20). It is lined by non-keratinized stratified squamous epithelium, the crypts of which contain basal keratinocytes, which function as traps for antigens and are susceptible to nuclear integration of HPV DNA (20, 31). Histologically, HPV-positive HNSCCs exhibit poorly differentiated, non-keratinized basaloid morphology (38-40).

In the western world, patients with HPV-related HNSCC are generally Caucasian, are younger, male, have a comparatively high socio-economic status, are non-smokers and non- or light drinkers (8, 41-46). There are important associations with sexual behavior, especially sexual debut at early age and a high number of lifetime vaginal and especially oral sex partners, these contributing to a 2 to 5 fold increase in the incidence rate (8, 47-

55). Although it seems that HPV-positive HNSCC is more likely to develop in Caucasians (including Australians), a significant prevalence has also been noted in the Asia Pacific populations (except Oceania), perhaps associated with changing sexual mores and the infiltration of western culture (53).

Molecular Pathogenesis of HPV-associated HNSCC:

It is widely accepted that HPV16 and HPV18 are responsible for approximately 70% of cancers of the uterine cervix (56) and are strongly associated with anal, vulvar, and penile cancers (20, 57, 58). However the exact biological mechanisms are not completely understood. The process is thought to commence with integration of HPV DNA into the host keratinocyte genome. Two of the early genes, *E6* and *E7*, are thought to be responsible for malignant transformation (Figure 1). Suppression of the transcriptional repressor gene *E2* of HPV allows continuous expression of *E6* and *E7*, which then function as oncogenes throughout the life of what become malignant keratinocytes (59). The E6 oncoprotein localized in the nuclear matrix targets and degrades the product of the cell's *p53* tumor suppressor gene, thus inhibiting *p53*-dependent cell cycle arrest & induced apoptosis (60, 61). E6 oncoprotein also enhances telomerase activity, favoring immortalization of affected keratinocytes. In addition, viral *E7* promotes cellular proliferation by down-regulating the cyclin-dependent kinase (CDK) inhibitors p21 & p27, and by inactivating Rb (retinoblastoma) protein (62, 63) by binding with its hypophosphorylated forms (pRb, p130, p107) (64-66). Thus, loss of both *p53* and Rb function ultimately leads to malignancy.

HPV-positive and HPV-negative HNSCC are distinctly different diseases. Understanding their molecular mechanisms is essential for effective bio-prevention and individualized biotherapies.

Materials and Methods:

Search Strategy: WEB of SCIENCE (Thompson Reuter, NY, USA), EMBASE, SciVerse Scopus (Reed Elsevier PLC, Amsterdam, Netherlands), MEDLINE and PubMed (US National Library of Medicine, MD, USA) were used to conduct a comprehensive literature search on HPV-related Head and Neck Cancer in the Asia

Pacific. The search was limited to English language and published studies from 1980 to May 2014. Both MeSH (Medical subject Headings) and more general search terms were used. MeSH terms were ‘cancer’, ‘neoplasm’, ‘squamous cell carcinoma’, ‘oral cancer’ and numerous permutations of ‘Head and Neck Cancer (HNC)’ and its subsites (‘oral cavity’, ‘oropharynx’, ‘nasopharynx’, ‘larynx’, ‘tonsils’, ‘tongue’) associating with ‘Human Papilloma Virus (HPV)’ and ‘South Asia’, ‘South-East Asia’, ‘East Asia’, ‘Oceania’ and individual countries (‘India’, ‘Bangladesh’, ‘Pakistan’, ‘Sri-Lanka’, ‘Nepal’, ‘Bhutan’, ‘Maldives’, ‘Afghanistan’, ‘Malaysia’, ‘Indonesia’, ‘Thailand’, ‘Vietnam’, ‘Cambodia’, ‘Philippines’, ‘China’, ‘Japan’, ‘Taiwan’, ‘Hong Kong’, ‘South Korea’, ‘North Korea’, ‘Australia’, ‘New Zealand’ and ‘Papua New Guinea’). A secondary search was also performed on the references cited in the selected articles.

Study Selection and Data Extraction: Data extraction was performed by all three authors, focusing on: country of origin, demographics of participants, number of cancer cases, proportion of HPV positive cancers in relation to all carcinomas at HNC sub sites, HPV detection methods, and HPV genotypes. Based on the CONSORT statement 2010 (Consolidated Standards Of Reporting Trials), Asia Pacific data were retrieved by reading the abstracts. In order to explore the possibility of a meta-analysis, we applied the additional criterion of case: control study design. PRISMA 2009 (Preferred Reporting Items for Systematic reviews and Meta-Analysis) guidelines were followed while selecting studies for meta-analysis (Figure 1).

Statistical Analysis: STATA v.12 (Stata Corp LP, College Station, Texas, USA) software was used. Firstly, to evaluate any significant difference, two-sided 95% confidence intervals (95% CI) were calculated by the ‘Wilson Score’ method in all 66 selected studies (Tables 2 and 3). For meta-analysis, dichotomous data of the numbers of HPV-positive and -negative head and neck cancers were analyzed by Forest plot. Then the odds ratios (OR) and 95% confidence intervals (CI) were calculated by the ‘Wilson Score’ method (67) for individual studies. The overall proportions were examined by meta-analysis to estimate the pooled prevalence of HPV in non-site specific HNSCC using the Mantel-Haenszel method. The Cochrane Chi-squared or I-squared statistic (I^2) - based Q statistic and a random effects model (REM) with the DerSimonian-Laird method

were used to assess the variation between study results (68, 69). Potential publication bias was assessed using funnel plots (70).

Figure – 1 : A cartoon of Human papillomavirus type 16 genome organization.

PRISMA Flow Diagram

Figure 2 : PRISMA flowchart of the selection process of studies on HPV- associated head and neck cancer form South Asia.

Table 1: Details of published studies from Asia-Pacific reporting HPV association with Head and Neck Cancer.

Authors/yr/ reference no.	Country	Habits Described	Age groups (Years)	Tissues collected	Tumor site(s) (ICD-10 for Head and Neck Cancer)	No. of cases	HPV positive/ no. of cases (overall %)	HPV positive (Control group)	HPV Genot types	DNA (HPV) Detection Methods (primers used)
Balaram P. et al (1995)(71)	India	Smokeless tobacco user (majority)	30 - 70	Formalin Fixed (FF) & Paraffin Embedded (PE)	Buccal mucosa (C06) Tongue (C01, C02) Floor of mouth (C04) Lower alveolus (C03) Unspecified (--)	60 11 6 10 4	42/60 9/11 (74%) 4/6 8/10 4/4	not given	16,18, 6 & 11	PCR (MY09/MY11 & GP5/GP6)
D'costa J. et al (1998)(6)	India	All are smokeless tobacco users (for 10-30 yrs)	22 - 70	FF & PE	Buccal mucosa (C06) Lower alveolus (C03) Tongue (C01, C02) Floor of mouth (C04) Hard palate (C05) Maxilla (--)	57 25 14 02 01 01	4/57 5/25 6/14 0/02 0/01 0/01	not given	16	PCR (MY09/MY11) & Southern Blot
Pillai M.R. et al (1999) (72)	India	not given	not given	FF & PE	Oral cavity (C01-C06)	61	39/61 (63.9%)	0 (10)	16,18, 6 & 11	<i>in situ</i> Hybridization & (p53, cyclin D1, Ki-67, E6 Immunocytochemistry)
Nagpal J.K. et al (2002)(73)	India	All are smokeless tobacco users (for 10 yrs)	≤ 50 & 50+	FF & PE	Buccal mucosa (C06) Gum (03) Tongue (C01, C02) Lip (C00) Maxilla (--) Mandible (--) Unspecified (--)	56 06 06 04 13 16 09	21/56 2/06 2/06 0/04 (33.63%) 1/13 9/16 2/09	7 (26)	16 & 18	PCR (MY09/MY11)
Jacob et al (2002)(74)	India	not given	not given	FF & PE	Larynx (C32)	44	15/44 (34%)	0 (8)	16	PCR (HPV 16L1, 18E1, 6E1, 11E1)
Kumar R.V et al. (2003)(35)	India	Smokers & Smokeless tobacco users	not given	FF & PE	Tonsil (C09) Base of tongue (C01)	20 21	5/20 (31.7%) 8/21	not given	16	<i>in situ</i> Hybridization & p53 immunocytochemistry
Jayasooriya P.R et al (2003) (75)	Sri Lanka	Smokeless tobacco Users (majority)	≤ 60 & 60+	FF & PE	Buccal mucosa (C06) Tongue (C01, C02) Lip (C00) Unspecified (--)	62 08 06 26	23/62 04/08 0/06 (37.25%) 11/26	not given	16,18, 45,66	PCR (GP5+/GP6+)
Koppikar P. et al (2004)(76)	India	Smokeless tobacco users (majority)	25 - 80	FF, PE & Exfoliated Buccal cells	Oral cavity (C01- C06) Oro-pharynx (C10) Hypo-pharynx (C12-C13) Larynx (C32) Unspecified (--)	83 06 01 02 08	28/83 2/06 0/01 0/02 (32%) 2/08	5(102)	16, 8, 11, 18 & 38	PCR (GP5+/GP6+)
Herreo Ret al. (2003)	India	Smokers & Smokeless tobacco users	Not specified	Fresh Frozen	Oral cavity (C00 – C06) Oropharynx (C10)	262 12	8/262 3/12	Not specified	16,18 & 35	PCR (GP5+/GP6+)

Authors/year / reference no.	Country	Habits Described	Age groups (Years)	Tissues collected	Tumor site(s) (ICD-10 for Head and Neck Cancer)	No. of cases	HPV positive/ no. of cases (overall %)	HPV positive (Control)	HPV Genoty pes	DNA (HPV) Detection Methods (primers used)
Mishra A. <i>et al</i> (2006)	India	not specified	52.9 – 63.7 (58.3)	Fresh Frozen	Lip (C00) Gingiva (C03) Buccal mucosa (C06) Tongue (C01, C02) Palate (C05)	4 11 12 36 3	18/66 (27%)	0 (10)	16	PCR (MY09/MY11)
Mitra S <i>et al</i> (2007)(77)	India	Smoker & smokeless tobacco users	≤ 40 & ≥ 60	Formalin Fixed (FF) & Paraffin Embedded (PE)	Buccal mucosa (C06) Tongue (C01, C02) Lip (C00) Oro-pharynx (C10) Tonsil (C09) Palate (C05) Larynx (C32) Maxilla (--) Mandible (--) Unspecified (--)	29 19 03 02 01 01 12 13 07 05	59/92 (64.1%) (Oral cavity – 34/55, Larynx – 10/12, Other location/Oro-facial – 14/25)	not given	16,18	PCR (MY09/MY11)
Bhattacharya N. <i>et al</i>. (2009)	India	Smoker & Smokeless tobacco users	8 – 85 (48)	Fresh Frozen	Oral cavity (C01- C06) Larynx (C32) Oro-facial (--)	200 19 17	120/200 12/19 (61 %) 12/17	not given	16,18	PCR (MY09/MY11)
Chaudhary AK. <i>et al</i>. (2010)	India	Smoker, Smokeless tobacco user & alcohol user	≤ 45 & >45 (58.2)	Fresh Frozen	Lip (C00) Oral cavity (C01- C06) Tongue (C01, C02) Palate (C05)	32 112 40 38	10/32 37/112 (32.4%) 15/40 10/38	not given	16,18	PCR (MY09/MY11) & Hybrid Capture – 2 assay (HC-2)
Castillo A. <i>et al</i> (2011) (78)	Pakistan	Smoker & Smokeless tobacco users	not given	FF & PE	Oral cavity (C01- C06)	48	27/48 (56%)	not given	16	PCR (SPF1/SPF2) & <i>in situ</i> Hybridization
* Kulkarni S.S. <i>et al</i> (2011)(79)	India	not specified	not given	Saliva samples	Oral cavity (C01- C06)	34	24/34 (70.59%)	255 (396)	16 & 18	PCR (Unspecified)
Elango K.J. <i>et al</i> (2011)(80)	India	Smoker, Smokeless tobacco users	≤ 45 & >45	FF & PE	Tongue (C01, C02)	60	29/60 (48.3%)	0 (46)	16	PCR (MY09/MY11 & GP5+ /GP6+)
Jalouli J. <i>et al</i> (2012)(81)	Sri Lanka	Smoker, Smokeless tobacco user & alcohol user	40 – 70	FF & PE	Tongue (C01, C02) Floor of mouth (C04) Unspecified oral cavity	03 02 15	6/20 (30%)	not given	16	PCR (MY09/MY11)
Jalouli J. <i>et al</i> (2012) (81)	India	Smoker, Smokeless tobacco user & alcohol user	40 – 70	FF & PE	Tongue (C01, C02) Floor of mouth (C04) Unspecified oral cavity	06 00 14	9/20 (45%)	not given	16	PCR (MY09/MY11)
Akhter M. <i>et al</i> (2013) (82)	Bangladesh	Smokeless tobacco users (majority) & smokers	not given	FF & PE	Unspecified (--)	34	1/34 (2.94%)	not given	16, 18, 6 & 11	PCR (MY09/MY11)
Bahl A. <i>et al</i> (2013) (36)	India	Smoker (majority), smokeless tobacco user & alcohol user	30 – 80	FF & PE	Base of tongue (C01) Tonsil (C09) Soft palate	75 25 05	14/75 10/25 0/05 (22.85%)	not given	16, 18, 31 & 33	PCR (MY09/MY11) & <i>in situ</i> hybridization

* The only study in South Asia, which used saliva samples.

Authors/yr/ reference no.	Country	Habits Described	Age groups (Mean age) yrs	Tissues collected	Tumor site(s) (ICD-10 for Head and Neck Cancer)	No. of cases	HPV positive/ no. of cases (overall %)	HPV positive (Control group)	HPV Genotypes	DNA (HPV) Detection Methods (primers used)
Saini R. <i>et al.</i> (2011)	Malaysia	Smokers, Smokeless tobacco or alcohol users	50 - 69	Fresh Frozen specimens	Lip (C00) Buccal mucosa (C06) Tongue (C01, C02) Gingiva (C03) Floor of mouth (C04) Palate (C05) Mandible (-)	01 27 29 17 07 08 03	0/01 15/27 16/29 (51.4%) 11/17 2/07 5/08 0/03	Not specified	16 (mostly) 1 8,31,33,35, 45,51,58, 6,11, 53 & 54	PCR (MY09/MY11 & GP5/GP6)
Lim KP. <i>et al.</i> (2007)	Malaysia	Smokeless tobacco users	Not specified	FF & PE	Buccal mucosa (C06)	20	20/20	Not specified	16 & 18	PCR (GP5+/GP6*)
Khovidhunkit SP. <i>et al.</i> (2008)	Thailand	Smokers, Smokeless tobacco or alcohol users	30 - 88	Formalin Fixed (FF) & Paraffin Embedded (PE)	Oral cavity (C01- C06)	32	1/32 (3.12%)	not given	16	PCR (MY09/MY11) & (HPV1003/HPV1004)
Wen S. <i>et al.</i> (1997)	China	Smokers or alcohol users	not given	FF & PE	Oral cavity (C01- C06)	45	14/45 (31.1%)	not given	16 & 18	PCR & Southern blot
Ma XL. <i>et al.</i> (1998)	China	Not specified	40 - 79 (60.8)	FF & PE	Larynx (C32)	102	60/102 (58.8%)	Not specified	16, 18, 33, 6 & 11	PCR (pU-1M/pU-2R) & Southern blot
Cao J. <i>et al.</i> (2000)	China	not given	not given	FF & PE	Oral cavity (C01- C06)	40	29/40 (72.5%)	?/20	16 & 18	PCR & p53 immunohistochemistry
Zhang ZY <i>et al.</i> (2003)	China	Smokers or alcohol users	29 - 84 (57)	FF & PE	Buccal mucosa (C06) Tongue (C01, C02) Gingiva (C03) Floor of mouth (C04) Palate (C05)	14 35 11 10 03	9/14 28/35 8/11 (74%) 6/10 3/3	Not specified	16 & 18	PCR (Primers for HPV types 16 & 18)
Wei Li. <i>et al.</i> (2003)	China	not given	37 - 72	FF & PE	Tonsil (C09)	16	0/16 (0%)	not given	none	PCR (GP5+/GP6+, CP65/70ct- CP66/69ct, FAP59/64 and A5/A10-A6/A8) & p53, p21, p27,pRb, cyclin D1 and p16 immunocytochemistry
Liu B. <i>et al.</i> (2010)	China	Not specified	Not specified	FF & PE	Larynx (C32)	84	29/84 (34.5%)	Not specified	16 & 18	PCR (type-specific HPV16 & 18 E7, and GP5+/GP6+)
Wei W. <i>et al.</i> (2012)	China	All are smokeless tobacco users (for 10 yrs)	40 - 65	FF & PE	Lip (C00) Oro-pharynx (C10) Hypo- pharynx (C12-C13) Larynx (C32)	09 05 15 64	1/09 2/05 6/15 (17.2%) 7/64	not given	16,18 & 52 1/P18-2)	PCR (P16-1,P16-2 & P18- 1/P18-2)

Authors/yr/ reference no.	Country	Habits Described	Age groups (Mean age) yrs	Tissues collected	Tumor site(s) (ICD-10 for Head and Neck Cancer)	No. of cases	HPV positive/ no. of cases (overall %)	HPV positive (Control group)	HPV Genotypes	DNA (HPV) Detection Methods (primers used)
Xu Y. <i>et al.</i> (2015)	China	Smokers, Smokeless tobacco or alcohol users	21 - 93	FF & PE	Hypo-pharynx (C12-C13) Larynx (C32)	20 159	1/20 (2.23%) 3/159	not given	16,33 & 11	PCR (PGMY-09/11) Followed by hybridization
Li W. <i>et al.</i> (2007)	Hong Kong	Not specified	Not specified	FF & PE	Tonsil (C09)	31	9/31 (29%)	Not specified	Not specified	PCR
Chen P.C. <i>et al.</i> (2002)	Taiwan	Smokers, Smokeless tobacco users	not given	FF & PE	Oral cavity (C01- C06)	29	24/29 (82.7%)	8(29)	16, 18, 6 & 11	PCR & <i>in situ</i> Hybridization
Chang JYF. <i>et al.</i> (2003)	Taiwan	Smokers, Smokeless tobacco or alcohol users	25 - 78 ()	Formalin Fixed (FF) & Paraffin Embedded (PE)	Lip (C00) Buccal mucosa (C06) Tongue (C01, C02) Gingiva (C03) Floor of mouth (C04) Palate (C05)	03 74 02 16 07 01	 51/103 (49.5%)	 6(30)	16, 18, 33, 6, 11, 32, 44, 53 & 68	PCR (MY09/MY11 & GP5+/GP6+)
Yang YY. <i>et al.</i> (2004)	Taiwan	Smokeless tobacco users	not given	FF & PE	Oral cavity (C01-C06)	37	4/37 (10.81%)	0(36)	16 & 18	PCR (MY09/MY11)
Kuo KT. <i>et al.</i> (2008)	Taiwan	Smokers, Smokeless tobacco or alcohol users	29 - 79 (51)	FF & PE	Tonsil (C09)	92	69/92 (75%)	Not specified	16, 18, 33, 35, 58, 66, & 69	PCR (MY09/MY11 & GP5+/GP6+)
Al-Swiahb NJ. et al. (2010)	Taiwan	Smokers, Smokeless tobacco or alcohol users	20 - 89 (59.2)	FF & PE	Base of tongue (C01) Tonsil (C09) Soft palate	274	45/274 (16.4%)	Not specified	16,18,31,6 & 11	PCR (MY09/MY11 & GP5/GP6)
Oh TJ. <i>et al.</i> (2004)	South Korea	Not specified	32 - 77 (58.2)	FF & PE	Tonsil (C09)	39	25/39 (64.1%)	3 (34)	16, 33, 58 & 6	DNA microarray (PCR based)
Shin KH <i>et al.</i> (2002)	South Korea	Not specified	Not specified	Not specified	Oral cavity (C01 - C06) Salivary gland (C07)	76 10	11/76 (14.5%) 4/10 (40%)	Not specified	16,18 & 33	PCR (type specific)
Kim SH. <i>et al.</i> (2007)	South Korea	Not specified	Not specified	FF & PE	Tonsil (C09)	52	38/52 (73.1%)	Not specified	16, 18, 33, 35 & 58	real time PCR (primers 16 E2 & 16F6, and probes 16E2 & 16E6) & immunohistochemistry
Ogura H. <i>et al.</i> (1991)	Japan	Not specified	Not specified	FF & PE	Oro-pharynx (C10) Larynx (C32)	39	1/11 3/28 (10.2%)	Not specified	16 & 18	PCR (Primers for HPV types 16 & 18)
Anwar K. <i>et al.</i> (1993)	Japan	Not specified	42 - 90 (66)	FF & PE	Larynx (C32)	43	16/43 (37.2%)	2/11	16, 18 & 33	PCR (type specific primers)
Shidara K. <i>et al.</i> (1994)	Japan	Not specified	Not specified	FF & PE	Larynx (C32)	45	11/45 (24.4%)	Not specified	16 & 18	PCR (type specific primers) & polymorphism analysis

Authors/yr/ reference no.	Country	Habits Described	Age groups (Mean age) yrs	Tissues collected	Tumor site(s) (ICD-10 for Head and Neck Cancer)	No. of cases	HPV positive/ no. of cases (overall %)	HPV positive (Control groups specified)	HPV Genotypes	DNA (HPV) Detection Methods (primers used)
Shindoh M <i>et al.</i> (1995)	Japan	Not specified	Not specified	FF & PE	Oral cavity (C01 – C06)	77	24/77 (31.2)	Not specified	16 & 18	PCR (type specific primers)
Chiba I. <i>et al.</i> (1996)	Japan	Not specified	Not specified	FF & PE	Oral cavity (C01-C06)	38	8/38 (21%)	Not specified	16	PCR (type specific primers)
Mineta H. <i>et al.</i> (1998)	Japan	Not specified	Not specified	Fresh frozen	Oral cavity (C01-C06) Oro-pharynx (C10) Hypo-pharynx (C12-C13) Larynx (C32) Nasal & paranasal sinus (--) Nasopharynx (C11)	14 13 16 26 19 10	3/14 5/13 (23.46%) 8/26 3/19 1/10	Not specified	16 & 18	PCR (type specific primers)
Nishioka S. <i>et al.</i> (1999)	Japan	Smokers	50 – 55 (52.3)	FF & PE	Oral cavity (C01-C06) Oro-pharynx (C10) Larynx (C32) Nose/sinus (--)	15 14 27 18	0/15 3/14 (14.86%) 5/27 3/18		16 & 18	PCR & Slot blot
Tsuhako K. <i>et al.</i> (2000)	Japan	Smokers or alcohol users	28 – 94 (61)	Formalin fixed	Lip (C00) Buccal mucosa (C06) Tongue (C01, C02) Floor of mouth (C04) Oro-pharynx (C10) Larynx (C32) Maxilla (--) Lower gum (--)	03 07 36 15 17 02 11 11	3/03 4/07 (56.86%) 16/36 11/15 9/17 2/02 6/11 7/11	not given	16, 18, 6 & 11	PCR & Southern blot
Shima K. <i>et al.</i> (2000)	Japan	Not specified	47 – 91 (70)	Fresh frozen	Buccal mucosa (C06) Gingiva (C03) Tongue (C01, C02) Floor of mouth (C04) Palate (C05)	3 10 27 3 3	34/46 (73.9%)	Not specified	16 & 18	PCR (type specific primers– pU-1ML,pU-2R)
Kojima A. <i>et al.</i> (2001)	Japan	not given	30 – 80 (61)	FF & PE	Buccal mucosa (C06) Tongue (C01, C02) Gingiva (C03) Floor of mouth (C04) Palate (C05)	05 27 16 04 01	2/05 18/27 13/16 (66%) 2/04 0/01	not given	38	PCR (primers for type 38) & Sequencing
Higa M. <i>et al.</i> (2002)	Japan	Smokers or alcohol users	(59)	Fresh frozen	Lip (C00) Buccal mucosa (C06) Floor of mouth (C04) Maxilla (--) Tongue (C01, C02) Gingiva (C03) Oro-pharynx (C10)	3 17 21 4 74 32 22	138/177 (78%)	Not specified	16, 18, 6 & 11	PCR (primers for E6 & E7)
Sugayama M. <i>et al.</i> (2003)	Japan	not given	39 – 80 (59.5)	FF & PE	Lip (C00) Buccal mucosa (C06) Tongue (C01, C02) Gingiva (C03) Floor of mouth (C04) Palate (C05)	07 07 25 32 13 02	0/07 4/07 9/25 (35%) 11/32 4/13 2/02	16(44)	16 & 18	PCR (Primers for HPV types 16 & 18)

Authors/yr/ reference no.	Country	Habits Described	Age groups (Mean age) yrs	Tissues collected	Tumor site(s) (ICD-10 for Head and Neck Cancer)	No. of cases	HPV positive/ no. of cases (overall %)	HPV positive (Control group)	HPV Genotypes	DNA (HPV) Detection Methods (primers used)
Shimizu M. et al. (2004)	Japan	Not specified	59 – 94 (61)	FF & PE	Lip (C00)	27	5/27 (18.5%)	10/30	58,75 & 76	Nested PCR (type specific)
Sugayama M. et al. (2006)	Japan	Smokers or alcohol users	40 – 91 (67.5)	FF & PE	Lip (C00) Buccal mucosa (C06) Floor of mouth (C04) Palate (C05) Tongue (C01, C02) Gingiva (C03)	04 06 09 02 18 27	24/66 (36%)	not given	16	PCR (primers for type 16)
Koyama K. et al. (2007)	Japan	not given	37 – 79 (58)	FF & PE	Buccal mucosa (C06) Tongue (C01, C02) Gingiva (C03)	02 11 07	2/02 11/11 07/07	not given	16, 18, 22, 38 & 70	<i>in situ</i> PCR (MY09/MY11) & ISH
Bhawal UK. et al. (2008)	Japan	Not specified	48 – 90 (67)	Fresh frozen	Oral cavity (C01-C06)	29	11/29 (38%)	Not specified	16	PCR (type specific primers)
Ishibashi M. et al. (2011)	Japan	not given	12 – 81 (59.2)	FF & PE	Oral cavity (C01-C06)	50	6/50 (12%)	not given	16, 31, 58 & 61	PCR (MY09/MY11 & GP5+/GP6+) & genotyping (microarray)
Deng Z. et al (2011)	Japan	Smokers or alcohol users	28 – 90 (64.8)	Snap Frozen specimens	Oral cavity (C01-C06) Oro-pharynx (C10) Hypo-pharynx (C12-C13) Larynx (C32) Maxillary sinus (-) Nasopharynx (C11)	25 44 46 16 10 09	9/25 22/44 6/46 (30.0%) 2/16 3/10 3/09		16 (mostly), 33, 58 & 39	PCR (MY09/MY11 & GP5+/GP6+)
Mizumachi T. et al. (2013)	Japan	Smoking history	40 & 81 (62.8)	FF & PE	Oro-pharynx (C10)	71	23/71 (32%)	not given	16, 18 & 58	Multiplex PCR with 16 different genotypes probe
Maruyama H. et al. (2014)	Japan	Smokers or alcohol users	<42 - ≥69	FF & PE	Oral cavity (C01-C06) Oro-pharynx (C10) Hypo-pharynx (C12-C13) Larynx (C32) Nasopharynx (C11)	57 163 171 77 25	0/57 56/163 6/171 (13.8%) 3/77 3/25		16 (mostly), 18,33,35 & 58	PCR (E6 & E7 consensus primers)
Deng Z. et al. (2014)	Japan	Smokers or alcohol users	28-95 (65)	FF & PE	Oral cavity (C01-C06) Oro-pharynx (C10) Hypo-pharynx (C12-C13) Larynx (C32) Nasopharynx (C11)	37 74 50 28 20	11/37 34/74 8/50 4/28 6/20	Not specified	16 (mostly), 33,35, 56, 58 & 67	PCR (MY09/MY11 & GP5+/GP6+)
Hong A. et al. (2010)	Australia	not given	34 – 90 (59.8)	FF & PE	Base of tongue (C01) Tonsil (C09) Other oropharynx (-)	48 166 52	17/48 74/166 (37%) 8/52	not given	16 (mostly), 18,35 & 59	Multiplex PCR with 21 different genotypes probe
Hong A. et al. (2012)	Australia	Smokers or alcohol users	31 – 89 (55)	FF & PE	Tonsil (C09)	411	185/411 (45.9%)	not given	16,18,33 & 35	Multiplex PCR with 21 different genotypes probe
Hong A. et al. (2012)	Australia	Smoking history	34 – 83 (58.2)	FF & PE	Base of tongue (C01) Tonsil (C09) Other oropharynx (-)	29 183 21	15/21 111/183 11/21	Not specified	16,18,33, 35 & 39	Multiplex PCR with 21 different genotypes probe

Authors/yr/ reference no.	Country	Habits Described	Age groups (Mean age) yrs	Tissues collected	Tumor site(s) (ICD-10 for Head and Neck Cancer)	No. of cases	HPV positive/ no. of cases (overall %)	HPV positive (Control group)	HPV Genotypes	DNA (HPV) Detection Methods (primers used)
Hong A. et al. (2013)	Australia	Smoking history	30 – 90 (58)	FF & PE	Base of tongue (C01) Tonsil (C09) Other oropharynx (–)	46 551 50	15/46 249/551 8/50	Not specified	16, 18 & 35	Multiplex PCR with 21 different genotypes probe
Antonsson A. <i>et al.</i> (2015)	Australia	Smokers or alcohol users	50 – 80 (56)	FF & PE	Oral cavity (C01-C06) Oro-pharynx (C10) Hypo-pharynx (C12-C13) Larynx (C32)	88 77 24 59	5/88 38/77 3/24 4/59	not given	16 (mostly), 18, 33 & 69	PCR (GP5+/GP6+) & sequencing

Figure 2 : Prevalence of HPV in head and neck squamous cell carcinoma by anatomical sites and countries of Asia Pacific.

Table 2: Studies of Head and neck cancer in the Asia-Pacific by major cancer sites and overall HPV prevalence:

Site	Geographic Location	Number of studies	Number of cases	Overall HPV prevalence (95% CI)
Oral cavity	South Asia, South East Asia, East Asia & Oceania	43	3081	38.91 (37.21 – 40.61)
Oropharynx	South Asia, East Asia & Oceania	22	2806	44.29 (25.29 – 63.29)
Larynx	South Asia, East Asia & Oceania	17	838	24.46 (22.99 – 25.93)
Overall			6725	39.36 (38.38 – 40.43)

Results:

From all the mentioned electronic data bases, we identified 67 studies that have reported prevalence of one or more HPV types, patients' demography, methods of tissue preservation, HPV detection methods, and provided information about anatomical subsites in Asia Pacific region. Details of all 67 studies are tabulated in Table 1. A total of 7288 cases from different countries of Asia-Pacific region include 3081 cases from Oral cavity (C00 – C06), 2806 cases from oropharynx (C10), 980 cases from the larynx (C32) and 322 cases from hypopharynx, the remaining 99 cases being from unspecified subsites of head and neck region. Fifty of these 67 studies were cross sectional and 17 were case control. Many did not have a control or comparison population. Only 17 studies were of case-control design. However, 16 of these 17 studies used tissue samples, one used saliva (). Tissues were either fresh-frozen or paraffin-embedded. Among those using tissue, 15 used PCR as HPV DNA detection method, the other *in situ* hybridization. Ultimately, fifteen case-control studies met our inclusion criteria for meta-analysis.

Approximately, 37.55 % (95% CI, 36.46 – 38.64) of total HNSCC specimens showed HPV positivity of any subtypes in Asia Pacific region. By cancer site, the overall pooled HPV DNA prevalence estimates 38.91% for Oral cavity (C00, C02 - C06), 44.29% for Oro-pharynx (C10, C09 & C11), 9.9% for hypopharynx (C12) and 27.04% for laryngeal cancer (C32). Our data analysis by anatomical site and geographical location shows the highest HPV prevalence in Oral cavity squamous cell carcinoma in South East Asia (48.61%), followed by East Asia (42.84 %), 36.66% in South Asia and lowest in Oceania (5.6%). In oropharyngeal cancer, HPV prevalence is significantly higher in Oceania (49.32%) compared to East Asia (38.70%). The lowest HPV prevalence in oropharyngeal cancer was noted in South Asia (25.74%). However, this region (South Asia) showed the highest HPV prevalence in laryngeal cancer (48.05%), where as the lowest HPV prevalence was detected in Oceania (6%). No data on HPV-associated oropharyngeal or even laryngeal cancer was available from South East Asia. Considerable heterogeneity was present between and within regions and countries (Fig-2).

HPV16 was the most common type detected in all the HPV DNA positive cases from Asia Pacific, accounted for 90%. Although it was found to be the most prevalent type, the

prevalence varied between sites and regions. HPV16 prevalence was higher in oropharyngeal cancer 75% compared to oral cavity cancer 70% and laryngeal cancer 67%. HPV18 was the second most common oncogenic type detected in this region. Other oncogenic HPV types were detected in at least one of all specimens; HPV31, 33, 35, 39, 45, 51, 52, 53, 58, 66, 68 & 70. In addition, some low risk and non-oncogenic types were also detected, including HPV6, 11, 38, 44, 54, 61, 69 and 22. Multiple HPV infections were rarely detected 15% and in most instances they were co-infected with HPV16.

The average age group of the patients was 57.3 years. In East Asia the patients' average age is slightly higher (60.8) compared to South East Asia (59 years), Oceania (56.9 years) and South Asia (52.53 years). Although most studies presented information about HPV prevalence stratified by sex, not much significant differences were notified between men and women. However, male predilection has been noted. Majority of the study populations were presented with stage III and stage IV disease and cases of moderately differentiated squamous cell carcinoma showed higher HPV infectivity as compared to well-differentiated or poorly differentiated cancer (ref.). All except 3 studies used PCR as HPV DNA detection method, two of these three, used ISH and the other used DNA microarray.

Meta-analysis of fifteen non-site specific case-control studies computed to an HPV prevalence of 40.92 % (469/1146; summary OR: 4.82; 95% CI: 3.62 – 6.42) (Figure 3). The overall odds ratio indicated that the risk for HPV-association was approximately 5 times more in HNSCC patients compared to non-cancerous control group in the Asia Pacific. Considerable heterogeneity was present across these studies ($I^2 = 68.8\%$). Meta-analysis stratified by anatomical site reported that HPV-association in more stronger for oropharyngeal cancer (OR: 14.66; 95%CI: 6.09 – 35.26), less stronger for oral cavity cancer (OR: 4.06; 95%CI: 3.05 – 5.39) and laryngeal cancer (OR: 3.23; 95%CI: 1.37 – 7.61). Although there was evidence of heterogeneity between in these effects for oral cavity cancer ($I^2 = 68.8\%$, $p = 0.000$), but no heterogeneity was noted for laryngeal cancer ($I^2 = 0\%$, $p = 0.553$) and oropharyngeal cancer ($I^2 = 0\%$, $p = 0.644$). However, it should be noted that the study effect size and number of samples were small in both oropharyngeal cancer and laryngeal cancer studies.

Discussion:

Since tobacco and areca nut/betel quid-related HNSCC has long been the major concern, few studies have been conducted on the Asia Pacific populations to determine HPV associations, especially in oropharyngeal cancer (except Australia). This review of studies from the Asia Pacific assessed the association between HPV and head and neck cancer in specific sites. The overall pooled prevalence in our selected 66 studies from the Asia Pacific region was (37.55%) concordant with the recent meta-analysis by Termine *et al.* but slightly higher than the other meta-analysis by Ndiaye *et al.*, those indicated a worldwide prevalence of 34.5% (95% CI; 28.4 - 40.6) and 29.5% (95% CI; 25.5 - 33.6) respectively (83). However, our analysis suggested a higher prevalence of HPV in oropharyngeal cancer (OPC) (44.29%; 95% CI, 25.29 – 63.29), compared to oral cavity cancer (OCC) (38.91%; 95%CI, 37.21 – 40.61) and laryngeal cancer (24.46 %; 95%CI, 22.99 – 25.93) in the Asia Pacific (Tables 2).

Although the prevalence of HPV-associated oropharyngeal cancer from our study on Asia Pacific shows similarity to the worldwide prevalence documented by Ndiaye *et al* (45.8%), the estimated association is much higher in Europe (73%) and in the North America (70%)(ref.). Significant heterogeneity of HPV-associated OPC was noted when analyzed by separate geographical locations of Asia Pacific region indicating 25.8%, 38.7% & 49.2% in South Asia, East Asia and Oceania respectively. The HPV-associated OCC prevalence noted to be higher in the Asia Pacific (38.91%) compared to other parts of the world; Europe (17.5%), North America (13.4%) and South America (33.1%) (). However, the prevalence becomes much higher if we exclude Oceania from the Asia Pacific (42.88%). Our findings on HPV-associated OCC prevalence support previous reviews from Kreimer *et al.* and Ndiaye *et al.* where it was reported to be higher in Asian populations; 33% and 43.4% respectively (25). Estimates for an association with cancer of the larynx are much lower, averaging ~25% in the Asia Pacific; with a wide range of 6% to 48.05%. However, similar variability has been suggested from other parts of the world, ranging from 9.4-27% in the USA, 4.4-52% in Europe and 32-37.3% in South America (37, 84-94). These differences could be explained by issues regarding number of

cases added in the study, types of collected specimens, methods of tissue preservation, techniques used for DNA detection, geographical location or habitual distribution.

Our meta-analysis was based only on 15 case-control studies, these being from Asia Pacific on non-site specific HPV-associated HNC, and this is clearly a limited database to generalise from. However, site-specific stratified meta-analysis indicated stronger HPV association with oropharyngeal cancer compared to oral cavity, laryngeal and non-site specific Head and neck cancers in this particular region. Such finding supports the fact about the oropharyngeal cancer is commonly associated with HPV even in the Asia Pacific.

Although the demography of HPV-associated HNC patients around the world emphasize young adult males with no/limited history of smoking or drinking, the majority of the cases from the Asia Pacific reviewed here indicate habitual use of tobacco in smoked or smokeless forms and alcohol use/abuse. A high incidence of HPV infection has also been noted in betel quid and tobacco chewers (6, 71, 73, 76). In both sexes, the incidence of HPV-associated HNC showed no significant difference in the Asia Pacific populations. Although two studies from India have shown a male predominance with male & female ratio reporting 11:1 and 7:1, however, both of these studies have issues with small sized study groups and unequal sex distribution in sample selection process (36, 77). Majority of HPV-associated HNC patients from the South Asia, South East Asia and East Asia are relatively younger with lower socio-economic background. The median age of patients from the Asia Pacific estimates ~ 57 years, relatively lower, compared to HPV-related HNSCC patients of Europe (50-59 and 60+ years) and the USA (57 – 63 years) (8, 95, 96). In addition, none of these studies from The Asia Pacific have illustrated about the sexual behavior of the HPV positive patients. Only one recent extensive study from north India has shown a strong association of higher number of lifetime sexual partners and high-risk sexual behaviors (oral and anal sex) with HPV positive oropharyngeal cancer group as compared to patients who are HPV negative (58% vs. 21%) (36). Another study from Australia shows a significant association of HPV with the head and neck cancer in homosexual men (men having sex with men) practicing oral-penile sex().

There are several limitations in our study. Although we have stratified our study by head and neck cancer subsites and by geographical regions, heterogeneity still persists across studies as it was based on literature reviews. Although the total number of patients included in meta-analysis was relatively large, however, the number of studies that could be combined to find out information about specific association was relatively small. This is one of the major limitations that could not be overcome by any statistical technique. Moreover, only fifteen studies out of sixty-six, used control subjects for the comparison of HPV-association. Misclassification of anatomical subsites of head and neck squamous cell carcinoma is of another concern. Analysis by anatomical subsites in the Asia Pacific showed that tonsils have the highest HPV prevalence in East Asia and Oceania. Whereas, a high prevalence of HPV association was reported for “tongue cancer” (6, 73, 77, 80, 81) in South Asia and South East Asia without separation of the oral tongue from the posterior third/base of tongue (C01). The latter is part of the oropharynx and, worldwide, has a higher prevalence of HPV-related HNSCC than the mobile tongue (97). Generally, infrequent association of HPV is noted with the mobile tongue (C02), floor of the mouth (C04), buccal mucosa (C03) and palate (C05) (27, 98). Those sites With this anatomical ambiguity, we considered tumors of the tongue as of oral cavity cancer, which may have resulted in overestimation of HPV association with the mouth (oral cavity), recording 38.91% (95%CI, 37.21 – 40.61). Such misclassification could also explain about the presence of heterogeneity in the meta-analysis that was performed on HPV –associated Oral cavity cancer. Further categorization of other anatomical subsites was also imprecise with terms like ‘Oral cavity’, ‘Mandible’, ‘Maxilla’, ‘others’ or even ‘Unspecified/Unknown site’ (Table 1). Therefore, an appropriate site-specific classification of head and neck cancer is warranted. In future studies, we strongly recommend the systematic use of detailed ICD10 (International classification of Diseases- revision 10) codes for all studies of HNC to avoid misclassification.

Information about the age, sexual behaviour and habitual distributions were not properly addressed in most of the studies across the Asia Pacific. Therefore, it was not possible to assess the role of sexual behaviour in HPV-positive HNSCC or even the co-relation between the HPV-positive HNSCC and tobacco (smoking/smokeless), areca nut or alcohol consumption as its risk factors. However, few studies from the South Asia and

East Asia have suggested a significantly higher association of HPV-associated HNSCC in smokers and betel quid chewers compared to non-smokers or non smokeless tobacco users. Another major confounding factor is the geographical location. HPV prevalence in OCC is considerably higher in Asia, especially, in South Asia, South East Asia and East Asia, compared to Europe, North America or even Oceania. Such association is opposite in case of HPV-associated OPC. Due to geographic heterogeneity, the present review should therefore be interpreted with caution. Although HPV-associated OPC is more commonly observed across the globe, our study has shown a lower prevalence of HPV-associated oropharyngeal cancer in South Asia compared to HPV-positive OCC. One of the major reasons could be misclassification of the anatomical sites. Moreover, biasness in sample collection, different types of sampling and detection methods could be other confounding factors. Since most of the HNSCC cases in South Asia are observed in the oral cavity of the patient due to extensive use of tobacco both in smoking and smokeless forms, majority of these studies included in this review collected oral cavity samples. Only 2 studies out of 20, reported the prevalence of HPV –association with oropharyngeal cancer. Therefore, more studies are required to understand the true prevalence of HPV-associated OPC in South Asian populations.

Selection of the type of specimen is another important factor to be taken in account while determining the association of HPV. The tissue biopsy represents more appropriate feature of oral mucosae compared to exfoliated cytology or even saliva, and this includes basal layer cells where HPV could be present in latent state (98). Transmission of the HPV is primarily through ‘skin to skin’ contact and integration of an oncogenic strain of the virus into the tissue of host genome is essential for cancer to supervene (99, 100). A large number of saliva samples from the general public, which would include exfoliated epithelial cells, have been screened across USA, and prevalence for carriage of high-risk HPV genotypes of 3.1% was found (101). In interpreting the public health implications of this finding, it would be important to know how many individuals cleared or retained this infection, and the proportion of such individuals in whom integration of the virus might have occurred (102).

Other limitations include variation in HPV DNA detection method and lack of standardized histo-pathological assessment. The method of detection of HPV may be an important source for heterogeneity across studies. The sensitivity and specificity of the method used to detect and type HPV is clearly critical in comparing studies due to the weak viral productivity in the upper aero-digestive tract mucosae. It is also important to remember that the techniques used also should be reproducible, subject to standardization, free from contamination and be economically viable. PCR assay could be the technique of choice as it is a sensitive, time-efficient, and requires minimal tissue, but is moderately expensive (103) and there is a risk of false positives. Inappropriate length of the consensus primer pair or over-amplification of the HPV DNA type-specific primers could be another reason that may result in excessive sensitivity with false positives from contamination (104): it is always important to ensure a clean and sterile environment while performing PCR. Further, recent studies have shown that combination of two sets of primers such as “PGMY/GP+” or “MY09/11” & “GP5+/6+” in nested PCR is more efficient for detection of HPV in oropharyngeal cancer tissues, which may have very low viral load (105-107). More recently, the E6/E7 mRNA detection method has been defined as the gold-standard test to exemplify the oncogenic role of HPV in the tumour. However, the sensitivity of this test could also be hampered by the use of paraffin embedded tissue as nucleic acid degradation may take place during the fixation process. Although real time quantitative PCR (RT-qPCR) could be a better choice for HPV DNA detection due to its high-throughput analysis, it would still be very expensive compared to conventional PCR which remains the preferred method especially for use in resource-poor settings. Recently, a commercial “Di-gene 2” kit (Hybrid capture 2) has been promoted to screen large numbers of samples in a short time frame. This, however, is currently very expensive where 100 tests would cost approximately 2000 USD (www.Qiagen.com, accessed on 12 February, 2014). There are other HPV DNA detection methods available including ISH and p16 immunohistochemistry. Both ISH and p16 assays are highly sensitive but lack specificity. A recent study by Boy et al. showed 12% HPV-positivity in oral squamous cell carcinoma with quantitative real time PCR, but zero with ISH on the same samples (108).

Management protocols for HNC patients differ from person to person depending on anatomical location, age, medical conditions or/and tumour stage (19, 109, 110). Recent reports indicate better survival and treatment outcomes for HPV-associated HNSCC patients compared to HPV-negative cancers (111-113). All these factors therefore need to be taken into account in treatment planning as de-intensified therapy can be applied to the HPV-positive subset of HNC patients, reducing treatment related non-specific toxicity and health care costs. Although vaccine against HPV-associated cervical cancer has already been well introduced to school going girls, however, effectiveness of such vaccine is not well documented in other HPV derived cancers. Recently, Few countries, such as USA, Canada, Australia have recommended male vaccination. If this vaccine proves to be effective, a substantial amount of HPV-associated HNSCC could be prevented.

Conclusion:

In the past 30 years, there has been growing evidence suggesting a significant involvement of HPV in head and neck squamous cell carcinoma. This meta-analysis covers available data from the Asia Pacific as a whole. It covers a region of the world where tobacco-related HNSCC is a major concern, but suggests that HPV-related cancers are also significant. The prevalence of high risk HPVs appears similar to other countries, however, more number comprehensive studies are necessary on oropharyngeal cancer especially from South Asia, South East Asia and East Asia for better the understanding of HPV association with head and neck cancer in these particular regions. Further studies using the best techniques available with site-specific classification (preferably, use of ICD10 codes) and on larger & better-defined populations with more appropriate control subjects & tissues are clearly needed. It would also be of value to screen populations at risk for carriage and persistence of high-risk genotypes, especially in subjects with existing oral potentially malignant disorders.

Conflict of interest statement:

The author(s) declare that they have no competing interests.

Authors' contributions:

MHS performed the initial literature searches and all authors agreed on selected papers. MHS also extracted data and prepared the meta-analyses, tables and diagrams. He wrote the first draft and agreed the final manuscript.

NM advised and contributed on molecular aspects and agreed the final manuscript.

NWJ contributed to data interpretation, manuscript revision and agreed the final manuscript.

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Figure legends:

Figure 1: A cartoon of Human papillomavirus type 16-genome organization.

This HPV16 genome is a double stranded circular DNA containing approximately 8000 base pairs with 3 regions: (1) early gene region containing open reading frames (ORF) for *E1*, *E2*, *E4*, *E5*, *E6* & *E7* genes that are responsible for trans-activation, transcription, replication and viral adaptation. *E6* & *E7* genes are viral oncogenes that contribute to carcinogenesis. (2) Late gene region encodes capsid proteins L1 and L2 that are responsible for viral DNA packaging and maturation and (3) one non-coding or long control region (LCR) which contains sequences for controlling viral transcription and replication.

Association of HPV in Head & Neck cancer in Asia Pacific

Association of HPV in Oral Cavity cancer in Asia Pacific

Association HPV with Oro-pharynx in Asia Pacific

Association of HPV in Laryngeal cancer in Asia Pacific