

A systematic literature review of studies reporting human papillomavirus (HPV) prevalence in esophageal carcinoma over 36 years (1982–2017)

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Abstract

A total of 203 reports published between 1982 and 2017 on the association between human papillomaviruses (HPV) and esophageal squamous cell carcinoma (ESCC), originating from 187 studies performed in 32 countries from six continents, were selected and reviewed. The selected studies included a total of 14,788 ESCC cases; the presence of HPV infection was detected in 30.9% of cases (95% CI 30.1–31.6%) and was the highest in regions with the highest incidence of ESCC. Across studies published in the last 6 years, *Alphapapillomavirus* species were detected in 31.1% of ESCCs (1,464/4,708, 95% CI 29.8–32.4%), of which 73.8% (1,080/1,464, 95% CI 71.5–76.0%) were positive for the presence of HPV16/18. HPV16 was by far the most common HPV type detected, accounting for 21.0% (799.5/3,803, 95% CI 19.8–22.4%) of the total number of ESCC cases investigated. Our results are in line with previously published studies, suggesting the etiological role of HPV in the development of a subset of ESCC cases. Although the association between HPV and esophageal adenocarcinoma (EAC) has been studied to a far lesser extent, some studies also suggest a potential etiological role of HPV in a subset of EAC cases.

Keywords: esophageal carcinoma, human papillomaviruses, HPV, prevalence

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Human papillomaviruses are etiologically associated with human cancers of several anatomical sites

Human papillomaviruses (HPVs) are a heterogeneous group of small non-enveloped viruses with a double-stranded circular DNA genome that cluster into the *Papillomaviridae* family. Based on the similarity of their L1 genes, HPVs are phylogenetically further classified into genera (> 60% similarity), species (60–70% similarity), types (> 90% similarity), subtypes (> 98% similarity), and viral variants (> 99% similarity of the complete genome sequence). According to the International Human Papillomavirus Reference Center, located at the Karolinska Institute in Stockholm, Sweden (http://www.nordicehealth.se/hpvcenter/reference_clones/), 221 HPV types, which cluster into five papillomavirus genera—*Alphapapillomavirus* (*Alpha*-PV), *Betapapillomavirus* (*Beta*-PV), *Gamma*-papillomavirus (*Gamma*-PV), *Mupapillomavirus* (*Mu*-PV), and *Nupapillomavirus* (*Nu*-PV)—have been identified and officially recognized as of August 15th, 2018.

In addition to asymptomatic infections, HPVs are etiologically associated with the development of several benign and malignant lesions of the mucosal and/or cutaneous epithelium. Based on their clinical relevance, approximately 40 HPV types from the *Alpha*-PV genus are sub-divided into two groups: high-risk (hr) and low-risk (lr) HPVs. HrHPV types (HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, and HPV59), considered group 1 carcinogens, are etiologically associated with more than 99% of cervical cancers, 70 to 90% of anal and vaginal cancers, 40% of vulvar cancers, 47% of penile cancers, and 25 to 30% of oropharyngeal cancers (reviewed in 1–4). On the other hand, lrHPV types (most commonly HPV6 and HPV11) are associated with more than 90% of anogenital warts and laryngeal papillomas (reviewed in 3). Despite the fact that *Alpha*-PVs cause the majority of clinically significant infections,

some HPV types (HPV5, HPV8, HPV20, HPV22, HPV 38, HPV76, and HPV92) from the *Beta*-PV genus can act as cofactors in the development of non-melanoma skin cancer in immunosuppressed patients (5). In addition, *Gamma*-, *Mu*-, and *Nu*-PVs are etiologically associated with a proportion of benign skin lesions (reviewed in 3).

Differences in epidemiology of esophageal cancer worldwide

According to GLOBOCAN 2012, esophageal cancer is the eighth most common cancer, with an estimated 456,000 new cases in 2012, representing 3.2% of the total number of new cancer cases worldwide (6). Moreover, esophageal cancer is the sixth most common cause of death from cancer, with an estimated 400,000 deaths in 2012, representing 4.9% of the total number of deaths caused by cancer worldwide (6).

Esophageal cancer usually develops in older patients, with a median age of 65 years, and only rarely in individuals under 30 (reviewed in 7 and 8). Worldwide, the incidence of esophageal cancer varies between 0.8 and 17.0 per 100,000 in males and 0.2 and 7.8 per 100,000 in females, suggesting that esophageal cancer is a male-predominant disease (323,000, or 70.8% of new cancer cases in 2012) (6). In addition, in low-income countries, males are at a twice greater lifetime risk of developing esophageal cancer in comparison to women, and the difference can be up to four times greater in some high-income countries (6).

In comparison to more developed regions, the incidence of esophageal cancer is more than four times higher in less-developed regions (6). Furthermore, up to 500-fold differences have been observed across different countries/areas and even between different ethnic groups within the same area (9). As shown in Table 1, in 2012, the estimated overall age-adjusted incidence rates

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standardized for the world population (ASR(w)s) per 100,000 were the highest in East Africa (Kenya) (17.6), China (12.5), South Africa (9.9), and Iran (8.6) (6), confirming the results of previous studies, which suggested that high-risk areas for esophageal cancer stretch from East Asia to Central Asia (the so-called esophageal cancer belt) and along the Rift Valley in East Africa into South Africa, with incidence rates as high as 246 per 100,000 (reviewed in 7 and 10). In 2012, 3,432 new esophageal cancer cases were diagnosed in both genders in Kenya. In the same year, 223,306 new esophageal cancer cases were recorded in China alone, confirming that China is still the most affected country worldwide (reviewed in 7), with esophageal cancer being the fourth most common cause of death from cancer, accounting for 9.8 and 7.4% of all cancer deaths among men and women, respectively (6). Interestingly, in comparison to urban areas (e.g., Beijing and Shanghai), the incidence of esophageal cancer is higher in rural areas of China (the highest in Henan province) (reviewed in 7). According to GLOBOCAN 2012, 3,871 and 5,343 new esophageal cancer cases were diagnosed in both genders in South Africa and Iran, respectively (6). Similar to in China, some parts of Iran (especially Turkmen Sahra) also have a higher incidence of esophageal cancer (reviewed in 7). As shown in Table 1, the population in high-income countries is at lower risk for development of esophageal cancer, with ASR(w)s per 100,000

of 3.5, 3.3, and 3.1 in Australia, Europe, and North America, respectively (reviewed in 6 and 7).

Although several histological subtypes of esophageal cancer have been described to date, esophageal squamous cell carcinoma (ESCC), arising from squamous epithelial cells, and esophageal adenocarcinoma (EAC), arising from glandular epithelial cells, are the most common, together representing more than 90% of all esophageal cancer cases. Whereas ESCC predominates in the upper and middle esophagus, EAC usually arises in the lower esophagus, near the gastric junction (reviewed in 11 and 12). The first study that evaluated the global burden of ESCC and EAC, respectively, reported that an estimated 398,000 new cases of ESCC and 52,000 new cases of EAC were diagnosed in 2012 (13). The male-to-female ratio of the probability of developing esophageal carcinoma was 4.4 and 2.7 for EAC and ESCC, respectively (13). Whereas the majority (79%) of ESCC cases occurred in less-developed regions (Southeast and Central Asia), the incidence of EAC was the highest in more developed countries (northern and western Europe, North America, and Oceania), where 46% of all EAC cases have been diagnosed (13). Interestingly, even though ESCC is still the predominant esophageal cancer subtype worldwide, in several high-income countries (Australia, Bahrain, Canada, Cyprus, Iceland, Ireland, Malta, the Netherlands, New Zealand,

Table 1 | HPV DNA prevalence in esophageal squamous cell carcinoma according to geographic region and country of origin (1982–2017).

Geographic region	Incidence of ESCC ASR (w) ^a	Risk of ESCC ^b	No. of samples tested	No. of HPV-positive samples	HPV prevalence (%)	Range of HPV positivity (%)	No. of reports ^c
East Africa (Kenya)	17.6	high	29	0	0		1
China	12.5	high	8,503	3,404	40.0	0–100	73
South Africa	9.7	high	641	165	25.7	2.2–66.7	13
– South Africa	9.9	high	549	163	29.7	8.8–66.7	12
– Zambia	9.1	high	92	2	2.2		1
Other Asia	N/A	high	2,527	536	21.2	0–100	44
– India	4.1	high	349	107	30.7	5.0–100	7
– Iran	8.6	high	937	208	22.2	0–49.4	13
– Japan	6.1	high	974	176	18.1	0–63.0	19
– Korea	2.9	low-medium	225	34	15.1	0–66.7	4
– Pakistan	4.1	N/A	42	11	26.2		1
Latin America	4.3	low-medium	816	136	16.7	0–100	13
– Brazil	6.1	low-medium	609	74	12.2	0–15.8	6
– Chile	3.2	low-medium	26	5	19.2		1
– Colombia	1.9	low-medium	102	25	24.5	0–34.0	3
– Mexico	1.0	low-medium	77	30	39.0	25.0–88.2	2
– Venezuela	1.2	low-medium	2	2	100		1
Australia	3.5	low-medium	370	23	6.2	1.0–50.0	4
Europe	3.3	low-medium	1,347	197	14.6	0–100	35
– Belgium	4.6	low-medium	23	3	13.0	4.8–100	3
– Bulgaria	1.6	low-medium	4	1	25.0		1
– Finland	2.3	low-medium	121	35	28.9	18.0–40.0	2
– France	3.8	low-medium	171	6	3.5	0–41.7	5
– Germany	4.0	low-medium	76	9	11.8	0–17.0	2
– Greece	0.8	low-medium	49	19	38.8	10.5–56.7	2
– Hungary	3.6	low-medium	26	6	23.1		1
– Italy	1.3	low-medium	136	20	14.7	0–47.1	5
– Netherlands	6.3	low-medium	124	0	0		2
– Poland	2.2	low-medium	56	28	50.0		1
– Portugal	3.1	low-medium	16	9	56.3		1
– Slovenia	2.1	low-medium	141	2	1.4	0–10.0	2
– Sweden	2.3	low-medium	314	36	11.5	0–16.0	3
– Turkey	3.6	low-medium	63	22	34.9	9.1–63.3	2
– United Kingdom	6.5	low-medium	27	1	3.7	0–100	3
North America	3.1	low-medium	369	79	21.4	0–100	19
North Africa (Egypt)	2.1	low-medium	50	27	54.0		1
Total according to geographic region			14,788	4,567	30.9	0–100	203

^aIncidence of ESCC in ASR (w): age-standardized incidence rate per 100,000 (6).

^bRisk of ESCC for individual geographic region (country) was set according to the latest GLOBOCAN 2012 report (6) and published literature (9, 22, 34).

^cTotal number of reports extracted from 187 studies included in the analyses. ASR(w) = age-standardized incidence rate standardized for the world population per 100,000; ESCC = esophageal squamous cell carcinoma; N/A = not available.

Norway, Sweden, the United Kingdom, and the United States) the incidence of EAC has already exceeded that of ESCC (13). Moreover, in the next 10 years, the burden of EAC is expected to increase rapidly, resulting in EAC being the predominant subtype of esophageal cancer at least in some high-income countries (14). Nevertheless, esophageal carcinoma will remain an important healthcare problem in less-developed regions, where 369,640 new cancer cases were diagnosed in 2012, in comparison to 86,144 in more developed regions (6).

In addition to epidemiological differences between ESCC and EAC, a recent study identified several molecular features that support the current histological differentiation of esophageal carcinoma (15). Based on the comprehensive molecular analysis of 164 esophageal carcinomas, Kim et al. proposed that ESCC is more similar to squamous cell carcinoma of the head and neck region than EAC, whereas EAC resembles a chromosomally unstable variant of gastric adenocarcinoma (15).

Unresolved etiology of esophageal cancer

As described above, esophageal cancer includes several different histological subtypes of cancer (reviewed in 11 and 12). In addition, inhabitants of low-income countries have a higher risk of developing esophageal cancer, especially ESCC, in comparison to those living in high-income countries (reviewed in 6 and 13). Thus the etiology of esophageal cancer is most likely multifactorial and is still unresolved (reviewed in 7). The most common risk factors proposed for EAC include obesity, tobacco smoking, gastroesophageal reflux disease, Barrett's esophagus, and a diet low in fruits and vegetables. Interestingly, the etiology of ESCC differs substantially between low- and high-endemic areas; ESCC in low-endemic areas is mostly caused by chronic cell damage as a consequence of tobacco smoking and high alcohol consumption, especially both risk factors together. On the other hand, in high-endemic areas, ESCC most probably develops as a consequence of synergistic effects of chemical risk factors (nitrosamines and their precursors, environmental pollutants, opium, alcohol, tobacco, processed red meat, and nutritional deficiency), physical risk factors (thermal damage to the esophageal mucosa, achalasia, and radiation), and biological risk factors (bacteria and their metabolites, which increase cellular proliferation and stimulate the inflammatory process; fungi, which produce mycotoxins with tumorigenic properties; and some viruses) (reviewed in 8, 7, 11 and 12). In addition, based on the results of three genome-wide association studies, which described several new susceptibility loci, it has been suggested that ESCC could also be attributed to some genetic factors (16).

Since the first report proposing an etiological role of HPV in the development of neoplasms of the esophagus, published in 1982 (17), the arguments for etiological association between HPV and a subset of ESCC cases are becoming stronger with time, with several studies published in the last 36 years.

Thirty-six years of confusing results on the etiological association between HPV and ESCC

In the last 36 years, over 200 geographically diverse peer-reviewed publications, spanning from case reports to case-control studies, have reported the prevalence of HPVs in patients with ESCC. Nevertheless, when considering each study separately, the results are inconclusive because the reported HPV prevalence in ESCC varies from 0 to 100%. Interestingly, HPV was not even mentioned as a

possible etiological agent in the development of a subset of ESCCs in two recent review articles published in *The Lancet* (11, 12), nor is the role of hrHPVs in association with ESCC recognized by the International Agency for Research on Cancer, in contrast to the role of HPVs in the development of various anogenital and oropharyngeal cancers of both genders (reviewed in 7). We therefore believe that periodical systematic reviews and meta-analyses can be of great help when critically evaluating and building knowledge concerning the potential role of hrHPV in the development of ESCC.

Summary of main systematic reviews and meta-analyses on the role of HPV in the development of ESCC

Following the first formal literature review of the role of HPV in the development of ESCC, which was published by our group in 1998 (18), Kari Syrjänen published several updated literature reviews in 2002, 2006, 2010, and 2013 (19–22) followed by others. Briefly, in the last systematic review and meta-analysis published by Syrjänen in 2013, 152 studies dating from 1982 to March 2012 and originating from 32 different countries were selected and analyzed. Out of 10,234 ESCC cases analyzed, 3,135 (30.6%) were HPV positive. However, the HPV prevalence varied greatly across different geographic regions, with the highest HPV prevalence recorded in regions with the highest incidence of ESCC: China (2,268/5,651; 40.1%), South Africa (176/468; 37.6%), and Latin America (80/357; 22.4%). Similarly, HPV prevalence was the lowest in ESCC low-incidence regions: North America: 8.0% (18/226), Australia: 8.1% (22/271), and Europe: 14.0% (132/944). The results suggested that the etiology of ESCC differs between low- and high-incidence regions, with HPV playing an important role only in high-incidence regions (22).

In 2014, our research group published an updated systematic review on the role of HPVs in the development of ESCC, which included 159 studies published between 1982 and August 2013, originating from 33 countries from five continents (7), using a slightly different approach from that of Syrjänen in 2013 (22). In our literature review, a total of 11,310 ESCC cases tested for the presence of HPV were analyzed and the overall prevalence of HPV infection was estimated at 30.3%, with important geographical differences, similar to what was obtained in Syrjänen's 2013 review (22). In addition, 42 studies, published between 2008 and 2013, including 4,014 ESCC patients from 17 countries worldwide, were eligible and critically assessed for type-specific HPV prevalence data. In these 42 studies, the overall *Alpha*-PV prevalence was 32.2% (1,291/4,014). The calculated combined HPV16/18 prevalence was 23.3%, with HPV16 and HPV18 found in 16.3% and 2.7% of ESCCs investigated, respectively. Moreover, the combined HPV16/18 prevalence among all HPV-positive cases was estimated at 73.7% (7).

In 2013, The Australian research group subsequently published a first global meta-analysis of case-control studies investigating the association between HPV and ESCC (9). A total of 21 case-control studies, dating from 1991 to 2010, including 1,223 and 1,415 patients with ESCC and control subjects, respectively, were analyzed in the meta-analysis. In contrast to 35% of *Alpha*-PV-positive ESCC cases, HPV DNA was detected in 27% of control cases, irrespective of the HPV detection method used: immunohistochemistry (IHC), in-situ hybridization (ISH), and/or polymerase chain reaction (PCR). Furthermore, this meta-analysis provided strong evidence of an etiological association between HPV and ESCC, with a calculated pooled odds ratio (OR) of 3.04 (95% confidence interval

(CI) 2.2–4.2). Interestingly, in comparison to countries with a high incidence of ESCC, the association was stronger in countries with a low to medium incidence of ESCC (OR 2.65, 95% CI 1.80–3.91 vs. OR 4.65, 95% CI 2.47–8.76) (9).

The geographical differences in HPV prevalence among ESCC cases observed in previous studies (7, 9, 22) were subsequently also confirmed in the meta-analysis by Liu et al., which critically addressed a total of 145 ESCC studies published between 1982 and 2012 (23). Among 6,912 ESCC cases, the HPV prevalence was estimated at 38.9% and was statistically significantly higher in China (44.0%) in comparison to other regions (31.2%) ($p < 0.05$). In addition, similar to Liyanage et al. (9), the meta-analysis by Liu et al. confirmed that ESCC is etiologically associated with HPV infection (OR 4.20, 95% CI 3.08–5.74) (23).

A comprehensive meta-analysis by Hardefeldt et al. selected a total of 132 studies published from 1986 to 2012 (24). The overall prevalence of HPV in ESCC cases was 24.8% (2,985/12,037, 95% CI 21.2–28.8%). Similar to previous studies (9, 23), Hardefeldt et al. found evidence of an increased risk of ESCC development in patients with HPV-positive cancers (OR 2.69, 95% CI 2.05–3.54) and also an increased risk associated with HPV16 infection (OR 2.35, 95% CI 1.73–3.19). Moreover, the meta-analysis confirmed geographical differences in the risk of ESCC development, with Asia (OR 2.94, 95% CI 2.16–4.00), and especially China (OR 2.85, 95% CI 2.05–3.96), characterized as high-risk regions (24).

In 2014, Li et al. published a systematic review with a meta-analysis that assessed 8,990 ESCC cases obtained from 76 studies (25). In addition to the overall HPV prevalence in ESCCs, which was 22.2% (95% CI 18.3–26.7%), the study also provided a type-specific HPV prevalence, with HPV16 being the most frequently detected HPV type, with an overall prevalence of 11.4% (95% CI 8.2–15.7%), similar to what was observed in previous studies (7, 24). In line with the previous studies (9, 23, 24), Li et al. also observed a significant association between ESCC and HPV infection (OR 3.32, 95% CI 2.26–4.87) and ESCC and HPV16 infection (OR 3.52, 95% CI 2.04–6.07) (25).

In 2013, Yong et al. published a meta-analysis of the etiological role of HPV16 and HPV18 in the development of ESCC, in which they analyzed 68 studies published between 1989 and September 2012 (26). The overall HPV16 and HPV18 positivity rates in ESCC cases were 11.7% (95% CI 7.74–16.21%) and 1.8% (95% CI 0.90–2.95%), respectively. In addition, a meta-analysis of 10 case-control studies, including 1,130 ESCC cases and 1,614 controls, revealed a significantly increased risk of ESCC in patients with HPV16 infection (OR 3.55, 95% CI 2.0–6.14), similar to previously published studies (24, 25). In contrast, based on an analysis of six case-control studies, including 750 ESCC cases and 1,356 controls, they showed that HPV18 infection is not associated with an increased risk of ESCC development (OR 1.25, 95% CI 0.46–3.43) (26).

Zhang et al. conducted a systematic review and a meta-analysis of HPV16 prevalence among Chinese patients with ESCC, including studies published from 2005 to July 2014 (27). A total of 3,429 ESCC cases, originating from 26 Chinese studies, were included in the meta-analysis. Although the estimated overall prevalence of HPV16 infection was 38.1% (95% CI 28.3–47.9%), significant differences were observed according to the geographical region, publication year, types of specimens tested, and HPV detection method used. Specifically, the HPV16 prevalence varied from 39.7% (95% CI 24.9–54.5%) in the northern part of China to 47.8% (95% CI 36.2–59.4%) in the southern part of the country. In comparison to studies published between 2010 and 2014, the

HPV16 prevalence was higher in studies that were published between 2005 and 2009 (36.2%, 95% CI 22.3–50.1% vs. 40.2%, 95% CI 0.238–0.565%). As expected, the observed HPV16 prevalence was higher in studies conducted on fresh-frozen tissue samples (43.3%, 95% CI 23.5–63.1%) than on paraffin-embedded tissues (36.5%, 95% CI 24.2–48.9%). Interestingly, the HPV16 prevalence also differed if HPV detection methods targeted different parts of the viral genome, with higher HPV16 prevalence observed when targeting E6/E7 genes in comparison to the L1 gene (47.4%, 95% CI 37.3–57.4% vs. 28.8%, 95% CI 18.0–39.6%) (27). Subsequently, the same research group conducted a meta-analysis of 10 case-control studies, selected from the original set of studies, together including 1,442 ESCC patients and 1,602 control subjects (28). With ORs ranging from 3.65 (95% CI 2.17–6.13) to 15.44 (95% CI 3.42–69.70) and a pooled estimate of 6.36 (95% CI 4.46–9.07), HPV16 was identified as a risk factor for the development of esophageal cancer in China (28). Furthermore, the same research group published a similar study on HPV18, including 19 studies with a total of 2,556 ESCC patients (29). The pooled HPV18 prevalence in ESCC cases was estimated at 4.1% (95% CI 2.7–5.5%) and ranged from 0% to 26.1%; however, the infection with HPV18 was not associated with an increased risk for ESCC development in China (29), confirming the results of the global analysis (26).

Michaelsen et al. conducted a systematic review of studies published between 1980 and July 2013 to assess whether overexpression of p16^{INK4A} can be used as a marker of transcriptionally active HPV in ESCCs. A total of 12 studies, including 1,383 ESCC cases, originating from 10 different countries, were included in the systematic review. HPV DNA were detected in 12% (161/1,347) of cases and 33.9% (209/617) of ESCC cases were p16^{INK4A} positive, suggesting that the two markers are unrelated (30). The results of this study were further confirmed by Wang et al. (31), who showed that p16^{INK4A}-positive expression was associated with lymph node metastasis and that p53-negative expression may be used as a reliable marker for HPV status in ESCC (31), and by Halec et al., who performed a meta-analysis of nine case-control studies, published up to December 2014, and additionally tested 116 ESCC tissue samples (32).

The systematic review with the largest number of ESCCs included so far—13,832 samples originating from 124 studies published up to July 2013—was conducted by Petrick et al. (33). Interestingly, the HPV prevalence rate primarily depended on the HPV detection method used, being the highest using L1 serology (32.2%, 95% CI 15.4–49.0%), followed by IHC (30.4%, 95% CI 18.5–42.3%), PCR (27.7%, 95% CI 23.4–32.0%), ISH (24.3%, 95% CI 15.9–32.6%), and Southern/slot/dot blot (17.6%, 95% CI 6.1–29.2%). In accordance with previous studies (7, 9, 22–24, 34), the detection rate of HPV varied across different geographical regions, with the HPV prevalence ranging from 15.6% (95% CI 7.3–23.8%) in Europe/Australia to 31.4% (95% CI 27.4–35.4%) in Asia. Differences in HPV prevalence were also observed between studies that were published prior to 1990 (10.3%, 95% CI 11.4–32.1%), between 1990 and 1999 (20.4%, 95% CI 14.9–25.9%), and after 2000 (31.0%, 95% CI 26.7–35.2%), and between studies including fewer than 60 ESCC cases (27.2%, 95% CI 22.6–31.8%) and studies with more than 60 ESCC cases (26.1%, 95% CI 21.1–31.1%). Similar to previous literature reviews (7, 24–26, 28), HPV16 was the predominant HPV type, with an estimated prevalence of 18.5% (95% CI 14.2–22.8%), when considering studies using PCR as an HPV-detection method (33).

The largest meta-analysis of case-control studies, which included 33 reports dating from between 1982 and 2014, with a total

of 2,430 ESCC patients and 3,621 control subjects, was published by Wang et al. (35). In agreement with Liyanage et al. (9), they similarly observed a higher HPV infection rate in the ESCC group (46.5%) than in the control group (26.2%) (OR 1.62; 95% CI 1.33–1.98). In addition, they also reported significant differences in the strength of association between HPV infection and ESCC due to use of different HPV detection methods, with the highest OR observed in studies based on IHC (1.69, 95% CI 0.96–2.96), followed by PCR (1.61, 95% CI 1.33–1.95), serology (1.28, 95% CI 0.54–3.04), and ISH (1.21, 95% CI 0.62–2.36) (35). Because the reported values in the largest meta-analysis of case–control studies differs from those observed previously by Liyanage et al. and Petrick et al. (9, 33), further studies are needed to clarify whether different HPV methodologies are confounding factors in studies investigating the etiological association between HPV and ESCC.

Taken together, the results of previously published systematic reviews and meta-analyses suggest that hrHPVs, particularly HPV16, could play an etiological role in the development of a subset of ESCC cases. However, due to substantial differences in the geographical distribution of (HPV-positive) ESCC cases and suggested differences in HPV detection rates due to study sizes, types of specimens tested, and HPV detection methods used, further studies are warranted.

To the best of our knowledge, no systematic reviews and/or meta-analyses, including a plethora of studies published between January 2015 and December 2017, are available in the peer-reviewed literature. As described above, our research group published the last systematic literature review on the topic in 2014, in which we assessed 159 studies published between 1982 and August 2013 (7). Here we provide an updated systematic literature review covering all eligible studies that were published until the end of 2017, with special emphasis on the most recent data.

An updated systematic literature review of studies reporting HPV prevalence in ESCC (1982–2017)

In order to identify eligible peer-reviewed studies, several web-based databases, including Medline/PubMed, Web of Science, Scopus, and Google Scholar, were searched for a combination of the following terms: *papillomavirus*, *human papillomavirus*, *HPV*, *epidemiology*, *esophagus*, *oesophagus*, *cancer*, and *carcinoma*. Following an initial search, which was performed on August 29th, 2017, the search was repeated on December 28th, 2017, and subsequently additional studies were singled out through the revision of reference lists of previously identified studies. To be eligible for this review, studies needed to fulfill the following criteria: i) a defined population of patients, ii) at least one patient diagnosed with ESCC, iii) well-described HPV detection method(s), iv) numbers of HPV-tested and HPV-positive samples provided, and v) results not published in duplicate/multiple publications. Date of publication, publication language, and choice of the HPV detection method were not limiting factors.

As shown in Table 1, a total of 203 reports originating from 187 studies and 32 countries from six continents (Africa, Asia, Australia, Europe, North America, and Latin America) published between 1982 and 2017 were selected and analyzed. Since our last systematic literature review article (7), 28 additional original and eligible studies have been published, with the majority of new studies originating from China (16/28; 57.1%) (Table 2).

The overall HPV prevalence among 14,788 ESCC cases was 30.9% (95% CI 30.1–31.6%) (Table 1), in the range that was as-

essed in previously published systematic reviews and meta-analyses (7, 9, 22). Although HPV prevalence ranged from 0 to 100% in the majority of regions, it was overall the highest in regions with the highest incidence of ESCC: China (40.0%, 95% CI 39.0–41.1%), South Africa (29.7%, 95% CI 26.0–33.6%), and several Asian countries, except China (21.2%, 95% CI 19.7–22.9%), confirming the results of previous studies (7, 22–24, 33). Among Asian countries, the overall HPV prevalence was the highest in India, Pakistan, and Iran (Table 1). Interestingly, no HPV-positive cases were observed in the single study originating from East Africa (Kenya), with the highest estimated worldwide incidence of ESCC in 2012 (6). In low to medium ESCC risk regions, HPV prevalence was the highest in North Africa (Egypt) (27/50, 54.0%, 95% CI 40.4–67.0%), followed by North America (79/369, 21.4%, 95% CI 17.5–25.9%), Latin America (136/816, 16.7%, 95% CI 14.3–19.4%), Europe (197/1,347, 14.6%, 95% CI 12.8–16.6%), and Australia (23/370, 6.2%, 95% CI 4.2–9.2%). In line with our previous literature review (7), in Latin American and European countries no association was observed between estimated HPV prevalence and the risk of ESCC (Table 1).

A review of type-specific prevalence in ESCC cases over the last 6 years

In the last 6 years, a total of 42 publications, originating from 12 different countries (Australia, Brazil, China, Greece, India, Iran, Poland, South Africa, Sweden, Turkey, the United States, and Zambia) from six continents, reported the type-specific HPV prevalence in ESCCs (Table 2). As shown in Table 2, in the vast majority of studies, HPV typing was performed by PCR-based methods, either using commercial *Alpha*-PV DNA-based tests or in-house consensus and type-specific PCRs. In two studies novel broader and more sensitive HPV typing methods were used: matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF MS) and Luminex technology (Luminex, Austin, TX, US) (45, 67). Older, less sensitive, and less specific HPV typing methods such as IHC and ISH were used in only one and five studies reviewed, respectively (40, 48, 72–74, 75), suggesting the predominance of more sensitive and specific PCR-based methods in the most recent studies.

In studies published in the last 6 years, the overall *Alpha*-PV prevalence was 31.1% (1,464/4,708, 95% CI 29.8–32.4%), ranging from 0 to 100% in individual studies (Table 2), similar to what was recorded in previous systematic reviews (7, 9, 22, 33, 34). In addition, in line with previously published reviews (7, 24–29, 33), the combined HPV16/18 prevalence among *Alpha*-PV-positive ESCCs was 73.8% (1,080/1,464, 95% CI 71.5–76.0%; Table 2), suggesting that any of the three currently approved prophylactic HPV vaccines have the potential to prevent more than two-thirds of all HPV-positive ESCCs. As shown in Table 2, the calculated combined general HPV16/18 prevalence among all ESCCs was 26.3% (1,080/4,113, 95% CI 25.0–27.6%), with HPV16 being by far the most common HPV type, accounting for 21.0% (799.5/3,803, 95% CI 19.8–22.4%) of all ESCCs tested, followed by HPV18 present in 5.5% of ESCCs (122.5/2,226, 95% CI 4.7–6.6%). Our results are in line with previously published studies (24–26, 28, 33, 36). In addition, several other HPV types (HPV6, HPV11, HPV31, HPV32, HPV33, HPV35, HPV39, HPV42, HPV43, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, HPV70, and HPV82) from the *Alpha*-PV genus were detected in ESCC tissue samples, mostly as coinfections with HPV16 and HPV18 (Table 2), similar to what was observed in our last literature review (reviewed in 7).

Table 2 | Studies reporting overall and type-specific HPV prevalence in esophageal squamous cell carcinoma in the last 6 years (2012–2017).

No. Region (country)	HPV detection method	No. of samples tested	No. of HPV-positive samples	HPV prevalence (%)	HPV types detected	No. of HPV 16/18-positive samples	No. of HPV 16-positive samples	No. of HPV 18-positive samples	Reference
1 China	PCR (SPF1/GP6+)	300	93	31.0	16, 18, HPV-X	70	69	1	Guo et al. 2012 (36)
2 China	PCR (TS-PCR)	150	55	36.7	16	55	55	0	Hu et al. 2013 (37)
3 China	PCR (GP5+/6+)	253	52	20.6	16, 18	52	49	3	Liu et al. 2013b (38)
4 China	PCR (MY09/11)	92	19	20.7	N/A	N/A	N/A	N/A	Wang et al. 2013 (39)
5 China	ISH (HPV16,18)	105	29	27.6	16	29	29	0	Cao et al. 2014 (40)
6 China	PCR (MY09/11, GP5+/6+)	66	44	66.7	16, 18	44	43.5	0.5	Chen et al. 2014 (41)
7 China	PCR (MY09/11)	183	58	31.7	6, 11, 16, 18, 35, 43, 52	54	53	1	Cui et al. 2014 (42)
8 China	PCR	78	60	76.9	6, 11, 16, 18, 31, 58	54	48	6	Liu et al. 2014 (43)
9 China	PCR	177	6	3.4	11, 16, 35	2	2	0	Teng et al. 2014 (44)
10 China	PCR, MALDI-TOF MS	89	46	51.7	16, 39, 51, 82	44	44	0	Dong et al. 2015 (45)
11 China	PCR (GP5+/6+, TS-PCR)	196	158	80.6	16, 18	158	N/A	N/A	Mehryar et al. 2015 (46)
12 China	PCR (MY09/11, GP5+/6+)	150	27	18.0	16, 32, 42, 56	22	22	0	Wang et al. 2015 (47)
13 China	IHC (HPV16)	103	65	63.1	16	65	65	0	Xi et al. 2015 (48)
14 China	PCR (GP5+/6+, TS-PCR)	361	204	56.5	16, 18, 31, 45	154	102	52	Zou et al. 2015 (49)
15 China	PCR (GP5+/6+, TS-PCR)	35	1	2.9	16	1	1	0	Halec et al. 2016 (32)
16 China	PCR (GP5+/6+, TS-PCR)	123	56	45.5	16	56	56	0	Wang et al. 2016b (31)
17 China	PCR (MY11/GP6+, GP5+/6+)	50	8	16.0	16, 32, 56	5	5	0	Wang et al. 2016c (50)
18 China	PCR (HPV16,18 TS-PCR)	192	67	34.9	16, 18	67	25.5	41.5	Zhang et al. 2016 (51)
19 China	PCR (MY11/GP6+, TS-PCR)	189	168	88.9	16, HPV-X	76	76	0	Li et al. 2017 (52)
20 South Africa (South Africa)	PCR (MY09/11, GP5+/6+)	114	10	8.8	18, HPV-X	8	0	8	Schäfer et al. 2013 (53)
21 South Africa (South Africa)	PCR (GP5+/6+, TS-PCR)	48	7	14.6	11, 16, 35, 45, 70	N/A	N/A	0	Halec et al. 2016 (32)
22 South Africa (Zambia)	PCR (SPF10-based commercial kit)	92	2	2.2	N/A	N/A	N/A	N/A	Kayamba et al. 2015 (54)
23 Other Asia (India)	PCR (MY09/11)	49	17	34.7	N/A	N/A	N/A	N/A	Gupta et al. 2012 (55)
24 Other Asia (India)	PCR (GP5+/6+)	60	3	5.0	N/A	N/A	N/A	N/A	Pandilla et al. 2013 (56)
25 Other Asia (India)	PCR (LA)	23	20	87.0	16, 18, HPV-X	4	3	1	Vaiphei et al. 2013 (57)
26 Other Asia (Iran)	PCR (TS-PCR, INNO-LiPA)	93	8	8.6	16, 18, HPV-X	4	3	1	Abdirad et al. 2012 (58)
27 Other Asia (Iran)	PCR (GP5+/6+, 16,18 TS-PCR)	92	0	0	-	0	0	0	Noori et al. 2012 (59)
28 Other Asia (Iran)	PCR (MY09/11)	177	49	27.7	16, 18, HPV-X	2	1	1	Yahyapour et al. 2013 (60)

Table 2 | Continued.

No. (country)	Region	HPV detection method	No. of samples tested	No. of HPV-positive samples	HPV prevalence (%)	HPV types detected	No. of HPV 16/18-positive samples	No. of HPV 16-positive samples	No. of HPV 18-positive samples	Reference
29	Other Asia (Iran)	PCR (GP5+/6+)	30	0	0	-	0	0	0	Haeri et al. 2013 (61)
30	Other Asia (Iran)	PCR (GP5+/6+, TS-PCR)	35	2	5.7	16, 33	N/A	N/A	0	Halec et al. 2016 (32)
31	Other Asia (Iran)	PCR (GP5+/6+, INNO-LiPA)	103	11	10.7	16, 18	11	6	5	Soheili et al. 2016 (62)
32	Other Asia (Iran)	PCR (MY09/11)	51	16	31.4	11, 16, 33, 35, 39, 45, 52, 56, 58, 59	N/A	N/A	N/A	Yahyapour et al. 2016 (63)
33	Latin America (Brazil)	PCR (GP5+/6+, INNO-LiPA, TS-PCR)	1	1	100	16	1	1	0	De Oliveira Mota et al. 2013 (64)
34	Latin America (Brazil)	PCR (MY11/GP6+, GP5+/6+)	264	34	12.9	16, 18, 66	26	25	1	Herbster et al. 2012 (65)
35	Latin America (Brazil)	PCR (MY09/11, GP5+/6+)	52	0	0	-	0	0	0	Antunes et al. 2013 (66)
36	Latin America (Brazil)	PCR (Luminex)	87	12	13.8	16, 18, 31, 51, 66	N/A	N/A	N/A	da Costa et al. 2017 (67)
37	Australia	PCR, (MY09/11, INNO-LiPA)	99	1	1.0	16	1	1	0	Liyanage et al. 2014 (68)
38	Europe (Greece)	PCR (MY09/11)	19	2	10.5	11, 31	0	0	0	Georgantzi et al. 2015 (69)
39	Europe (Poland)	PCR (MY09/11)	56	28	50.0	16, 18, HPV-X	N/A	N/A	N/A	Dabrowski et al. 2012 (70)
40	Europe (Sweden)	PCR (GP5+/6+)	204	20	9.8	16, HPV-X	13	13	0	Löfdahl et al. 2012 (71)
41	Europe (Turkey)	PCR (GP5+/6+, TS-PCR), ISH	33	3	9.1	16, 39	1	1	0	Türkay et al. 2016 (72)
42	North America (US)	ISH (HPV6,11,16,18,31,33,51)	9	0	0	-	0	0	0	Landau et al. 2012 (73)
43	North America (US)	ISH (HR-HPV)	6	1	16.7	N/A	N/A	N/A	N/A	Doxtader and Katzenstein 2012 (74)
44	North America (US)	ISH (HPV16,18)	19	1	5.3	16, 18	1	0.5	0.5	Ludmir et al. 2014 (75)
	Total		4,708	1,464	31.1		1,080	799.5	122.5	
	HPV16/18 prevalence among all HPV-positive samples						73.8			
	Total no. of HPV16/18 samples tested		4,113				26.3			
	Calculated HPV16/18 prevalence									
	Total no. of HPV16 samples tested		3,803							
	Calculated HPV16 prevalence								21.0	
	Total no. of HPV18 samples tested		2,226							
	Calculated HPV18 prevalence								5.5	

IHC = immunohistochemistry; INNO-LiPA = INNO-LiPA HPV Genotyping Extra test (Fujirebio, Ghent, Belgium); ISH = in-situ hybridization; HPV-X = unspecified HPV type(s); LA = Linear Array HPV genotyping test (Roche Molecular Systems Inc., Alameda, CA, US); Luminex = Luminex technology (Luminex Corp., Austin, TX, US); MALDI-TOF MS = matrix-assisted laser desorption/ionization time-of-flight mass spectrometer; PCR = polymerase chain reaction (the primers used are provided in parenthesis); TS-PCR = type-specific PCR; N/A = not available; -- = not applicable.

HPV infection is associated with prognosis of patients with ESCC

In a recent case-control study, which included 103 ESCC cases and 54 controls obtained from Chinese patients between September 2008 and December 2009, Xi et al. (48) confirmed the results of previous studies showing a high prevalence of HPV infection among ESCC patients in China (7, 22–24, 27, 33). Moreover, they showed that HPV16 infection and high phosphatidylinositol 3-kinase (PI3K) expression levels are independently negatively correlated with 3-year and 5-year overall survival and progression-free survival and can therefore be used as markers for predicting disease outcome in patients with ESCC (48). The same research group performed a subsequent study investigating the HPV16-mediated mechanisms linked with the development and progression of ESCC and showed that HPV16 E6 and E7 proteins can promote cancer stem-like cells' phenotype in ESCC cells by activating the PI3K/Akt signaling pathway, which can potentially be targeted in treatment of ESCC (76).

The role of HPV in the development of EAC

In addition to obesity, smoking, gastroesophageal reflux disease, Barrett's esophagus, and a diet low in fruits and vegetables, traditionally described as the risk factors for the development of EAC (reviewed in 11 and 12), some research groups report that hrHPV infection could also be involved in the etiopathogenesis of a subset of EAC cases (reviewed in 77 and 78). The cumulative overall HPV prevalence obtained in six studies published between 2000 and 2016, including a total of 404 EAC cases, was 7.7% (31/404, 95% CI 5.5–10.7%) (79–85). Rajendra et al. (Rajendra et al. 2013) performed a case-control study, including 261 patients with Barrett's esophagus, Barrett's dysplasia, and EAC and 51 healthy controls. In comparison to the control group, the HPV prevalence was significantly higher in Barrett's dysplasia (68.6%, incidence rate ratio (IRR) 2.94, 95% CI 1.78–4.85, $p < 0.001$) and EAC (66.7%, IRR 2.87, 95% CI 1.69–4.86, $p < 0.001$). In addition, 75/81 (92.6%) HPV-positive patients were infected with hrHPVs (HPV16, HPV18). Moreover, when testing for HPV E6/E7 mRNA expression using a nested reverse transcription PCR, expression of mRNA for these two most important HPV oncogenes was demonstrated in 9/22 (40.9%) and 9/15 (60.0%) of Barrett's dysplasia and EAC cases, respectively, whereas all controls and patients with Barrett's esophagus were without measurable oncogene expression ($p < 0.001$). Furthermore, Rajendra et al. showed a strong association between disease severity, the presence of HPV DNA, and expression of E6/E7 mRNA and p16^{INK4A} (OR 62.2, 95% CI 12.4–311, $p < 0.001$) (85). The same research group subsequently performed a whole exome sequencing analysis of HPV-positive and HPV-negative EAC cases and revealed the genomic differences between both groups (86). Namely, HPV-positive cases harbored 50% less non-silent somatic mutations in comparison to HPV-negative cases (1.31 mutations/Mb vs. 2.56 mutations/Mb, $p = 0.048$), similar to what was observed previously in other head and neck cancers as well as cervical cancer (87, 88). In addition, Rajendra et al. confirmed the results of their previous study (89), showing the absence of aberrations in the gene encoding the tumor suppressor protein p53 (wild-type) among HPV-positive cases, in contrast to HPV-negative tumors, which frequently (up to 50%) contained p53 mutations (86). In their most recent study, Rajendra et al. complemented their previous results and concluded that the active role of HPV in

the development of Barrett's dysplasia and EAC can be indirectly determined by detection of wild-type p53 and aberrations of the retinoblastoma protein (pRB) pathway (90).

Conclusion

Esophageal cancer is the eighth most common cancer and sixth most common cause of death from cancer worldwide (6). The etiology of esophageal cancer is most likely multifactorial and is still unresolved. Several studies performed in the last 36 years have suggested HPV as a potential risk factor for the development of the two most frequent histological subtypes of esophageal carcinoma: ESCC and EAC.

To the best of our knowledge, this literature review covers the most complete list of studies on the association between HPV and the development of esophageal carcinoma published in peer-reviewed journals between 1982 and 2017. The overall HPV prevalence identified in our review was 30.9% and 7.7% among 14,788 ESCC and 404 EAC cases, respectively. Infections with HPV16 and HPV18 were the most common, suggesting that more than two-thirds of all HPV-positive esophageal carcinomas could theoretically be prevented using any of the three current prophylactic HPV vaccines, although only if proved to also work against non-genital HPV-related cancers or their precursors.

In order to provide conclusive evidence that hrHPVs are definitive causative factors of ESCC, we need more studies. In our opinion, a well-designed large case-control study with sufficient power to indisputably ascertain HPV rates in ESCC cases compared with controls without ESCC is the most practical way forward (7). The proposed large case-control study should preferably be an international collaboration bringing together the best researchers from all continents, and should use a mandatory uniform HPV DNA (and possibly HPV RNA) testing methodology for all tissue samples. In a perfect scenario, all tissue samples should be processed (e.g., cut from paraffin blocks) and tested for HPV DNA in a single central laboratory with long experience in dealing with archival clinical specimens (7). ESCC tissue samples and tissue samples of normal esophageal mucosa should be collected from countries/areas covering the entire spectrum of ESCC incidence rates, from extremely low-incidence areas to areas with the highest incidence rates of ESCC, with minimal variations in specimen retrieval and storage. The study should not use tissue adjacent to the ESCC lesion (histologically normal esophageal tissue from resection specimens of ESCC) as "controls" because there is a high potential for cross-contamination and spread of HPV from tumor tissue to adjacent non-malignant esophageal tissue, creating false-positive results in the detection of HPV DNA in the non-tumor tissue (7, 91). Finally, the case-control study should collect data on potential confounders and effect modifiers, and these should be adjusted for when examining the effect of HPV. These include at least age, gender, smoking, alcohol consumption, family history of esophageal cancer, pre-existing immunosuppression prior to cancer diagnosis, a history of thoracic irradiation, socioeconomic status, diets high in processed red meat, consumption of hot food and beverages, pickled foods, and diets low in fresh fruit and vegetables (7).

In conclusion, in order to provide conclusive evidence that HPV is a definitive causative factor of a subset of ESCC, any future case-control study to address the HPV-ESCC relationship would need to be substantially larger and planned in a substantially different way than any of the currently published studies.

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