

# Human papillomavirus tumor infection in esophageal squamous cell carcinoma

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**Abstract:** The association between human papillomavirus (HPV) and esophageal squamous cell carcinoma (ESCC) has been recognized for over three decades. Recently, multiple meta-analyses have drawn upon existing literature to assess the strength of the HPV-ESCC linkage. Here, we review these analyses and attempt to provide a clinically-relevant overview of HPV infection in ESCC. HPV-ESCC detection rates are highly variable across studies. Geographic location likely accounts for a majority of the variation in HPV prevalence, with high-incidence regions including Asia reporting significantly higher HPV-ESCC infection rates compared with low-incidence regions such as Europe, North America, and Oceania. Based on our examination of existing data, the current literature does not support the notion that HPV is a prominent carcinogen in ESCC. We conclude that there is no basis to change the current clinical approach to ESCC patients with respect to tumor HPV status.

**Keywords:** Esophageal cancer; squamous cell carcinoma; human papillomavirus (HPV); p16/INK4A

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## Introduction

Human papillomavirus (HPV) is a circular, double-stranded DNA virus with established oncogenic potential in the setting of multiple malignancies, most prominently squamous cell carcinoma of the cervix (1). The established role of HPV in squamous cell cancers of other sites, including the anus and the oropharynx, has resulted in a growing literature aiming to assess the biological and clinical roles of HPV tumor infection in a diversity of cancers. Evidence dating back over three decades has indicated an association between HPV infection and esophageal squamous cell carcinoma (ESCC) (2,3). Recent meta-analyses have assessed the incidence and etiological role of HPV-ESCC tumor infection. This review aims to provide clinicians with a summary of the current HPV-ESCC literature and clinical recommendations regarding HPV infection in this disease.

## HPV-ESCC infection rates

Perhaps the most well-studied and yet controversial issue with regard to HPV infection in the setting of ESCC is the overall rate of HPV infection in these tumors. The question of whether, or to what extent, HPV infection occurs in ESCC has been the subject of ongoing debate. Dozens of studies have attempted to address this question using a variety of techniques, controls, and patient populations, as will be discussed below. More recently, a number of meta-analyses and systematic reviews have been published, attempting to address this question. *Table 1* summarizes these, providing an overall rate of HPV-ESCC infection identified across studies. While these meta-analyses draw upon much of the same primary literature, each utilizes differing criteria with regard to the type of studies included. As illustrated in *Table 1*, worldwide HPV-ESCC infection rates range from 11.7% to 38.9%. Of note, one of these

**Table 1** Tabulation of recent meta-analyses and systematic reviews of HPV infection in ESCC

Authors	Publication year	Citation	Overall percentage of HPV-positive ESCC cases (%)	Overall number of ESCC cases	Notes
Liu <i>et al.</i>	2013	(4)	38.9	6,912	
Liyanage <i>et al.</i>	2013	(5)	35.0	1,223	
Syrjänen	2013	(3)	30.6	10,234	
Yong <i>et al.</i>	2013	(6)	11.7	5,755	HPV-16 only
Hardefeldt <i>et al.</i>	2014	(7)	24.8	12,037	
Li <i>et al.</i>	2014	(8)	22.2	8,990	
Michaelsen <i>et al.</i>	2014	(9)	12.0	1,347	
Petrick <i>et al.</i>	2014	(10)	27.7	13,832	By PCR only; ISH rate =24.3%

HPV, human papillomavirus; ESCC, esophageal squamous cell carcinoma; ISH, in situ hybridization; PCR, polymerase chain reaction.

analyses provides separate data for high-risk HPV types 16 and 18 (HPV-16 and HPV-18); these authors report an 11.7% infection rate for HPV-16, and a 1.8% infection rate for HPV-18, but do not provide pooled data for both high-risk HPV types (6). Issues pertaining to strain-specific HPV identification will be discussed further below.

The wide range of HPV-ESCC infection rates reflects the variability in the primary literature, where even large-scale case-control studies have reported infection rates between 0% and 78% (11,12). While many reasons for this variability have been postulated, geographic variation remains among the strongest predictors for observed disparities in infection rates (3). There are wide geographic differences in the overall incidence of ESCC, with high-incidence countries (e.g., China and Iran) reporting one-hundred-fold higher rates of ESCC compared with low-incidence countries (e.g., Australia and the United States) (13). Low-incidence countries have ESCC rates of approximately 2.5 per 100,000, whereas high-incidence countries have rates as high as 250 per 100,000 (13,14). Interestingly, in high-ESCC-incidence countries, the HPV tumor infection rate is also significantly higher relative to low-ESCC-incidence countries (3). Multiple meta-analyses have demonstrated this difference, with reported rates of HPV-ESCC tumor infection in China on the order of 32.8-63.6% (3-5,7). Low-incidence regions, including North America, have substantially lower reported rates of HPV-ESCC infection (Table 2). For North America alone, rates range between 8.7% and 16.6% (3,6,7,10).

Given these geographic differences in HPV-ESCC infection rates, an important caveat is that worldwide HPV prevalence estimates are biased by the number of

cases and studies from specific regions. In most meta-analyses, investigators report the majority of studies used in their analyses are from Asia alone, a high-risk region (3,4,6,7,10). This publication bias toward high-incidence regions complicates interpretation of the overall reported HPV-ESCC prevalence rates in Table 1. We therefore included Table 2 to facilitate a geographically-specific interpretation of the reported HPV-ESCC infection rates primarily in a Western population. One meta-analysis, for instance, calculated an overall HPV prevalence of 30.6%, but a region-specific infection rate of 10.1% for Canada and the United States (3). The differences between reported worldwide and low-incidence-region HPV-ESCC rates, even within the same meta-analysis, demonstrates the degree to which geographic publication bias affects the reported overall infection rates. Why this asymmetric geographic distribution exists, however, remains largely unanswered. Some evidence has suggested that environmental exposures and nutritional deficiencies might provide an etiological basis for higher ESCC incidence in the developing world (3,13).

### Methodological considerations

Despite evidence that geography accounts for much of the variability observed in the primary literature measuring HPV-ESCC infection rates, another area of concern relates to the different methods used to evaluate HPV status in patient samples (3,7). Historically, methods have included histology, Southern blotting, dot blot hybridization, HPV L1 protein serology, in situ hybridization (ISH), and polymerase chain reaction (PCR), with most contemporary

**Table 2** Tabulation of recent meta-analyses and systematic reviews of HPV infection in ESCC in low-incidence regions

Authors	Publication year	Citation	Region-specific percentage of HPV-positive ESCC cases (%)	Region or countries	Notes
Syrjänen	2013	(3)	10.1	Canada and USA	
Yong <i>et al.</i>	2013	(6)	4.6	Europe	HPV-16 only
			10.5	North America	HPV-16 only
Hardefeldt <i>et al.</i>	2014	(7)	18.8	Europe	
			15.2	North America	
			8.7	USA	
			15.7	Oceania	
Li <i>et al.</i>	2014	(8)	14.0	Europe and the Americas	
Petrick <i>et al.</i>	2014	(10)	15.6	Europe	
			16.6	North America	

HPV, human papillomavirus; ESCC, esophageal squamous cell carcinoma.

studies utilizing ISH or PCR. Comparing these two, PCR has high specificity but also a high false positive rate, whereas ISH has high specificity but low sensitivity (15). Several reviews have proposed that variability in reported HPV-ESCC infection rates may be at least partially explained by HPV detection method (3,13,16,17). One recent systemic review found the overall HPV-ESCC prevalence varied significantly between less-used methods, from 17.6% for the now-outdated Southern blot technique to 32.2% for HPV L1 serology (10). However, as shown in *Table 1*, this review found that the two most commonly-used methods, PCR and ISH, demonstrate similar overall HPV-ESCC rates of 27.7% and 24.3%, respectively, with no statistically significant difference between the PCR and ISH HPV prevalence rates (10). Along these lines, other meta-analyses have concluded that detection method does not account for the variability in reported HPV-ESCC infection rates, as this variability persists even when examining studies all utilizing the same detection method (3,5,10). Collectively, these analyses suggest it is unlikely that choice of detection method accounts for a significant portion of the observed HPV prevalence variability.

Since variability persists within studies using the same detection method, it is worthwhile to examine any potential intra-method technical factors that may influence reported HPV prevalence rates. Looking at the most prevalent detection technique, PCR, one methodological issue is the lack of a ‘gold standard’ primer set for use in this approach (6). In PCR-based studies, investigators have utilized both type-specific and broad-spectrum primers;

type-specific primers tend to amplify short segments of DNA, making these primers more sensitive than broad-spectrum primers which amplify longer DNA stretches (6). Consistent with this, one meta-analysis showed that HPV-16/18 prevalence is higher when type-specific primers were used (versus broad-spectrum primers) (6). Even when controlling for these two broad categories of primers, issues remain with the PCR detection method, including which specific primer set is used in a given study, as well as rapidly-advancing PCR technologies.

The discussion regarding PCR primers leads to another concern in these studies: the selection of HPV strains to be probed. Over 100 HPV strains have been identified, with only a fraction of these implicated in the development of cancer, termed high-risk strains. It is worth noting that the high-risk and low-risk distinctions were broadly developed based on literature from cervical carcinoma. These designations were validated by data from oropharyngeal and anal cancers, where the vast majority of HPV-positive squamous cell carcinoma lesions are caused by the previously-identified high-risk HPV genotypes (notably HPV-16, -18, and -33) (1,18-20). The variation between studies as to which HPV strains are interrogated results in potential selection bias, affecting estimated HPV-ESCC prevalence rates. There is also an assumption in the literature that the same high-risk genotypes identified in other settings should also be considered high risk in ESCC. If HPV is an etiologic agent in the setting of ESCC, it may have a similar or dissimilar strain profile to that of other disease sites; this phenomenon has been observed

previously, where the HPV strain profile differs based on cancer site (18-20). In esophageal cancer, one meta-analysis described a significant association between HPV-16 and ESCC, but not HPV-18 (6). As shown in *Table 1*, this analysis reported an overall HPV-16 prevalence rate of 11.7%, vs. 1.8% for HPV-18 (6). This is consistent with other systematic reviews showing HPV-16 as the most commonly identified strain in HPV-ESCC infection across multiple methods (10). Regarding HPV-18, however, there is some disagreement within the literature; while the analysis above cites no significant HPV-18 association with ESCC, other studies have identified HPV-18 as the second most prevalent HPV-ESCC genotype (21-24). The HPV strain profile for ESCC remains elusive, with some reviews suggesting a heavily HPV-16-predominant profile as in the case of oropharyngeal lesions (6,19). However, several studies examining multiple HPV genotypes across ESCC samples found virtually no incidence of HPV-ESCC infection, irrespective of genotype (25,26). Future studies may benefit from standardizing detection method and expanding the selection of HPV strains being probed.

Other methodological and study-design considerations complicate data interpretation. In many studies, tissue samples derived from endoscopic biopsies may lead to sampling error or insufficient tissue for testing (27). Tissue stability and quality may also be affected by overall DNA concentration, solution pH, use and duration of formalin fixation, temperature, and type of tissue storage (28). Conflicting reports have suggested that formalin-embedding might enhance or reduce rates of HPV detection in ESCC samples (6,28,29). Several groups have also raised concern over sample quality control and potential contamination, as HPV fomites can be found on medical surfaces and are often resistant to commonly used detergents. Potential contamination of samples would complicate any HPV-ESCC analysis (10). Of note, among the studies with the most rigorous methodological controls, one large series from a particularly high-incidence region within China found essentially no HPV-ESCC correlation (26). Regarding study-design, case-control methods have traditionally been used to assess potential links between cancer and environmental factors. One analysis drawing upon case-control studies alone found a three-fold increase in the risk of ESCC with HPV infection (5). Focusing on case-control literature is likely of particular importance given that HPV detection rates in normal esophagus have been reported as high as 58.9% in some studies, and as low as 0% in others (11,23). Lastly, most series have relatively small sample sizes, likely

making most underpowered to answer questions of HPV-ESCC prevalence (10).

### **Etiological role of HPV in ESCC**

Given the diversity of HPV genotypes and their relative contributions to oncogenesis in established clinical paradigms such as cervical cancer and head and neck cancers (HNC), it is clear that measuring the oncogenic activity of HPV infection is critical to understanding the etiological role of HPV-ESCC infection (1). To this end, the p16/INK4A (p16) biomarker has been used as a measure of HPV oncoprotein activity. HPV E7 protein inactivates retinoblastoma protein, consequently releasing E2F transcription factors, which then promote both cell-cycle progression and p16 expression (30). Immunohistochemical evidence of p16 overexpression has been widely used as a correlate for HPV oncogenic activity in cervical cancer and dysplasia (31,32). In oropharyngeal lesions, over 90% of high-risk HPV-positive lesions also overexpress p16 (19). Although 10-20% of p16-positive HNC cases are HPV-negative, it is noteworthy that p16 overexpression is a positive prognostic factor, irrespective of HPV status (20,33-37). Together, these data highlight the role of p16 overexpression in other sites as a functional correlate for HPV-mediated oncogenesis (20,31).

Although there are many potential pitfalls in determining rates of HPV tumor infection, including issues of geography, HPV detection method, and others, none of these address the issue of biological significance of HPV-ESCC infection. Assessing functional correlates for HPV oncoactivity is particularly important in the context of multiple HPV-ESCC case-control studies showing HPV infection rates of greater than 50% in the normal esophagus (12,23). Recent meta-analyses have not evaluated p16 overexpression or any other functional correlate for HPV oncoactivity to characterize the potential etiological role of HPV infection in ESCC (5,8). The available data, as a whole, are markedly different from the oropharyngeal and cervical carcinoma literature. Studies of HPV infection in ESCC have shown little to no agreement between p16 overexpression and HPV-positivity. As described previously, over 90% of HPV-positive oropharyngeal cancers are p16 overexpressing (19), but the association between p16 and HPV in ESCC remains limited (*Table 3*). With one exception (42) the data presented in *Table 3* suggest a low rate of double-positive (HPV-positive and p16-overexpressing) ESCC lesions, around or below 5% of all cases (21,25-27,38-41). These

**Table 3** Tabulation of studies examining both p16 overexpression and HPV positivity in ESCC

Authors	Publication year	Citation	Overall rate of HPV-ESCC infection (%)	Percentage of HPV+ cases that are p16+ (%)	Overall rate of ESCC cases both HPV+ and p16+ (%)	Region or country	Notes
Castillo <i>et al.</i>	2006	(21)	29	11-22	3.2-6.4	Colombia and Chile	
Shuyama <i>et al.</i>	2007	(38)	32	16	5.1	China	
Antonsson <i>et al.</i>	2010	(39)	3.6	50	1.8	Australia	
Koshiol <i>et al.</i>	2010	(26)	1	0	0	China	
Malik <i>et al.</i>	2011	(40)	Not assessed	Not assessed	0	USA	Only assessed HPV in p16+ cases; p16+ in 34% of ESCC, all of which were HPV-negative
Herbster <i>et al.</i>	2012	(41)	13	20	2.6	Brazil	
Löfdahl <i>et al.</i>	2012	(27)	10	24	2.4	Sweden	
Cao <i>et al.</i>	2014	(42)	28	86	23.8	China	
Teng <i>et al.</i>	2014	(25)	3.4	17	0.6	China	

HPV, human papillomavirus; ESCC, esophageal squamous cell carcinoma.

conclusions are supported by a recent systematic review that demonstrated p16 overexpression to be an unreliable marker for HPV status in ESCC, with an odds ratio of HPV-positivity in a p16-overexpressing ESCC lesion of 1.07 [95% confidence interval (CI), 0.70-1.62] (9).

These data lead to questions regarding the etiological significance of HPV infection in ESCC. Despite studies from other sites confirming the role of p16 overexpression as a reliable marker for HPV oncoactivity, might HPV still be playing a carcinogenic role in ESCC without induction of p16 overexpression? Several hypotheses have been proposed suggesting this may be the case. One hypothesis states that ESCC carcinogenesis may involve a high rate of p16 promoter methylation, thereby inhibiting p16 expression even in the setting of oncogenic HPV infection. One study supporting this hypothesis found over 70% of ESCC cases with loss of p16 expression secondary to promoter methylation, as compared to another study demonstrating p16 loss in only 20% of oropharyngeal lesions (43,44). Nevertheless, a lack of correlation between p16-overexpression and HPV oncoactivity in ESCC would be unusual given that p16 is used clinically as a proxy for HPV carcinogenic activity in multiple other sites (20,31). One systematic review, drawing upon multiple case-control studies, attempted to address the question of HPV oncoactivity in ESCC using another method. These

investigators utilized HPV-16 and -18 E6 and E7 protein serology to assess the functional association between ESCC and HPV (45). High-risk HPV E6 and E7 protein serology is a highly-specific marker for HPV-driven squamous cell carcinomas of multiple sites, including the cervix and oropharynx (45-49). Synthesizing their findings, the investigators found a limited association between HPV and ESCC based on serology. This led them to conclude that HPV may contribute to the etiology of a small subset of ESCC, but is unlikely to represent an important oncogenic risk factor (45).

Collectively, p16-overexpression and HPV serological data appear to indicate that despite reported rates of HPV infection in ESCC, HPV may not play a significant oncogenic role in ESCC. This is complicated by geographic variation highlighted previously, wherein regions with the highest rates of ESCC also have the highest rates of HPV prevalence and HPV-ESCC infection. The geographic picture correlating HPV with ESCC may suggest a causal link between the two. However, this geographic correlation is taken with caution as studies even from the same geographic regions report tremendous variability in HPV-ESCC infection rates (12,26). Contemporary literature appears to indicate that HPV likely does not play a significant etiological role in the vast majority of ESCC cases, particularly in low-incidence regions.

**Table 4** Tabulation of studies examining overall survival (OS) in HPV-positive versus HPV-negative ESCC lesions

Authors	Publication year	Citation	Summary of findings	Statistical measures
Furihata <i>et al.</i>	1993	(50)	Decreased OS in HPV-positive tumors	Not available
Hippeläinen <i>et al.</i>	1993	(51)	No significant difference in OS	Not available
Dreilich <i>et al.</i>	2006	(22)	No significant difference in OS	P=0.72
Antonsson <i>et al.</i>	2010	(39)	No significant difference in OS	P=0.17; HR 0.9 (95% CI, 0.3-2.8)
Cao <i>et al.</i>	2014	(42)	Increased OS in HPV-positive tumors	P=0.004; HR 0.31 (95% CI, 0.14-0.68)

HPV, human papillomavirus; ESCC, esophageal squamous cell carcinoma; HR, hazard ratio; CI, confidence interval.

### Clinical relevance and recommendations

While HPV does not appear to be a significant etiologic agent in ESCC, is it possible HPV infection might offer prognostic significance in esophageal cancer? Several investigators have evaluated the prognostic role of HPV-ESCC infection. However, in aggregate, these data are conflicting (*Table 4*). In one series, HPV infection in ESCC was shown to be a poor prognostic indicator (50). In contrast, another recent series showed improved overall and disease-free survival in ESCC patients with HPV-positive tumors (42). Several other studies still have failed to show any significant association between HPV infection and patient survival (*Table 4*) (22,39,51). This is in contrast to oropharyngeal lesions, where HPV-positivity has been consistently shown to be a strong positive prognostic factor in patient outcomes (19,34,52,53). The outcomes data for HPV in ESCC, however, remains both limited and conflicting, precluding definitive interpretation.

Another clinical question is whether a specific subset of ESCC patients may be more likely to have HPV-positive lesions. Preliminary evidence suggests HPV-positive ESCC may be more common among patients that are less than 55 years old, male, of higher BMI, or smokers (39,54). Other studies have examined whether location of the primary ESCC lesion within the esophagus predicts HPV prevalence rates. The notion that upper esophageal lesions may have higher incidence of HPV infection is grounded in data demonstrating higher risk of HPV-positive ESCC in patients with prior or concurrent HPV-positive HNC (55). However, studies investigating differential HPV rates in proximal versus distal esophageal lesions have concluded that no association exists between primary esophageal tumor location and HPV prevalence (27,56).

There have been concerns about whether clinicians should test for HPV in patients with ESCC. Based on the evidence described above, it currently appears unlikely that HPV is

clinically or etiologically relevant for ESCC. We currently do not recommend that ESCC patients be tested for HPV tumor infection outside the context of a research study. At present, there is no clear indication to change clinical practice or treatment strategy for ESCC lesions based on HPV status.

### Conclusions

The role of HPV in the setting of ESCC remains ill-defined. Worldwide, HPV-ESCC tumor infection rates are highly variable, such that HPV prevalence correlates strongly with high-ESCC-incidence regions. In countries such as the United States, HPV-ESCC infection rates are low, on the order of 5-15%. Geographic differences and methodological heterogeneity complicate interpretation of the current studies, leading to variable conclusions. In particular, future investigations should rely upon case-control paradigms, given the high rates of HPV infection observed in the normal esophagus. Regardless of HPV-ESCC infection rates, p16 overexpression and HPV serological data do not presently support a definitive etiological role for HPV in ESCC. Similarly, clinical data do not suggest a prognostic role for HPV infection in these lesions. Although this issue warrants further investigation, there is currently no basis to change the clinical approach to ESCC patients.

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