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Review Article

Prevalence of human papillomavirus in patients with nasopharyngeal carcinoma: a meta-analysis

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Abstract: Background: The human papillomaviruses (HPVs) are causally associated with the tumorigenesis of several types of cancers. However, HPV prevalence in patients with nasopharyngeal carcinoma (NPC) has not previously been systematically reviewed. Therefore, we performed a meta-analysis to estimate the HPV prevalence in patients diagnosed with NPC and to assess the potential etiological significance of HPVs. The goal of this study was to quantitatively summarize published data to evaluate the effects of HPV infection on the pathogenesis of NPC. Methods: The PubMed, Embase, Web of Science, Science Direct, Ovid, Wiley Online Library, and Cochrane Library databases, as well as several Chinese databases (China National Knowledge Infrastructure (CNKI), Chinese Chong Qing VIP, Chinese Wan Fang and China Biology Medicine databases) were searched to identify all relevant studies. For case-control studies, the odds ratios (ORs) and 95% confidence intervals (CIs) were computed. For studies providing World Health Organization (WHO) classifications and HPV subtypes, the corresponding pooled ORs and 95% CIs were also calculated. The Stata 12.0 software was used for the Meta-analysis. Results: Thirty-nine studies were included in the meta-analysis, involving a total of 1748 cases of NPC and 289 control cases. The pooled HPV prevalence was 21% among all of the NPC patients (95% CI: 17%, 26%; I^2=89.4%; P<0.001). A pooled OR of 4.77 (95% CI: 1.69, 13.45) was calculated based on the 11 case-control studies (I^2=69.7%; P<0.001). Moreover, the prevalence of HPV was higher in cases outside of China than in cases from regions in China (23% vs 19%; I^2=95.0%; P<0.001). The HPV prevalence was 33.6% (24/66) in patients with a WHO type I NPC, and 27.9% (115/402) in patients with a WHO type II/III NPC. The pooled OR of 2.638 (95% CI: 0.984, 7.072) was not statistically significant. In addition, the pooled prevalence of HPV16 and HPV18 were 10.5% and 1.9%, respectively, and the pooled OR was 2.26 (95% CI: 1.28, 3.99). Conclusions: Our study suggests that HPVs play a potential role in the pathogenesis of NPC. In addition, this study expands the knowledge of the molecular mechanisms underlying NPC tumorigenesis and suggests precautionary measures.

Keywords: Human papillomaviruses (HPVs), nasopharyngeal carcinoma (NPC), meta-analysis

Introduction

Nasopharyngeal carcinoma (NPC) is a rare malignant cancer, responsible for approximately 50,000 deaths worldwide per year [1]. The average incidence of NPC is less than 1% in the population worldwide [2]. These estimates indicate that NPC is a relatively uncommon type of cancer. However, the incidence of NPC is highest in Southeastern Asia, particularly in Southern China. NPC is considered to be an endemic carcinoma that has a notably higher risk among specific geographic regions and ethnic groups [2, 3].

The World Health Organization (WHO) classifies NPC into the following 3 histological subtypes: type I (keratinizing), type II (non-keratinizing) and type III (undifferentiated). In Southeastern Asia, most NPC tumors are type III (95%), while types I and II occur in a minority of cases (2% and 3%, respectively) in 2005 [4-6]. Despite these differences, the precise etiology of NPC has not yet been elucidated. The etiology of NPC involves environmental factors, genetics, and infectious agents. The Epstein-Barr virus infection may primarily account for the high incidence of NPC tumorigenesis in certain geographical areas [7]. Moreover, the human papil-

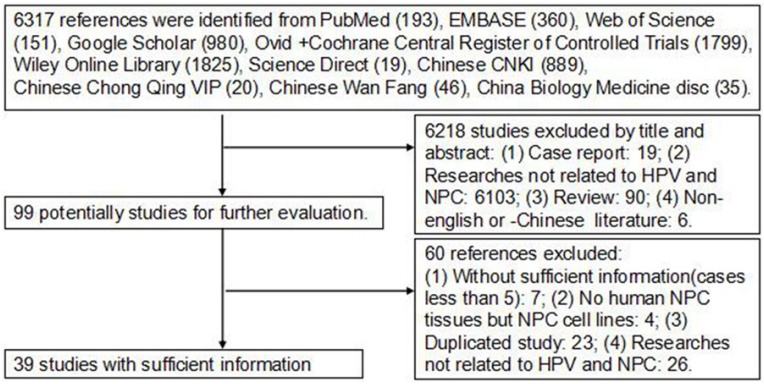

Figure 1. The flow diagram of search and screening process, as well as the amount of screened, excluded, and inclusion criteria publications.

lomaviruses (HPVs), a class of double-stranded DNA viruses with various subtypes, have a reported association with the occurrence of NPC [4, 6, 8]. Infection with low-risk types of HPV, such as types 6 and 11, is highly correlated with condyloma acuminatum [9]. In contrast, high-risk types of HPV, such as types 16 and 18, are strongly implicated in the genesis of human cervical carcinoma and even breast cancer [8, 10]. In addition, studies in recent years have demonstrated a correlation between HPV and upper respiratory tract and upper gastrointestinal tumors. The HPV infection is related to tumorigenesis of the larynx, accessory sinus and other head and neck cancers, namely oropharynx and oral cavity [11-13]. Simultaneously, numerous studies have suggested a link between HPV status and an increased risk of NPC. In this meta-analysis, based on previous studies, we described the relationship between HPV infection and NPC and expected to provide new clues to the etiology, prevention and treatment of NPC.

Materials and methods

Search strategy

The PubMed, Embase, Web of Science, Science Direct, Ovid, Wiley Online Library, and Cochrane Library databases, as well as several Chinese Databases (China National Knowledge Infrastructure, Chinese Chong Qing VIP, Chinese Wan Fang and China Biology Medicine databases) were searched to identify all relevant articles published on or before February 2, 2016, by using the following search strategy: (HPV OR human papilloma virus) AND (nasopharynx OR nasopharyngeal OR NPC) AND (adenocarcinoma OR carcinoma OR cancer OR neoplasm OR tumor OR neoplasm* OR malignan*).

Study inclusion and exclusion criteria

In this meta-analysis, all of the studies had to meet the following inclusion criteria: (1) studies had to estimate the prevalence of HPV in NPC cases where the classification was unspecified or provide sufficient information; (2) studies were not case reports, review articles, meeting abstracts, unpublished reports or letters; and (3) studies had to investigate HPV DNA in human NPC tissue (studies were excluded if they investigated NPC cell lines instead of NPC tissue collected from patients diagnosed with NPC, and the NPC tissue used to detect HPV DNA could have been collected in the following three formats: Formalin-fixed paraffin-embedded (FFPE) tissue, fresh frozen (FF) tissue or a nasopharyngeal swab); (4) studies were in English or Chinese; and (5) studies used the polymerase chain reaction (PCR) or in situ hybridization (ISH) methods to detect HPV; (6) Total cases of each studies should not be less than 5. The major exclusion criteria were as follows: insufficient information; duplicate publications; non-human studies; and publications in the format of letters, editorials, abstracts, reviews, case reports, expert opinions, and meta-analyses.

In addition, if two or more studies were published by the same group on the same case series, we selected the study with the largest sample size. The search was performed independently by two investigators, and disagreements were resolved by discussion or by consulting with the third investigator (shown in **Figure 1**).

Data extraction

The following items were extracted from all eligible studies: name of the first author, published year, country of origin of the subjects, anatomical site, language, sample size (N), HPV DNA test methods and materials, the number of cases and controls, as well as the HPV subtypes, pathological differentiations and WHO classifications of the NPC.

Table 1. The characteristics of the 39 included studies (1748 cases in total)

First author	Year of publication	Country	Language	Sample size (N)	HPV DNA detection method	Biological materials	Case		Control		HPV subtypes
							HPV (+)	Total	HPV (+)	Total	
*Kassim SK [45]	1998	Egypt	English	<50	PCR	FFPE	5	20	0	10	HPV16
Giannoudis A [46]	1994	Greece	English	>50	PCR	FFPE	12	63	NA	NA	NA
Tyan YS [47]	1993	China	English	<50	PCR	Other	14	30	NA	NA	HPV6, 11, 16, 18, 33
Dogan S [28]	2013	USA	English	>50	ISH	FFPE	6	63	NA	NA	HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52
Atighechi S [14]	2014	Iran	English	<50	PCR	FFPE	9	41	NA	NA	HPV18, 16, 11, 6
Punwaney R [4]	1999	USA	English	<50	PCR	FFPE	7	30	NA	NA	HPV6, 11, 16, 18
Hørding U [24]	1994	Denmark	English	<50	PCR	FFPE	4	15	NA	NA	HPV6, 11, 16, 18
Robinson M [27]	2013	UK	English	>50	PCR	FFPE	11	67	NA	NA	HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 66
Barwad A [48]	2011	India	English	<50	PCR	Other	1	20	NA	NA	HPV16, 18
Chow CW [49]	2007	Australia	English	<50	PCR	FFPE	0	5	NA	NA	NA
Deng Z [50]	2014	Japan	English	<50	PCR	FFPE	6	20	NA	NA	HPV16, 33, 35, 56, 58
Rassekh CH [21]	1998	USA	English	<50	PCR	FFPE	9	17	NA	NA	HPV16, 33
*Huang CC [16]	2011	China	English	<50	PCR	FFPE	15	43	17	40	HPV16, 18
Laantri N [18]	2011	Morocco	English	>50	PCR	FFPE	24	70	NA	NA	HPV16, 18, 31, 33, 35, 59
Lin Z [29]	2014	China	English	>50	PCR	FFPE	5	108	NA	NA	HPV11, 16
Lo EJ [19]	2010	USA	English	<50	ISH	FFPE	5	30	NA	NA	HPV16, 18
Maruyama H [51]	2014	Japan	English	<50	PCR	FFPE	3	25	NA	NA	HPV13, 16
Maxwell JH [20]	2010	USA	English	<50	PCR	FFPE	4	5	NA	NA	HPV16, 18, 59
Mirzamani N [52]	2006	Iran	English	<50	ISH	FFPE	4	20	NA	NA	HPV6, 11, 16, 18
Singhi AD [30]	2012	USA	English	<50	ISH	FFPE	4	45	NA	NA	HPV16, 18
Stenmark MH [31]	2014	USA	English	>50	PCR	FFPE	18	61	NA	NA	NA
Walline HM [22]	2013	USA	English	<50	PCR	FFPE	8	18	NA	NA	HPV16, 18, 39, 59
Wilson DD [32]	2014	USA	English	<50	PCR	FFPE	4	13	NA	NA	NA
*Prabha B [26]	2006	India	English	>50	PCR	FFPE	31	103	1	26	NA
*Zhou BC [53]	2003	China	Chinese	>50	PCR	FFPE	20	90	0	11	NA
*Lin CY [54]	2000	China	Chinese	<50	PCR	FFPE	3	37	0	20	HPV16, 18
*Yang F [6]	2014	China	Chinese	>50	PCR	FFPE	2	70	0	25	HPV18, HPV70
Chen XS [15]	2012	China	Chinese	>50	PCR	Other	8	107	NA	NA	HPV16, 18, 32, HPV52, 58, HPV68
Ye Q [23]	2000	China	Chinese	<50	PCR	Other	2	14	NA	NA	HPV16, HPV18
Cui WM [55]	2005	China	Chinese	<50	PCR	FFPE	0	47	NA	NA	NA
Wang YD [56]	2013	China	Chinese	<50	ISH	FFPE	0	29	NA	NA	NA
*Jin HF [17]	1999	China	Chinese	<50	PCR	FFPE	16	30	0	30	HPV16, HPV18, HPV5
Wang DH [57]	1997	China	Chinese	>50	PCR	FFPE	10	66	NA	NA	HPV16/18
*Jiang LZ [25]	2009	China	Chinese	>50	PCR	FFPE	35	56	0	12	HPV16
*Huang ZQ [58]	2010	China	Chinese	>50	PCR	Other	55	150	3	50	HPV16, 18
*Chen JS [59]	1996	China	Chinese	<50	PCR	FFPE	7	48	0	18	NA
*Chen BF [60]	1994	China	Chinese	>50	PCR	FFPE	14	58	13	47	NA
He JH [61]	2003	China	Chinese	<50	PCR	FFPE	0	5	NA	NA	NA
Wu RC [62]	2014	China	Chinese	<50	ISH	FFPE	0	9	NA	NA	NA

*: case-control study; FFPE: formalin-fixed paraffin-embedded tissue; Other: including fresh frozen (FF) tissue and nasopharyngeal swab; NA: not available; PCR: polymerase chain reaction; ISH: in situ hybridization.

Statistical analysis

The pooled HPV prevalence and odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the association between HPV infection and the occurrence and development of NPC. STATA version 12.0 was used to analyze all of the included studies using the mate mod-

Table 2. The HPV prevalence in subgroups using a stratified analysis

Subgroup	R	LCI	UCI	HPV prevalence
Infection				
EBV	0.604	0.497	0.711	60.4%
HPV	0.227	0.17	0.283	22.7%
HPV subtypes				
HPV16	0.105	0.054	0.156	10.5%
HPV18	0.019	0.005	0.032	1.9%
Other HPVs	0.035	0.016	0.055	3.5%
Areas				
Europe and America	0.24	0.17	0.32	24%
Asia	0.19	0.14	0.25	19%
Other areas	0.26	0.12	0.4	26%
Country				
Non-China	0.23	0.17	0.28	23%
China	0.19	0.13	0.25	19%
Sample size				
>50	0.22	0.14	0.29	22%
≤50	0.21	0.15	0.27	21%
HPV test method				
PCR	0.24	0.18	0.29	24%
ISH	0.08	0.03	0.13	8%
Materials				
FFPE	0.21	0.16	0.26	21%
Others	0.21	0.17	0.26	21%
WHO classification				
WHO-I	0.336	0.226	0.445	33.6%
WHO-II/III	0.279	0.152	0.406	27.9%

R: ratio; LCI: lower confidence interval; UCI: upper confidence interval.

Table 3. The characteristics of the 11 case-control studies

First author	Year of publication	Case (N) 705		Control (N) 289	
		HPV (+)	Case (total)	HPV (+)	Control (total)
Kassim SK [45]	1998	5	20	0	10
Huang CC [16]	2011	15	43	17	40#
Prabha B [26]	2006	31	103	1	26
Zhou BC [53]	2003	20	90	0	11
Lin CY [54]	2000	3	37	0	20
Yang F [6]	2014	2	70	0	25#
Jin HF [17]	1999	16	30	0	30
Jiang LZ [25]	2009	35	56	0	12
Huang ZQ [58]	2010	55	150	3	50#
Chen JS [59]	1996	7	48	0	18
Chen BF [60]	1994	14	58	13	47

#: normal tissue (the remainder is tissue that was adjacent to the tumor or inflamed tissue).

ule "meta" or "metan" command. Estimates, standard errors, and 95% CIs were used to calculate the HPV prevalence percentages in all of the studies. ORs and 95% CIs were measured in 11 case-control studies, and the pooled HPV prevalence and 95% CIs were calculated in all of the 39 studies. We logarithmically transformed all prevalence estimates, which necessitated adding a correction factor of 0.5 to both the numerator and denominator for a reported prevalence of 0. The pooled estimates were computed with the Mantel-Haenszel method, assuming a fixed effects model, or with the random effect model of the DerSimonian and Laird method. When significant heterogeneity occurred in the pooled estimates across studies, a random effect model was considered. In addition, the heterogeneity was described using the I^2 statistic. To analyze the heterogeneity across each study, meta-regression models were estimated using the "metareg" command. Begg's Test ("metabias") was used to diagram funnel plots and to describe the publication bias of funnel plot asymmetry (publication bias). The "metainf" command was utilized to assess the influence of each individual study on the effect of the pooled estimate. The "metareg", "metabias" and "metainf" commands were performed on the 11 case-control studies. A p-value< 0.05 was considered to be statistically significant. The source of heterogeneity was explored using the following techniques: sensitivity analysis, subgroup analysis, meta-regression or the random-effects model.

Results

Eligible studies

The flow chart represents the process of selecting the studies included in this meta-analysis. Based on the primary search strategy, a total of 6317 related publications were identified. After examining the titles and abstracts, we deemed 99 references eligible according to the selection and inclusion criteria. Through a strict investigation of the 99 complete articles, 60 studies were excluded, including 7 studies that did not include sufficient infor-

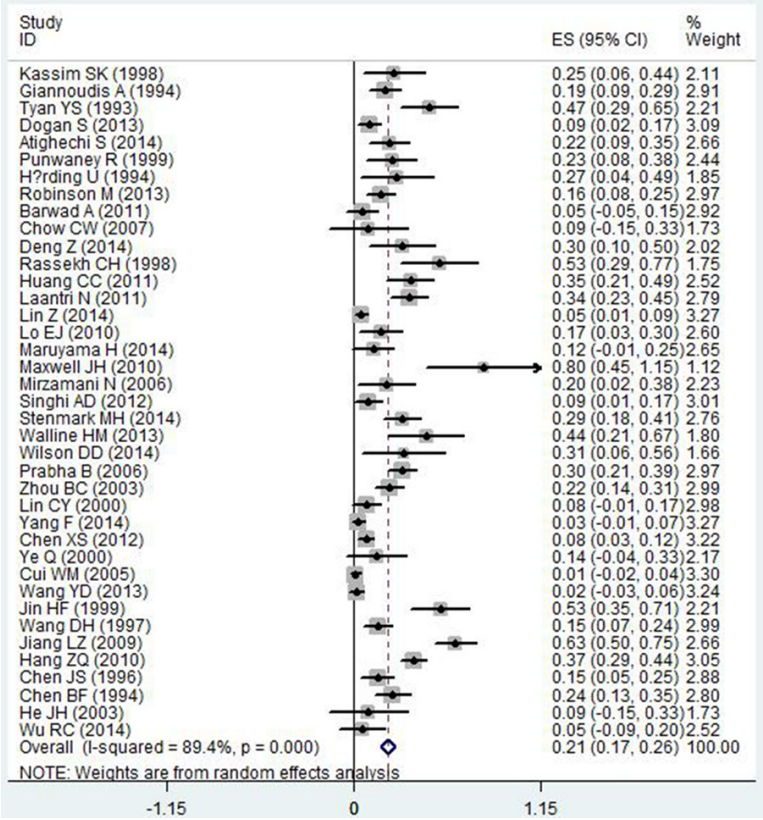

Figure 2. Overall association between HPV infection and risk of NPC. Forest plot of the pooled prevalence of HPV in patients with NPC based on 39 studies. NOTE: Weights were from the random effect analysis. ES, effect size.

mation, 4 studies that used NPC cell lines, 23 duplicate studies and 26 irrelevant studies. As a result, 39 eligible studies were included in this meta-analysis, including 24 studies written in English and 15 studies in Chinese, involving 1748 cases of NPC and 289 controls. Eleven were case-control studies, and the remainders were case-only studies. The 11 case-control studies included 705 cases and 289 corresponding controls (shown in **Tables 1** and **3**).

Study characteristics

Among the 39 studies, 18 were conducted in China. The material used in the detection procedures included FFPE tissue, FF tissue and nasopharyngeal swabs. A PCR-based technique was used to detect HPV DNA in 33 of the studies, while 6 studies used ISH to detect HPV genes (**Table 1**). In addition, nine studies provided the prevalence of HPV for the different WHO classifications of NPC. Some studies specified the specific types of HPV (HPV16 and 18). The main information provided in the studies is presented in **Table 1**. The sample sizes of the 39 studies ranged from 5 to 150.

Results

The overall HPV prevalence in patients with NPC

The HPV prevalence was represented as decimals over the forest plots. **Figure 2** showed the HPV prevalence and 95% CI estimates from all of the 1748 NPC cases, based on the DerSimonian and Laird (D+L) method with a random effect model. The pooled HPV prevalence was 0.21 (95% CI: 0.17, 0.26), which is equal to a proportion of 21% (95% CI: 17%, 26%) (**Table 1**; **Figure 2**). Heterogeneity between the studies was apparent (I^2= 89.4%, P<0.001). The 11 case-control studies had a pooled OR of 4.77 (95% CI: 1.69, 13.45), based on the D+L method with a random effect model, which was statistically significant (z=2.95, P=0.003) (**Table 3**; **Figure 3**). This indicated that the HPV prevalence in NPC cases was more than four-fold of that in the corresponding control cases, but the heterogeneity across the studies should not be ignored (I^2=69.7%, P<0.001).

The HPV prevalence in subgroups stratified by country, sample size, HPV test method and materials used

All of the studies could be stratified by country, sample size, HPV test method and materials used (**Tables 1** and **2**; **Figure 4**). The pooled HPV prevalence in Europe and America, Asia, and other areas were 24%, 19%, and 26%, respectively, and HPV prevalence was higher in studies from outside of China than those from regions in China (23% vs 19%). Oddly, the pooled HPV prevalence was identical in studies using FFPE and in those using other tumor specimens (21% vs 21%). The PCR method yielded a higher rate of HPV infection than the ISH method (24% vs 8%). There was no signifi-

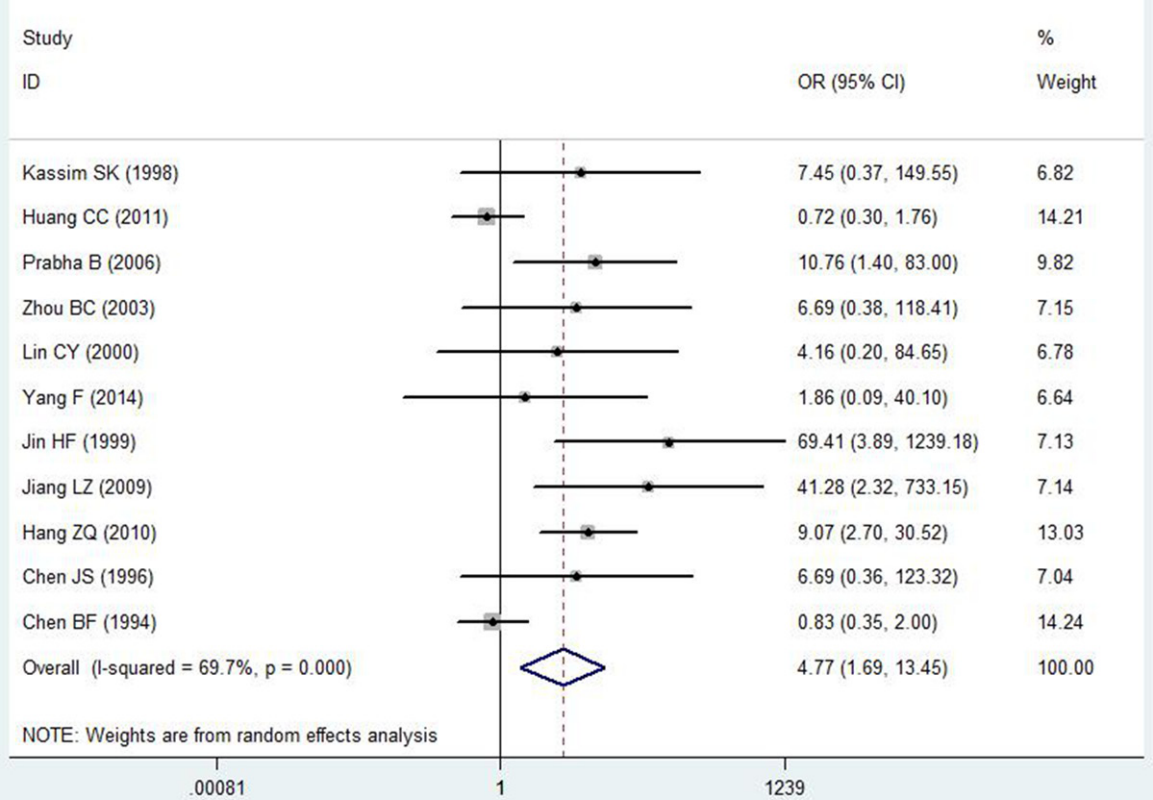

Figure 3. A pooled OR of the 11 case-control studies. For the 11 case-control studies, the estimates of the odds ratios (OR) and the 95% confidence intervals (CI) were plotted in a forest plot. NOTE: Weights were from the random effect analysis.

cant difference between large samples (≥50 cases) and small samples (<50 cases), with HPV prevalence of 22% and 21%, respectively.

HPV prevalence and clinicopathological parameters in patients with NPC

HPV subtypes: Eleven studies provided data on specific HPV types (including at least HPV16 and HPV18) [6, 14-23]. The prevalence of HPV16, HPV18 and other HPV subtypes were 10.5%, 1.9%, and 3.5%, respectively. The prevalence of HPV16 and HPV18 had a significant OR of 2.26 (95% CI: 1.28, 3.99). HPV16 had a two-fold higher risk than HPV18 of NPC pathogenesis.

WHO classification: Nine studies provided concrete information on the HPV prevalence among different NPC WHO classifications [4, 16, 18, 19, 21, 24-27]. The HPV prevalence was 33.6% (24/66) in patients with a WHO-I NPC, and 27.9% (115/402) in patients with a WHO II/III NPC. The pooled OR was not statistically significant (2.638, 95% CI: 0.984, 7.072; z=1.93, P=0.054).

HPV prevalence and p16 gene mutation: Ten studies included in this meta-analysis [4, 19, 20, 22, 27-32], involving 440 cases of NPC, provided information on the relationship between HPV prevalence and the p16 gene (36.8% vs 20.2%). The pooled OR of 1.83 (95% CI: 1.00, 3.36) was not statistically significant, which indicates that the p16 gene mutation may not be a biomarker for the prevalence of HPV in patients with NPC.

EBV status in patients with NPC: Twenty-eight studies provided concrete information on the prevalence of EBV in patients with NPC. The pooled EBV prevalence was 60% (**Figure 4**).

Sensitivity analysis, meta-regression and publication bias in the 11 case-control studies

A sensitivity analysis indicated that no individual study could significantly influence the pooled effect estimate. A meta-regression analysis was performed and indicated that the source of the heterogeneity across the studies was not due to any of the following covariates: country, sample size, HPV test method and

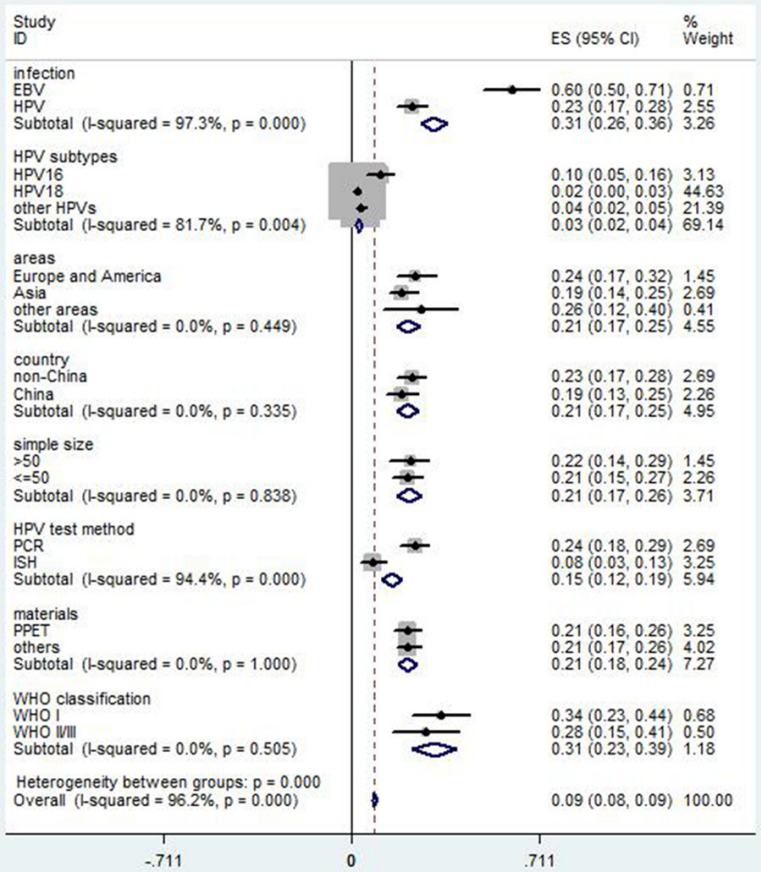

Figure 4. The HPV prevalence in subgroups. The estimate of the HPV prevalence and the 95% confidence intervals (CI), as decimals instead of percentages, were plotted in a forest plot (random effect model). All searches were stratified by area, country, sample size, HPV test method and materials. Twenty-eight studies provided data on EBV status, 11 studies provided data regarding HPV subtypes, and nine studies provided WHO classification information.

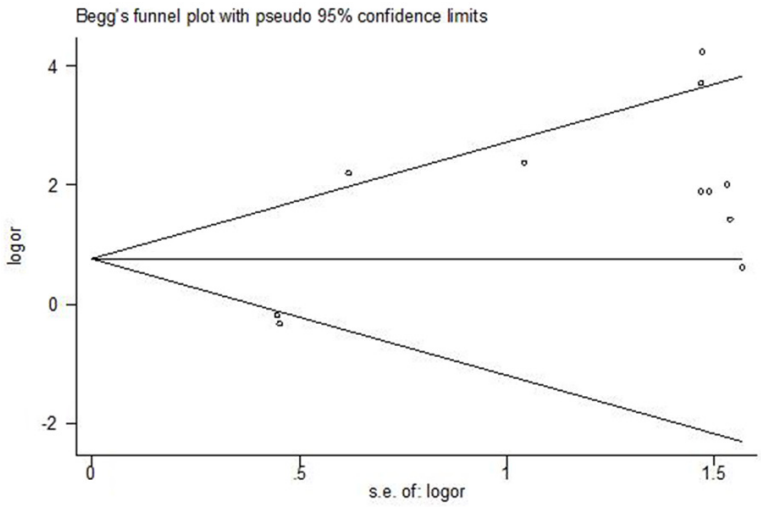

Figure 5. Begg's test funnel plot with pseudo 95% confidence limits. Begg's test for publication bias (continuity corrected: z=0.31, P=0.755), no evidence of publication bias.

materials. Begg's tests were used to test for publication bias (**Figure 5**). There was no evidence of any publication biases (continuity corrected z=0.31, P=0.755).

Discussion

Due to a lack of specific clinical symptoms, NPC is commonly diagnosed at later stages; however, early detection and treatment of NPC can increase survival [33-35]. Preventing the risk factors that contribute to NPC can allow for an earlier diagnosis and a favorable prognosis for this malignancy. The precise etiology of NPC involves many potential factors such as occupational exposure, tobacco smoke, viral infections, Southeast Asian descent, and nitrosamine consumption [36-38]. It is generally acknowledged that Epstein-Barr virus infection is a high-risk factor for NPC [39-41]. Additionally, HPV infection is also thought to be a risk factor for NPC, although this view is controversial [19]. However, HPV can induce both the expression of tumor-associated proteins and atypical hyperplasia of the mucosal epithelium, consequently causing cancer.

Today, it is widely accepted that head and neck cancers are related to HPV [42]. In the past decade, an increasing number of studies have reported a role for HPV in NPC, one of the head and neck cancers. Based on these studies, we conducted a meta-analysis to investigate the associations between the presence of HPV and NPC worldwide and also to determine factors that can influence this relationship.

This meta-analysis highlights that the prevalence HPV incr-

eased the risk of NPC by more than four-fold (OR=4.77). The overall pooled prevalence of HPV was 21%, which provides strong evidence for a potential role for HPV in the etiology of NPC. However, some factors may have influenced the variability of the results evaluating the association of HPV infection and NPC. There was heterogeneity between the studies that should not be ignored. We used a stratified analysis, sensitivity analysis and meta-regression to find the source of the heterogeneity, but we failed to determine the exact causes. HPV16 is the most dangerous of the HPV subtypes and increases the risk of NPC more than the other subtypes. In this study, we investigated 10 studies containing information about the presence of HPV and p16 and concluded that the p16 overexpression observed in NPC is not predictive of HPV status in patients with NPC.

Oddly, we found the pooled HPV prevalences in Europe and America, Asia, and other areas were 24%, 19%, and 26%, respectively, and HPV prevalence was higher in studies from outside of China than those from regions in China (23% vs 19%). The finding may be related to the regional or ethnic variations of HPV infection.

Recently, two vaccines have been used to prevent HPV-related cancers. The quadrivalent vaccine Gardasil and the bivalent vaccine Cervarix have been recently developed and approved for use to prevent HPV [42]. Surprisingly, some studies have reported that patients with HPV-positive cancers have a better prognosis than those with HPV-negative cancers, which include the head and neck cancers [43, 44]. We expect that using these vaccines to prevent patients with NPC from getting an HPV infection will increase survival.

However, there are still some limitations to this study. First, the control group should include normal tissue instead of tissue adjacent to tumors because of the comparatively higher prevalence rates of HPV in tissue adjacent to tumors, which makes it unsuitable to use as control tissue. Second, we cannot ignore the effects of contamination in the specimens, which can directly affect the detection of HPV DNA. The contamination from oral secretion is not rare clinically. Third, the 39 studies included in our meta-analysis involved only 11 case-control studies, with relatively small sample sizes. In addition, we should acknowledge the limitations related to the heterogeneity of studies and risk of bias, particularly among the smaller studies. Finally, the quality of all the 11 case-control studies was assessed based on Newcastle-Ottawa Scale standard conditions. As a result, 11 studies were qualified, and another 29 case-only studies were not assessed yet. Generally, the research included in the present study was appraised as being up to the standard.

In conclusion, this meta-analysis has confirmed a link between NPC and HPV infection. Furthermore, it has demonstrated an increased risk of developing NPC in patients infected with HPV16 compared to those infected with other HPV subtypes.

Disclosure of conflict of interest

None.

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