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ORIGINAL ARTICLE

Clinicopathological features of Epstein-Barr virus-associated gastric carcinoma: A systematic review and meta-analysis

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Summary

Purpose: *The purpose of this meta-analysis was to explore the clinicopathological characteristics of Epstein-Barr virus (EBV)-associated gastric carcinoma (AGC) based on qualified studies.*

Methods: *PubMed, Cochrane and Embase databases updated to November 2017 were searched by the index words to identify relevant studies, including qualified randomized controlled trials, cohort studies or case-control studies. Studies were also identified by tracking reference lists. The meta-analysis included relative risks (RR), mean difference (MD) along with 95% confidence interval (95% CI) to analyze the main outcomes.*

Results: *A total of 35 studies were included in this meta-analysis with 7540 cases and 950 Epstein-Barr virus associated gastric cancer (EBVaGC) cases. The results indicated the EBV infection rate was significantly higher in males than in females (OR, 0.42; 95%CI 0.35-0.52), and the EBV infection rate was significantly higher in the diffuse type than in intestinal type (OR, 1.56; 95%CI 1.24-1.97). Besides, there was no marked association of EBV infection rate with lymph node metastasis (OR, 0.73; 95%CI 0.41-1.29), age (OR, 0.78; 95%CI 0.61-1.00) and pathologic tumor stage (OR, 1.11; 95%CI 0.90-1.38). The results of funnel plot, Begg's and Mazumdar's rank test, and Egger's test all showed no significant publication bias.*

Conclusions: *In this meta-analysis, EBV infection rate was significantly higher in males and in diffuse cancer type. However, there was no marked association with lymph node metastasis, age and pathologic tumor stage. Thus, EBV-positive gastric cancer has distinct clinicopathological features.*

Key words: *meta-analysis, Epstein-Barr virus, gastric carcinoma*

Introduction

Reports have been shown that EBVaGC has unique clinicopathological and molecular characteristics with high occurrence among Caucasian and Hispanic populations [1]. EBVaGC mainly occurs in young and males and it generally shows diffuse histological type and is frequently identified in the body and cardia. EBVaGC has been proved to be highly associated with lymphoepithelial carcinoma (LEC), remnant cancer, and a CpG island methylatorphenotype (CIMP)-high status, whereas no relation was proved regarding the depth of invasion, lymph node metastasis, clinical stage, TP53 expression, or *H.pylori* infection.

EBV is a member of gamma sub-family of Herpesviridae. EBV is one of the viruses that causes human tumors with latent infection capability like other herpesviruses. A previous study [2] has indicated that the occurrence of several tumors, such as nasopharyngeal carcinoma (NPC), Hodgkin's disease, and Burkitt's lymphoma, are associated with EBV infection. A recent study [3] has found that the development of gastric cancer is associated with EBV infection. Although there is an association of EBV infection with the occurrence of some gastric cancers, the prevalence of EBV-associated gastric cancer (EBVaGC) that has been reported from dif-

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ferent regions of the world varies greatly and their clinicopathological features also differ [4-10].

Therefore, in this paper, we pooled the trial data from published papers concerning EBVaGC in which *in situ* hybridization (ISH) was applied for detection of EBV. A meta-analysis was performed to determine the distribution frequency of EBVaGC among the gastric cancer specimens and the relationship between EBVaGC and clinicopathological features of gastric cancer [11,12].

Methods

Search strategy

The following electronic databases were searched from their inception to November 2017: The Cochrane, PubMed, Embase, for all the qualified trials concerning the clinicopathological characteristics of EBVaGC. The reference lists of all articles were also searched for additional available studies. The articles were searched independently by two investigators, and a third investigator was involved to reach an agreement.

Study selection

The studies that met the following criteria were included in our review: (1) the articles analyzed the association between EBV and gastric carcinoma; (2) the research individuals harbored gastric carcinoma without others serious diseases; (3) the articles described the clinicopathological and molecular characteristics of patients; (4) the publications were available in English and Chinese.

Studies with the following criteria were excluded: (1) repeat publications, or shared content and results; (2) case report, theoretical research, conference report, systematic review, meta-analysis, expert comment, economic analysis; (3) the outcomes were not relevant; (4) lack of clinicopathological data.

Data extraction and quality assessment

Two independent investigators extracted the data based on predefined criteria. Differences were settled by discussion with a third reviewer. Analyses of data were registered for all the included studies and consisted of two parts: basic information and main outcomes. The authors' name, the type of samples, the number of cases, the number of EBV-positive specimens were extracted as basic information. Clinical outcomes, such as the estimated odds ratios for gender, lymph node metastasis, histological type, age and pathologic tumor stage of EBVaGC were utilized separately in the studies' analyses. The studies were reviewed independently by two reviewers. Any difference arising was resolved by discussion.

Statistics

All statistical analyses were performed using the STATA 10.0 (TX,USA). X^2 and I^2 tests were used to assess the statistical heterogeneity of clinical trial results and determine the analysis model (fixed-effect model or random-effect model). The heterogeneity was acceptable when x^2, p value was 0.05 and the value of I^2 was >50% assessed by random-effects model. It was defined as homogeneous data when the x^2, p value was >0.05 and the value of I^2 was <50% assessed by fixed-effects model. The continuous variables were expressed as the mean ± standard deviation and compared by mean difference (MD). The categorical variables were calculated as percentages and compared by relative risk (RR) or odds ratio (OR). The relationship between gender, lymph node metastasis, histological type, age, pathologic tumor stage and EBVaGC were analyzed by OR and 95%CI.

Results

Characteristics of the included studies

A total of 1985 studies were identified by the indexes. During the preliminary screening of the titles and abstracts, 1918 articles were excluded, leaving 67 articles for further selection. After full-texts screening, 32 articles were excluded due to the following reasons: repeat publications (2), economic analysis (7), has no clinical outcomes (17), theoretical or review research (6). Finally, 35 records [4-38] were included in our meta-analysis with 7540 cases, among which 950 cases were EBVaGC (12.60%). The detailed search process is presented in Figure 1. The main characteristics of included studies are summarized in Figure 1. The basic information included the authors' names, the type of samples, the number of cases, and the number of EBV-positive specimens.

Gender

Thirty-three studies reported the relationship between gender and EBVaGC. There were 7241 cases with gastric cancer, and 923 cases were EBV-positive (12.75%); EBV was positive in 789 out of 4799 males (16.51%) and in 134 out of 2442 females (5.49%). Based on the x^2, p value (p=0.087) and I^2 tests value (I^2 =26.2%), we chose the fixed

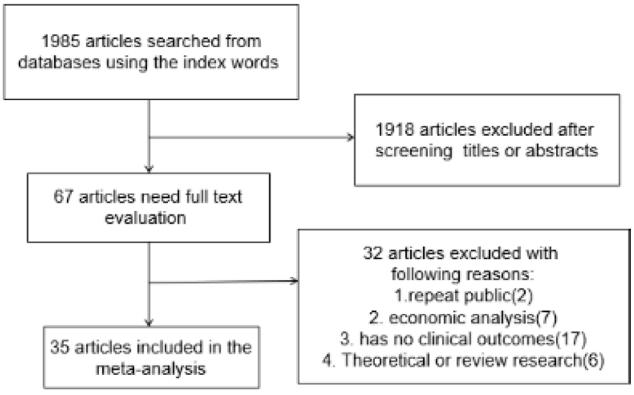

Figure 1. Flow diagram of the literature search and selection process.

effect model to analyze the relationship between gender and EBVaGC, and the male cases were selected in the reference group. The pooled results showed the EBV infection rate was significantly higher in males than in females (OR, 0.42; 95%CI 0.35-0.52, Figure 2).

Lymph node metastasis

Twelve studies reported the relationship between lymph node metastasis and EBVaGC. There were totally 1934 cases with gastric cancer, and 441 (22.80%) were EBV-positive; EBV was positive in 165 out of 945 cases (17.46%) with lymph node metastasis and in 276 out of 989 (27.91%) cases without lymph node metastasis. Based on x^2, p value (p=0.280) and I^2 test value (I^2=71.3%), we chose the random effect model to analyze the relationship between lymph node metastasis and EBVaGC, while the cases with lymph node metastasis were selected in the reference group. The pooled results showed no significant difference of EBV infection rate in cases with or without lymph node metastasis (OR, 0.73; 95%CI 0.41-1.29, Figure 3).

Histological type

Twenty-three studies reported the relationship between histological type and EBVaGC. There were totally 4533 cases with gastric cancer, and 414 (9.13%) were EBV-positive; EBV was positive in 158 out of 2166 cases of intestinal type (7.29%) and in 256 out of 2367 cases with diffuse type (10.82%). Based on the x^2, p value (p=0.000) and I^2 test value (I^2=23.8%), we chose the fixed effect model to analyze the relationship between histological type and EBVaGC; the cases of intestinal type were selected in the reference group. The pooled results showed the EBV infection rate was significantly higher in the diffuse type than in intestinal type (OR, 1.56; 95%CI 1.24-1.97, Figure 4).

Age

Sixteen studies reported the relationship between age and EBVaGC. There were totally 3182 cases with gastric cancer, and 314 (9.87%) were EBV-positive; EBV was positive in 192 out of 1804 (10.64%) cases younger than 65 years and in 122 out of 1378 (8.85%) cases older than 65 years. Based on the x^2, p=0.051 and I^2 =42.9%, we chose the fixed effect model to analyze the relationship between age and EBVaGC. Patients younger than 65 years were selected in the reference group. The pooled results showed no significant relationship between EBV infection rate and age (OR, 0.78; 95%CI 0.61-1.00, Figure 5).

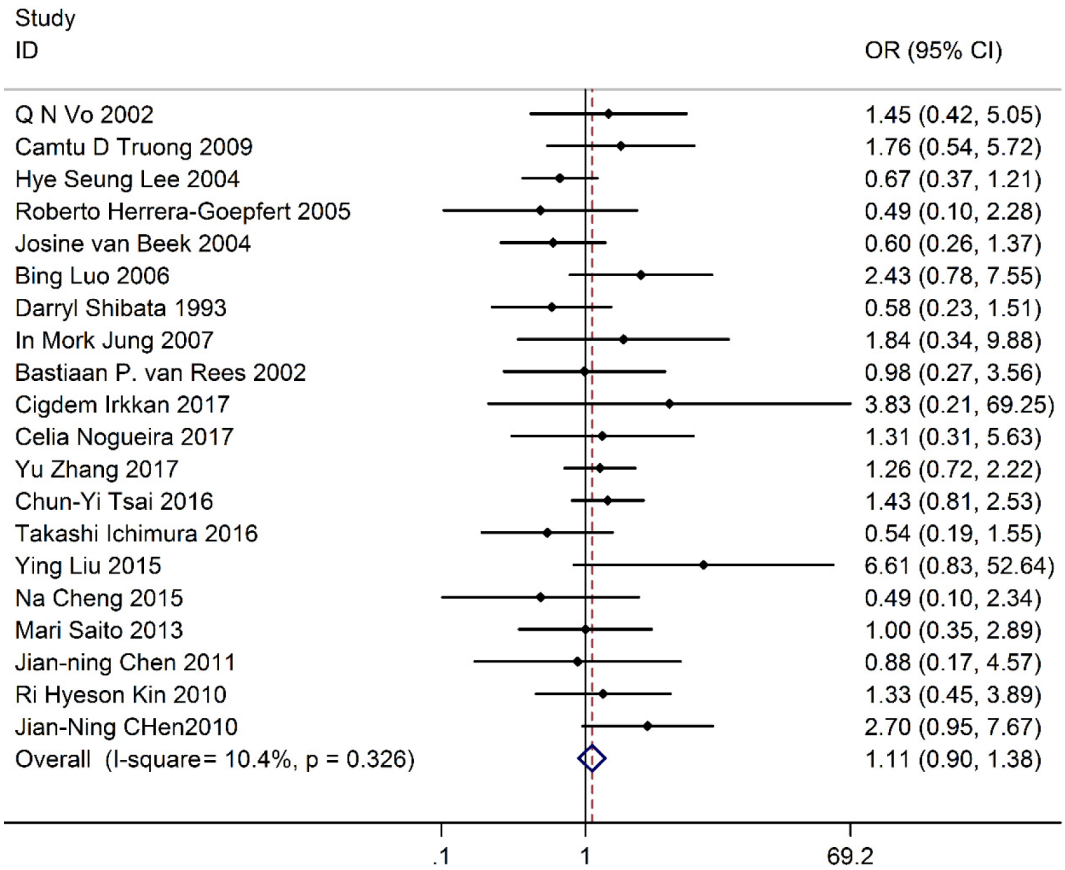

Figure 2. Forest plot showing estimated odds ratio for gender of EBV-associated gastric cancer.

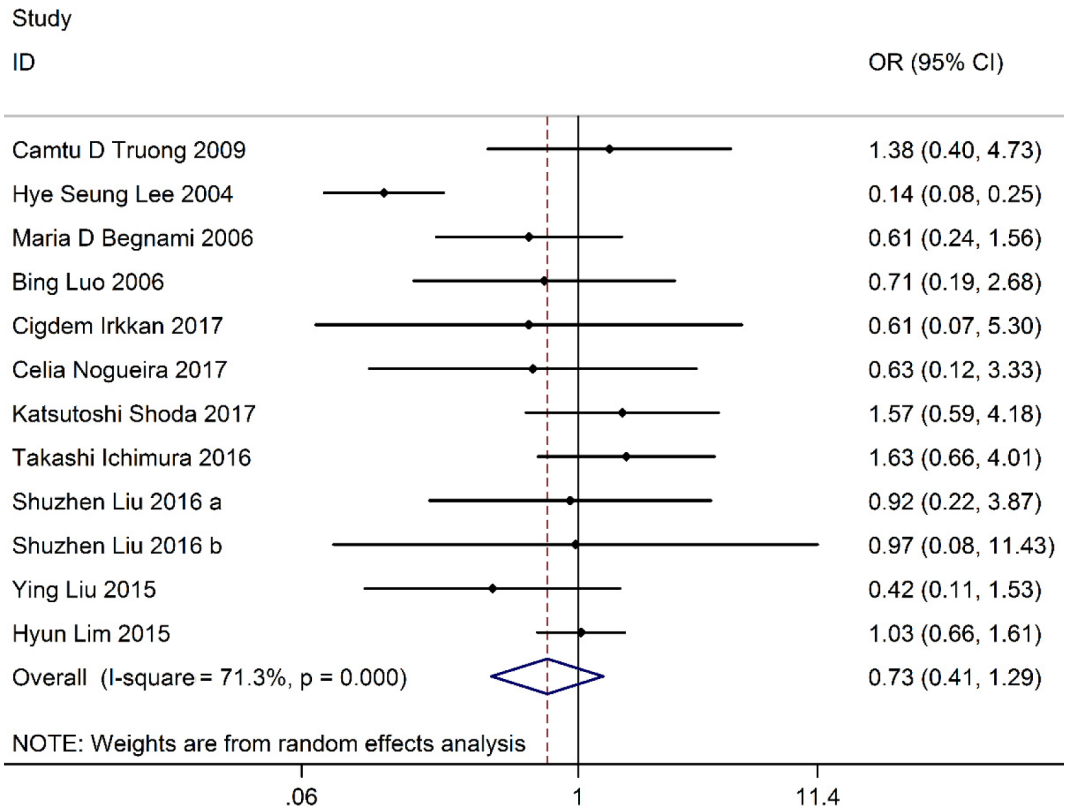

Figure 3. Forest plot showing estimated odds ratio for lymph node metastasis of EBV-associated gastric cancer.

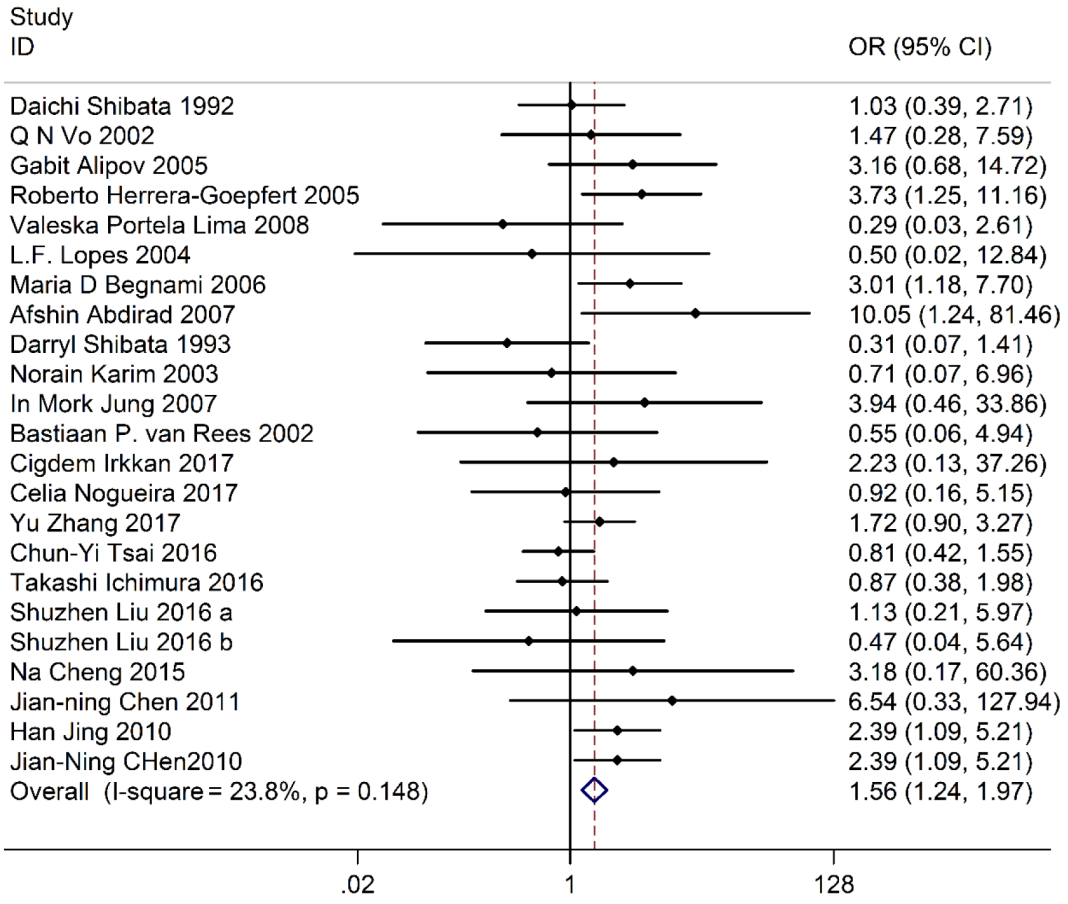

Figure 4. Forest plot showing estimated odds ratio for histological type of EBV-associated gastric cancer.

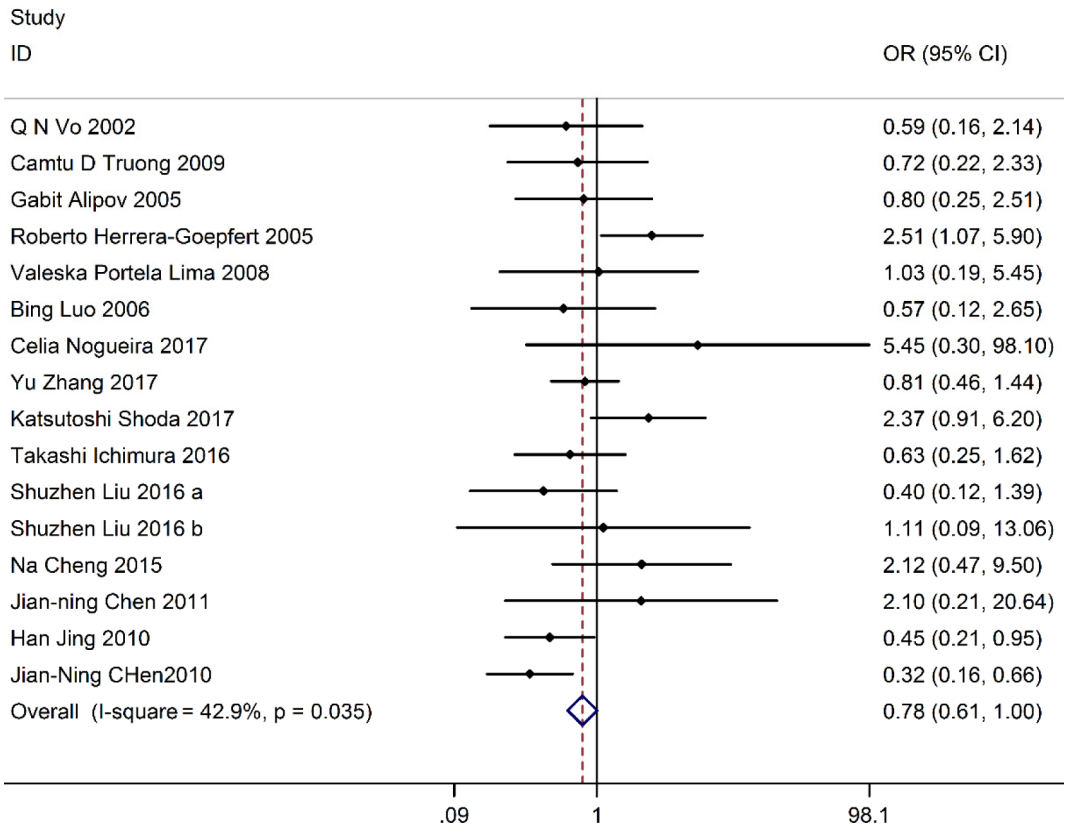

Figure 5. Forest plot showing estimated odds ratio for age of EBV-associated gastric cancer.

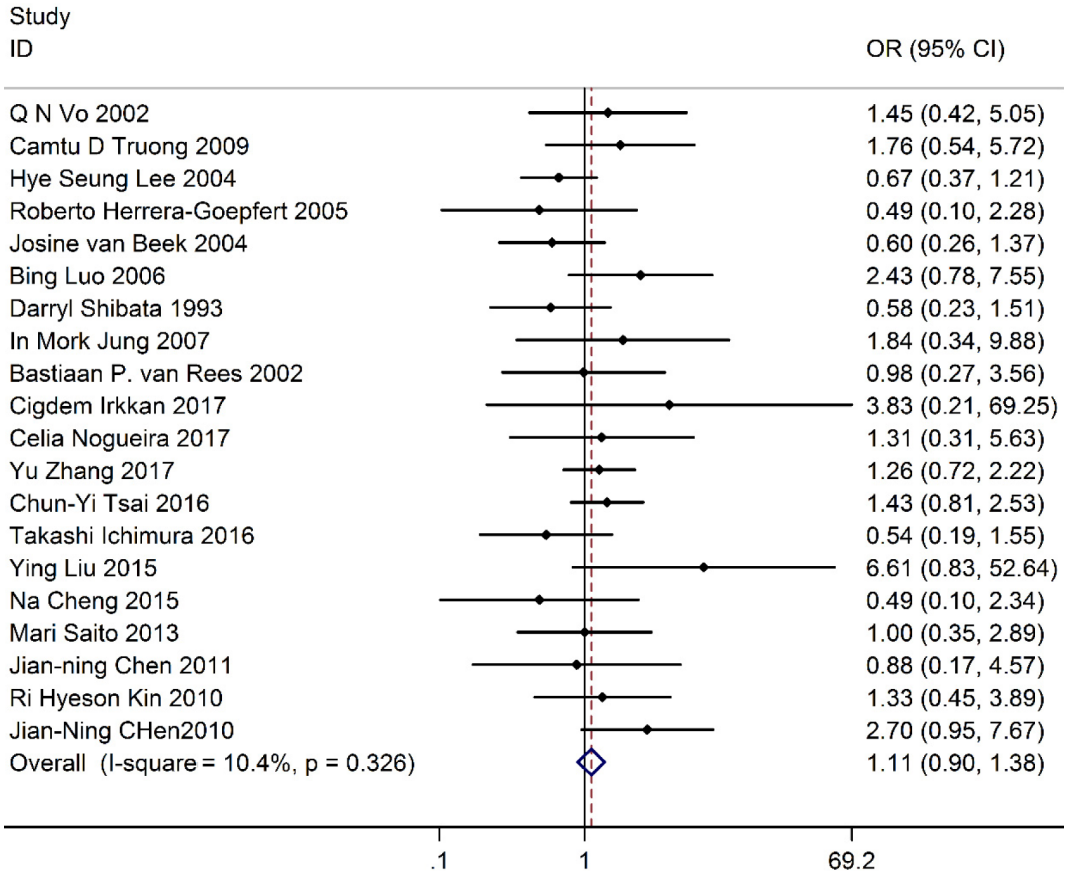

Figure 6. Forest plot showing estimated odds ratio for pathologic tumor stage of EBV-associated gastric cancer.

Pathologic tumor stage

Twenty studies reported the relationship between pathologic tumor stage and EBVaGC. There were totally 4960 cases with gastric cancer, and 480 (9.68%) were EBV-positive; EBV was positive in 230 out of 2337 (9.84%) cases with stage I or II and in 250 out of 2623 (9.53%) cases with stage III or IV. Based on x^2, p=0.325 and I^2=10.4%, we chose the fixed effect model to analyze the relationship between pathologic tumor stage and EBVaGC. The cases with stage I or II were selected in the reference group. The pooled results showed no significant difference of EBV infection rate between stage I or II and stage III or IV (OR, 1.11; 95%CI 0.90-1.38, Figure 6).

Quality assessment and potential bias

Based on the mentioned criteria, 35 articles were included in the meta-analysis. The quality assessment and potential bias were assessed by funnel plot, Begg and Mazumdar's rank test, and Egger's test. The funnel plot for log OR in traffic density of included studies was notably symmetrical, suggesting no significant publication bias (Figure 7). Meanwhile, significant symmetry was detected by Begg and Mazumdar's rank test (Z=0.65, p=0.515). However, the Egger's test showed that there was no significant publication bias (p=0.191).

Discussion

Previous studies with similar meta-analyses have been performed to explore the clinicopathological characteristics of EBVaGC. Li ShuYing et al. [39] performed a meta-analysis of 22 research papers, with a total of 5475 cases with gastric cancer, of whom 411 cases were EBV-positive (7.5%). Among the EBV-positive gastric cancer cases, the detection rate was 11.1% in males and 3.0% in females. Compared with EBV-negative gastric cancer, EBV-positive gastric cancer had decreased rate of lymph node metastasis. In the EBV-positive gastric cancers, the diffuse type rate was 8.1%, and the intestinal type rate was 8.0% based on histology. The examined specimens included stored paraffin blocks and fresh specimens that were surgically removed, and their EBV positive rates were 7.9% and 6.5% respectively. In terms of geographic distribution, the detection rate of EBV-positive gastric cancer was 9.4% in USA, 6.1% in Asia and 9.1% in Europe. Meta-analysis showed that EBV infection occurred only in gastric cancer tissue cells and was significantly associated with the patient gender, lymph node metastases, the location where tumor tissue generated and the geographic distribution, but was not significantly associated with the patient histological types of tumor and the types of specimens (p>0.05). JuHan Lee et al. [40] included 48 studies that encompassed a total of 9738 patients. The frequency of EBVaGC was 8.8%, and was significantly associated with ethnicity. It mainly occurred in young and in men. Interestingly, EBVaGC was more prevalent in Caucasian and Hispanic populations than in Asians. EBVaGC was frequently identified in the cardia and body, and was generally of diffuse histological type. EBVaGC was highly prevalent in patients with lymphoepithelial carcinoma. This tumor was closely associated with remnant cancer and a CpG island methylator-high status, but with no *H.pylori* infection, TP53 expression, and p53 mutation. In addition, EBVaGC was not significantly associated with the depth of invasion, lymph node metastasis or clinical stage. The clinicopathological and molecular characteristics of EBVaGC are quite different from those of conventional gastric adenocarcinoma. Gonzalo Carrasco-Avino et al. [41] have summarized all studies of Epstein Barr virus-associated gastric carcinoma in the Americas, focusing on host characteristics, environmental associations and phylogeographic diversity of Epstein-Barr virus strains. In the Americas, the prevalence of EBVaGC was 11.4%, more frequent in males, and portrayed predominantly diffuse-type histology. EBERs, EBNAs, BARTs and LMP are the highest expressed genes, and their variations in healthy individuals may explain the phylogeographic diversity of EBV across the region. Gastric cancer cases harbor exclusively the western genotype (subtype D and kept Xho I site), suggesting a disrupted co-evolution between the pathogen and its host. EBVaGC molecular subtype cases from The Cancer Genome Atlas displays PIK-3CA gene mutations, amplification of JAK2, PD-L1 and PD-L2 and CpG island methylator phenotype,

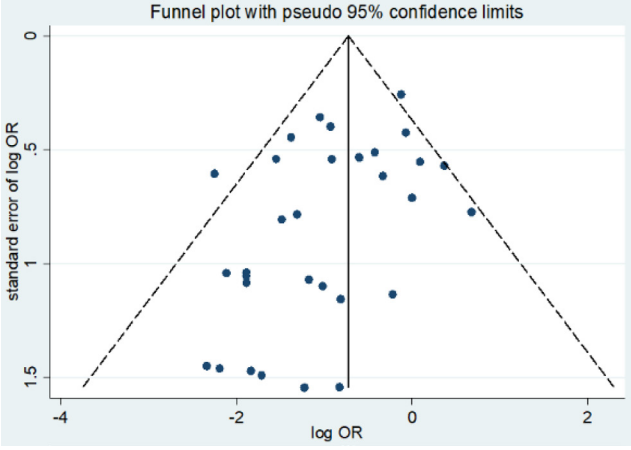

Figure 7. Funnel plot of studies included in the meta-analysis.

leading to more extensive methylation of host and viral genomes than any other subtypes from the study. Environmental conditions include negative- and positive-associations with being firstborn child and smoking, respectively. A marginal association with *H.pylori* has also been reported. Lymphoepithelioma-like carcinoma is associated with EBV in 80-86% of the cases, most of which have been included as part of EBVaGC series (prevalence 1.1-7.6%). Whether or not these cases represent a variant of EBVaGC is matter of discussion. We proposed novel research strategies to solve the conundrum of the high prevalence of EBVaGC in the Americas.

Several limitations in this analysis were present and included: (1) different design of s were included; (2) differences in the inclusion criteria and exclusion criteria for cases; (3) cases with previous disease and treatments were unavailable; (4) different types of samples; (5) all the included studies were English or Chinese articles, being a source of bias; (6) pooled data were used for analysis, and individual patients' data were unavailable, which prevented us from performing more comprehensive analysis.

In conclusion, this systematic review and meta-analysis suggest that EBV infection rate is significantly higher in males and in diffuse type, however, it is not significantly associated with lymph node metastasis, age and pathologic tumor stage. Further studies with high quality are warranted to verify the clinicopathological characteristics of EBV-associated gastric carcinoma.

Conflict of interests

The authors declare no conflict of interests.

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