Meta-analysis Shows That Prevalence of Epstein–Barr Virus-Positive Gastric Cancer Differs Based on Sex and Anatomic Location

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BACKGROUND & AIMS: Epstein–Barr virus (EBV) has been causally associated with cancer; some gastric carcinomas have a monoclonal EBV genome in every cancer cell, indicating that they arose from a single infected progenitor cell. However, the proportion of EBV-positive gastric carcinomas is uncertain, and the etiologic significance is unknown. **METHODS:** We conducted a meta-analysis of 70 studies including 15,952 cases of gastric cancer assessed by in situ hybridization for EBV-encoded small RNA. **RESULTS:** The pooled prevalence estimate of EBV positivity was 8.7% (95% confidence interval [CI]: 7.5%–10.0%) overall, with a 2-fold difference by sex: 11.1% (95% CI: 8.7%–14.1%) of gastric cancer cases in males vs 5.2% (95% CI: 3.6%–7.4%) of cases in females. Tumors arising in the gastric cardia (13.6%) or corpus (13.1%) were more than twice as likely to be EBV-positive as those in the antrum (5.2%; $P < .01$ for both comparisons). EBV prevalence was 4 times higher (35.1%) for tumors in postsurgical gastric stump/remnants. Over 90% of lymphoepithelioma-like carcinomas were EBV positive, but only 15 studies reported any cases of this type; prevalence did not significantly differ between the more common diffuse (7.6%) and intestinal (9.5%) histologies. EBV prevalence was similar in cases from Asia (8.3%), Europe (9.2%), and the Americas (9.9%). **CONCLUSIONS: EBV-positive gastric cancers greatly differ from other gastric carcinomas based on sex, anatomic subsite, and surgically disrupted anatomy, indicating that it is a distinct etiologic entity. Epidemiologic studies comparing EBV-positive and -negative gastric cancers are warranted to investigate EBV's role in gastric carcinogenesis.**

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Despite its decline in incidence during the 20th century, gastric cancer (GC) remains the fourth most commonly diagnosed cancer and the second leading cause of cancer-related mortality worldwide.[1] Risk of gastric cancer is now believed to be modulated by a complex interaction between *Helicobacter pylori* and a myriad of human genetic polymorphisms, as well as a number of other environmental and lifestyle factors.[2–4]

Epstein–Barr virus (EBV) is a ubiquitous γ-1 herpes virus usually acquired during childhood via salivary transmission, which establishes a life-long persistent infection of B cells in over 90% of adults.[5] EBV is an established cause of Burkitt lymphoma, sinonasal angiocentric T-cell lymphoma, immunosuppression-related lymphoma, Hodgkin's lymphoma, and nasopharyngeal carcinoma.[6] The oncogenic effects of the virus are likely exerted via the expression of EBV nuclear antigens (EBNAs) and latent membrane proteins (LMPs), which interact with a number of tumor suppressor genes and signaling pathways.[7–10]

EBV is known to be present in a small percentage of gastric carcinomas; estimates vary widely, but EBV-positive GC has been reported to constitute between 2% and 16% of cases.[11] In EBV-positive cases, virtually 100% of the carcinoma cells contain EBV nucleic acid sequences,[12] and the EBV terminal repeat sequences are always uniform.[13–15] These observations imply that the tumor arose from a single EBV-infected cell and that the EBV genome was retained during malignant transformation and proliferation. Moreover, EBV is routinely detected in an uncommon histologic entity, undifferentiated lymphoepithelioma-like gastric carcinoma (also known as medullary carcinoma), the microscopic appearance of which resembles nasopharyngeal lymphoepithelioma.[16,17]

Recent reviews[18,19] have qualitatively described some of the epidemiologic and clinicopathologic features of Epstein–Barr virus-associated GC. However, to date, there has not been a formal overview of published prevalence estimates. We therefore undertook a rigorous meta-analysis of papers demonstrating EBV tumor positivity using the demonstrated gold standard (in situ hybridization). This type of formal meta-analysis technique using a random effects model allowed our prevalence estimate to include consideration of within- and between-study variation in estimating the overall prevalence of EBV-positive GC and assessing variation by regional, clinical, and tumor characteristics.

Abbreviations used in this paper: EBV, Epstein–Barr virus; GC, gastric cancer; EBNAs, EBV nuclear antigens; LMPs, latent membrane proteins.

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Materials and Methods

We used PubMed software to search Medline (US National Library of Medicine, Bethesda, MD) using the following search terms: "Epstein Barr Virus AND gastric cancer," "EBV AND gastric cancer," "Epstein Barr Virus AND stomach cancer," "EBV AND stomach cancer" for studies listed on or before September 30, 2008. Eligibility criteria for inclusion were (1) studies must have ascertained EBV status of gastric tumor tissue using (EBV-encoded RNA) EBER in situ hybridization (the accepted gold standard in determining EBV positivity in tumor tissue), and (2) studies had to report prevalence of EBV positivity in unselected GC cases or provide enough information to calculate this estimate.

A total of 407 papers were identified and their titles and abstracts reviewed for relevance. One hundred fifty-seven papers were discounted as irrelevant and 64 as duplications from a single population already represented. Twenty-eight papers were excluded because of patient selection (thereby making calculation of true prevalence impossible), 17 had not used EBER in situ hybridization, 19 were in languages other than English, and 56 were found to be review articles. Thus, 70 papers (papers are in Supplementary References) met the inclusion criteria and were abstracted for prevalence data. Of the 63 studies that were included in the analyses of adenocarcinoma (defined from here onwards as primary GC tumors that are not stump/remnant cancers), 12 studies also included lymphoepithelioma-like gastric carcinoma tumors, and 4 included stump/remnant cancers. In addition, 3 studies were included that exclusively described lymphoepithelioma-like gastric carcinoma as were 5 describing stump/remnant cancers only. Separate analyses were conducted for gastric adenocarcinoma (63 studies), lymphoepithelioma-like gastric carcinoma tumors (15 studies), and stump/remnant cancers (9 studies). One publication clearly differentiated between ethnic Japanese and non-Japanese GC cases in Brazil[20] and is, therefore, included in the meta-analysis of gastric adenocarcinoma as 2 separate studies.

The following data were abstracted as available: first author, year of publication, sample size, EBV prevalence (or EBV-positive cases), sex, country of origin, regional group (Asia, Europe, Americas), histologic type (Laurén classification[21]), and tumor anatomic subsite (cardia, middle/corpus, or antrum).

Statistical Analysis

Meta-analyses were performed with Stata version 10 (StataCorp, College Station, TX), using the "metan" command.[22] Summary estimates (percentage prevalence), standard errors, and 95% confidence intervals (CIs) were calculated, using the Wilson method,[23] for each study. Because the meta-analysis technique assumes normally distributed data, we logarithmically transformed all prevalence estimates,[24] which necessitated adding a correction factor of 0.5 to both numerator and denominator[25] for reported prevalence of 0.

We first computed pooled summary estimates using the Mantel-Haenszel method assuming a fixed effects model.[26] However, because we found significant heterogeneity in prevalence estimates across studies, we also employed the random effect model of DerSimonian and Laird[27] and focus on those results in our presentation. Heterogeneity was described using the I^2 statistic, which represents the approximate proportion of total variability in point estimates that can be attributed to heterogeneity[28]:

$$I^2 = \frac{\tau^2}{\tau^2 + \sigma^2},$$

where σ^2 denotes the within-study variance, and τ^2 denotes the between-studies variance component.

Meta-regression models were estimated using the "metareg" command in Stata v10.1, to analyze associations of EBV prevalence in GC with national incidence rates, study size, and study quality. Incidence rates of GC among males were obtained from GLOBOCAN estimates for individual countries[29]; national incidence was treated both as a continuous variable and as a categorical variable, comparing countries in the top quintile (>21.7 cases per 100,000 population) to all other countries.[1] Study size was categorized according to whether the prevalence estimate was based on more than or less than 100 GC cases. Although we have no direct measure of "quality" across reports, we calculated a surrogate measure based on the number of variables (0–3) included among the following: (1) sex, (2) anatomic subsite, and (3) histologic type.

Meta-analytic assumptions were assessed with Egger's test ("metabias") of funnel plot asymmetry (publication bias). This test identified no evidence of publication bias ($P = .49$). The influence of individual studies on the summary effect estimate was analyzed using the "metainf" command,[30] which graphically compares meta-analytic estimates computed by omitting each study in turn. None of the included studies appeared to dominate the overall meta-analysis.

Results

A total of 70 studies were chosen for inclusion in the meta-analysis; these represented hospital cancer case series, together reporting a grand total of 15,952 GC cases. The earliest study was published in 1992[15] and the most recent studies in 2008[31,32]; the largest study included 2966 GC cases,[33] and the smallest included 19 cases.[34] The majority of the 70 studies included originated in Asia (45/70), with a similar number of studies from Europe (12/70) and the Americas (13/70). Of the 70 studies included, 47 provided information on patients' sex.