Epstein-Barr Virus Infection and Increased Sporadic Breast Carcinoma Risk: A Meta-Analysis

Qing'e Jin Jianrong Su Donghui Yan Shanna Wu

Department of Clinical Laboratory, The Second Clinical Medical College of Capital Medical University, Beijing Friendship Hospital, Beijing, China

Significance of the Study

- We evaluated the relationship between Epstein-Barr virus (EBV) infection and breast carcinoma (BC) risk in 16 studies with 1,279 patients and 814 controls and found that EBV infection was significantly associated with BC risk.
- The findings may contribute to furthering our understanding of the role of EBV in the pathogenesis of BC and optimize therapeutic strategies for the treatment of BC.

Keywords

Epstein-Barr virus · Breast cancer · Meta-analyses

Abstract

Objective: An association of Epstein-Barr virus (EBV) infection with breast carcinoma (BC) risk has so far been disputed in the literature. Therefore, we performed a meta-analysis to clarify this relationship. **Materials and Methods:** An electronic database search for eligible case-control studies was performed using PubMed, Embase, Web of Science, the Cochrane Library, CNKI, and Wanfang Data until May 17, 2018. The pooled OR and 95% CI were used to estimate the relationship between EBV infection and BC risk using a fixed or random-effects model depending on heterogeneity. Subgroup analysis and meta-regression were used to explore the heterogeneity. Publication bias was assessed using Egger's and Harbord's tests. **Results:** A total of 16 studies with 1,279 patients and 814 controls were reviewed based on our inclusion and exclusion criteria. Compared with the control

group, EBV infection had a significant association with BC risk (OR 4.75, 95% CI 2.53–8.92, $p < 0.01$) with significant heterogeneity observed ($I^2 = 65.3\%$). The subgroup analysis revealed that region and tissue type might explain potential sources of heterogeneity. The sensitivity analyses yielded stable results. No significant publication bias was observed. **Conclusion:** The current results suggest that EBV infection is significantly associated with increased risk of BC.

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Introduction

Breast carcinoma (BC) is the most common malignancy that affects women worldwide [1]. The high disease burden highlights the need to better understand its etiology, but conventional risk factors such as genetic variation, hormones, and environmental factors only account for approximately 50% of the causes. A possible viral etiology from Epstein-Barr virus (EBV), mouse mammary

tumor virus (MMTV), bovine leukemia virus, and human papillomavirus (HPV) has been proposed. EBV is an oncogenic human herpesvirus persisting with lifelong latent infection in the majority of infected people [2]. The virus has been well known as a causative agent for several types of tumor, such as nasopharyngeal carcinoma, lymphomas, and oral squamous cell carcinoma [3].

Since 1995, the association of EBV infection with BC risk has been studied [4]. However, controversial results were reported. Several mechanisms for oncogenic EBV and BC risk have been investigated, indicating a potentially important role for EBV infection in BC pathogenesis. Thus, a meta-analysis was performed to evaluate the relationship.

Materials and Methods

Search Strategy

Our meta-analysis was performed in compliance with the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) statement guidelines [5]. A computerized literature search on PubMed, Embase, Web of Science, and the Cochrane Library for English-language publications, as well as on CNKI and Wanfang Data for Chinese-language publications, was conducted until May 17, 2018. The MeSH words used were the following keywords: (((“Epstein-Barr virus” [Title/Abstract]) or “EBV” [Title/Abstract]) or “human herpesvirus 4” [Title/Abstract]) or “HHV4” [Title/Abstract]) and (((“breast neoplasms” [Title/Abstract]) or “breast carcinoma” [Title/Abstract]) or “breast cancer” [Title/Abstract])). The titles and abstracts of the articles were carefully checked independently by two authors (Q.J. and S.W.) to determine if the full texts should be obtained.

Inclusion and Exclusion Criteria

The extracted data were required to meet the following criteria: (1) designed as a case-control study; (2) confirmed BC by histopathological diagnosis; (3) evaluated EBV infection by detecting the expression level (DNA, RNA, or protein) in tissue samples; (4) use of PCR, quantitative PCR, in situ hybridization (ISH), and immunohistochemistry (IHC); (5) only sporadic, not familial BC; and (6) full text available.

Studies meeting more than one of the following criteria were excluded: (1) animal research, in vitro study, case report, review, editorial, or commentary; (2) the available data could not be extracted by calculation from the article or by contacting the authors; and (3) duplicate publication.

Data Extraction

The data were extracted independently by two authors (Q.J. and S.W.) using a predesigned form. Discrepancies were discussed and resolved within the research team. The following information was recorded: first author; publication year; journal name; country of origin (Asia, Africa, Europe, North America, South America, and Oceania); numbers of cases and controls; detection method and marker; sample type (paraffin-embedded tissue or fresh/frozen tissue); and control type.

Quality Assessment

The quality of the papers was assessed independently by two authors (Q.J. and S.W.) according to the Newcastle-Ottawa Scale (NOS) [6], where a score of ≥ 6 indicates moderate-to-high quality.

Statistical Analysis

The statistical analysis was carried out using Stata software (version 14.1). Forest plots were generated to evaluate the pooled effects. Heterogeneity was assessed using Cochran's Q test and the I^2 statistic with a significance level of $p < 0.10$ or $I^2 > 50\%$. Depending on the heterogeneity test results, a fixed-effects or a random-effects model was adopted for the next analyses. Subgroup analysis and meta-regression were conducted when heterogeneity was evident based on vital variables (study location, tissue type, and publication year). A sensitivity analysis was performed to estimate the influence of a single study on the pooled ORs. Egger's test and Harbord's weighted linear regression test were used to assess publication bias. All p values were two-sided, and the significance level was 0.05.

Results

Study Selection

A total of 609 articles were selected from the databases. 560 citations were excluded due to duplication, irrelevant literature, being a review, or including no controls, and the remaining 49 articles were screened using the full text. Sixteen studies comprising 1,279 cases and 814 controls were finally included in the meta-analysis according to the eligibility criteria (online suppl. Fig. S1; see www.karger.com/doi/10.1159/000502131 for all online suppl. material).

Study Characteristics and Quality Assessment

The 16 included studies were published between 1995 and 2018 [7–22]. The prevalence of EBV infection ranged from 0% [17] to 60.0% [13] and the overall EBV positivity was 28.5%. The number of included BC patients ranged from 40 [20] to 144 [9] per study. The EBV prevalence was highest among Asian subjects and lowest among North American subjects. There was no significant difference in EBV infection by year of publication. The characteristics of the included articles are summarized, and the quality of the papers scored, in Table 1. The NOS scores ranged from 6 to 8, indicating a relatively high quality.

Statistical Pooling between EBV Infection and Risk of BC

The pooled ORs showed that EBV infection had an association with increased BC risk (OR 4.75, 95% CI 2.53–8.92, $p < 0.01$) in the random-effects model due to sig-

Table 1. Detailed information and quality assessment of included studies in the meta-analysis

First author [Ref.]	Year	Study location	Number of EBV cases		Number of EBV controls		Detection method	Detection marker	Tissue type	Control type	NOS score
			+	-	+	-					
Pai [7]	2018	India	25	58	0	7	ISH	EBERs	PE	Benign breast diseases	7
EI-Naby [8]	2017	Egypt	10	32	6	36	Nested PCR, IHC	EBNA-1, LMP-1	PE	Benign breast diseases	7
Fessahaye [9]	2017	Eritrea	40	104	6	57	PCR	EBERs, LMP-1	PE	Normal breast tissues	6
Richardson [10]	2015	New Zealand	24	46	9	61	qPCR	EBNA-1	Fresh/FT	Normal breast tissues	7
Yahia [11]	2014	Sudan	49	43	12	38	PCR, ISH	LMP-1, EBERs	Fresh/FT	Normal breast tissues	7
Peng [13]	2014	China	60	40	16	34	PCR-MS, ISH	EBERs	Fresh/FT	Benign breast diseases	8
Mohammadizadeh [12]	2014	Iran	6	74	0	80	IHC	LMP-1	PE	Normal breast tissues	6
Morales-Sánchez [15]	2013	Mexico	4	82	0	65	Nested PCR	EBV DNA	Fresh/FT	Normal breast tissues	7
Khabaz [14]	2013	Jordan	24	68	3	46	PCR, IHC	EBER-2, EBNA-1	PE	Benign breast diseases	7
Zekri [16]	2012	Iraq	14	36	0	20	PCR, IHC, ISH	EBNA-1, LMP-1, EBERs	PE	Normal breast tissues	6
Hachana [18]	2011	Tunisia	33	90	0	123	PCR	EBV DNA	Fresh/FT	Normal breast tissues	8
Kadivar [17]	2011	Iran	0	100	0	42	PCR, IHC	EBERs, EBNA-2, LMP	PE	Benign breast diseases	6
Joshi [19]	2009	India	28	23	0	30	IHC	EBNA-1	PE	Benign breast diseases	7
Fawzy [20]	2008	Egypt	10	30	0	20	PCR, IHC	EBNA-1	PE	Benign breast diseases	7
Kalkan [22]	2005	Turkey	13	44	19	36	PCR	EBV DNA	PE	Benign breast diseases	6
Preciado [21]	2005	Argentina	24	45	0	48	PCR, IHC	EBER, EBNA-1	PE	Benign breast diseases	7

EBV, Epstein-Barr virus; ISH, in situ hybridization; IHC, immunohistochemistry; EBER, EBV-encoded small nonpolyadenylated RNA; EBNA, EBV-determined nuclear antigen; LMP, latent membrane protein; PE, paraffin-embedded tissue; NOS, Newcastle-Ottawa Scale; FT, frozen tissue.

nificant heterogeneity between studies ($I^2 = 65.3\%$). One of the studies [17] was excluded after data analysis due to the absence of EBV in both groups. A forest plot of the effect sizes and 95% CIs is shown in Figure 1.

Heterogeneity, Subgroup Analysis, Meta-Regression, and Sensitivity Analyses

To explore the heterogeneity between studies, subgroup analysis and meta-regression were performed. Region (Asia/Africa and Europe/America) and tissue type (paraffin-embedded and fresh/frozen tissues) were chosen as variables by group. The results revealed that study location and tissue type could partly account for the heterogeneity. The pooled ORs (95% CIs) for the respective

regions were 4.98 (2.82–8.77) and 3.57 (0.64–19.99), and those for the respective tissue types were 5.62 (2.03–15.54) and 4.16 (2.17–7.97; online suppl. Fig. S2). The meta-regression analysis revealed that the publication date did not influence heterogeneity ($p = 0.760$). The sensitivity analyses revealed that no single study significantly changed the recalculated pooled ORs (online suppl. Fig. S3).

Publication Bias

A funnel plot revealed a relatively symmetrical distribution (online suppl. Fig. S4). No publication bias was observed by Egger's test ($p = 0.111$) or Harbord's test ($p = 0.468$) (Fig. 2).

Fig. 1. Forest plot of the association between Epstein-Barr virus (EBV) infection and increased breast carcinoma (BC) risk. Sixteen studies were selected for the meta-analysis. One was excluded automatically after the data analysis due to the absence of EBV in both groups. The random-effects model was chosen for the heterogeneity. A 4.75-fold (95% CI 2.53–8.92) increased BC risk with EBV infection was shown.

Fig. 2. Estimation of publication bias by Egger's test (**a**) and Harbord's weighted linear regression test (**b**). The two kinds of method showed no significant publication bias ($p = 0.111$ and $p = 0.468$, respectively).

Discussion

Based on all the outcomes and our analysis, EBV infection had a significant association with increased BC risk. The high incidence of BC worldwide has aroused interest in a possible viral etiology. EBV infected more than 90% of the population worldwide. In 1995, Labrecque et al. [4] detected EBV in epithelial cells of BC. Since then, EBV infection has been investigated as an etiological agent of BC, and the results have been controversial [4, 23]. The inconsistency could be attributed to several aspects: a

study design that involved a specific histological type of BC, differences in methodologies or techniques (PCR, ISH, and IHC), dissimilarities in the archival materials (paraffin-embedded and fresh/frozen tissues), and ethnic origin [24].

Arbach et al. [25] found that EBV genomes were heterogeneously distributed in morphologically identical tumor cells. Another study revealed that EBV was localized to infiltrated lymphocytes rather than malignant breast cells [26]. Therefore, to depend solely on PCR-based approaches to determine the relationship between EBV in-

fection and BC risk is inappropriate. Indeed, several different techniques should be performed in a single experiment to verify each other's results. Huo et al. [27] performed a pooled analysis of 10 studies on EBV infection and BC risk published during 1995–2008. They found a significant increase in breast malignancy risk among patients with EBV infection (OR 6.29, 95% CI 2.13–18.59), with a high heterogeneity of 75.9%. Their research used only the PCR technique to detect EBV infection. Since 2012, we have reviewed few reports on the relationship between EBV infection and BC risk.

Our results are consistent with those of Huo's study. In our meta-analysis, 11 of the 16 included studies utilized ISH or IHC or both. Thirteen of the 16 studies, published between 2009 and 2018, represent the latest research progress. Only 3 papers were published during 2005–2008, which were also included in the study by Huo et al. [27]. Moreover, our study had a larger case number, making it more convincing. Heterogeneity is a common problem in meta-analyses, since it could weaken the reliability of the results. The results of our subgroup analysis indicate that geographic region and tissue type could partially account for the heterogeneity observed; otherwise, in the meta-regression analysis, the publication date had no influence on the heterogeneity. No publication bias was observed, and the sensitivity analyses also showed stable results.

It is known that oncovirus coinfection could play an important role in human oncogenesis. Research on Pakistani BC patients found that coinfection with EBV and high-risk HPVs suggested a possible synergistic carcinogenic effect [28]. An MMTV-like virus has been a major suspect as a causative virus for BC over 50 years. EBV, HPV, and MMTV gene sequences are present and coexist in BC patients [29]. Thus, oncovirus coinfection of BC

patients also was an important issue to explore. In the future, more attention should be paid to the interaction of oncoviruses during the occurrence of BC.

Although we evaluated the association between EBV infection and BC risk with reliable statistical results, several limitations of the current study should be addressed. The results could be attributable to technical challenges and methods, the study design used with different histological types, and ethnicity, as the majority of the populations studied were Asian and African.

Additional high-quality research with a wider reach and larger sample sizes is required to further confirm the relationship. Treatment for BC still does not take into account the status of infection with EBV and related viruses in most areas of China. Promising therapeutic approaches based on EBV status should be further explored.

Conclusion

Based on the current meta-analysis, we suggest that EBV infection is statistically significantly associated with increased BC risk. The findings will help in understanding the role of EBV in the pathogenesis of BC and in optimizing therapeutic strategies for BC.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors declare no conflicts of interest.

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