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Epstein-Barr Viral Infection and the Risk for Breast Cancer: A Systematic Review

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ABSTRACT

Background: The prevalence of breast cancer has increased and has currently become one of the most common cancers. Although the majority of the world's population is infected with Epstein Barr Virus (EBV) during their lives, the severity of symptoms varies and not everyone infected with EBV is diagnosed with cancer. EBV might increase the risk for breast cancer either by activating the HER2/HER3 signaling cascades or by creating a state of prolonged immune stimulation.

Materials and Methods: A systematic search of several electronic databases including PubMed, ScienceDirect, Cochrane, EBSCOhost, JSTOR, and Scopus, following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines was conducted. The primary outcome of this review was to assess the prevalence of people with breast cancer that had a prior EBV infection.

Results: For this review, 24 case-control studies were accepted. Our analyses included 1.989 breast cancer cases versus 1.034 control cases. EBV was found to be present in 27.9% of breast cancer cases versus 8.02% found in the normal breast tissue of controls. All affected people were women with a mean age was 48.19 years. The most common type of breast cancer found in EBV-infected tissues was invasive breast cancer. Cases were reported sporadically in a wide geographical distribution, and the prevalence varied from 4.6% - 64.1%.

Conclusions: A previous EBV infection might be associated with a higher risk for breast malignancy. The most common type is invasive cancer. It mainly affects women and geographical variances are observed. More studies are necessary to elucidate the role of EBV in the mechanisms of breast cancer. Also, it is crucial to improve the prevention and treatment strategies.

Keywords: Epstein - Barr virus (EBV); Systematic review; Breast carcinoma; Breast cancer; Epstein–Barr virus

INTRODUCTION

In 2020, the World Health Organization (WHO) reported that breast cancer was the most frequent cancer worldwide with 2.3 million cases diagnosed and 685,000 breast cancer-related deaths[1]. Given the high prevalence of breast cancer in women, it is crucial to identify any novel risk factors that are associated with it to facilitate early diagnosis, treatment, and prevention. An increasing curiosity in studying viruses with oncogenic properties in the recent past has been observed. It is reported that 20% of overall cancers are of viral etiology[2]. Over the years, many oncogenic viruses have been discussed as being related to breast cancer[3]. Among those, Mouse Mammary Tumor Virus (MMTV), Epstein Barr Virus (EBV), and Human Papilloma Virus (HPV) have been the most prevalent types[3]. Each of these

viruses, with oncogenic potential, has been identified and reported to have been identified in malignant breast tissues. A comprehensive survey conducted in 2014 reported a total of 143,000 deaths globally due to EBV-attributed malignancies, making approximately 1.8% of all cancer deaths[4].

Different modalities can be utilized to identify viral genomic data in tissue, which include polymerase chain reaction (PCR) and in-situ hybridization (ISH) [5]. PCR could be very poor in differentiating between cancer cells and lymphocytes; therefore, viral genomic sequences should be extracted from malignant breast tissues using a combination of ISH and PCR to yield better results[6]. Tissue preparation could also affect the detection of EBV DNA in breast tissue. In a study conducted in dogs there was not a reported association between EBV and their mammary tumors; however, the authors were able to detect for a first time EBV DNA in canine mammary tumors. This suggests the viral detection might be affected by the quality and quantity of DNA extracted from paraffin-embedded tissues[7]. Despite these efforts in improving EBV detection, the evaluation of oncogenic viruses in all breast cancers is challenging due to very low viral loads.

Even though these oncogenic viruses' exact mechanisms of action are unclear, some studies have demonstrated that oncogenic viruses like EBV promote oncogenic activity through *HER2* and *HER3* pathway cascades[8]. The pathophysiology of EBV, leading to different cancers such as nasopharyngeal and gastric cancer, or Hodgkin's lymphoma and Burkitt's lymphoma it is well established. However, over the past 15-20 years, researchers have correlated its relationship with breast cancer.

EBV, a human herpesvirus 4, belongs to the herpes virus family. It is the most commonly found human viruses worldwide and spreads primarily through saliva[9]. Almost 95% of the world's adult population has been infected with in EBV in a lifetime[10]. There is an increased incidence in younger compared to older women, suggesting that younger women are more sexually active, and their chances of EBV transmission are higher[3]. Although an association between EBV and its oncogenic abilities has been previously established, it was only recently that its relationship with breast cancer was determined[11]. Normal breast tissues contain lymphatic cell lines which can make them susceptible to infection on direct contact with EBV[12] . First, it was Labrecque et al. who isolated EBV in the epithelial cells of cancerous breast tissue where EBV was sequenced in 21% of the 91 studied breast cancer patients[13]. Additionally, although the pathogenesis of EBV in lymphoma is thought to differ from that in breast cancer, studies have reported increased incidence of Hodgkin's lymphoma and breast cancer[14]. With these properties in mind, EBV could potentially be used as a tumor marker for the early detection of breast cancer. In view of this controversial topic and the lack of sufficient data, our research aims to evaluate the prevalence and potential association of EBV in breast cancer patients.

Physiopathology

EBV has been found in breast cancer tissues, however its not well elucidated how this virus contributes to pathogenesis and progression of breast malignancies.[15]However, different mechanisms have been proposed trying to describe the pathogenesis and this virus role on progression to cancer. Most of these mechanisms have been linked to the existence of the viral proteins that are expressed upon infection including Epstein-Barr virus nuclear antigens (EBNAs) and latent membrane proteins (LMPs).[16]The viral proteins may modulate host proteins in associated malignancies. The key proto-oncogenes and tumor suppressors in various EBV-associated malignancies are E-cadherin, PD-L1, c-Myc, p53[17].

EBV-associated neoplasm affect both immune-compromised patients from organ transplantation or immunosuppressive treatment, as well as immune-competent hosts.[18]The virus is thought to be associated with sporadic breast cancer, as presence of EBV genetic material was found in breast tumor tissue, but not in normal tissue[19].

EBV infection activates the HER2/HER3 signaling cascades, predisposing breast epithelial cells to malignant transformation[20]. There is a significant increase noted of Apolipoprotein B mRNA editing enzym**e** **(**APOBEC)-mediated mutagenesis in HER+/HER2 metastatic breast tumors, versus early-stage primary breast cancer[21]. APOBEC enzymes are

catalytic polypeptide-like enzymes normally activated during innate immune responses. It is shown to inhibit MMTV infections and viral replication in mice, therefore abnormal expression may predispose MMTV infection. Furthermore, deletions and inactivating mutations in *APOBEC3B* are also thought to be associated with breast cancer development. Specifically, a deletion polymorphism in this gene cluster is associated with an increased risk for breast cancer[20].

Another possible mechanism for breast cancer development could be due to a "delayed" primary EBV infection (Figure. 1). This can lead to a strong host response against a ubiquitous, normally asymptomatic infection, which can result in a state of prolonged immune stimulation and elevation of tumor necrosis factor (TNF)-alpha and interleukin (IL)-6. Resultant stimulation of aromatase activity drives the conversion of androstenedione to estrone in adipose tissue, ultimately increasing the risk of breast cancer development[14].

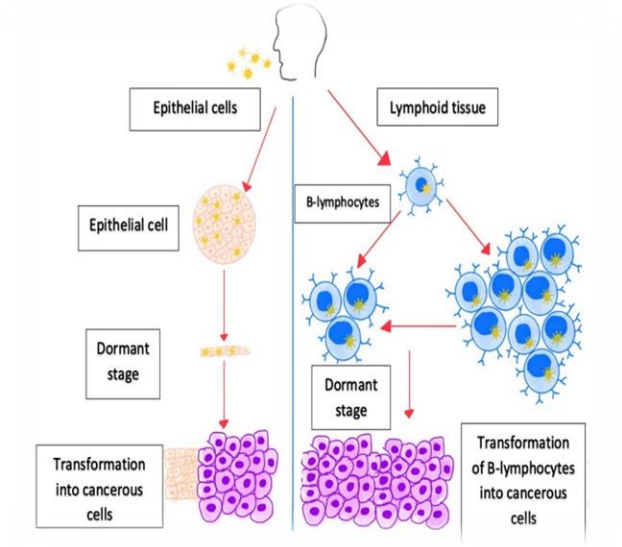

Figure 1: The role of EBV in pathogenesis of breast cancer

Materials and Methods

Search Strategy and Study Selection

The following systematic review was reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[22] (Figure 2). The review was conducted from December 10th, 2021 until May 1st, 2022.

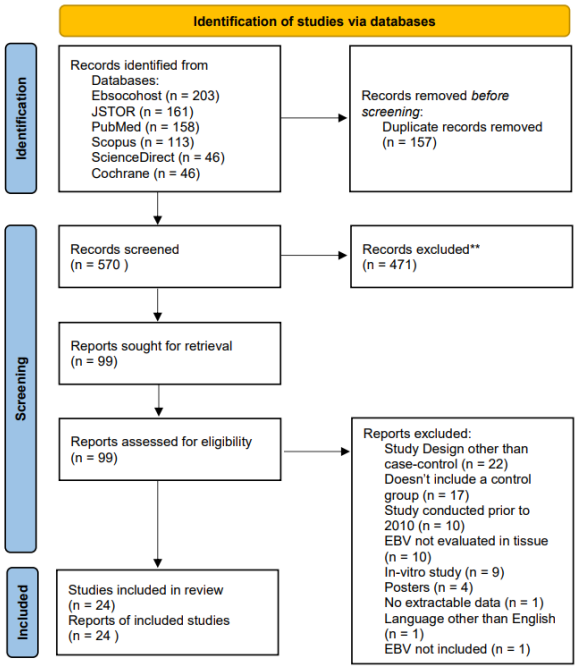

Figure 2: PRISMA Flow Chart

Multiple electronic databases were searched for relevant peer-reviewed articles including PubMed, ScienceDirect, Cochrane, EBSCOhost, JSTOR and Scopus. Medical Subject Headings [Mesh] terms and the Boolean operators (AND/OR] were used to develop the search strategy. "Epstein-Barr virus" OR "EBV" OR "human herpesvirus 4" OR "HHV4" AND "breast neoplasm" OR "breast carcinoma" OR "breast cancer" were used for the search.

Two authors independently screened selected articles by title and abstract to determine if the full texts should be retrieved using the software Rayyan[23]. Eligible full-text articles were obtained and reviewed by the same two authors. Any conflicts that arose during the screening process were resolved by a third author. Bibliography lists of eligible articles were manually screened for any relevant studies that were missed during the electronic search.

Selection Criteria and Quality Assessment

Full-text articles were considered eligible if they fulfilled the inclusion criteria: (1) case-control studies; (2) confirmed breast cancer diagnosis by histopathological technique; (3) analyzed EBV infection in tissue by detecting the expression level (DNA, RNA, or protein); (4) use of PCR, ISH, quantitative PCR, and immunohistochemistry (IHC)

to detect EBV in tissue samples; (5) only sporadic breast cancer; and (7) article written in the English language. Articles were excluded based on exclusion criteria: (1) animal study, *in-vitro* design, case reports, reviews, or editorials; (2) the relevant information could not be extracted by calculation from the article and/or its supplementary files, or by contacting the authors; (3) study published before year 2010; and (4) duplicate publication (5) studies published in language other than English.

Data Extraction and Study Outcomes

Data extraction was done by three authors (AI, MV, EL) independently using an extraction spreadsheet from Google Sheets. The following data were extracted: 1) author's last name, 2) publication year, 3) country the study was conducted in, 4) patient and control characteristics (sample size and age), 5) tumor characteristics (tumor type, grade, and histological type), 6) detection methods and markers, 7) tissue type, and 8) the number of EBV positive tissue samples in patient and control groups. The primary outcome of this review was to assess the prevalence of EBV in breast cancer tissue. The second outcome included the geographical distribution of EBV-positive breast cancer cases.

The risk of bias assessment for each eligible full-text article was assessed using the Newcastle–Ottawa scale for case-control studies[24]. Two independent reviewers assessed the methodology quality of each study included. In case of a disagreement, reviewers reached consensus by discussion with a third reviewer. We considered 7 out of 9 stars to be a low risk of bias, 4–6 stars to be a moderate risk and less than four stars to be a high risk of bias (Table 1).

RESULTS

Figure 2 depicts in detail the flow of study selection and screening following the PRISMA guidelines. We updated the search for new articles published on January 2022. A total of 727 articles were identified following an electronic database search of which 157 duplicates were removed. Following title and abstracts screening, 99 fulfilled the inclusion criteria and were retrieved for full-text screening. Out of these, a total of 75 studies were excluded for reasons highlighted in Figure 1. Hence, a total of 24 studies were included in the final analysis.

Study and Patient Characteristics

The characteristics of included studies and patients in the systematic review are summarized in **Table 1.** Overall, 24 case-control studies with either prospective and/or exploratory studies were included. This study involved research conducted worldwide, namely in Syria[25], Jordan[26], Australia[27–28], USA[29], Iran[30–37], Eritrea[38], Croatia[39], Iraq[40,41], Saudi Arabia[42], Portugal, Egypt[41,43], Pakistan[44], India[45], New Zealand[46], and Sudan[47]. The study design used in all studies was case control. The ages of the female breast cancer patients in each study ranged from 23-70 years old and were age-matched with the control subjects. Types of breast cancer were invasive ductal, mixed, in-situ ductal, with invasive breast cancer being the most common. Common histopathologic features of breast cancer included invasive ductal carcinoma (IDC), ductal carcinoma-in-situ (DCIS), invasive lobular carcinoma (ILC), ductal, lobular, medullary, and mucinous, with one study reporting a recurrent tumor.[40] A majority of the subjects had breast cancers in stages 1 to 3, with a few in the 4th stage. Breast tissues were preserved as paraffin-embedded tissue or frozen tissue. These were then run using various detection methods (PCR, IHC, ISH, tissue microarray (TMA), reverse-transcriptase polymerase chain reaction (RT-PCR) chromogen ISH, RT-qPCR, and nested PCR) to detect for the presence of EBV markers such as *LMP-1, EBNA-1, EBNA-2, EBER* probe, EBV assay, *BamH1*, *MiR-218, BHFR1* region, *BZLF1, Gp220, EBER-2, anti-ZEBRA* antibodies, *IFN*-gamma, TNF-alpha, *BXLF-1*, and CD21 against EBV membrane receptor.

All included studies reported findings for 1989 cases of breast cancer cases versus 1034 control cases. EBV infection was prevalent in 27.9% of the 1989 breast cancer cases versus 8% of the controls. EBV positive breast cancers were most commonly invasive (68%). The mean age of affected subjects was 48.19 years. According to data reported, there was a total of 555 or 27.9% EBV in cancer diagnosed patients versus a total of 83 or 8% of EBV in the positive control group. The highest and the lowest prevalence of EBV among patients with breast cancer were observed in the

Sudan and Mexico populations, at 64.1%[47] and 4.6%[48] respectively. Our findings show that EBV infection leads to a 4.41-fold increase in the odds of breast cancer development versus the control group.

DISCUSSION

EBV remains one of the most common viruses found in humans. Recently it was revealed that almost 50% of children have been exposed at a young age, and almost 95% of the adult population has been affected by EBV[49]. The majority of infections in humans are asymptomatic but can cause long-term health consequences such as cancer. Research has been continually linking viruses to the development of different cancers, and various oncogenic viruses have been strongly associated with the development of breast cancer[3]. EBV infection activates the HER2/HER3 signaling cascade, predisposing breast epithelial cells to malignant transformation. EBV EBNA genes are responsible for tumor growth and metastasis and can affect the mesenchymal transition of cells[50]

In this systematic review of literature, we found that 24.6% of breast cancer patients have EBV genetic material in their tumors. This is in line with a recent meta-analysis that showed the prevalence of EBV in malignant breast cancer was 26.4%[51]. However, the EBV's prevalence in malignant breast cancer tissue appears to vary widely, with some studies reporting a prevalence as low as 0% and others as high as 90%[46,52]. A major reason behind the wide variation in prevalence is the utilization of different techniques to identify EBV genes.

The techniques used to detect EBV DNA vary in sensitivity, with certain PCR primers more sensitive to viral proteins than others do. Huo et al. analyzed common primers used to detect EBV material in PCR assays. Of 14 genome fragments, they found EBER2 and LMP-1 in EBV detection to be significantly higher and lower, respectively. Bam H1W was the most frequently used region, while BXLF1 demonstrated a high prevalence rate of EBV in breast cancer[53]. Another study targeting EBER and LMP-1 found a higher sensitivity for detection of EBV genome signals with EBER primers. Given that literature describes EBV positive tumor infiltrating lymphocytes potentially producing false positive PCR results, EBER-ISH, which eliminates this result, has been considered a gold standard technique to detect EBV material[38]. In an interesting finding by Lorenzetti et al, however, EBERs ISH was pronounced an unsuitable method to apply in breast carcinoma. The authors instead highlighted the actions of LMP2A and suggested that it may down regulate LMP1 expression and could be the cause of traditionally low EBV detection in breast tumors when using only LMP1 and EBERs transcripts[54]. In addition, the higher prevalence detected in recent studies could be due to improved detection methods such as high-sensitivity ISH which allows viral detection even when only incomplete viral remnants are available in the breast tissue[55].

It is poorly understood how EBV could affect different kinds of breast cancer. Heng et al. reported that young women(10-22 years of age) with infectious mononucleosis (IM) were at less risk for progressing to invasive breast cancer versus women who never had infectious mononucleosis[56]. However, this study was only using a questionnaire and the presence of EBV in breast cancer tissue was not evaluated[56]. Aboulkassim et al. explored the presence of EBV in 108 breast cancer tissues in women in Syria using PCR and tissue microarray analysis[25]. They found that EBV was present in 51.85% of breast cancer samples and the expression of the LMP1 gene of EBV was associated with an invasive breast cancer phenotype[25]. Hussein et al. report that the types of breast cancers associated with EBV infection varied but invasive breast cancer was the most commonly found[40]. This is supported by Bonnet et al., who were able to find EBV presence in 51% of the tumors by using PCR. In majority (90%) of the cases they studied, EBV viral genome was not found in the healthy tissue, close to the tumor (p<.001). The virus was not only found specifically in tumor cells, but furthermore it was associated with the most aggressive tumors[57]. Ballard et al., reported that EBV infection was present in 42.5% of cases of invasive ductal carcinoma and 36.2% cases of invasive lobular carcinoma (p=0.518). This shows that EBV infection is equally found present in the ductal and lobular tumor types[58].

Of interest, data reported by Fessahaye et al. pointed to possible differences in population predisposition

and EBV-associated breast cancer epidemiology. They found that EBV was associated less with tumors diagnosis in Eritrea compared to their neighboring Sudan[38]. This hypothesis has been supported by Sinclair et al. who showed evidence for the presence of EBV in breast cancer biopsies more concentrated in specific geographic regions[15]. A higher association in samples from Asia and South America was observed in two other studies versus a lower association with EBV in samples from the USA and Western Europe[15]. In our review, EBV prevalence in breast cancer varied widely among countries and geographic areas. However, the establishment of a cause is beyond the scope of this review currently.

LIMITATIONS

This systematic review is not without limitations. A major limitation is the different methods used to detect EBV DNA in breast tissue among the included studies. The observed heterogeneity in methodology and populations among studies did not allow for a meaningful qualitative analysis. Additionally, only a limited number of studies used control tissue from the same patient while others compared tissue from women without breast cancer or benign lesions to those with breast cancer. Finally, our review was limited to only English language and peer reviewed articles.

CONCLUSION

Based on our systematic review findings, we conclude that EBV infection may be related to an increased breast cancer risk. Although the oncogenic properties of EBV in the pathogenesis of breast cancer are not yet well understood, a previous EBV infection is associated with a higher risk for breast malignancy. Further research is recommended to understand the pathogenesis and optimize treatment strategies for breast cancer.

Abbreviations

Epstein Barr Virus (EBV); World Health Organization (WHO); Bovine Leukemia Virus (BLV); Mouse Mammary Tumor Virus (MMTV); Human Papillomavirus (HPV).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. World Health Organization. Breast cancer. Who.int. Published 2021. Accessed April 20, 2022. https://www.who.int/news-room/fact-sheets/detail/breast-cancer.

2. Vokes EE, Liebowitz DN, Weichselbaum RR. Nasopharyngeal carcinoma. The Lancet. 1997;350(9084):1087-1091.

3. Lawson JS, Heng B. Viruses and breast cancer. Cancers (Basel). 2010;2(2):752-772.

4. Khan G, Hashim MJ. Global burden of deaths from Epstein-Barr virus attributable malignancies 1990-2010. Infect Agents Cancer. 2014;9(1):38.

5. Sterchi DL. Molecular Pathology—In Situ Hybridization. In: Theory and Practice of Histological Techniques. Elsevier; 2008:537-558.

6. Fina F, Romain S, Ouafik L, et al. Frequency and genome load of Epstein–Barr virus in 509 breast cancers from different geographical areas. Br J Cancer. 2001;84(6):783-790.

7. Roa López GA, Suárez JJ, Barato P, et al. Lack of association between Epstein–Barr virus and mammary tumours in dogs. J Vet Res. 2018;62(3):309-315.

8. Lin JH, Tsai CH, Chu JS, et al. Dysregulation of HER2/HER3 Signaling Axis in Epstein-Barr Virus-Infected Breast Carcinoma Cells. J Virol. 2007;81(11):5705-5713.

9. Longnecker R, Neipel F. Introduction to the human γ-herpesviruses. In: Arvin A, Campadelli-Fiume G, Mocarski E, et al., eds. Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis. Cambridge University Press; 2007.

Accessed April 25, 2022. http://www.ncbi.nlm.nih.gov/books/NBK47397/
10. Hoover K, Higginbotham K. Epstein Barr Virus. In: StatPearls. StatPearls Publishing; 2022. Accessed April 29, 2022. http://www.ncbi.nlm.nih.gov/books/NBK559285/
11. Afzal S, Fiaz K, Noor A, et al. Interrelated Oncogenic Viruses and Breast Cancer. Front Mol Biosci. 2022;9:781111.
12. Speck P. Infection of Breast Epithelial Cells With Epstein-Barr Virus Via Cell-to-Cell Contact. Journal of the National Cancer Institute. 2000;92(22):1849-1851.
13. Labrecque LG, Barnes DM, Fentiman IS, et al. Epstein-Barr virus in epithelial cell tumors: a breast cancer study. Cancer Res. 1995;55(1):39-45.
14. Yasui Y, Potter JD, Stanford JL, et al. Breast cancer risk and "delayed" primary Epstein-Barr virus infection. Cancer Epidemiol Biomarkers Prev. 2001;10(1):9-16.
15. Sinclair AJ, Moalwi MH, Amoaten T. Is EBV Associated with Breast Cancer in Specific Geographic Locations? Cancers. 2021;13(4):819.
16. Kang H, Kiess A, Chung CH. Emerging biomarkers in head and neck cancer in the era of genomics. *Nat Rev Clin Oncol*. 2015;12(1):11-26.
17. Fierti AO, Yakass MB, Okertchiri EA, et al. The Role of Epstein-Barr Virus in Modulating Key Tumor Suppressor Genes in Associated Malignancies: Epigenetics, Transcriptional, and Post-Translational Modifications. Biomolecules. 2022;12(1):127.
18. Kushekhar K, van den Berg A, Nolte I, et al. Genetic Associations in Classical Hodgkin Lymphoma: A Systematic Review and Insights into Susceptibility Mechanisms. Cancer Epidemiol Biomarkers Prev. 2014;23(12):2737-2747.
19. Kazemi Aghdam M, Nadji SA, Khoddami M, et al. Epstein-Barr Virus and Breast Carcinoma in Iran. Jundishapur J Microbiol. 2017;10(10).
20. Lawson JS, Salmons B, Glenn WK. Oncogenic Viruses and Breast Cancer: Mouse Mammary Tumor Virus (MMTV), Bovine Leukemia Virus (BLV), Human Papilloma Virus (HPV), and Epstein–Barr Virus (EBV). Front Oncol. 2018;8:1.
21. Lefebvre C, Bachelot T, Filleron T, et al. Mutational Profile of Metastatic Breast Cancers: A Retrospective Analysis. Mardis ER, ed. *PLoS Med*. 2016;13(12):e1002201.
22. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71.
23. Ouzzani M, Hammady H, Fedorowicz Z,et al. Rayyan—a web and mobile app for systematic reviews. *Syst Rev*. 2016;5(1):210.
24. Wells G, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Accessed February 23, 2022. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp
25. Aboulkassim T, Yasmeen A, Akil N, et al. Incidence of Epstein–Barr virus in Syrian women with breast cancer: A tissue microarray study. *Human Vaccines & Immunotherapeutics*. 2015;11(4):951-955.
26. Al Hamad M, Matalka I, Al Zoubi MS, et al. Human Mammary Tumor Virus, Human Papilloma Virus, and Epstein-Barr Virus Infection Are Associated With Sporadic Breast Cancer Metastasis. Breast Cancer (Auckl). 2020;14:117822342097638.
27. Antonsson A, Bialasiewicz S, Rockett RJ, et al. Exploring the Prevalence of Ten Polyomaviruses and Two Herpes Viruses in Breast Cancer. Buck CB, ed. *PLoS ONE*. 2012;7(8):e39842.
28. Glenn WK, Heng B, Delprado W, et al. Epstein-Barr Virus, Human Papillomavirus and Mouse Mammary Tumour Virus as Multiple Viruses in Breast Cancer. Mahieux R, ed. *PLoS ONE*. 2012;7(11):e48788.
29. El-Naby NEH, Hassan Mohamed H, Mohamed Goda A, et al. Epstein-Barr virus infection and breast invasive ductal carcinoma in Egyptian women: A single center experience. *J. Egypt. Natl. Cancer Inst*. 2017;29(2):77-82.
30. Torfi F, Kamani F, Tavakolian S, et al. Evaluation of association between the frequency of Epstein–Barr virus and cytomegalovirus infection in healthy and tumoral breast samples. *Gene Rep*. 2021;24(2):101208.
31. Reza MA, Reza MH, Mahdiyeh L, et al. Evaluation Frequency of Merkel Cell Polyoma, Epstein-Barr and Mouse Mammary Tumor Viruses in Patients with Breast Cancer in Kerman, Southeast of Iran. *Asian Pac J Cancer Prev*. 2015;16(16):7351-7357.
32. Sharifpour C, Makvandi M, Samabafzadeh A, et al. Frequency of Epstein–Barr Virus DNA in Formalin-fixed Paraffin-embedded Tissue of Patients with Ductal Breast Carcinoma. *Asian Pac J Cancer Prev*. 2019;20(3):687-692.
33. Mostafaei S, Kazemnejad A, Norooznezhad AH, et al. Simultaneous Effects of Viral Factors of Human Papilloma Virus and Epstein-Barr Virus on Progression of Breast and Thyroid Cancers: Application of Structural Equation Modeling. *Asian Pac J Cancer Prev*. 2020;21(5):1431-1439.
34. Golrokh Mofrad M, Kazeminezhad B, Faghihloo E. Prevalence of Epstein–Barr virus (EBV) in Iranian Breast Carcinoma Patients. *Asian Pac J Cancer Prev*. 2020;21(1):133-137.
35. Mohammadizadeh F, Zarean M, Abbasi M. Association of Epstein-Barr virus with invasive breast carcinoma and

its impact on well-known clinicopathologic parameters in Iranian women. *Adv Biomed Res*. 2014;3(1):140.
36. Dowran R, Joharinia N, Safaei A, et al. No detection of EBV, BKV and JCV in breast cancer tissue samples in Iran. *BMC Res Notes*. 2019;12(1):171.
37. Charostad J, Nakhaei M, Azaran A, et al. MiRNA-218 Is Frequently Downregulated in Malignant Breast Tumors: A Footprint of Epstein-Barr Virus Infection. *Iran J Pathol*. 2021;16(4):376-385.
38. Fessahaye G, Elhassan AM, Elamin EM, et al. Association of Epstein - Barr virus and breast cancer in Eritrea. *Infect Agents Cancer*. 2017;12:62.
39. Gupta I, Jabeen A, Al-Sarraf R, et al. The co-presence of high-risk human papillomaviruses and Epstein-Barr virus is linked with tumor grade and stage in Qatari women with breast cancer. Hum Vaccin Immunother. 2021;17(4):982-989.

40. Hussein AA. Molecular detection of Epstein Barr Virus in Women with Breast cancer. *J Fac Med Baghdad*. 2013;55(2):144-148.
41. Zekri ARN, Bahnassy AA, Mohamed WS, et al. Epstein-Barr virus and breast cancer: Epidemiological and Molecular study on Egyptian and Iraqi women. *Journal of the Egyptian National Cancer Institute*. 2012;24(3):123-131.
42. Khabaz MN. Association of Epstein-Barr virus infection and breast carcinoma. Arch Med Sci. 2013; 9(4): 745–751.
43. Metwally SA, Abo-Shadi MA, Abdel Fattah NF, et al. Presence of HPV, EBV and HMTV Viruses Among Egyptian Breast Cancer Women: Molecular Detection and Clinical Relevance. Infect Drug Resist. 2021; 14: 2327–2339.
44. Naushad W, Surriya O, Sadia H. Prevalence of EBV, HPV and MMTV in Pakistani breast cancer patients: A possible etiological role of viruses in breast cancer. Infect Genet Evol. 2017 Oct;54:230-237.
45. Pai T, Gupta S, Gurav M, et al. Evidence for the association of Epstein-Barr Virus in breast cancer in Indian patients using in-situ hybridization technique. *Breast J*. 2018;24(1):16-22.
46. Richardson AK, Currie MJ, Robinson BA, et al. Cytomegalovirus and Epstein-Barr Virus in Breast Cancer. *PLoS One*. 2015;10(2):e0118989.
47. Yahia ZA, Adam AA, Elgizouli M, et al. Epstein Barr virus: a prime candidate of breast cancer aetiology in Sudanese patients. *Infect Agent Cancer*. 2014;9(1):9.
48. Morales-Sánchez A, Molina-Muñoz T, Martínez-López JLE, et al. No association between Epstein-Barr Virus and Mouse Mammary Tumor Virus with Breast Cancer in Mexican Women. *Sci Rep*. 2013;3:2970.
49. Dunmire SK, Hogquist KA, Balfour HH. Infectious Mononucleosis. In: Münz C, ed. *Epstein Barr Virus Volume 1*. Vol 390. Current Topics in Microbiology and Immunology. Springer International Publishing; 2015:211-240.
50. Lawson JS, Glenn WK. Catching viral breast cancer. *Infect Agents Cancer*. 2021;16(1):37.
51. Farahmand M, Monavari SH, Shoja Z, et al. Epstein–Barr virus and risk of breast cancer: a systematic review and meta-analysis. *Future Oncol*. 2019;15(24):2873-2885.
52. Kadivar M, Monabati A, Joulaee A, et al. Epstein-Barr Virus and Breast Cancer: Lack of Evidence for an Association in Iranian Women. *Pathol Oncol Res*. 2011;17(3):489-492.
53. Huo Q, Zhang N, Yang Q. Epstein-Barr Virus Infection and Sporadic Breast Cancer Risk: A Meta-Analysis. *PLoS ONE*. 2012;7(2):e31656.
54. Lorenzetti MA, De Matteo E, Gass H, et al. Characterization of Epstein Barr Virus Latency Pattern in Argentine Breast Carcinoma. *PLoS ONE*. 2010;5(10):e13603.
55. Hu H, Luo ML, Desmedt C, et al. Epstein–Barr Virus Infection of Mammary Epithelial Cells Promotes Malignant Transformation. *EBioMedicine*. 2016;9:148-160.
56. Heng YJ, Love S, DeHart JC, et al. The association of infectious mononucleosis and invasive breast cancer in The Health of Women (HOW) Study®. 2022; 29(4):731-739.
57. Bonnet M, Guinebretiere JM, Kremmer E, et al. Detection of Epstein-Barr Virus in Invasive Breast Cancers. J Natl Cancer Inst. 1999;91(16):1376-81.
58. Ballard AJ. Epstein–Barr virus infection is equally distributed across the invasive ductal and invasive lobular forms of breast cancer. Pathol Res Pract. 2015; 211(12):1003-5.

Table 1. Detailed information and quality assessment of all included studies

Author (Year)	Location	Study Design	Number of cases	Number of controls	Number of EBV positive cancer tissue	Number of EBV positive control	Type of Breast Cancer	Tumor grade	Tissue type (paraffin-embedded tissue/ frozen tissue)	Detection Method	Detection Marker	Newcastle-Ottawa Scale*
Aboulkassim et al., 2015[25]	Syria	case control	108	13	56	8	invasive breast cancer (84), in situ (24)	N/A	paraffin-embedded tissue	PCR, tissue microarray, IHC	IHC: LMP-1 IHC: EBNA-1	9
Al Hamad et al., 2020[26]	Jordan	case control	100	50	24	3	invasive breast cancer (95) and in situ (5)	Grade I = 12 Grade II = 56 Grade III = 32	Paraffin-embedded tissue	RT-PCR, chromogen ISH	EBNA 2 ISH: EBER probe	7
Antonsson et al., 2012[27]	Australia	case control	54	10	5	0	invasive breast cancer (52); mixed (1); DCIS (1)	Grade I = 10 Grade II = 23 Grade III = 20	Frozen tissue	RT-PCR	EBV assay	8
Charostad et al., 2021[37]	Iran	case control	51	51	6	1	N/A	Early (I/II) = 32 Advanced (III/IV) = 19	Frozen tissue	Nested PCR, RT-qPCR	MiR-218	7
Dowran et al., 2019[36]	Iran	case control	150	150	0	0	Invasive	Grade I = 35 Grade II = 67 Grade III = 48	Paraffin-embedded tissue	PCR	BHFR1 region	8
El-Naby et al., 2017[29]	USA	case control	42	42	10	6	Invasive	Grade I/II = 28 Grade III = 14	Paraffin-embedded tissue	Nested PCR, IHC	EBNA-1, LMP-1	5
Mohammadizadeh, et al., 2014[35]	Iran	case control	80	80	6	0	Invasive	Grade I = 5 Grade II = 40 Grade III = 32	Paraffin-embedded tissue	IHC	LMP-1	9
Fessahaye et al., 2017[38]	Eritrea	case control	144	63	47	6	Invasive	N/A	Paraffin-embedded tissue	PCR, ISH and IHC	EBER LMP-1	9

Glenn et al., 2012[28]	Australia	case control	standard PCR: 50, in-situ PCR: 27	standard PCR: 40, insitu PCR: 18	39	20	Invasive (64), in situ (13)	NA	Fresh frozen DNA extract, human milk epithelial cell DNA, formalin-fixed specimens	Standard PCR, in situ PCR	EBNA-1	9
Gupta et al., 2021[39]	Croatia	case-control	70	16	25	0	Triple-negative breast cancer	Grade I = 0 Grade II = 11 Grade III= 59	Paraffin-embedded tissue	Thermo Scientific GeneJET FFPE DNA Purification Kit, PCR	EBNA1 EBNA2 LMP-1	7
Hussein et al., 2013[40]	Iraq	case control	22	10	11	0	invasive (12), infiltrative (8), recurrent (2)	Grade I=4, Grade II=16, Grade III=2	Paraffin-embedded tissue	ISH	EBER	7
Khabaz et al., 2013[42]	Saudi Arabia	case control	92	49	24	3	Invasive	Grade I=10, Grade II=32, Grade III=39; Grade III=7	Paraffin-embedded tissue	PCR	EBER 2, BNLF-1, EBNA 2, Gp220, EBNA-1	6
Metwally et al., 2021[43]	Egypt	case control	80	30	23	0	Invasive	Grade II=75,	paraffin-embedded tissue, fresh tissue samples, WBC	PCR	EBNA-1 BXLF-1 BamH1-K LMP-1	5
Mofrad et al., 2020[34]	Iran	case control	59	11	4	0	Invasive	I=4, II/III=55	FFPE	PCR	EBV-EBNA, GAPDH	8
Morales-Sanchez et al,, 2013[48]	Mexico	case control	86	65	4	0	Invasive (79), in-situ (7)	na	FFPE	PCR	Raji, Daudi and B95-8 cells	6
Mostafaei et al., 2020[33]	Iran	case control	83	31	50	11	Invasive, in-situ	NA	FFPE	PCR	EBNA-2, LMP-1, LMP-2A, EBER 1, EBER 2	8
Naushad et al., 2017[44]	Pakistan	Case-	250	15	61	0	Primary invasive	grade 1 112 (44.8%)	paraffin embedded	PCR	PCR: EBNA2	4

		Control					breast cancer	grade 2 113 (45.2%) grade 3 25 (10%)	(FFPE) block		F and EBNA2	
Pai et al., 2018[45]	India	Case - Control	83	7	25	0	Primary invasive breast cancer	Grade II 4 (4.8%) Grade III 79 (95.2%)	paraffin embedded tissues	ISH	ISH: EBER	8
Reza et al., 2015[31]	Iran	Case - Control	100	100	8	0	Primary invasive breast cancer	grade I (35%), grade II (42%), and grade III (23%)	Paraffin embedded block	PCR	PCR: EBER	4
Richardson et al., 2015[46]	New Zealand	Case - Control	70	70	24	9	N/A	Grade I: 8.5% Grade II: 29.6% Grade III: 62.0%	Frozen tissue	PCR	PCR: EBNA-1	6
Sharifpour et al., 2019[32]	Iran	Case - Control	37	35	10	4	Invasive (34) In situ (3)	Grade I: 12 Grade II: 13 Grade III: 12	Paraffin-Embedded Tissue	PCR	PCR: EBNA 3C	7
Torfi et al., 2021[30]	Iran	Case - Control	46	46	2	0	N/A	(I–II: 48%); (III–IV: 52%)	Not mentioned	PCR	PCR: EBNA-1	7
Yahia et al., 2014[47]	Sudan	Case - Control	92	50	59	12	Primary invasive breast cancer	Not mentioned	Paraffin blocks and frozen tissue	PCR & ISH	PCR: EBNA-4 and LMP-1	6
Zekri et al., 2012[41]	Egypt and Iraq	Case - Control	90	40	32	0	Primary invasive breast cancer	Grade I: 3, Grade II: 60, Grade III: 19	Paraffin blocks	PCR & ISH & IHC	PCR: EBNA-1 and LMP-1 ISH: EBER IHC: CD21 against EBV membrane receptor and LMP-1	7