Investigating the relationship between Epstein-Barr virus and breast cancer in Dhi-Qar province

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ABSTRACT

Introduction: According to previous reviews, breast cancer is one of the most common malignant tumors among women. Although the causes of breast cancer are many and not fully understood, exposure to Epstein-Barr virus (EBV) can be suggested as a risk factor for breast cancer. Studies since 1995 have reported that the EBV virus is directly involved in the development of breast cancer.

The aim of the study: is to evaluate the presence of EBV in patients suffering from breast cancer in Dhi Qar Governorate.

Methods: This study was conducted in which we used 40 paraffin-embedded tumor tissues and 40 tumor-free breast tissues from women with this type of breast cancer in Dhi Qar Governorate. After DNA extraction using the salting out method and amplifying the betactin gene, all samples were screened for EBV DNA using the PCR (polymerase chain reaction) method. Data were analyzed using the chi-square test using SPSS 16 software.

Results: EBV was detected by PCR in 20 of 40 (50%) breast cancer specimens and 5 of 40 (12.5%) control specimens. The chi-square statistic for analyzing EBV infection in tumour and normal samples was 0.000, which indicates a significant relationship between breast cancer and EBV infection in Dhi Qar Governorate.

Conclusion: The presence of the EBV gene in a large subset of women with breast cancer in Dhi Qar city shows that EBV can be one of the causes of breast cancer, but more studies are needed to prove the relationship between the virus and breast cancer.

Keywords: Breast cancer, Epstein-Barr virus, polymerase chain reaction, Dhi Qar Governorate

Introduction: -Breast cancer is a significant public health problem worldwide and the second leading cause of death in the United States. Most cancer death statistics in men and women are associated with lung cancer, followed by breast cancer in women (50%) (5). Breast cancer is one of the most common cancers in women (0). Although the prevalence of breast cancer in Asian women is lower than in women in Western countries, the trend of its prevalence is increasing. Breast cancer is also the most common

type of cancer among Iraqi women (3). The cause of breast cancer is not completely known, but genetic background and hormonal influences are thought to play an important role in its development (1,0). According to the International Agency for Research on Cancer, 51-04% of cancer cases are related to infection (6). Other risk factors for breast cancer include race, breast tissue density, lifestyle, infertility (7), late menopause and early menstruation (1). Finally, exposure to common viruses such as Epstein-Barr virus (EBV), murine mammary tumor virus (MMTV), and human papillomavirus (HPV) has also been suggested as a risk factor for breast cancer (9). In 1960, Epstein-Barr virus was discovered as the causative agent of the disease in humans (Burkitt's lymphoma) (54). Studies since 1991 have reported that EBV is involved in the development of breast cancer (55). Epstein-Barr virus is a DNA virus of the herpesvirus family (50). The genome of this virus is 510 kb and contains approximately 544 coding genes (53) and causes infectious mononucleosis in humans (50). EBV infects approximately 94% of the world's population and usually carries a long-term and asymptomatic infection (50). This virus is a B lymphotropic agent and is associated with B lymphocyte-associated cancers (0). EBV has also been reported with some malignancies such as Burkitt's lymphoma, Hodgkin's lymphoma, and nasopharyngeal carcinoma (51, 56). The EBV lymphocyte count is 0-64 lymphocytes per million It infects lymphocytes in the blood and leads to

The infection is hidden (1). If the virus is controlled by the host immune response, latent virus infection remains in B cells and healthy people carry the viral genome in their B cells (53). Latent infections are mainly transmitted through saliva, but can also be transmitted through sexual contact (54). Most EBV infections appear to occur during infancy or early childhood (57). The results of various studies indicate that a logical relationship can be established between breast cancer and viral contamination, the most important of which is the presence of the EBV virus in breast tissue and its ability to transmit to the milk of some women, which stimulates the growth of EBV. Breast milk cells with EBV DNA transfection and the presence of related lymphomas. With EBV in the breast, EBV has also been observed in benign breast tumors in women whose immune systems are suppressed, and in some cases in vitro, breast epithelial cells can be associated with direct contact with EBV lymphoblasts (53). The possibility of an association of invasive ductal and lobular breast cancer with EBV has been raised by Labreque et al. It led to further studies in this area (51). In different studies, different methods for EBV identification have been investigated and the results have been controversial. It has been reported that using the PCR method to identify viral DNA was positive in up to 14% in breast samples. However, studies using immunohistochemistry or in situ hybridization methods confirmed the presence of the virus in only one percent of these cases (51). PCR and real-time PCR have been proposed as the most sensitive methods for detecting EBV and these methods have been used frequently (59). In this study, we identified the virus in breast cancer samples and healthy breast tissue samples by PCR method to determine whether the virus plays a role in causing breast cancer in Iraqi patients or not, so that if the results are positive, it can be used in It is also used for prevention and treatment.

Materials and methods

Sample preparation and DNA extraction: 04 paraffin block samples of breast cancer and 04 paraffin block samples of healthy breast tissue were collected from hospitals and pathology laboratories in Dhi Qar Governorate. The selected pieces related to the years 5390 to 5391 AH. The age group of cancer patients was from 35 to 94 years, divided into three groups (14-35, 15-74, and 75-94 years). The largest number of cancer patients were between the ages of 15-74 years, and an attempt was made to select normal people. Control samples must be chosen more carefully to consider this age distribution. Using a microtome, 6–54 6-µm-thick slices were prepared from paraffin blocks and poured into 1.5-ml microtubes without DNase/RNase. After deparaffinization of samples using xylene (MERK/Germany) and absolute ethanol, genomic DNA was extracted using the salting-out method. The extraction method was such that 144 µL of buffer and 04 to 04 µL of proteinase K were added to each sample, and the samples were then vortexed, spun, and placed in a water bath at 11°C overnight (if later since the tissue was not completely digested , Proteinase K was added again and incubated at 11°C for another day and

night. Next, 0.44 µL of five M NaCl was added to each sample, and after centrifugation at 50,000 rpm for one minute, the supernatant solution was transferred to a new tube. The sediment containing protein and salt was discarded. The same volume of cold isopropanol was added to the supernatant solution transferred to another vial and mixed well by stirring. The microtubes were placed in a freezer at -04 C for 1 hour to increase the working efficiency. It was then centrifuged at 50,000 rpm for 4 minutes, and the supernatant was discarded. The sediment was washed with 74% ethanol and centrifuged at 50,000 rpm for 1 minute. This step was repeated twice until Wash the DNA completely Finally, after completely drying the sediment at temperature The chamber was dissolved in an appropriate amount of water (to dissolve the DNA in water, the extracted DNA was placed in a water bath at 11°C for 34 minutes). Samples are kept in a refrigerator at -04°C until PCR.

Evaluation of the purity and quality of the extracted DNA: The concentration and purity of the extracted DNA were determined using the NanoDrop device. The optical absorption ratio (optical density) was measured for wavelengths from 064 nm to 014 nm and samples that showed this ratio between 7.5 and 0 were used for PCR. Samples suitable for PCR were identified by amplifying the beta-actin gene with a primer specific for that gene (Table 5). To perform the reaction, a 0.01 µl mixture for each sample was prepared according to the instructions provided for the Mastermix PCR by Cinagen. First, they were heated at 90°C for 1 min, then subjected to PCR for 34 cycles, including annealing at 91°C for 1 min, primer annealing at 65°C for 14 s, and elongation at 70°C. It was placed for 01 seconds. Finally, they were left at 70°C for 54 minutes to ensure the product was fully amplified. PCR products were analyzed on a 1.5% agarose gel up to a 565 bp range Watch the game. Polymerase chain reaction (PCR) was performed to check for the presence of Epstein-Barr virus, and all samples that were positive for the beta actin gene were retested to identify and diagnose EBV. At this point, since the potential virus must replicate in the samples, this is why an Epstein-Barr virus primer is needed. The sequence of this primer is shown in Table 0. For PCR samples, 54 µl of PCR Mastermix (Ampliqon Company), 1.4 µl each of forward and reverse primers, and 0.4 ng of template DNA were poured into a 0.4 ml microtube, followed by water Deionized. The final volume of the reaction was brought to 04 µl .

Statistical: For statistical analysis, the data is according to the test Chi-square, SPSS 16 and association intensity were analyzed using Cramer's test.

the findings Extracting DNA and examining its quality: After extracting DNA from cancerous and non-cancerous tissues, its quality is examined. The results showed that all samples had acceptable absorption and concentration of light with the NanoDrop device. The optical absorption is 014/064 in these samples.

It was observed between 1.5 and 0. Next, a PCR reaction was performed with the aforementioned DNA with primers specific for the housekeeping beta-actin gene. All samples were qualitatively adequate and reproduced the desired range 565 (Figure 5).

The temperature program for PCR for the EBV gene includes an initiation step at 90°C for 1 minute, 04 cycles including an annealing step for two strands of DNA at 90°C for 1 second, ligation of primers at 10°C for 1 second and elongation at 70°C for 1 second and finally at 70°C for 54 minutes to ensure complete elongation of the product. For confirmation, PCR products were analyzed on a 1.5% agarose gel until a band of 097 bp was observed. At all stages, to detect possible contamination from the positive control (genomic DNA virus), a negative control (water) was used.

Performing a PCR reaction to check for the presence of the Epstein-Barr virus: PCR results from genomic DNA

Cancer and control samples with the help of EBV-specific primers showed that out of 04 breast cancer tissue samples, 04 cases (14%) and out of 04 tissue samples Healthy, one case (1.50%) was positive (Figure 0).

Figure 4: PCR results for some cancer tissue samples using Epstein-Barr virus gene specific primers. Well 7: 100bp molecular weight marker (fermentation), Well 4: Positive control (virus), Well 9: Negative control (water), Wells 2 to 77: Cancer samples containing EBV virus.

Statistical analysis: For statistical analysis, the data were analyzed according to the chi-square test of SPSS 16. Chi-square results Considering that the significance level (sig) is less than 441.4, the difference between the two groups is significant and the value of the correlation coefficient was 4.041 by Cramer's test and the value of the decision criterion was smaller than 41.4 Therefore, the null hypothesis is rejected, i.e. between breast cancer and... There is an important relationship between the Epstein-Barr virus

Table 1 : sequence of beta-actin gene primers and its characteristics

primer	Sequence 5-3	Size	junction temperature	GC Percent	PCR
Gone (Act F)	AGACGCAGG ATGGCATGGG	19	62\41	62\16	161
Retur (Act R)	GAGACCTTCAA CACCCCAGCC	21	62\93	61\90	161

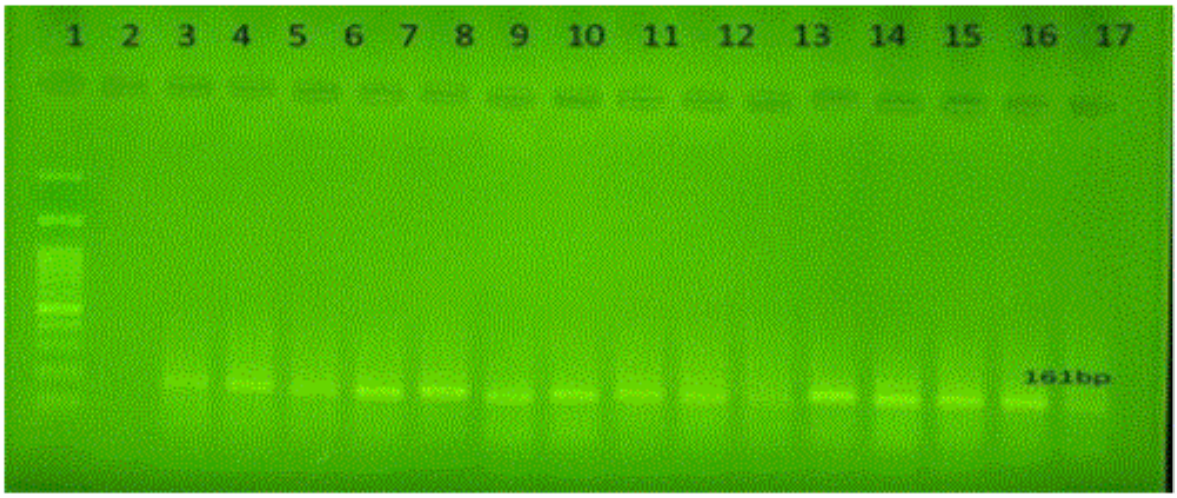
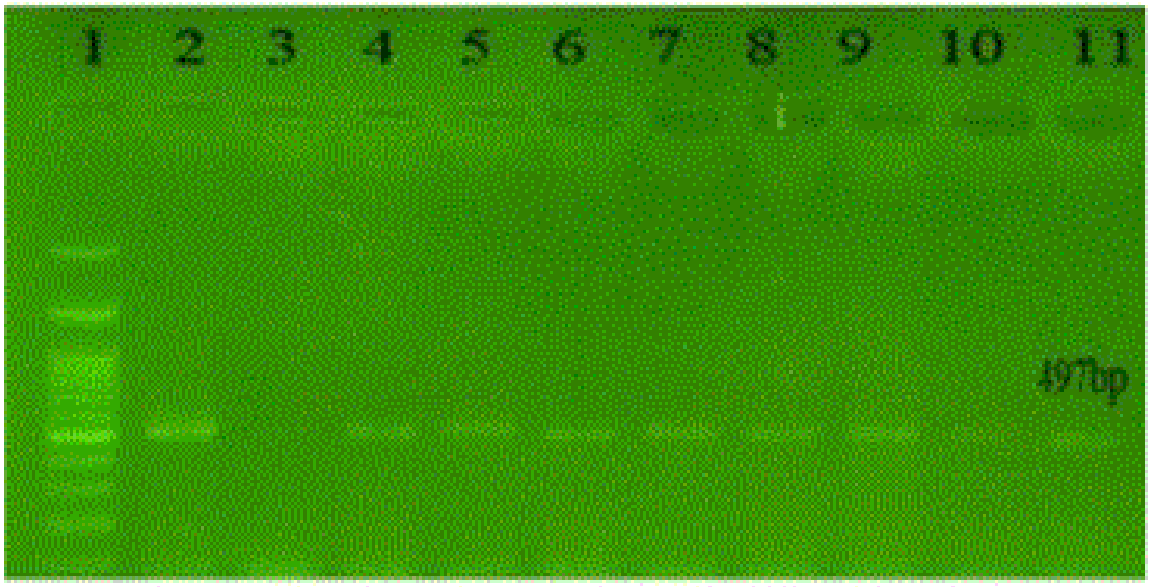


Figure 1: Electrophore model. PCR product of beta-actin gene in cancer and control samples. 100bp molecular weight well, well 2: negative control (water), wells 3 to 12, cancer samples, wells 13 to 17, control samples



Some PCR samples: the results of 4 forms of cancer tissue using: 7 markers of Epstein-Barr virus gene specific primer. Wells: positive control (virus), 4 (fermentase), 100 bp well, molecular weight: sample 77 to 2: negative control (water), well 9 cancer wells that had EBV virus.

Discusses

Despite recent scientific advances, cancer remains the second leading cause of death after cardiovascular disease. Currently, one-third of all cancers affecting women are breast cancer. Breast cancer, after lung cancer, is the leading cause of cancer-related deaths in women and is the cause of 59% of cancer-related deaths in women (01). Breast cancer is a multifactorial and potentially fatal disease, with many environmental factors playing a role, in addition to genetic factors, keeping in mind that genetic factors and common risk factors only affect a small percentage of patients. The need to address other risk factors has been identified There is no escape from it (50). One of the risk factors in Among other factors causing this cancer, we can mention cancer-causing viruses. These are viruses that have the ability to change the shape of cells and thus lead to uncontrolled proliferation of target cells. This high prevalence causes a tumor or cancer. The possibility that viruses play a role in the development of breast cancer was first proposed in 1930 by John Bittner and colleagues. They observed that an unknown agent in mouse milk caused mammary tumors in developing children, and this unknown agent was later identified as mouse mammary tumor virus (MMTV) (57). In 1991, for the first time, the presence of EBV DNA in breast cancer was reported by Labrecque and colleagues (04). Then several years later, in 1999, Bonnett and others announced that the Epstein-Barr virus could play a role as a contributing factor in the development of various malignancies such as several types of cancer. In this study, the presence of the EBV virus in breast cancer was verified by PCR, and it was found that the EBV gene is present in 15% of tumors, while the virus was not detected in 94% of healthy people, as well as by Southern blot. Analysis of the presence of EBV genome in confirmed breast tumors (05). In 2004, using immunohistochemical techniques and examining the level of antibodies against Epstein-Barr virus nuclear antigen (EBNA-1), conducted by Deepti Joshi and colleagues on rural Indian women with breast cancer, they showed that out of 11 cases in samples of patients with malignant breast cancer and 63 cases associated with benign breast cancer As a control group, the level of antibodies in the samples of malignant patients was significantly higher compared to the control group. These patients also

showed a stronger immune response to 1-EBNA(00). In 2015, Mazzone and colleagues investigated the recurrence of EBV infection in 596 patients with breast cancer using RT-PCR. EBV DNA in 61 cases (0.33%) He was. According to the results of this test, the patients who attended They were Epstein-Barr virus positive, and their tumor samples had a more aggressive phenotype reported in previous studies. The most interesting thing is that most of these patients were estrogen receptor negative and the expression of thymidine kinase activity was higher in the virus-infected samples. EBV(03). In 2004, a study conducted using immunohistochemistry, in situ hybridization, and PCR techniques on 4 Egyptian women and 14 Iraqi women with breast cancer (two different nationalities) by Al-Zarki and colleagues showed that EBV was present in 10% of Egyptian women and 14% of Egyptian women. Of Iraqi women, only 0.4% of the control group contained EBV. As a result, they reported that there is a statistically significant relationship between the presence of EBV and breast cancer (00). In a study conducted by Jung Myun Bae and colleagues in Korea in 2016, a total of 04 case-control studies were selected, and the number of people in the case and control groups was 5907 and 5454, respectively. The results of this meta-analysis showed that EBV infection can increase the risk of breast cancer (01). In some studies, it may not be possible to conclude a statistically significant relationship between breast cancer and EBV, such as the study by Herrmann and Nedobetek in 2004 (06). Or the study conducted by Tarabizadeh et al.

The presence of this virus was greater in cancer samples (07). As mentioned, during the past decade, the relationship of EBV to breast cancer has been continually debated despite the persistent presence of EBV genetic material in 15% of breast cancer tumors. These discrepancies are due to the failure of some researchers to identify EBV in cancer (56). This disagreement may also arise from Differences in methods used to detect the virus Differences in the analysis of proteins from EBV or be nucleic acid (04)

Conclusion

In this study, our results showed the presence of the EBV gene in subtype

It showed a large group of breast cancer cases in women in Dhi Qar Governorate. This is consistent with studies that reinforce the relationship between viral infection and breast cancer, so EBV may play a role in the development of breast cancer. Given new approaches in treating EBV-associated malignancies, it is hoped that a significant proportion of invasive breast cancer will be treated with antiviral drugs. Our findings show that Epstein-Barr virus could be one of the causes of breast cancer. Based on our research, among 04 cancer samples, mostly associated with ductal carcinoma, 04 cases were positive for the diagnosis of EBV (14%), that is, if only five positive cases were reported out of 04 healthy samples. These results suggest that EBV may play a role in carcinogenesis. But more studies are definitely needed to determine its mechanism in the pathogenic process. Or it may seem that viruses can play a role in the development of tumors, or that in cases of breast cancer in which the estrogen receptor is negative, they are the cause of this tumor. On the other hand, for people of different nationalities, different statistics show that the genetic background of people in... Exposure to this virus can also be considered one of the influencing factors, so it is suggested that the virus is present at different stages of pathogenesis, in certain types of breast tumors, and in different nationalities with different backgrounds. Genetically different, further investigations should be conducted.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin 2016; 66(1):730.
2. Wu Y, Sun W, Feng J. Antiangiogenic therapy in the management of breast cancer. Asia Pac J Clin Oncol 2013; 9(2):110-6.
1. Arshive.
2. Otaghvar HA, Hosseini M, Tizmaghz A, Shabestanipour G, Noori H. A review on metastatic breast cancer in Iran. Asian Pac J Trop Biomed 2015; 5(6):429-33.
3. Hippocrate A, Oussaief L, Joab I. Possible role of EBV in breast cancer and other unusually EBV-associated cancers. Cancer Lett 2011; 305(2):144-9.

4. Chu PG, Chang KL, Chen YY, Chen WG, Weiss LM. No significant association of Epstein-Barr virus infection with invasive breast carcinoma. AmJPathol [Internet]. 2001;159:571–8.
5. Alibek K, Kakpenova A, Mussabekova A, Sypabekova M, Karatayeva N. Role of viruses in the development of breast cancer. Infect Agent Cancer 2013; 8(1):32.
6. Key TJ, Verkasalo PK, Banks E. Reviews Epidemiology of breast cancer 1865; 44(0):133-40.
7. Bonnet M, Guinebretiere JJ-M, Kremmer E, Grunewald V, Benhamou E, Contesso G, et al. Detection of Epstein-Barr Virus in Invasive Breast Cancers. JNCI J Natl Cancer Inst 1999; 91(16):1376-81.
8. Wang T, Chang P, Wang L, Yao Q, Guo W, Chen J, et al. The role of human papillomavirus infection in breast cancer. Med Oncol 2012; 29(1):48-55.
9. Joshi D, Buehring GC. Are viruses associated with human breast cancer? Scrutinizing the molecular evidence. Breast Cancer Res Treat. 2012;135(1):1– 15.
10. Marrão G, Habib M, Paiva A, Bicout D, Fallecker C, Franco S, et al. Epstein-Barr virus infection and clinical outcome in breast cancer patients correlate with immune cell TNF-α/IFN-γ response. BMC Cancer 2014; 14(1):665.
11. Ribeiro-Silva A, Ramalho LNZ, Garcia SB, Zucoloto S. Does the correlation between EBNA-1 and p63 expression in breast carcinomas provide a clue to tumorigenesis in Epstein-Barr virus-related breast malignancies? Brazilian J Med Biol Res 2004; 37(1):89-95.
12. Glaser SL, Hsu JL, Gulley ML. EpsteinBarr virus and breast cancer: state of the evidence for viral carcinogenesis. Cancer.
13. Epidemiol Prev Biomarkers 2004; 13(5): 688-97.
14. Fawzy S, Sallam M, Mohammad Awad N. Detection of Epstein-Barr virus in breast carcinoma in Egyptian women. Clin Biochem 2008; 41(7-8):486-92.
15. Arbach H, Viglasky V, Lefeu F, Guinebretière J-M, Ramirez V, Bride N, et al. Epstein-Barr virus (EBV) genome and expression in breast cancer tissue: effect of EBV infection of breast cancer cells on resistance to paclitaxel (Taxol). J Virol 2006; 80(2):845-53.
16. Murray PG. Epstein–Barr virus in breast cancer: artefact or aetiological agent? J Pathol 2006; 209(4):427-9.
17. Lawson JS, Heng B. Viruses and breast cancer. Cancers (Basel) 2010; 2(2):752-72.
18. Herrmann K, Niedobitek G. Epstein– Barr virus-associated carcinomas: facts and fiction. J Pathol 2003; 199(2):140-5.
19. Fadavi P, Rostamian M, Arashkia A, Shafaghi B, Niknam HM. Epstein-barr virus may not be associated with breast cancer in Iranian patients. Oncol Discov 2013; 1(1):3.
20. Labrecque LG, Barnes DM, Fentiman IS, Griffin BE. Epstein-Barr virus in epithelial cell tumors: a breast cancer study. Cancer Res 1995; 55(1):39-45.
21. Bonnet M, Guinebretiere J-M, Kremmer E, Grunewald V, Benhamou E, Contesso G, et al. Detection of Epstein-Barr virus in invasive breast cancers. J Natl Cancer Inst 1999; 91(16):1376-81.
22. Joshi D, Quadri M, Gangane N, Joshi R, Gangane N. Association of Epstein Barr virus infection (EBV) with breast cancer in rural Indian women. PLoS One 2009; 4(12):e8180.
23. Mazouni C, Fina F, Romain S, Ouafik L, Bonnier P, Brandone JM, et al. EpsteinBarr virus as a marker of biological aggressiveness in breast cancer. Br J Cancer 2011; 104(2):332.
24. Zekri A-RN, Bahnassy AA, Mohamed WS, El-Kassem FA, El-Khalidi SJ, Hafez MM, et al. Epstein-Barr virus and breast cancer: epidemiological and molecular