



## **A STUDY AIMED AT THE RELATIONSHIP BETWEEN BREAST CANCER AND THE EPSTEIN-BARR VIRUS**

Alaa Aodhaib Lahmood

College of Health and Medical Technologies,  
National University for Science and Technology, Thi-Qar-IRAQ  
alaaod90@gmail.com

Noor K. Abed Al-Abadi

College of Health and Medical Technologies,  
National University for Science and Technology, Thi-Qar-IRAQ  
noorkadhimabed@gmail.com

Ameer Qusay Abbas

Al-Furat Al-Awsat Technical University,  
Technical Institute, Babylon, Iraq  
Ameer.abbas.iba1@atu.edu.iq

### **Abstract**

#### **Background and purpose:**

Breast cancer is one of the most common malignancies in women in the world. Early diagnosis of this cancer It is the key factor for its treatment. Breast cancer is a multistage disease in which the virus can play a role slow. EBV is known as an important factor in causing human cancers. This study aims to determine the relationship between EBV And breast cancer was done.

#### **Materials and methods:**

67 paraffin blocks from breast cancer tissue and 53 paraffin blocks from non-cancerous breast tissue (26 samples fibroadenoma and 27 fibrocystic samples) were collected as controls. After extracting DNA with a kit (Roche company), All the samples were checked for the presence of betaglobin gene and suitable samples were checked for the presence of EBV-DNA by the method PCR (Polymerase Chain Reaction) were evaluated and the data were analyzed with statistical tests.



### Results :

Out of 67 breast cancer tissue samples, 2 cases were negative for beta globin gene and were discarded. Other examples of 65 breast cancer samples, 23 cases (38.35 percent) and 11 cases (75.20 percent) of 53 control samples (7 cases of fibroadenoma (92.26%) and fibrocystic 4 cases (81.14%) were positive for EBV-DNA. A total of 118 samples were examined 34 cases (81.28 percent) were observed to be positive, statistically speaking, Kramer's index for EBV infection in cancer samples and The witness was 0.46, which indicates a significant relationship ( $p = 0.01$ ).

**Conclusion** We noticed that there is an important relationship between EBV and breast cancer. In my opinion, there is evidence of a possible connection and this is what our study aims to do.

### Introduction

Currently, cancer after heart diseases- Vascular is the second cause of death is (1). Breast cancer is the second leading cause of death Cancers are considered to be approximately one out of every eight women. She is suffering from breast cancer, which often leads to Complete removal of breast tissue, chemotherapy, radiation treatment and hormone therapy (2). breast cancer It is a type of cancer that arises from breast tissue. Mainly the inner layer of milk glands or lobules (ducts for It involves milk transfer (3). Primary risk factors To find cancer, including age (4), infertility or Lactation (5), high level of hormones (6, 7), race, Economic situation and iodine deficiency in the diet report has been (8-10). Researchers seek to identify others Paths that cause disease. Breast cancer is a multistage disease Viruses can develop in one of these several stages have a role in disease (11). Viruses in The spread of different types of cancers play a role (12). Recently, the theory of viral etiology regarding physiopathology Breast cancer has been proposed. In addition to theories in The case of cancer induction by several infectious agents with Suppression of the immune system by transfer from animal to Human or direct or indirect effect on oncogenes has been (13). The most important DNA viruses that are Oncogenics have been identified in humans, including viruses Hepatitis B, papillomaviruses and EBV are (11). EBV is one of the eight human herpes viruses The first time by Epstein and Barr in 1964 It was identified. The researchers identified it as the cause of lymphoma It was common in certain parts of East Africa. they knew Clinical symptoms of this lymphoma by Dennis Burkitt stated (14). In addition to Burkitt's lymphoma





Africans in AIDS-related lymphoma and Hodgkin's lymphoma It was also identified in transplant patients. This is the DNA of the virus Oncogenic, related to other malignancies such as lung cancer. Stomach cancer (11) Cancers similar to telial lymphoma in the glands Salivary and thymus (16,15) cell-T lymphoma is also known. has been (16). Epstein Barovirus was found in Shiransan. Cells can grow after EBV-DNA transfer Shiri stimulates the human body from the other side of the cells Normal breast epithelial also with direct contact with this virus. They get infected (17). Apparently, oncogenic mechanisms EBV in lymphatic cells (leads to lymphoma) and epithelial cells (which lead to nasopharyngeal cancer and other cancers) are different from each other (17).

More than 90% of the world's young population is affected by this virus They are contaminated. Infected people for a long time of life They themselves are carriers of this virus. EBV through contact Transmitted saliva, during acute infection, epithelium Corresponding paving pollutes the larynx, but later on during the latent infection inside the B lymphocytes takes (18). The relationship between EBV and breast cancer It is under discussion. Some reports of EBV prevalence in Breast cancer is caused by radiation from 21-51% while other researchers are able to identify it in There were no breast cancer tissue samples (18). Communication The etiology between EBV and the disease has not been determined yet and its role in changing the shape of cells is unknown It remains. In this study, the identification of the virus in Breast cancer samples and normal breast tissue samples It has been done by PCR method to determine whether The role of viruses in the etiology of breast cancer in Iraqi patients Is there or not? so that if the results are positive, you can use it It is also used for prevention, diagnosis and treatment.

### **Materials and methods**

Sample preparation and DNA extraction 67 paraffin block samples for breast cancer and 53 biopsy samples Paraffin block of non-cancerous breast tissue (fibroadenoma and Fibrocystic) witnessed from Al-Hussein Al-Alimi Hospitals and 13 from Bint Al-Huda Hospital in Nasiriyah Non-cancerous samples were linked to individuals Who are not affected by any type of malignant tumor anywhere in the body. The selected blocks relate to the years 2020 There were 9 suns and the average age of the operated people They were 48 years old (minimum 33 years and maximum 56 years). The age range of the control subjects was also selected, with cancer cases It was similar. Using a microtome device, many slices 10 micron thin, 4 blocks Paraffin was prepared in 1.5 ml tubes Eppendorf was cast without RNase/DNase. for every A sample of disposable

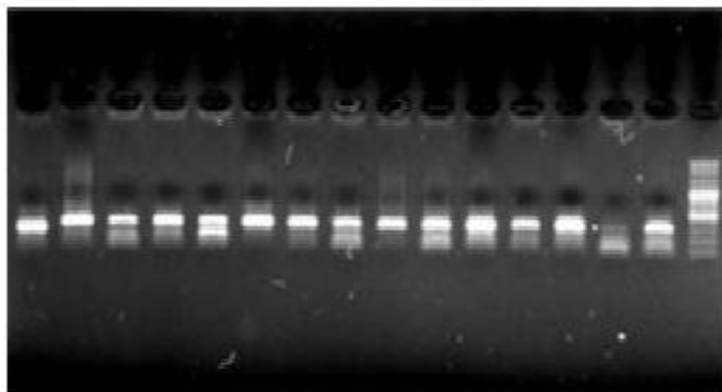




gloves, a new razor and applicator was used. After deparaffinizing with Xylene and Absolute ethanol, DNA using the company's extraction kit According to the agenda provided by the company, 1 Roche It was extracted. The extraction method was as follows First, deparaffinized tissues in 100 microliters of lysing buffer tissue, 16  $\mu$ l SDS 10% and 40  $\mu$ l Proteinase K was incubated at 55 degrees Celsius until The tissue should be completely lysed. After adding 325 microliters Binding Buffer and 325  $\mu$ l absolute ethanol, mixed He was enslaved on a pillar. Then wash it three times Removal of excess material was done with the help of washing buffer. DNA attached to the column with 50 microliters of eluted buffer Washing was done and the time to do PCR was frozen It was kept at -20 degrees Celsius. After DNA extraction, all samples with Primer 4PCO 20, GH betaglobin gene with combinations and F:5' - GCTCGGTGCCTTTAGTGATGG-3'

R:5' -CGATCCTGAGACTTCCACACTG-3' PCR. were cut to the length of 250 pairs of zinc The gel can be observed. Suitable samples for multiplication PCR was determined on betaglobin amplification. increase

The parts of this gene are suitable for the reaction environment PCR showed. To do the reaction, mixed 20 microliters was prepared for each sample. First, in the temperature They were heated at 95 degrees Celsius for 5 minutes and then for 45 cycles under the PCR process, including the destruction of 95 degrees Celsius for 30 seconds, connecting the primers in 66 degrees Celsius for 40 seconds and elongation of the piece at a temperature of 72 degrees centigrade for 40 seconds They took then at the end for a cycle also in the temperature They were set at 72 degrees Celsius for 5 minutes. PCR products for confirmation, on agarose gel 1.5% were electrophoresed to a band of 250 pairs. can be seen (picture number 1).



**(Figure 1:** Because of excessive beta-globin in this cancerous system .And watch: Chahak 1 ta 10 Namoura hi cancer, Chahak 11 ta 15 This growth is non-cancerous (see), with 16 markers)





Injam and Akneesh PCR for EBV Tamami Namonhay with stabilizing taglobin, in my opinion DNA-EBV multiplication. In Harmona's opinion, please Microtube 0/2 ml, 12/5 µl (Ampliqon, Denmark) company (Master mix 1 micro-litre of this liquid primer. And µg1DNA extraction has been extracted from it Distilled final volume, containing 20 microliters of resin Tighten and tighten, reassure yourself that it is secure with six pushes. Its tube, it is in the device of the thermocycler of the decision of Gravtend. Increase cracking with a specialized EBV primer

F: 5' -AGT CTG GGA AGA CAA CCA CA-3')

(ACA CCA ACT AT-3' & R: 5' -CCC GCC TAC

Comprehensive, initial sabotage stage at 94 degrees Santegrade For a minute and 40 cracks, including destroying the ring in the car Damai 94 degrees Santigrade Bray 30 seconds, Chespeden Primer at 60 degree Sanitary Gradient for 30 seconds and Long turn, 72 degrees centigrade, for 30 seconds and then turn. It's over again, at 72 degrees, 5 minutes Baqi memandand ta atmanan get shud ke ke complete crop It's been a long time. Specifications of PCR products in Roy Jal Agars 1/5 drops

210 bp Roy all Ashkar Jardad. Complete the stages of Bray Shanasayi

It is possible that the control is installed (and the virus is very severe).

Lymphoblastoid response (and exiled control) (August Bhagai DNA

) Used Shad. 5 growing buffet crab orchard 23 suppliers (35/38 pounds) and Az 53 Names Watch 11 Resources (20/75 Demos) Sticky Shaddand.

Please see what you have learned about fibroadenoma and fibrocystic Dashtend decision. 26 Namunah Vibro Adunuma Uri plural Shaddah Bud

Here is the number of 7 resources (26/92 lessons) installed. 27 cm Fibrocystic Niz 4 Suppliers (14/81 drops) Contains EBV-DNA body. Make a sum of all this growth 118

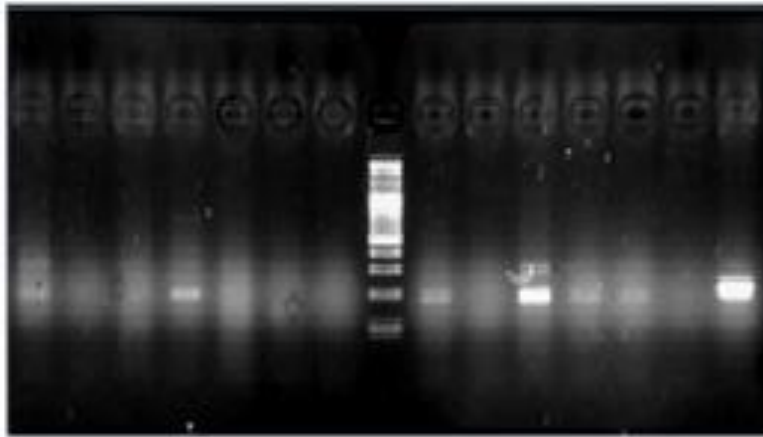
watts per 34 hours (28/81) DrsD) with a Roche PCR fixative. Photography session 2

The enumeration of the number is fixed, and the negative in the canar control is fixed (The virus is very severe due to its lymphoblastoid response) And negative control (az

aab bhajay dna using shad) Because of Dada Shadhand. Hemgouni 2 1 Pray Annaliese Amari Dadha, Az Azmoun X Take advantage and be assured that the 99 lessons are

personalized and clear. Virus Epstein Bar and cancer garden link to the presence of Dard.





**Figure: 2** In order to obtain the results obtained from his growth census, verified and Menfi Bimaran cancerous and witness: Az Ta:6 witness: (Shamara 1 and 4 are affirmative and the rest are negative), jahak: 7 nmuna as a negative syllable, Jahk 8: Marker, Az Jahk 9 Ta 14 Namouna Bimaran Canceri (Shamara) 9, 11, 12, 13 are affirmed and the rest are negative.) Jahak 15: Namuna Control is affirmed. (DNA to get rid of viruses)

### **Discuss:**

It is possible that viruses can play a role in the etiology Breast cancer has played a role for the first time. Bittner and colleagues (1936) proposed (19). They are in They found an unknown factor that could She developed breast cancer as an adult. This Unknown factor later mouse mammary tumor virus (17) Get the name (Mouse mammary tumor virus) The role of viruses in breast cancer since its discovery The virus was brought up, but the genome sequence of the viruses in Humans were found, it did not show that they could directly enter have a carcinogenic role (12). In a research by Speck and colleagues (2003) was conducted on 22 categories. A cell obtained from breast cancer has the presence of EBV The PCR method was investigated, but none of them No viral DNA was found in the samples (20). They are a negative reason They justified their results in such a way that the virus He can participate in oncogenesis, then there To leave means to operate with the Run and Hit mechanism. Observation of EBV in the form of stable episome and virus reduction In transformed cell lines without the phenotype The change in their shape will decrease, this is an indication It is the role of EBV in many tumor viruses The negative cannot be ignored (21,20). In the study by Sherman and colleagues, 1 case out of 115 The studied case of breast cancer was positive for EBV. This finding has been reported with up to 48% positive results There was a contradiction. They are two reasons for the high results Positively, others suggested that there is a hidden contamination The lymphocytes were EBV because



PCR could not detect it Differentiating neoplastic cells and infected lymphocytes and the other is to use high multiplication cycles to the limit It was 80 cycles that led to positive results.

It becomes more (22). In total, certain evidence of connection EBV and breast cancer by identifying EBV genes Breast cancer has been obtained. About 27 studies It has been done on EBV and breast cancer, which has 22 cases It was based on PCR analysis. EBV sequences in 18 The case of these studies is known. 5 cases of this Studies based on IHC immunohistochemistry and techniques situ hybridization of ISH that EBV in none It has not been identified in these studies. Only in three studies

Use normal female breast tissue as a control EBV was not detected in two of these studies In one of them, 23% in cancer samples 35% of the control samples have been diagnosed. From Where the evaluation of the virus is based on techniques Identification of viral proteins is difficult for two reasons Low virus and that EBV in latent state is minimal expression

It has protein, it is difficult (17), so we use the method We used PCR. The results obtained from this research, Similar to the results obtained by other researchers, e.g Mazouni and colleagues (2011) who (2.33 percent of cases

positively identified) (23) Perkins and colleagues (2006) (46% reported positive (24), Bonnet et al (1999) (positively identified in 51% of tumors)

and even in another report by Arbach and (2006) presented colleagues, EBV genome copy number It has been calculated in breast cancer samples (16,15). Our findings show that Epstein is probably infected with the virus can be considered as one of the existing pollution factors Breast tissues were taken into consideration. More reviews Epidemiological, biological and molecular studies are necessary The intervention mechanism of this virus in the cancer process Be turned on. Due to the increase in the number of related diseases EBV infection, production of vaccine against this virus

It becomes very important to avoid diseases in this way It prevented malignancies related to this virus.

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