

# Relation of Epstein Barr virus with Interleukin-6 Level among Women with Breast Cancer in Ramadi City

Arkan Abdullah Abbas<sup>1</sup>, Israa Hashim Saadoon<sup>1</sup>

<sup>1</sup>Department of Microbiology, College of Medicine, Tikrit University, Tikrit, Iraq

## Abstract

The study aimed at evaluating the relation of Epstein Barr virus (EBV) with level of interleukin-6 (IL-6) in women with breast cancer. The study was carried out in Ramadi city from 12th of January to 12th of September 2018, and included a total of 95 breast cancer women who admitted to oncology clinic of Ramadi Teaching Hospital whose ages were between 25-70 years. Patients were investigated for detection of EBV by using Real Time PCR and interleukin-6 (IL-6) by ELISA technique. The rate of breast cancer women with EBV was 29.47%. The highest rate of breast cancer women was within the age group 40-49 years with no significant relation between breast cancer and age. The study showed that the highest means of IL-6 level (151.50 pg/ml) were found in breast cancer women without EBV. The highest rate of breast cancer women was from rural areas.

**Keywords:** Breast cancer, EBV, IL-6, Ramadi.

## Introduction

Breast Cancer (BC) is one of the most important neoplasia among women. It was recently suggested that biological agents could be the etiological cause (1). Epstein-Barr virus (EBV) is a ubiquitous in that infecting more than 90% of adult population worldwide. Epstein-Barr virus has been linked to the development of variety of human malignancies. Epstein-Barr virus and human papilloma virus (HPV), which are DNA viruses, were reported to be linked with 38% of all virus-associated cancers (2). A viral etiology for several malignancies has been suggested. One of the risk factors for development of breast carcinoma, which is the leading malignancy in women all over the world, is proposed to be a viral infection; hence recognition of the causative issues is essential for proper management (3).

Elevated interleukin 6 (IL-6) and interleukin 10 (IL-10) serum concentration, are strongly associated with breast cancer and correlate with clinical stage of disease. Interleukins may stimulate cancer cells growth and contribute to locoregional relapse as well as metastasis (4). Interleukin-6 plays an important role in the process of inflammation, particularly in the transition from acute to chronic inflammation. In breast cancer, IL-6 has been shown to inhibit the growth of cancer cells but promote the development of metastases (5,6).

## Material and Method

Across-sectional study was carried out in Ramadi city from 12th of January to 12th of September 2018, and included 95 women with breast cancer whose ages were between 25-70 years old. These patients admitted to oncology clinic of Ramadi Teaching Hospital.

Blood samples were taken from breast cancer women. Samples were examined by immunological methods, enzyme linked immuno sorbent assay (ELISA) for detection interleukin-6 (IL-6) and molecular technique (Real time PCR) which included DNA amplification of Epstein Barr virus based on the specific primers.

Seven and half ml of blood was collected by vein puncture using vacutainer tubes from each patient enrolled in this study. Blood samples were placed into two sterile test tubes, in one of them 2.5 ml of blood was put in test tube containing anticoagulant ethylene diamine tetra acetic acid (EDTA) and used for DNA extraction of EBV. The second part of sample (5ml) was placed in plain tubes left for 30 minutes at 37 °C then was centrifuged at 3000 round per minute (rpm) for 15 minutes then the clot was removed and the remain re-centrifuged at 3000 rpm for 10 min and the obtained sera were then aspirated using automatic micropipette and transferred into two clean test tubes, for serological

tests. Label was fixed on each test tube which then stored in deep freeze at -20°C for late serological testing for determination the level of IL-6 by using ELISA technique.

For DNA extraction, kit was purchased from Gene Aid(USA) company for molecular detection of EBV by Real Time PCR using Anatolia Gene Works(Turkey). Detection of IL-6 was done by using ELISA kit Elabscience (China), which depends on the Sandwich-ELISA principle.

### Statistical Analysis

Computerized statistically analysis was performed using T-Test probability. The P value >0.05 was

considered statistically significant, and for result which its P value was less than 0.01 was considered highly significant, while for those which its P value greater than 0.05 was considered statistically non-significant.

### Finding

A total of 95 breast cancer women, their age ranged between 20-89 years old, were investigated for detection of EBV by using real time PCR and estimation the level of IL-6. The present study revealed that EBV was found in 29.47% of women with breast cancer, as shown in Table 1.

**Table 1: Frequency of EBV in women with breast cancer.**

EBV	Breast Cancer Women	
	No.	%
Positive	28	29.47
Negative	67	70.53
Total	95	100

The current study showed that the highest rate of EBV infections (12.63 %) was found in women with breast cancer within the age group 40-49 years, as shown in Table 2.

**Table 2: Distribution of EBV infection in women with breast cancer according to their age.**

Age Group (Years)	No. of Women with Breast Cancer Patients	EBV Infection	
		No.	%
20-29 (No:0)	2	0	0
30-39 (No:3)	19	5	5.26
40-49 (No:15)	43	12	12.63
50-59 (No:38)	23	9	9.47
60-69 (No:27)	5	1	1.05
70-79 (No:6)	1	0	0
80-89 (No:6)	2	1	1.06
Total (No:95)		28	29.47

The present study revealed that there was a negative correlation between EBV infection and age of women with breast cancer, but the difference was statistically non-significant (R value: -0.132), as shown in Figure 1.

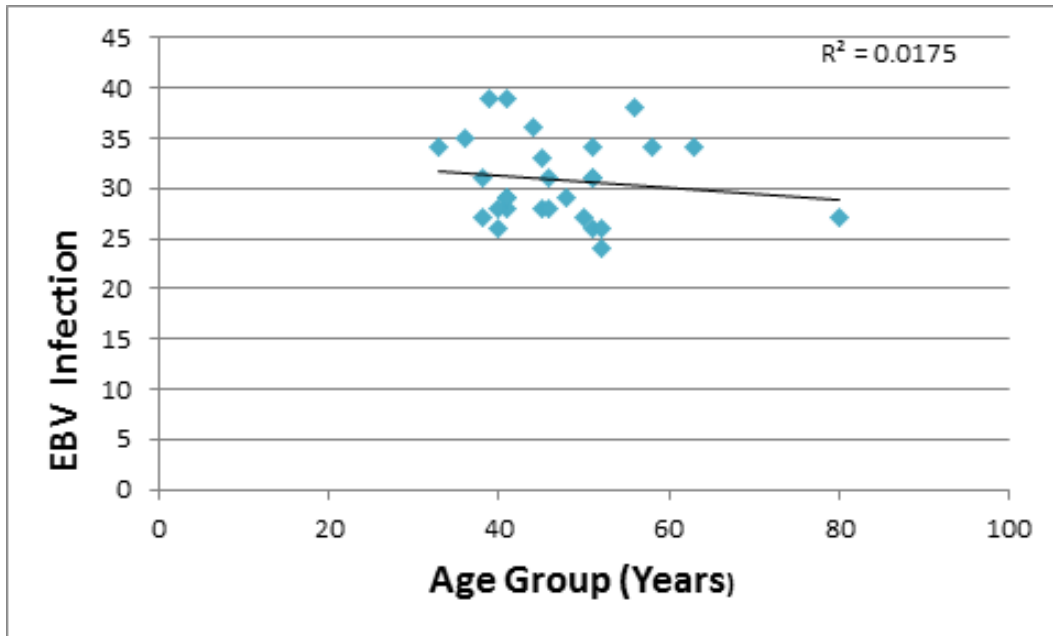


Figure 1: Negative correlation between EBV infection and age of women with breast cancer.

The present study revealed that the highest rate of women with breast cancer and EBV infection was from rural areas, as shown in Table 3.

Table 3: Distribution of EBV infection according to residence of women with breast cancer.

Residence	No. of Patients	EBV Infection	
		No.	%
Rural	51	14	14.73
Urban	44	14	14.74
Total	95	28	29.47

The present study revealed that the mean of IL-6 was higher in women with breast cancer without EBV infection as compared with those infected with EBV, as shown in Table 4.

Table 4: Relation of EBV infection with level of IL-6 among women with breast cancer.

EBV Infection	No. of Women with Breast Cancer	IL-6		P value
		Mean	SD	
+ve	28	76.46	±70.71	0.74
-ve	67	81.61	±62.69	

The present study revealed that there was a positive correlation between EBV infection with IL-6 among women with breast cancer but the difference was statistically non-significant between them (R value: 0.084).. Figure 2.

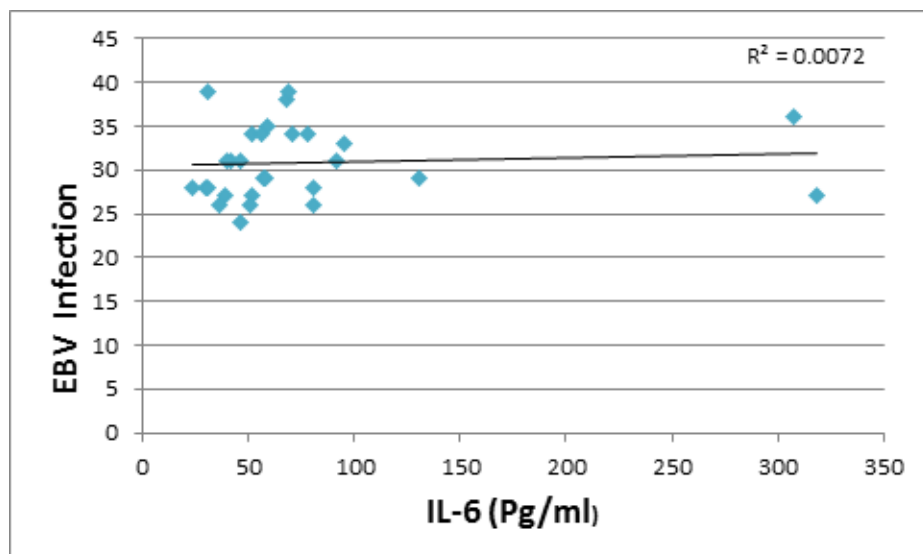


Figure 2: Positive correlation between EBV infection with IL-6 among women with breast cancer.

### Discussion

Breast cancer is a public malignancy and a major cause of morbidity in women. The present study revealed that EBV was detected in 29.47% of women with breast cancer. Numerous studies have reported a relationship between EBV and breast cancer. Zekri et al (7) revealed that EBV was found in 28% of Iraqi women with breast cancer and 45% of Egyptian. Lorenzetti et al (8) indicated that 31% was positive for EBV and in agreement with 31% to 52% EBV association which described in several geographic locations (9,10,11,12,13,14,15). Preciado et al (10) revealed that EBV was detected in 31% of tumor samples analyzed. No EBNA-1 labeling was obtained from samples without tumor. Fawzi et al (11), reported that EBV-DNA has been detected in 20% of breast cancer samples, using PCR targeting sequences specific for the Bam HI-W region of EB and refutes all studies that deny the relationship between breast cancer and Epstein-Barr virus infection (16,17).

The current study showed that the highest rate of EBV infections (12.63 %) was detected in those within the age group 40-49 years. Richardson et al (18), showed that the mean age of breast cancer women was 48 years with most in the 40-54 year age-group. It has been reported that the rate of EBV infection was higher in women less than 50 years than those older (19). According to American Cancer Society, age of the patient is an important factor both for the occurrence and management of the cancer with 95% of all new breast cancer cases occur in women aged 40 years (20). Al-Khafaji (21) revealed that the peak frequency was

recorded in the age of 50 years. The results reported that risk of breast cancer increases with age (22). Epstein – Barr virus [EBV] in Arab countries including Iraq was with high rate of infection (23). Epstein –Barr virus infection was linked with the development of breast cancer (24). However, other study was carried out in Taiwan detected EBV in 6.5% to 35.25% in breast cancer tissue (25). In addition, EBV detection rate in breast cancer tissue was the lowest in USA (18.27%) and the highest in Asia (35.25%) (26).

The current study revealed that the highest rate of women with breast cancer was from rural areas. The study of epidemiology of breast cancer in Indian women showed that the higher breast cancer is the major cause of morbidity and mortality among females ranking number one among females in Indian metropolitan cities like Delhi, Kolkatta, Pune and Thi'puram, Bangalore and Mumbai and in Northeast. Factors as marital status, location (urban/rural), BMI, breast feeding, waist to hip ratio, low parity, obesity, alcohol consumption, tobacco chewing, smoking, lack of exercise, diet and environmental factors were major risk factors in India leading to increasing incidence cancer. Activities play a role among urban and rural women in the development of breast cancer. The more time spent on household activities further reduced the breast cancer risk (27).

The present work revealed that the means of IL-6 level was higher in women with breast cancer without EBV infection than those with EBV infection. Cytokines may play a role in pathophysiology of neuropsychiatric of the immune and neuro-endocrine

system. Pro-inflammatory cytokines have been associated with depression in persons with cancer during treatment and fatigue in survivors of breast cancer (28,29). Victoret al(30) confirm the association of levels IL-6, and IL-10 in breast cancer patients with or without receiving chemotherapy. In breast cancer, high levels of the inflammatory cytokine IL-6 have been associated with disease survival and treatment resistance (31,32). A study on interleukin-6 gene promoter and influence of -174G/C polymorphism on breast cancer revealed that there was no association or trend of association between -174G/C polymorphism of IL-6 promoter gene and breast cancer diagnosis or prognosis(33).

### Conclusions

The study concluded EBV may play a role in the development of breast cancer and may be an etiology or it may induce immunosuppression that enhance the development of breast cancer. The mean age of breast cancer women was 54.5 years, The highest rate of breast cancer women was within the age group 40-49 years, with no significant relation among breast cancer patients and their age concerning EBV infection. There was a highest rate of rural than urban residence among breast cancer women, concerning EBV infection. The mean of IL-6 level was higher in women with breast cancer without EBV than those with EBV infection.

**Ethical Clearance:** taken from hospital and patients.

**Conflict of Interest:** Nil

**Source of Funding :** Nil

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