



Association of Epstein - Barr virus infection with Breast Cancer in Kirkuk city

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ABSTRACT

Breast cancer is one of the most common cancers in afflicted Iraqi women. All studies in recent years have indicated that Epstein - Barr virus (EBV) is associated with certain cancers, including breast cancer. The early detection of breast cancer is the best solution to control the disease. The purpose of this study is to reveal the relationship between EBV and breast cancer in Kirkuk city women. The study included the blood sampling of female with breast cancer in the ages of 23 to 88 years for the period from September 2018 to April 2019. The total number of samples was 100 blood samples for women with breast cancer and 10 blood samples of healthy women did not have any diseases in the breast as control to serological analysis was done using the ELISA device to detect EBNA-1IgM and EBNA-1 IgG in all sample. The results showed that (76%) of breast cancer women sample were positive for EBNA-1 IgG While IgM appeared negative in all samples, The highest incidence of EBNA-1 IgG in female with breast cancer in the current study was aged (56 to 65) years 22% .

Conclusions: Our findings suggest that EBV may have a role or a relationship with breast cancer.

Introduction

Cancer is one of the most important health problems in the world today, it is one of the five main causes of death, and it can start in any part of the human body, where cells are characterized by irregular divisions and multiplication [1]. Iraqi Ministry of Health / Iraqi Cancer Register for 2014 reported that the number of cases of breast cancer has been increasing since the 1991 war, and breast cancer is one of the most prevalent among women according to the recorded statistics [2]. Breast cancer arises from several factors, but the actual factor causing breast cancer is not determined, so epidemiological studies have done a study that identified various factors that have been associated with breast cancer risk factors [3]. These factors include environmental factors, family history of the disease, age progression, gene mutations, late menopause, certain cancer-related diseases, and treatment of hormonal therapies, infection with which viruses develop and arise from cancer [4]. The most important risk factors are viral factors which are considered infectious agents of humans and linked to the incidence of cancer in the body. Studies have

shown that the rate of 17-20% of cases of cancer is due to a viral infection[5]. In 1995, 21% of breast cancer samples were taken and EBV was detected in the samples after the discovery, several studies were conducted that showed EBV injury in women with breast cancer [6]. Human cytomegalovirus (HCMV) and Epstein -Barr virus (EBV) could potentially involve in breast cancer [7].

Materials and methods

Cases and Controls

Woman with breast cancer were recruited from those who attended the Kirkuk Specialist Center in Kirkuk from September 2018 to the end of April 2019. They ranged in age from 23 to 88 years. A total of 100 patients were included and 10 as control and verbal informed consent were taken from each woman in the study prior to enrollment. Data collected using a pre-defined questionnaire and information collected by direct interview for each participant

Serology

Enzyme linked immunosorbent assay technique of IgG and IgM antibodies against EBNA-1 were

performed on serum samples of cases and controls using the commercial ELISA kit (Demeditec GmbH, Germany). The test was performed using the manufacturer's instructions. Briefly, 100µl of standard dilute were pipetted in micro test wells and incubated for 60 min at 18-25°C at room temperatures. After subsequent washing, a 100 µl conjugated solution was added and incubated for 30 minutes at 18-25 °C at room temperature. After washing 100 µl the substrate solution was added and incubated again (20 min at 18-25 °C) in a dark place, the reaction was stopped by adding a 100 µl stopping solution and reading the extinction values at 450 nm. Determination of antibody activity was performed using cut-off value to compare the results of the patients, and measure the levels of IgG and IgM antibodies. Serums were examined for all samples in the public health laboratory.

Result

100 cases of female with breast cancer were listed in various stages in the Kirkuk city and 10 healthy women who did not suffer from breast diseases (controls) in the study. Using the manufacturer's identification (IgG, IgM) EBNA-1, controls was negative serum for IgM and IgG. The positive percentage of female with breast cancer for EBNA-1IgG was 76% and the negative percentage was 24%. No positive EBNA-1 IgM was observed for women with breast cancer, our results show that the expression EBNA-1IgG appears in a large proportion of breast cancer samples in ages (65-56), reaching 22% illustrated in Table (1) and figure (1).

Table 1: Distribution of Positive and Negative EBNA- 1 IgG Among Breast cancer Women According to Age Groups.

Age (year)	No. Breast Cancer	EBNA-1 IgG			
		Positive	%	Negative	%
23 - 35	10	7	7	3	3
36 - 45	25	19	19	6	6
46 - 55	25	15	15	10	10
56 - 65	25	22	22	3	3
66 - 75	6	6	6	0	0
76 - 88	9	7	7	2	2
Total	100	76	76	24	24

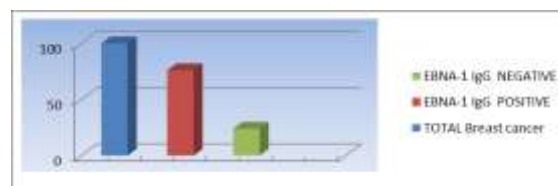


Fig. 1: Detection the positive and negative for EBNA-1 IgG among Breast Cancer Women, (Blue color shows the total number of cases of breast cancer, which are 100 samples, while the red color shows a positive number of EBNA-1 IgG for women with breast cancer and green color shows the number of cases of EBNA-1 IgG negative for women with breast cancer).

Regarding the stages of cancer, EBNA-1IgG showed a significant increase in the stageII of (33%) and in the stage III it reached (16%) and decreased in stage 0 (4%) illustrated in table 2. Our results showed that Breast Cancer cases the result was highly significant ($p < 0.05$).

Table 2: Distribution of Positive and Negative EBNA-1 IgG among Breast Cancer woman according to stage of cancer

Seroprevalence	Stages									
	stage 0		stage 1		stage II		stage III		Stage IV	
	No.	%	No.	%	No.	%	No.	%	No.	%
EBNA-1 IgG + Ve	1	1	16	16	33	33	16	16	10	10
EBNA-1 IgG - Ve	3	3	3	3	9	9	4	4	5	5
Total	4	4	19	19	42	42	20	20	15	15

Our results showed for risk factor according to the information obtained from for each patient including marital status, smoking and occupation showed that EBNA-1 IgG was prevalence in married with Breast Cancer with percentage of 56% while it was 20% in

unmarried women with Breast Cancer. The study showed that the marital status not demonstrated a significant effect of determined EBNA-1IgG, the result was highly significant ($p < 0.01$), showed Table3.

Table 3: Frequency Positive and Negative EBNA-1 IgG among Breast Cancer Women According to Marital Status.

Seroprevalence	Marital status							
	Married				Unmarried			
	Number of Breast Cancer cases	%	Number of Control cases	%	Number of Breast Cancer cases	%	Number of Control cases	%
EBNA-1IgG (Positive)	56	56	0	0	20	20	0	0
EBNA-1IgG (Negative)	21	21	6	6	3	3	4	4
Total	77	77	6	6	23	23	4	4

No relationship was notice between smooking and EBNA-1IgG in breast cancer women that non-smoker womens EBNA-1IgG percentage reach 70% it was higher than smoker women (6%). smokers was lower

than non-smokers then EBNA-1 was higher in non-smokers, the result was highly significant ($p < 0.05$), showed Table 4.

Table 4: Distribution of Positive and Negative EBNA-1 IgG among Breast Cancer Women According to the Smoking.

EBNA-1IgG	Smoking							
	Smokers				Non -smokers			
	Number of Breast Cancer cases	%	Number of Control cases	%	Number of Breast Cancer cases	%	Number of Control cases	%
Positive	6	6	0	0	70	70	0	0
Negative	3	3	0	0	21	21	10	10
Total	9	9	0	0	91	91	10	10

Regarding occupation, the results were 24% positive in EBNA-1IgG for working women with breast cancer and (52%) positive EBNA-1IgG for non –

working women with breast cancer, as shown in Table 5. The result was highly significant ($p < 0.05$).

Table 5: Frequency Positive and Negative EBNA-1 IgG among Breast Cancer Women According to Occupation.

EBNA-1IgG	Occupation							
	occupied				not- occupied			
	Number of Breast Cancer cases	%	Number of Control cases	%	Number of Breast Cancer cases	%	Number of Control cases	%
Positive	24	24	0	0	52	52	0	0
Negative	11	11	9	10	13	13	1	1
Total	35	35	9%	10	65	65	1	1

Discussion

One of the most common cancers among women in the world is breast cancer [8]. It is one of the most deadly cause in 11 regions of the world [9]. Several risk factors may be a cause of breast cancer, of these factors viral infection [10]. One of the most common types of virus spread in the world Epstein Barr virus (EBV) [11]. Epstein Barr virus which can be a cause of breast cancer [12, 13].

This study was conducted on samples taken from women with breast cancer from the Center of Oncology in Kirkuk city. The total number of blood samples of patients with breast cancer was 100 samples and 10 samples were from healthy women. In the current study, ELISA technique was used to detect (EBNA-1 IgM and EBNA-1 IgG), which showed that 76% of blood samples for breast cancer patients had an EBNA-1 IgG was positive. Our finding agree's [14] reported that 13 out of 22 (40 %) with breast cancer cases were positive for EBV. In another study designed by [15] they showed that Epstein–Barr virus is present in 56 (51.85%) of breast cancer samples in Syrian women. There are also studies that are disagreeing with our study. In a study carried out by [16] and [17], in a samples taken from female with breast cancer in Iran, these studies showed that EBV infection in Iranian women with breast cancer may not plays an important role as causes of breast cancer.

Table (1) the rate of EBNA-1 IgG and EBNA-1 IgM seroprevalence among 10 control group and 100 samples breast cancer woman's by ELISA technique,

and the effect of age on female with breast cancer was as follows: The higher positive EBNA -1 IgG in breast cancer woman was among age group 56 - 65 years (22 %), secondly was age group (36 - 45 years) 19 (19%), the other age groups came as, 46 - 55 years (15 %) and (23 - 35 years) (76 - 88 years) 7 (7%). This finding agrees with a study done by Mezher and his coworker (2017) [14] whom found that the infection with EBV among Breast cancer women was 66.6% among age group (59 years). Also the lower percent of EBV infection found in the breast cancer women in age (66 - 75 years) 6%

Table (2) the rate of IgG of EBNA-1 seropositive was the highest in the stage II, which showed 33% the women infected with the EBNA-1 IgG, women followed by the III stage, which showed 16% women infected with the EBNA-1 IgG, followed by the I stage the rate of infection with the EBNA-1 IgG was 19%, while the women with breast and the IV stage, the rate of infection with the EBNA-1 IgG was 10%, the last stage is (0), which was the rate of EBNA-1 IgG infection was 1%.

The study showed that breast cancer cases, it was in the advanced stages that mean delayed diagnosis of breast cancer, This finding agrees with a study done by Alwan and his coworker [18] whom found that the cause of breast cancer progression is due to the fact that the low and middle-income countries among these countries of Iraq do not have a strong capacity for costs to obtain medical attention and also lack of knowledge and awareness regarding breast cancer [18].

The study showed that, married women with breast cancer was 77% and the number of women married with EBNA-1 IgG was 56%, as for unmarried women with breast cancer who were 23%, the EBNA-1 IgG ratio was 20%. The study showed that the rate of infection is high with married more than unmarried.

And studies that have agreed with us done by Alwan and his coworker (2019) whom found the percentage of women married with breast cancer was 93.8% and unmarried women with breast cancer was 13% [18].

The study did not agree with our study was Hinyard and his coworker (2017) the study showed that unmarried women were more likely to have breast cancer and more deaths than married women [19]. In another study, Lafta and his coworker (2013) found that marital status was not a risk factor for breast cancer [20]. Another study was conducted by Gomez and his coworker (2016) on women in California in the United States in a comparison between married and unmarried women with breast cancer found a rate of breast cancer for married 50.6%, unmarried by 39.1% in this study, the risk ratio for unmarried was higher than that of married [21]

Table (4) showed the results of the study of women who are smoker and have breast cancer 9% and positive for EBNA-1 IgG 6% while women who were not smokers and had breast cancer were 91% and positive for EBNA-1 IgG 70%. In our study, smokers were lower than non-smokers then EBNA-1 IgG was higher in non-smokers. The study that was agreed with our study was by Gatea and Wadi (2018) in Baghdad, Iraq, where the study showed that the number of women with breast cancer smoked 34 out of 93 samples and the number of women with breast cancer non-smoker 59 out of 93 [22]. And another study by Ahmed with Ruanduzy in (2017) in the city of Erbil the study showed that only 40 smoker

women out of 549 samples taken from women with breast cancer [23]. Despite the small number of smokers and the demographic Iraq, which lives by the population, but several studies, have shown that smoking is one of the risk factors that can cause cancer, smoking was associated before or after diagnosis of breast cancer and increase in mortality from breast cancer and as a risk factor[24].

Table (5) showed the occupation may be a risk factor for breast cancer and there has been a significant difference in the frequency of different professions among patients and controls. The rate of non-working patients was higher in the group of patients with breast cancer 65% than controls 1% and IgG of EBNA-1 52%. Also, the frequency of workers in the group was 35% Controls 9% and IgG of EBNA-1 rate 24%. We found that occupational association was not associated with an increased risk of breast cancer especially female workers through our study was less than non-working women. The rate of our study did not agree with Johnsson and his coworker [25] who found that occupational association of risk factors for breast cancer especially those younger than 55 years.

The study agreed with Hashim and his coworker [26] for samples taken from 200 Iraqi women with breast cancer found that occupational association was not a risk factor for breast cancer where female workers were 14.0% and non-working was 86.0 % .

Finally, the study showed that IgG of EBNA-1 appeared in a wide range of breast cancer cases, which makes it a risk factor and a causative factor in breast cancer, the role of EBV, whether a promoter of breast cancer or progress of breast cancer, will require studies to determine the effective role of EBV for appropriate prevention and treatment. Early detection may follow advanced therapeutic studies in the EBV vaccine

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العلاقة بين إصابة بفيروس ابشتاين بار وسرطان الثدي في مدينة كركوك

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الملخص

سرطان الثدي هو احد اكثر انواع السرطان شيوعا في نساء العراق، اشارت جميع الدراسات التي اجريت في السنوات الاخيرة الى ان فيروس ابشتاين بار مرتبط ببعض انواع السرطان ومن سرطان الثدي، الاكتشاف المبكر لسرطان الثدي هو الحل الامثل للسيطرة على المرض. الغرض من الدراسة الحالية هو الكشف عن العلاقة بين فيروس ابشتاين بار وسرطان الثدي عند النساء في مدينة كركوك. شملت الدراسة اخذ عينات الدم من نساء مصابات بسرطان الثدي من سن (23 الى 88) سنة للفترة من ايلول 2018 لغاية نيسان 2019، بلغ العدد الاجمالي للعينات 100 عينة دم لنساء مصابات بسرطان الثدي و10 عينات دم من نساء اصحاء لم يكن لديهن اي مرض في الثدي وتمت المقارنة بين المجاميع بالتحاليل المصلية وذلك باستخدام تقنية اليزا حيث تم التحري عن الاجسام المضادة للفيروس (EBNA-1 IgM EBNA-1 IgG)، اظهرت النتائج ان 76% من مجموع عينات الدم للنساء المصابات بسرطان الثدي كانت ايجابية للأضداد EBNA-1 IgG بينما اظهرت الدراسة الحالية نتائج سالبة للأضداد EBNA-1 IgM وكانت اعلى نسبة اصابة بفيروس ابشتاين بار في المصابات بسرطان الثدي ضمن الدراسة الحالية اللاتي تراوحت اعمارهن بين 56 الى 65 سنة (22%).