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Nuclear Co-Localization of Expressional Products of BRCA-2 and Epstein Barr Virus- Latent Membrane Protein-1 Genes: An Immunohistochemical Study of Breast Cancers Tissues from a Group of Iraqi Female Patients.

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Abstract : Background: Globally, and including Iraq, breast cancer is the commonest malignancy among women. BRCA1 and BRCA2 are human genes producing tumor suppressor proteins to help repairing damaged DNA and ensuring the stability of the cellular genetic material. A possible viral etiology was recognized in relation to the development and progression of breast cancers, among them mouse mammary tumor virus (MMTV), the Epstein-Barr virus (EBV) and the human papilloma (HPV) have prevailed linkages to these cancers.

Objective: To analyze the rate of EBV-LMP1 infection in the breast tissues in association with defects and / or mutations in BRCA-2 gene, by assessing the endogenous levels of the total expressed BRCA-1- as well as LMP-1 protein products, and their relations to the differentiation of primary invasive breast cancer tissues.

Patients and methods: Fifty-four (54) formalin-fixed, paraffin- embedded breast tissues were obtained in this study; (34) biopsies from breast cancers (BC) and (20) from apparently normal breast autopsies as a control group. Detection of protein expressional products of LMP-1 gene of Epstein Barr Virus as well as BRCA-2gene was done by HRP/DAB immune-enzymatic antigen detection system using specific rabbit- anti-human primary antibodies for EBV-LMP -1as well as defected or mutated BRCA-2 protein products.

Results: Detection of LMP-1 - immunohistochemical (IHC) reactions in tissues with BC was observed in 11 out of 34 (32.4%), while in healthy breast tissues in the control group was detected in 10% (2 out of 20). Detection of BRCA-2- protein- immunohistochemical (IHC) reactions in tissues with BC was observed in 14 out of 34 (41.2%), while none of the examined healthy breast tissues in the control group revealed such IHC- reactions. The difference between the percentages of BRCA-2- as well as LMP1 proteins detection in BC tissues & control group was statistically significant (<0.05). Among breast cancer tissues that showed score II of IHC reactions for BRCA2, 60% have well differentiated grade; and 50% of those tissues that have score I- IHC reactions showed moderate differentiated grade and lastly, 66.7% of the BC tissues which showed score III have presented as poorly differentiated BC tissues. However, statistical significant differences between the frequencies of EBV-LMP1 and BRCA-2- immunohistochemical reactions were neither observed in relation to the age of these breast cancer patients nor to the grade of invasive breast cancer tissues (P value > 0.05).

Conclusions: Our results indicate that the EBV might contribute to the development of subset of breast tumors. The present results of the rates of defects or mutations in the BRCA-2- genes in relation to the grade of breast cancer tissues also could point for their occurrence and contribution as early events in breast carcinogenesis.

Keywords : Breast cancer; LMP 1;Defects / Mutations; BRCA-2,IHC.

Introduction:

Breast carcinoma is the commonest malignancy among women in developed and developing countries including Iraq[1,2]. Local studies in Iraq have demonstrated that most breast cancer patients present in advanced stages with a likely prevalence of more aggressive tumour forms[3,4]. Many risk factors have been associated with breast cancer development and progression including a possible viral etiology[5].Although various reproductive and hormonal factors have been identified as risk factors for breast cancer, yet these factors together do not explain more than fifty percent of all cases of breast cancer[6].Researchers are thus prompted to consider other routes and risk factors, including a possible viral etiology, breast cancer pathogenesis. Three viruses that could possibly related to human breast cancers are: mouse mammary tumor virus (MMTV), the Epstein-Barr virus (EBV) and the human papilloma (HPV)[7].

Epstein Barr Virus is one of the eight known human herpesviruses. Its genome is a linear, double stranded DNA, about 170kb in length. Latently infected cells contain the genome as a circular plasmid in the nucleus. The terminal repeat (TR) sequences are present at both ends of the linear form of the genome and these repeats mediate the circularization in the infected cell, An unusually large tandemly repeated DNA sequence in the genome of EBV is known as the major internal repeat (IR1). The IR1 site divides the EBV genome into long and short unique sequences (UL and US). These sequences are filled with closely packed genes[8].In addition, the EBV genome contains a viral cytokine, vIL-10, that was pirated from the host genome. This viral cytokine can prevent macrophages and monocytes from activating T-cells are required for EBV dependent transformation of B-cell[9].

Since then, a large number of studies have detected EBV infection in patients with breast carcinoma. A series of studies that adopted non-breast-cancer control groups have also been performed[10,11],and several mechanisms and hypotheses about the association between EBV infection and breast carcinoma have been developed[12-14].In the growth program of EBV expresses all the nine known latent proteins: the six EBV nuclear antigens (EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, and EBNALP), three latent membrane proteins (LMP1, LMP2A, and LMP2B), and the non-polyadenylated EBV RNAs (EBERs)[15]. In the default program, EBNA1, LMP1, LMP2A, and the EBERs are expressed, providing necessary signals that are thought to allow infected lymphoblasts to differentiate into memory B cells[16].

In the latency program, which has a much more restricted pattern of viral gene expression, very few viral genes are expressed. The transcript for LMP2A has been consistently detected, and recent reports suggest the EBERs are also expressed. This low level of viral gene expression allows persistence of the virus in resting recirculating memory cells in a way that is nonpathogenic and not detectable by the immune system[17]. Production of infectious virus is the essential feature of fourth and final gene expression program found in humans latently infected with EBV[16].

The viral oncogene latent membrane protein 1 (LMP1) plays an essential role in the pathogenesis of a number of EBV-associated malignancies; oncogenicity (promotion of cell transformation, survival, and invasion); production of angiogenic factors as well as in vivo formation of the neovasculature (rapid tumor cell expansion and metastasis) ; and via modulation of immune genes related to inflammation and Ag presentation, LMP1 has a crucial role for the in vivo immunogenicity for tumor promotion and progression , especially in cancers (18). EBV and MMTV were reported in 50% and 37% of breast cancer cases ,respectively[19]. In 1995, the Epstein-Barr virus (EBV), an ubiquitous herpes virus, was found in 21% of 91 breast cancers[20].

Some researchers believed that EBV infection may play a role in the early stages of breast carcinogenesis and elevate breast cancer risk[21].Moreover, EBV infection might be a latent factor in the development of certain types of breast carcinoma[22]. However, statistical data from studies have varied widely. This inconsistency could be largely attributable to several problems: technical challenges in detecting and localizing the EBV in tumor cells, study designs that involved a specific histological type of breast carcinoma, and the lack of an epidemiological perspective that could clarify the inconsistencies in EBV prevalence across studies[23].

Among human tumor suppressor genes, the BRCA-1 and BRCA-2 genes are ubiquitously found in all humans where their genes and proteins are called breast cancer type 1 and 2 susceptibility genes and proteins, respectively[24]. Certain variations of the *BRCA1* gene lead to an increased risk for breast cancer as part of a hereditary breast - ovarian cancer syndrome[24].

On the hand, BRCA2 mutation carriers are at increased risk of breast cancers in males and females, and also of ovarian, prostatic, pancreatic, gall bladder, bile duct and stomach cancers as well as melanoma. Biochemical, genetic and cytological studies have revealed parallelism of BRCA1 and BRCA2 phenotypes and in turn have suggested a commonality of in their multiple functions. BRCA1 and BRCA2 proteins are involved in control of homologous recombination and double strand break repair in response to DNA damage[25].

Researchers have identified hundreds of mutations in the *BRCA1* gene, many of which associated with an increased risk of cancer. Women with an abnormal BRCA1 or BRCA2 gene have up to an 80% risk of developing breast cancer by age 90; increased risk of developing ovarian cancer is about 55% for women with BRCA1 mutations(26). Approximately 50% to 65% of women who have a deleterious mutation in *BRCA1* could develop breast cancer by age 70, and 35% to 46% could develop ovarian cancer by age 70. Approximately 40% to 57% of women with a deleterious mutation in *BRCA2* will develop breast cancer by age 70, and 13% to 23% will develop ovarian cancer by age 70[26].

The present study was proposed to unravel the rates of both LMP-1-EBV infection as well as defects and / or mutations in BRCA-2 gene in the breast tissues and their relations to the differentiation grades of primary invasive breast cancer tissues.

Materials and Methods:

Study Groups:

This retrospective research enrolled a number of (54) formalin-fixed, paraffin embedded breast tissue blocks which comprised both patients and control samples where their ages have ranged from 32 to 71 years. These paraffin-embedded samples were related to the archives of the period from 2011 till 2016 of the major hospitals and private histopathological laboratories in Kerbela, Babylon, and Al-Najaf provinces. Their diagnoses were based primarily on the accompanied pathological reports of each corresponding tissue blocks. This group of blocks has included (34) biopsies from patients who had undergone surgical operation or biopsies for their breast cancers (BC) and (20) autopsies from apparently normal breast tissues control group. Then these breast tissues were properly subjected to both fixation and paraffin embedding so as to be used for this research as an age-matched groups.

Laboratory methods:

Slide Preparation:

Tissue sectioning has been conducted following the trimming of these tissue blocks at the histopathological department of Teaching laboratories / Al- Sadar Medical City (Al- Najf) &Al_Hilla hospital teaching and a second confirmatory histopathological re– examination of the obtained blocks was done and one 4 mm thick-tissue section was mounted on an ordinary glass slide and stained with hematoxyline and eosin, while other 4 mm thick-tissue sections were stuck onto positively charged slide to be used for detection of EBV-LMP 1& BRCA2 – antigens using Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC kit (Lot. Number: ab64264, Abcam, UK), an immunoenzymatic antigen detection system for immunohistochemistry techniques, using specificRabbit Monoclonal primary Anti-EBV Latent Membrane Protein 1 antibody [Lot. Number: D24-G; ab136633], (Abcam, UK) and Anti- BRCA2 Protein antibody [Lot. Number:(ab27976, Abcam, UK) .The detailed methods of performing IHC reactions with these antibodies were conducted according the manufacturing company instructions and were done in the Research Laboratories of the Clinical Communicable Diseases Research Unit, at College of Medicine, University of Baghdad as well as in the Advanced Microbiology Research Laboratory at College of Science, University of Babylon.

Immunohistochemical Analysis:

The proper use of the IHC- detection kits gives, under light microscope, an intense brown signal at specific sites in positive test tissues in referring to that specifically expressed protein. The signals were evaluated using × 100 lenses for counting the positive cells. The IHC intensity and percentage scores were based on the intensity of positive signals as well as the number of cells that gave these signals, respectively.

Counting the positive cells in 10 different fields of 100 cells for each sample and the average percentage of positive cells within the 10 fields was determined. A scale of 0-3 was used to indicate the relative intensity, where 0 is corresponding to no detectable IHC reaction, and 1, 2, 3 are equivalents to low, moderate, and high intensity of reaction, respectively. The results were assigned to the following score categories: 1%–25% (score 1), 26%–50% (score 2) or > 50% (score 3)[22].

Statistical Analysis:

The T test, ANOVA test, or Chi square were specified for statistical examination of each results in this research work. These statistical analysis were done by using version-19 of the SPSS program and Excel application.

Results:

Distribution of patients with malignant breast tumors according to their Age . Archival specimens enrolled in this study were related to female patients with breast cancers, their mean age was (52.7+ 8.5 years) while the mean age of those who have apparently healthy tissues was (62.7 + 7.2 years) (Table 1).

Table (1): Studied groups according to the mean age .

Study Groups	N	Mean (years)	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Patients	34	52.7	8.5	1.5	49.5	55.9	38.00	69.00
Control	20	62.7	7.2	2.2	58.1	67.3	54.00	76.00
Total	54	55.6	9.3	1.5	52.7	58.4	38.00	76.00

	t-test for Equality of Means						
	Independent Samples Test:					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Age	-3.579	40	.001	-10.00000	2.79444	-15.64777	-4.35223

II. Histopathological Grading of Breast Carcinomas:

According to their Scarf-Bloom-Richardson system(SBR) for grading of breast cancers(Table 2) ,the results of present study show that poorly differentiated grade breast carcinomas constituted 44.1% (15 of total 34 cases) , whereas cases with moderately and well differentiated grades constituted 32.4% (11 out of 34cases) and 23.5% (8 out of 34 cases), respectively .The statistical analysis of grading distribution of breast carcinoma shows significant differences ($p<0.05$)between poorly differentiated grade and well differentiated grade, while non-significant difference was noticed between poorly differentiated and moderately differentiated breast carcinomas .

Table (2) :Tumor Grading of Breast cancers group.

%	N	Breast Cancer Grades(Differentiated)
23.5	8	Well *
32.4	11	Moderately **
44.1	15	Poorly
100.0	34	Total

* Significant differences when well grade compared to poorly grade.
**Non significant difference when moderate grade compared to poorly grade.

III. EBV-LMP 1 -Associated Breast cancers:

Thesignals of EBV-LMP 1 immunohistochemical reactions were detected as brown discoloration at the antigenic sites that were detected by their specific primary antibodies (Figure 1). Table (3) shows the positive results of EBV-LMP 1 - immunohistochemical (IHC) reactions, where 32.4% (11 of total 34) breast cancers showed positive signals while 2 out of 20 (10%) in control group has presented with such positive signals. The statistical analysis shows no significant differences among patient and control groups (p =0 .117).

Table (3): Frequency of EBV-LMP 1 immunohistochemical reactions among the study groups

			EBV		
			Positive	**Negative**	**Total**
Type	Patient	Count	11	23	34
		% within Type	32.4%	67.6%	100.0%
	Control	Count	2	18	20
		% within Type	10%	90%	100.0%
Total		Count	13	41	54
		% within Type	24.1%	75.9%	100.0%

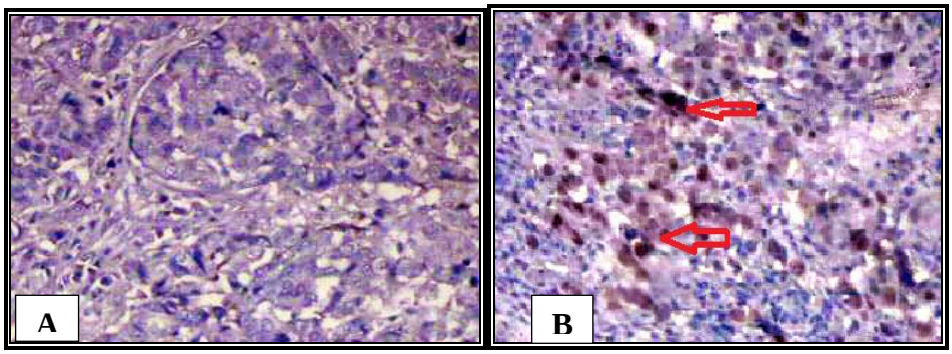

Figure 1: Immunohistochemical reactions of EBV-LMP 1 using specific primary antibodies for EBV-LMP 1 and biotinylated -labeled anti-EBV; Stained with HRP/DAB (brown) and counter stained by hematoxyline (Blue).

A. Invasive breast ductal carcinoma with negative EBV-LMP 1 –IHC reaction(40X). B. Invasive breast cancer with positive EBV-LMP 1 –IHC reaction that revealed moderate score and high signal intensity (40X).

IV. Correlation of EBV infection with Age of Patients:

There was no statistical significant difference between the frequencies of EBV-LMP 1 immunohistochemical reactions according to the age of the study groups (Table 4).

Table (4): Frequency of EBV-LMP 1 immunohistochemical reactions according to the age of the study groups.

	EBV-LMP1	N	Mean (Year)	Std. Deviation	Std. Error Mean
Age	Positive	11	50.6667	7.57188	2.18581
	Negative	23	53.9545	8.99988	1.91878

V. Correlation of EBV infection with Grading of Breast Carcinoma:

It was found that the percentage of EBV-LMP 1 - IHC test reactions in breast cancer tissues with well grades constituted (42.86%) followed by moderate grade (50.0%) and poorly grade (0%). The statistical analysis according to the grading distribution EBV-LMP 1 - IHC reactions in breast carcinoma shows non-significant differences (Figure 2).

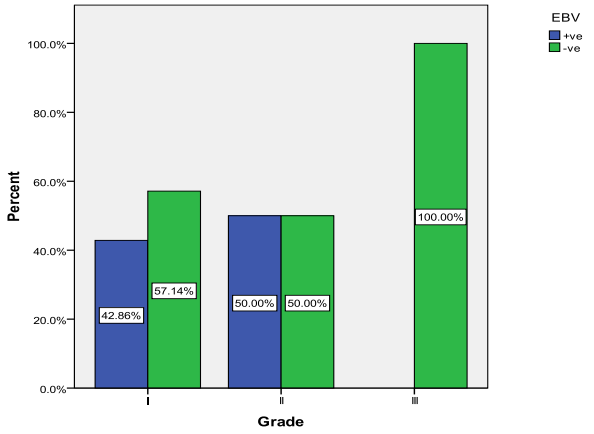

Figure 2: Distribution of EBV-LMP 1 immunohistochemical reactions according to the grading of breast cancers.

VI. Frequency distribution of immunohistochemistry results of BRCA-2 protein expression according to the signal scoring.

The signals of immunohistochemical reactions for BRCA-2antigen with their specific primary antibodies were observed as brown discoloration at the specific antigenic sites of these reactions (Figure 3). The positive-signal of BRCA-2 - immunohistochemical reactions were found in 41.2% % (14 out of total 34)breast cancers while no tissue in the control group has showed such IHC signals. The l Chi-Square analysis shows significant difference between the patients and control groups (<0.05) Table (5).

Table (5): Frequency of BRCA-2 immunohistochemical reactions among the study groups

				GROUP			Valid Percent	Cumulative Percent
The Marker				**Patients**	**Control**	**Total**		
BRCA-2		Positive	Count	14	0	14		
			%	41.2%	0.0%	25.9%	41.2	41.2
		Negative	Count	20	20	40		
			%	58.2%	100.0%	74.1%	58.2	100.0
	Total		Count	34	20	54		
			%	100.0%	100.0%	100.0%	100.0	

		Value	df	Asymp. Sig. (2-sided)
BRCA-2	Pearson Chi-Square	7.800	1	.003

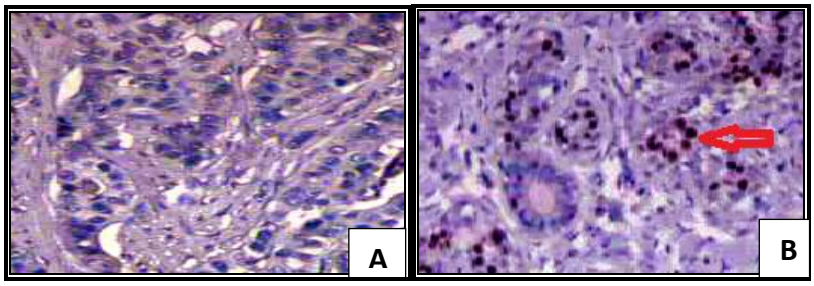

Figure 3: Infiltrative Breast Cancer Showing The Results of Immunohistochemistry Staining Protein Expression Using Biotinylated Anti-BRCA2 Protein Antibody; Stained By DAB-Chromogen (Brown) and Counter Stained By Mayer's Heamatoxylin (Blue).

A- Breast Cancer with negative staining for BRCA-2 antigen .

B-BRCA2-IHC-reaction with moderate signal score and strong signal intensity (40x).

VII. Correlation of BRCA2 protein expression with Grading of Breast Carcinoma:

In the present study, 14 tissues out of 34 showed positive BRCA 2 -IHC reactions, where 60% of these breast cancer tissues with score II of IHC reaction for BRCA- 2 have well differentiation grade; and 50% of breast tissues that have score I of IHC reaction for BRCA 2 have moderate differentiation. Lastly, 66.7% of the BC tissues cases that showed score III have presented with poor differentiation. Statistically, the overall results of BRCA 2– IHC scoring in relation to tumor grading showed non- significant differences ($p>0.05$) (Table 6).

Table (6): Correlation of BRCA 2 -IHC scoring results with the grading of breast cancers .

P	Breast Cancer Grading						State Of Signal Scoring BRCA2	
	Poorly differentiated (n=15)		Moderately differentiated (n=11)		Well differentiated (n=8)			
	%	N	%	N	%	N		
0.48 [N.S]	80	12/15	45.5	5/11	37.5	3/8	Negative	
	20	3/15	54.5	6/11	62.5	5/8	Positive	
	33.3	1/3	50	3/6	40	2/5	I	Scoring
	25	0/ 3	33.3	2/6	60	3/5	II	
	66.7	2/ 3	16.7	1/6	0.00	0/5	III	

Discussion

Breast cancers have constituted one third of the Iraqi registered female cancers ranking the top of the commonest ten cancers[27]. Role of EBV in breast carcinogenesis is still controversial; yet, unraveling its relationship to this cancer is important for a better understanding of viral participation in the multifactorial carcinogenesis as well as for early detection and the prevention of such cancer[28].Regarding this group of 34 breast cancers Iraqi patients included in this study; the age was ranging between 38-69 years while the mean age was 52.8 + 8.6 years. The present results are consistent with many Iraqi as well as global reports that breast malignant tumors are usually targeting females aged over forty[29-31].Aging is generally a risk factor increasing the possibility of malignancy in the breast epithelial tissues. The present results could also point for the importance of age factor in the carcinogenesis of this group of breast cancer tissues along with the prolonged effect of exposure of breast tissues to the hormonal changes[32].Many high oncogenic-risk genotypes of human papilloma viruses such as HPV 16, 18, 31 and 33 were localized breast cancers in a recent Iraqi study by Ali et al (2014)[33] and was noticed this significant association of such HPV types in invasive breast carcinomas potentiating a possible relationship with the pathology as well as grade of the breast cancers[34].However, the role of Epstein-Barr virus (EBV) in the etiology of breast cancer is still controversial. The current study, as such was conducted to highlight a possibility of a role of such virus in breast carcinogenesis of Iraqi female patients with breast cancer.

The present results have shown EBV positivity in (32.4%) of BC tissues, as represented by EBV-LMP 1 - immunohistochemical reactions, versus 13.3% (2 out of 15) of breast tissues in the control group.Latent membrane protein 1 (LMP1) is the major oncogenic product of EBV; its role is well documented in malignant diseases[35].The latency II program of EBV, expressing LMP1, LMP2, and EBNA1, is observed in most EBV-associated pathologies[36].

The LMP1 is constitutively active and lead to induce the activation of NF-κB, which regulates the main biological process in cells[37].As such, NF-κB-induced genes involved in survival include anti-apoptotic proteins, such as cellular FLIP, Bcl-2, A20, c-IAPs, and TRAFs[38]. Constitutive NF-κB activation contributes to the transformation of cell lines by LMP1[39].Although EBV was put among group-1 carcinogens by IARC Working Group[40], its prevalence varied markedly with the associated cancers, (about 10% in gastric carcinoma to nearly 100% in nasopharyngeal carcinoma)[41-43]. This virus may affect cell growth in more than one way depending on the differences in the patterns of expressed EBV genes. In addition, this virus alone is not a sufficient for breast carcinogenesis but could represent an additional as well as important epidemiological risk factor that could play a critical role in that process. Here in, and upon EBV infection, deregulated MYC expression was observed as it helps in EBV-driven cell proliferation. These findings may enable the infected cells to evade Cytotoxic T Lymphocyte (CTL)-mediated immune surveillance (44).The introduction of activated MYC gene into an EBV-transformed cell line in which EBNA2 was rendered null was shown to be capable of inducing continuous proliferation of these cells in the absence of functional LMP1 and EBNA2. Therefore, the possibility that MYC can substitute for LMP1 and EBNA2[45]and rearranged defective EBV genomes have been detected in some sporadic BL tumors[46].

EBV infection was found in approximately 52% in breast cancer, which is similar to its presence in breast cancer in Middle Eastern women, based on Kalkan*et al*.[47], Zekri*et al*.,[18]and Hachana*et al*.,[48] studies. In parallel, it was reported that the presence of EBV in breast cancer is associated with more aggressive phenotype. The current results are comparable to the results of Zekri*et al*., (2012)[28]; PCR detected LMP1 in 11/50(22%) of 50 studied Iraqi cases and in 12/40(30%) of their 40 Egyptian studied invasive breast carcinomatous tissues.

The global geographical differences might be related in part to many demographic features and genetic background, however, other reasons were recognized for the differences in the results reported in the literature such as variation in the numbers of tested samples and sensitivity of the used technical methods[49].

EBV is considered to play an etiological role in the multifactorial breast cancer development, as that role in the nasopharyngeal carcinogenesis and other strongly related cancers, and since this virus has an analogous role of HPV (which is also regarded as a group-1 carcinogen and as classified by IARC Working Group) in the cervical carcinogenesis when compared to the role in HPV- associated breast cancers[22, 26], it would be reasonably as well as possibly expected to detect the virus at an early stage of this disease, and that EBV would be found at least in some normal tissues[26].

The current finding of viral DNA in a group of healthy breast specimens could, to a certain extent, support the proposition that the virus might have a role in the etiology of a subpopulation of patients with breast cancer. On other hand, the presence of EBV alone is not sufficient to implement the full carcinogenesis process and that it is logic to believe that further changes would accumulate over time in a to cause that cancer and as such prompting to recommend further large cohort studies to explore the viral role as well as each other contributing factors. Most of Iraqi patients with breast cancer were diagnosed at younger age and clinically presented with late stage and more aggressive cancers[50]. Other researchers have also reported tumors in younger women were of higher grade and had more vascular invasion compared to tumors in the older women[29].

Our results have shown that EBV positivity in BC tissues are consistent to those results (28%) of Zekri*et al*.,[28]regarding their studied Iraqi patients, but are less than the results in their Egyptian counterparts(45%). These observations might be related to the difference in viral distributions according to demographic and population characteristic that reflected as difference in the presence of EBV in breast cancer tissues in each studied patients and in each studied population[51].

Regarding the association between EBV and tumor aggressiveness, many recent studies have found that EBV is correlated with human invasive carcinomas including breast and nasopharyngeal[52-54]. Earlier investigations have reported that LMP1 and EBNA1 onco-proteins of EBV enhanced cancer progression and metastasis of nasopharyngeal malignancy though the initiation of the epithelial-mesenchymal transition (EMT) phenomenon,[54, 55] a crucial event during cancer metastasis progression[56]. For these aforementioned notes and based on the findings of this study and others, we believe that the presence of this EBV in breast cancerous tissues could enhance cancer progression (possibly through the initiation of EMT event via EGF-receptor and/or Akt signaling pathways) as previously described in gastric and nasopharyngeal carcinomas;[54, 57].Grading of breast cancers in this research was done according to Nottingham modification of the Scarff Bloom Richardson(SBR) system[58]. Histopathological grading is an important as risk parameter in assessment of breast cancer patients[59]. It has been noticed that the 10-year survival rates for breast cancers patients with grade I is around 80% whereas 45% in grade III[58].

The results of this study show that the percentage of EBV-LMP 1 - antigens in the breast cancer tissues was found to decrease with the proceeding of the grading of these cancers. Similarly, the EBV-LMP 1 - negative counterpart tissues have decreasing trend of grades, too. The association of EBV positivity with the examined breast cancer grade shows no statistical significant difference. These results are in line with the results of Zekri*et al*.,[28] who also found no correlation between EBV positivity and tumor grades of their studied patients with breast cancers from two different Arabic populations .The present results also support the findings of Yang et al[60]; herein the EBV might have a role in enhancing the possibility of breast oncogenesis although not directly be involved in that oncogenic process. However, the authors of this study, along many other authors, believe that further studies are required to elucidate the role as well as the pathogenesis of EBV in breast cancers, that is in the line of importance of EBV vaccine which is presently under clinical trial investigations for a better understanding of the association between EBV infection and breast cancer initiation and progression.

In the present study, the results have shown BRCA-2- protein- IHC reactions positivity in 14 out of 34 (41.2%), while none of the examined healthy breast tissues in the control group revealed such IHC- reactions.

The composite BRCA2 cDNA sequence assembled consisted of 11,385 bp, but did not include the polyadenylation signal or poly(A) tail. Conceptual translation of the cDNA revealed an ORF beginning at nucleotide 229 and encoding a protein of 3,418 amino acids. There was no signal sequence at the end of terminus, and there were no obvious membrane-spanning regions[61].Frans*et al.*,[62] was found Two novel deletions were identified: a deletion of exon 8 ,and exons 20–22. In addition, two duplications were found: a duplication of exon 13 and a novel duplication of exons 21–23. In contrast, *BRCA2* mutation-associated breast cancers are more similar to sporadic breast cancer, although they are more likely to be estrogen receptor positive[63, 64].

Many oncologists have found not yet possible to draw evidence-based conclusions about the association between *BRCA1* and/or *BRCA2* mutation carrier ship and breast cancer prognosis, for the heterogeneity of the reported results that precluded a clear conclusions regarding the contribution of *BRCA1/2* status and tumor features to a worse survival. However, treatments may be different for *BRCA1* and *BRCA2* mutation carriers compared to non-carriers, because of difference in the pathological features of these tumors in the studied carriers (S10 Supporting Information, part A)[65, 66] where the therapy response of tumors in *BRCA1/2* mutation carriers could be better compared to the response in non-carriers[67].

In the current study, it was found 62.5% of breast cancer tissues that revealed BRCA2 mutation have well grades, followed by moderate grade (54.5%) and poor grade (20%).In this study, the percentage of BRCA2 was found to decrease with the proceeding of the grading of breast cancer. Their BRCA2-negative BC counterparts tissues were found to have a similar decreasing trend of grades of BC.

By analogy, the grades of BRCA1-related breast cancers have been shown to be consistently elevated with grade 3 comprising 61.3–81.5% of cases versus 22–27% of hospital-based comparison series[68, 69]. In this report, 65% of tumors from BRCA1 patients were of high grade. The higher proportion of grade 3 tumors has been reported for BRCA1- related breast cancers (70), yet the survival has been reported as comparable or better[71]. The hereditary breast cancers, on other hand, present at a slightly younger age[72], and was found that the young age be an independent adverse risk factor[73,74]. Thus, grade 3 in BRCA1-related breast cancer may have a different implication for prognosis than in other breast cancer cases[75].

Bordeleau and colleagues review article[76] has demonstrated that the overall prognosis of *BRCA*-associated breast cancer was similar to breast cancer not associated with *BRCA* mutations. In 2010, Lee and colleagues[77], found that *BRCA-1* mutation carriers had significantly lower short-term and long-term overall survival rates (OSR) relative to non-carriers while both short-term and long-term OSR of *BRCA2* carriers did not differ from non-carriers.

The systematic and evidence-based analysis of all studies published to date have found that only two factors seem to explain part of the heterogeneity;(1) misclassification bias for studies which had not tested the comparison (‘non-carriers’) group, and the proportion of incident cases (S11 Supporting Information, panels C and D) and (2) might be related to the population differences (i.e. different mutations),where differences in completeness of follow-up and differences in consideration of contralateral breast cancer and prophylactic surgeries[78].

From our results we can conclude that EBV might contribute to the development of subset of breast tumors. The present results of the rates of defects or mutations in the BRCA-1- genes in relation to the grade of breast cancer tissues also could point for their occurrence and contribution as early events in breast carcinogenesis.

References

1. Dumitrescu RG, Cotarla I. Understanding breast cancer risk – where do we stand in 2005? J Cell Mol Med. 2005;9:208–221.
2. Globocan (2008) International Agency for Research on Cancer, WHO, Lyon, IARC Press, 2010.
3. Glaser SL, Hsu JL, Gulley ML. Epstein-Barr virus and breast cancer: state of the evidence for viral carcinogenesis. Cancer Epid. Biomarkers Prev 2004;13:688–97.

4. Al-Alwan NA. Breast cancer: demographic characteristics and clinico-pathological presentation of patients in Iraq. East Mediterr Health J. 2010 Nov;16(11):1159–64.
5. Al-Alwan NA. DNA proliferative index as a marker in Iraqi aneuploid mammary carcinoma. East Mediterr Health J. 2000 Sep-Nov ;6(5-6):1062–72.
6. Pal T, Permuth-Wey J, Betts JA, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. *Cancer* 2005; 104(12):2807–16.
7. NanciePetrucelli , Mary B Daly, and Gerald L Feldman.(2013).*BRCA1* and *BRCA2* Hereditary Breast and Ovarian Cancer.Copyright © 1993-2016, University of Washington, Seattle.GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.
8. Aidan Dolan, Clare Addison, Derek Gatherer, Andrew J. Davison, Duncan J. McGeoch. The genome of Epstein–Barr virus type 2 strain AG876. Virology 350 (2006) 164–170.
9. Gary J Lindquester, Kimberly A Greer1, James P Stewart and Jeffery T Sample.Epstein-Barr virus IL-10 gene expression by arecombinant murine gammaherpesvirus in vivo enhances acute pathogenicity but does not affect latency or reactivation. Herpesviridae 2014, 5:1.
10. Serraino D, Piselli P, Scognamiglio P. Viral infections and cancer: epidemiological aspects. J BiolRegulHomeost Agents.2001;15:224–228.
11. Labrecque LG, Barnes DM, Fentiman IS, Griffin BE. Epstein-Barr virus in epithelial cell tumors: a breast cancer study. Cancer Res. 1995;55:39–45.
12. Bonnet M, Guinebretiere JM, Kremmer E, Grunewald V, Benhamou E, et al. Detection of Epstein-Barr virus in invasive breast cancers. J Natl Cancer Inst. 1999;91:1376–1381.
13. Subramanian C, Robertson ES. The metastatic suppressor Nm23-H1 interacts with EBNA3C at sequences located between the glutamine- and proline-rich domains and can cooperate in activation of transcription. J Virol.2002;76:8702–8709.
14. Trabelsi A, Rammeh S, Stita W, Mokni M, Mourou A, et al. Detection of Epstein-Barr virus in breast cancers with lymphoid stroma. Ann BiolClin. 2008;66:59–62.
15. Nanbo A,and Takada K. The role of Epstein-Barr virus-encoded small RNAs (EBERs) in oncogenesis.RevMed Virol2002; 12: 321-326.
16. Deshpande, S Badve, N Kidwai and R Longnecker .(2002). Lack of Expression of the Epstein-Barr Virus (EBV) Gene Products, EBERs, EBNA1, LMP1, and LMP2A, in Breast Cancer Cells. Lab Invest, 82:1193–1199.
17. Thorley-Lawson D.A. EBV persistence in vivo. Invading and avoiding the immune response. In: Medveczky P.G., Friedman H., Bendinelli M., editors. Herpesviruses and Immunity. Plenum Press; New York: 1998. pp. 207–229.
18. Haiyan Lei, Tianwei Li, Bingjie Li, Shien Tsai, Robert J. Biggar, Francis Nkrumah, Janet Neequaye,Marina Gutierrez,SidneiEpelman, Sam M. Mbulaiteye,Kishor Bhatia, and Shyh-Ching Lo . Epstein-Barr virus from Burkitt Lymphoma biopsies from Africa and South America share novel LMP-1 promoter and gene variations.Sci Rep. 2015; 5: 16706.
19. Lawson, J.S., Tran, D., Rawlinson, W. D. (2001). From Bittner to Barr: a viral, diet and hormone breast cancer aetiology hypothesis. Breast Cancer Res.,3(2):81-85.
20. Labrecque LG, Barnes DM, Fentiman IS, Griffin BE. Epstein-Barr virus in epithelial cell tumors: a breast cancer study. Cancer Res. 1995;55:39–45.
21. Yasui Y, Potter JD, Stanford JL, Rossing MA, Winget MD, et al. Breast cancer risk and “delayed” primary Epstein-Barr virus infection. Cancer Epidemiol Biomarkers Prev. 2001;10:9–16.
22. Trabelsi A, Rammeh S, Stita W, Mokni M, Mourou A, et al. Detection of Epstein-Barr virus in breast cancers with lymphoid stroma. Ann BiolClin. 2008;66:59–62.
23. Hsu JL, Glaser SL. Epstein-barr virus-associated malignancies: epidemiologic patterns and etiologic implications. Crit Rev OncolHematol.2000;34:27–53.
24. Pal T, Permuth-Wey J, Betts JA, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. *Cancer* 2005; 104(12):2807–16.
25. Nancie Petrucelli, Mary B Daly and Gerald L Feldman.Hereditary breast and ovarian cancer due to mutations in*BRCA1* and *BRCA2*. *Genetics in Medicine* (2010) 12, 245–259.
26. WelcshPiri L, King Mary-Claire: BRCA1 and BRCA2 and genetics of breast and ovarian cancer. Human Molecular Genetics.2001; 10:7705-713.
27. Iraqi Cancer Board; Iraqi Cancer Registry Center: Results of Iraqi Cancer Registry. Iraqi Ministry of Health (editor). Baghdad. Iraq. 2012: Pp.44 and 131.

28. Zekri AR, Bahnassy AA, Mohamed WS, El-Kassem FA, El-Khalidi SJ, Hafez MM, Hassan ZK.(2012). Epstein-Barr virus and breast cancer: epidemiological and molecular study on Egyptian and Iraqi women.J Egypt NatlCanc Inst.;24(3):123-31.
29. Al-Alwan NA. DNA proliferative index as a marker in Iraqi aneuploid mammary carcinoma. East Mediterr Health J. 2000 Sep-Nov ;6(5-6):1062–72.
30. Elkum N, Dermim S ,Ajarim D ,et al ; Being 40 or younger is an independent risk factor for relapsein operable breast cancer patients :The Saudi Arabia experience BMC Cancer ., 2007.
31. Al-KhafajiK,AttuG.and AL –Kurri j .Pathological staging of breast cancer .A study of 220 patients.TheIragi post graduate medical journal.2004;January.3(1).P:38-4.
32. Pike MC, Spicer DV, Dahmoush L, Press MF. Estrogens, progestogensnormal breast cell proliferation, and breast cancer risk. Epidemiol Rev. 1993;15(1):17–35.
33. Ali,S.H.M. Al-Alwan N.A.S. 2 and Al-AlwanySh.H.M.: Detection and genotyping of human papillomavirus in breast cancer tissues from Iraqi patients. Eastern Mediterranean Health Journal 2014; Vol. 20, No. 6 : 229-234.
34. Yasmeen A, Bismar TA, Kandouz M, Foulkes WD, Desprez PY, Al Moustafa AE. E6/E7 of HPV type 16 promotes cell invasion and metastasis of human breast cancer cells. Cell Cycle. 2007.
35. Rickinson A, Kieff E. 2007. Epstein-Barr virus, p 2655–2700 *In* Knipe DM, et al., editors. (ed), Fields virology, 5th ed Lippincott Williams & Wilkins, Philadelphia, PA
36. Tsang NM, et al. 2003. Presence of the latent membrane protein 1 gene in nasopharyngeal swabs from patients with mucosal recurrent nasopharyngeal carcinoma. Cancer 98:2385–2392.
37. Karin M, Lin A. 2002. NF-κB at the crossroads of life and death. Nat. Immunol. 3:221–227
38. Suzuki Y, Nakabayashi Y, Nakata K, Reed JC, Takahashi R. 2001. X-linked inhibitor of apoptosis protein (XIAP) inhibits caspase-3 and -7 in distinct modes. J. Biol. Chem. 276:27058–27063.
39. Kaye KM, Izumi KM, Kieff E. 1993. Epstein-Barr virus latent membrane protein 1 is essential for B-lymphocyte growth transformation. Proc. Natl. Acad. Sci. U. S. A. 90:9150–9154.
40. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Epstein - Barr virus and Kaposi's sarcoma Herpesvirus/Human Herpesvirus8. Lyon:IARC;1997.
41. Lee J.H., Kim S.H., Han S.H., An J.S., Lee E.S., Kim Y.S. Clinicopathological and molecular characteristics of Epstein–Barr virus–associated gastric carcinoma: a meta–analysis. J. Gastroenterol. Hepatol. 2009;24:354–365.
42. Akiba S., Koriyama C., Herrera–Goepfert R., Eizuru Y. Epstein–Barr virus associated gastric carcinoma: epidemiological and clinicopathological features. Cancer Sci. 2008;99:195–201.
43. Koriyama C., Akiba S., Minakami Y., Eizuru Y. Environmental factors related to Epstein–Barr virus–associated gastric cancer in Japan. J. Exp. Clin. Cancer Res. 2005;24:547–553.
44. Yanai H., Murakami T., Yoshiyama H., Takeuchi H., Nishikawa J., Nakamura H., Okita K., Miura O., Shimizu N., Takada K. Epstein–Barr virus–associated gastric carcinoma and atrophic gastritis. J. Clin. Gastroenterol. 1999;29:39–43.
45. Nemerow G.R., Mold C., Schwend V.K., Tollefson V., Cooper N.R. Identification of gp350 as the viral glycoprotein mediating attachment of Epstein–Barr virus (EBV) to the EBV/C3d receptor of B cells: sequence homology of gp350 and C3 complement fragment C3d. J. Virol. 1987;61:1416–1420.
46. Tanner J., Weis J., Fearon D., Whang Y., Kieff E. Epstein–Barr virus gp350/220 binding to the B lymphocyte C3d receptor mediates adsorption, capping, and endocytosis. Cell. 1987;50:203–213.
47. Kalkan A., Ozdarendeli A., Bulut Y., Yekeler H., Cobanoglu B., Doymaz MZ.. Investigation of Epstein-Barr virus DNA in formalin-fixed and paraffin- embedded breast cancer tissues. Med PrincPract 2005; 14:268-71.
48. Hachana M, Trimeche M, Ziadi S, Amara K, Gaddas N, Mokni M, Korbi S. Prevalence and characteristics of the MMTV-like associated breast carcinomas in Tunisia. Cancer Lett. 2008;271: 222–230.
49. Blancato J, Singh B, Liu A, Liao DJ and Dickson RB : Correlation of amplification and overexpression of the c-myc oncogene in high-grade breast cancer: FISH, in situ hybridization and immunohistochemical analyses. British Journal of Cancer (2004) 90, 1612 – 1619.
50. Alwan N (2010). Breast Cancer: Demographic Characteristics and Clinicopathological Presentation of Patients in Iraq. Eastern Mediterranean Health Journal, 16:1073–1078.
51. Elkum N, Dermim S ,Ajarim D ,et al ; Being 40 or younger is an independent risk factor for relapsein operable breast cancer patients :The Saudi Arabia experience BMC Cancer ., 2007.

52. Glenn WK., Heng B., Delprado W., Iacopetta B., Whitaker NJ., Lawson JS.. Epstein-Barr virus, human papillomavirus and mouse mammary tumour virus as multiple viruses in breast cancer. PLoS One 2012; 7:e48788.
53. He JR, Tang LY, Yu DD, Su FX, Song EW, Lin Y, et al. Epstein-Barr virus and breast cancer: Serological study in a high-incidence area of nasopharyngeal carcinoma. Cancer Lett. 2011;309: 128–36.
54. Kong QL., Hu LJ., Cao JY., Huang YJ., Xu LH., Liang Y., Xiong D., Guan S., Guo BH., Mai HQ., Chen QY., Zhang X, et al. Epstein-Barr virus-encoded LMP2A induces an epithelial-mesenchymal transition and increases the number of side population stem-like cancer cells in nasopharyngeal carcinoma. PLoSPathog 2010;6:e1000940; PMID:20532215;
55. Horikawa T., Yoshizaki T., Kondo S., Furukawa M., Kaizaki Y., Pagano JS.. Epstein-Barr Virus latent membrane protein 1 induces Snail and epithelial-mesenchymal transition in metastatic nasopharyngeal carcinoma.Br J Cancer 2011; 104:1160-7.
56. Al Moustafa AE., Achkhar A., YasmeenA.. EGF-receptor signaling and epithelial-mesenchymal transition in human carcinomas. Front Biosci (Schol Ed) 2012; 4:671-84.
57. Raab-TraubN.. Novel mechanisms of EBV-induced oncogenesis. CurrOpinVirol 2012; 2:453-8.
58. Al-KhafajiK,AttuG.and AL –Kurri j .Pathological staging of breast cancer .A study of 220 patients.TheIragi post graduate medical journal.2004;January.3(1).P:38-4.
59. Pike MC, Spicer DV, Dahmoush L, Press MF. Estrogens, progestogensnormal breast cell proliferation, and breast cancer risk. Epidemiol Rev. 1993;15(1):17–35.
60. Yang J., Mani S.A., Donaher J.L., Ramaswamy S., Itzykson R.A., Come C., Savagner P., Gitelman I., Richardson A., Weinberg R.A. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell. 2004;117:927–939.
61. Tavtigian, S. V., Simard, J., Rommens, J., Couch, F., Shattuck-Eidens, D., Neuhausen, S., Merajver, S., Thorlacius, S., Offit, K., Stoppa-Lyonnet, D., Belanger, C., Bell, R., and 37 others. The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds. Nature Genet. 12: 333-337, 1996.
62. Frans B. L. Hogervorst, Petra M. Nederlof, Johan J. P. Gille, Cathal J.et al.Large Genomic Deletions and Duplications in the *BRCA1* Gene Identified by aNovel Quantitative Method. CANCER RESEARCH 63, 1449–1453,, 2003.
63. Bordeleau L, Panchal S, Goodwin P. Prognosis of BRCA-associated breast cancer: a summary of evidence.Breast Cancer Res Treat. 2010;119:13–24.
64. Atchley DP, Albarracin CT, Lopez A, et al. Clinical and pathologic characteristics of patients with BRCA-positive and BRCA-negative breast cancer. J ClinOncol. 2008;26:4282–4288.
65. Honrado E, Osorio A, Palacios J, Benitez J. Pathology and gene expression of hereditary breast tumors associated with BRCA1, BRCA2 and CHEK2 gene mutations. Oncogene. 2006;25: 5837–5845.
66. Phillips KA. Current perspectives on BRCA1- and BRCA2-associated breast cancers. Intern Med J. 2001;31: 349–356.
67. Foulkes WD. BRCA1 and BRCA2: chemosensitivity, treatment outcomes and prognosis. Fam Cancer. 2006;5: 135–142.
68. Plakhins G, Irmejs A, Gardovskis A, Subatniece S, Liepniece-Karele I, Purkalne G, et al. Underestimated survival predictions of the prognostic tools Adjuvant! Online and PREDICT in BRCA1-associated breast cancer patients. Fam Cancer. 2013.
69. Bayraktar S, Gutierrez-Barrera AM, Liu D, Tasbas T, Akar U, Litton JK, et al. Outcome of triple-negative breast cancer in patients with or without deleterious BRCA mutations. Breast Cancer Res Treat. 2011;130: 145–153.
70. Eccles D, Simmonds P, Goddard J, Coultas M, Hodgson S, et al. Familial breast cancer: an investigation into the outcome of treatment for early stage disease. Fam Cancer. 2001;1: 65–72.
71. Robson ME. Clinical considerations in the management of individuals at risk for hereditary breast and ovarian cancer. Cancer Control. 2002;9: 457–465.
72. Budroni M, Cesaraccio R, Coviello V, Sechi O, Pirino D, Cossu A, et al. Role of BRCA2 mutation status on overall survival among breast cancer patients from Sardinia. BMC Cancer. 2009;9: 62 .
73. Chappuis PO, Kapusta L, Begin LR, Wong N, Brunet JS, Narod SA, et al. Germline BRCA1/2 mutations and p27(Kip1) protein levels independently predict outcome after breast cancer. J ClinOncol. 2000;18: 4045–4052.
74. Dersimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7: 177–188.

75. Narod SA, Foulkes WD. BRCA1 and BRCA2: 1994 and beyond. Nat Rev Cancer. 2004;4: 665–676.
76. Bordeleau L, Panchal S, Goodwin P. Prognosis of BRCA-associated breast cancer: a summary of evidence.Breast Cancer Res Treat. 2010;119: 13–24.
77. Lee EH, Park SK, Park B, Kim SW, Lee MH, Ahn SH, et al. Effect of BRCA1/2 mutation on short-term and long-term breast cancer survival: a systematic review and meta-analysis. Breast Cancer Res Treat. 2010;122: 11–25.
78. Broek AJ, Schmidt MK, van 't Veer LJ, Tollenaar RA, van Leeuwen FE.(2015). Worse Breast Cancer Prognosis of *BRCA1/BRCA2* Mutation Carriers: What's the Evidence? A Systematic Review with Meta-Analysis.PLoS One. 2015 Mar 27;10(3):e0120189.

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