Int.J.Curr.Microbiol.App.Sci (2016) *5*(9): 1-13

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 9 (2016) pp. 1-13**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.509.001

Evaluation of the Association of Epstein barrvirus with Breast Carcinoma in Egyptian Patients

Niveen El-Wakeel[1]*, Azza Abdel-Aziz[2], Hosam Roshdi[3] and Fayez Shahatto[4]

[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Egypt
[2]Department of Pathology, Faculty of Medicine, Mansoura University, Egypt
[3]Department of Vascular Surgery, Faculty of Medicine, Mansoura University, Egypt
[4]General Surgery, Faculty of Medicine, Mansoura University, Egypt
**Corresponding author*

Keywords

Epstein barr virus, Egyptian patients, breast carcinoma, EBNA-1, CD21.

Article Info

Accepted:
08 August 2016
Available Online:
10 September 2016

A B S T R A C T

Infection with EBV virus is a frequent event. However, the reason why it has the ability to exert an oncogenic impact on some individuals while sparing others remains poorly understood. Studies revealed a heterogeneous prevalence of the association of EBV and BC in different localities. Most of these studies were in Western countries and the USA. These studies couldn't explain the difference. We are trying to predict whether an association of EBV and the BC is present in our locality. Eighty subjects were enrolled in the study; 40 patients (with BC) and 40 age and sex matched controls. All the participants' breast and blood specimens were subjected to Rt-PCR assay, EBNA-1 immuno histochemistry and EBNA-1 IgG assessment by ELISA. Twenty (50%) patients with BC were positively detected by IHC of EBNA-1 while, no cases were detected in the control group, (P-value; <0.05); Thirty (75%) patients with BC were positive for EBNA-1 gene by Rt-PCR, whereas, only 10 (25%) had this gene in the control group, (P- value; <0.05). Twenty five (62.5%) patients with BC were positive by ELISA while, only 3 cases (7.5%) were positive in the control group, (P- value; <0.05). EBV was more frequent in BC cases than in the controls and in the invasive than the non-invasive histopathological grades of BC cases. The association of EBV with BC was frequent in younger age groups. Rt-PCR was more sensitive and specific than IHC and ELISA in assessment of EBV presence. A larger number of cases and healthy controls with different, ages, races and localities and with a combination of different assays is recommended for evaluating EBV association with BC.

Introduction

Breast carcinoma (BC) is the second leading cause of deaths in women after lung cancer and it is the most common cancer among women worldwide (23% of all new cancer cases) (American Cancer Society, 2010). It was the first presented cancer to the National Cancer Institute, Cairo (17.5% of cancer cases) (El Saghir *et al*., 2007). Among the causal factors implicated, Epstein-Barr virus (EBV) was found in 21% of 91 BC in mid 1990s (Labrecque *et al*., 1995).

There were many evidences that suggest the association between the BC and the EBV; high incidence of male breast cancers in EBV endemic Mediterranean countries, morphological similarities between medullary BC and nasopharyngeal carcinoma and the high incidence of the BC and the Hodgkin's lymphoma in which the EBV is a main contributor (Hippocrate *et al*., 2011; Mant *et al*., 2004).

The aggressive pattern of breast cancers were more associated with EBV (Mazouni *et al*., 2011). EBV induces epithelial cell growth. EBV encodes BARFO which activates HER2 and HER3 signaling cascades that promote the oncogenic activity in the cultured BC (Lin *et al*., 2007). The relationship between BC and EBV could be of potential importance not only for better understanding of BC etiology, but also for early detection, prevention of BC and treatment.

Immunohistochemistry (IHC) can be used in order to localize and target EBV antigens. Enzyme-labeled antibodies are used to bind specific antigens in tissues samples or cultured cells. After adding in substrate of enzyme it generates insoluble or high-electronic density particles that could be localized under light microscope or electronic microscope. Compared to immunofluorescence staining, immune-enzymological staining has more accurate localizing, better contrast ratio, and samples are able to be stored for a long time (Chu *et al*., 2001).

In Polymerase Chain Reaction (PCR) study, the importance of amplification cycles was suggested to be of possible significance for the detection of EBV in breast cancers.

Two general targets have been used in attempts to assess EBV in breast tumor specimens: viral products such as the EBV-encoded small RNAs (EBER); or EBNA-, a nuclear protein essential for maintaining the viral genome and the viral genome DNA itself (Brink *et al*., 2000; Fina *et al*., 2001; Stevens *et al*., 2005; Arbach *et al*., 2006).

Real-time PCR (Q-PCR) used to quantify the copy numbers of the EBV genome in biopsy specimens to show that BC cells harbor the viral genome. However, even in EBV-positive tumor samples, many tumor cells do not contain EBV genomes and that the breast carcinomas are highly heterogeneous in terms of genome content and distribution.

Moreover, using reverse transcription-PCR (RT-PCR), to detect EBNA-1 and LMP-1 transcripts in EBV-positive tumors is an important reliable method. The findings raise the possibility that although EBV is likely to have an etiologic role in the genesis of BC, the virus also, might contribute to tumor progression. Moreover, the potential impact of EBV in BC progression was evaluated by estimation of resistance to chemo-therapeutic agents (Arbach *et al*., 2006). The detection of EBV DNA by PCR methods in BC has ranged from 51% to 6.

Serological assays have a useful clinical diagnostic utility. However, indirect immunofluorescence assay is not well standardized, time-consuming, and ill-suited for big-scale assessment, or automated usage. Enzyme-linked immunosorbent assay (ELISA) techniques conveyed a promising alternative with advantage for automation and large screening. In all cases two-marker testing required testing by two separate assays. In addition, synthetic peptides provide the potential of being chemically defined, conveying reproducible large-scale serving at low costs, with improved assessment standardization (Cox *et al*., 2010).

Infection with EBV virus is a frequent event and the reason why it has the ability to exert an oncogenic impact on some individuals while sparing others remains poorly understood, in addition, most of the studies performed to date to assess the association of EBV infection and BC were mostly form Western countries and so far, few studies have been reported from Egypt, Therefore, we studied 40 cases of breast carcinomas for evidence of EBV infection evaluating the association of EBV with BC in different tumor grades in our locality where we could depend on this association on prediction and prophylaxis against BC.

Patients Design and Methods

This study was conducted on 80 subjects that were admitted to the surgical departments in Mansoura University Hospitals (MUH), in the period from January, 2015to December, 2015. Their age ranged from20to 70years old and all of them were female.

The subjects enrolled in the study were divided into two groups, first group, was 40 patients diagnosed pathologically as BC and second group was, 40age and sex (all are females) matched subjects included as a control group with benign breast tumors. Tumor biopsy and serum samples were collected from both patients and control groups, in accordance with and approval of the local ethical committee.

All patients were newly diagnosed and none of them had a previous history of BC or any other malignancy. All control subjects and patients eligible for enrollment in the study were subjected to full history taking and physical examination. At the time of sample collection of breast biopsies, fat was removed, proper gross examination and dissection was done and breast tissues were divided into two parts;

The first part was submitted for routine histopathological examination and histo-prognostic grading, histo-prognostic Scarff-Bloom-Richardson classification (SBR) were implicated using the relevant criteria. Each patient was classified according to the TNM (tumor-nodes-metastasis) system. Sections of 4 um thickness have been cut from formalin fixed paraffin embedded blocks for routine H&E in Pathology department of Mansoura Faculty of Medicine, Mansoura, Egypt. Other sections were prepared on charged slides for for immune-histochemical staining EBNA-1, CD21. Examination of three tumor slides from each specimen was done on an Olympus CX31 light microscope. Pictures were obtained by a PC-driven digital camera (Olympus E-620). The computer software (Cell, Olympus Soft Imaging Solution GmbH) allowed morphometric analysis to be performed.

The second section was cut into a sterile Eppendorf tube and preserved as fresh tissues at -40^{o} C for subsequent DNA extraction and PCR.

Exclusion Criteria

Patients with post-neoadjuvent mastectomy, BC of the other side, patients with associated other malignancies, previous history of patient or family malignancies. Also, uncooperative subjects and those who refused the consent.

The breast tissues of both cases and controls were subjected to the following:

Routine histopathological examination: were performed for each tumor in Pathology department of Mansoura Faculty of Medicine, Mansoura, Egypt.

Immunohistochemistry (IHC)

Immunohistochemical analysis for EBNA-1, and CD21 with a labelled streptavidin-biotin-peroxidase complex technique was performed on tissue sections from 40 cases of breast carcinoma and that of controls (total; 80). We used the Monoclonal antibody EBNA-1 (Dako, Denmark) and mouse monoclonal antibody for CD21 (CD21 A-3, Santa Cruz biotechnology, California, USA) against EBV membrane receptor. Heat-induced epitope retrieval using 100 mmol/L ethylenediaminetetra-acetic acid buffer (EDTA) (pH 8.0) for 20 minute. Detection kit used was high sensitive kit (DakoCytomation envision +dual link system peroxidase code K4061) using DAB as chromogene. The slides were counterstained with hematoxylin. Proper positive and negative controls were performed. Sections of known EBV-positive classical Hodgkin's disease were used as positive controls for EBNA-1 and CD21 (Pan *et al*.). As a negative control, sections were stained without the addition of a primary antibody. As for the immunohistochemistry assessment, Slides were scanned.

Ten cellular areas selected (i.e. the so-called hot spots) and evaluated at X400 magnification by pathologist. Assessment of EBNA-1 positive staining was interpreted as nuclear or granular nuclear while assessment of CD21 was based on a membranous or membrano-cytoplasmic pattern. Cases were considered positive with >10% stained cells (8, 18).

DNA extraction for PCR

DNA was extracted using QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) using the manufacturer's instructions.

Polymerase chain reaction (PCR)

Real time-PCR was done to detect EBNA-1 expression as previously described by Stevens *et al.*, 2005 (13);using two primers that targets EBV nuclear antigen (EBNA-1); Forward primer: (5′- GCCGGTGTGTT CGTATATGG-3′) and Reverse primer: (5′-CAAAACCTCAGCAAATATATGAG-3′). We prepared the DNA Master Hybridization Probes mix stock solution and PCR master mix then 15 µL of PCR master mix were pipetted to a precooled capillary placed in an adapter in the cooling block then we added 5 µL of isolated DNA to the 15 µL PCR mix in the capillary then placed capillaries in the carrousel in Light Cycler centrifuge.

The serum samples from both controls and BC patients were obtained for EBV-antibody detection using ELISA (Greiner Labortechniek, Germany)

Serum samples were obtained from all cases and controls for measurement of anti-EBNA-1 immunoglobulin IgG.The well plate has been precoated with EBV antigens to bind cognate antibodies. Controls or test samples are added to the wells and incubated. Following washing, a horseradish peroxidase (HRP) labelled anti-Human IgG conjugate is added to the wells, which binds to the immobilized EBV-specific antibodies. Production of a blue color product was produced then changes to yellow after adding an acidic stop solution. The density of yellow coloration is directly proportional to the amount of EBV-immunoglobulin captured in plate (De Paschale *et al*., 2012; Fachiroh *et al*., 2006).

Statistical analysis

The collected data were coded, processed

and analyzed using SPSS program; version 16 for windows. Numerical data (age) were presented as mean ± Standard Deviation (SD) or median and range. Qualitative variables such as, EBV viral markers for Egyptian patients were expressed as percentage and frequencies. *P*-value less than 0.05 was considered to be statistically significant.

Ethical considerations

Study protocol was submitted for approval by IRB (Institutional Review Board).

Approval of the managers of hospital in which the study was conducted and informed verbal consent was obtained from each participant sharing in the study.

Confidentiality and personal privacy were respected in all levels of the study.

Results and Discussion

Twenty (50%) patients with BC were positively detected by IHC of EBNA-1 as nuclear staining in more than 10% of tumor cells figure(1) while, no cases were detected in the control group, (P- value; <0.05); Table(1). The CD21 markers were immuno histochemically detected in 15(37.5%) patients as membranous and cytoplasmic staining in more than 10% of tumor cells; figure(2) and not detected in control groups.

Thirty (75%) patients with BC were positive for EBNA-1 gene by PCR whereas, only 10 (25%) had this gene in the control group, (P-value; <0.05).

Twenty five (62.5%) patients with BC were positive by ELISA while, only 3 cases (7.5%) were positive in the control group, (P- value; <0.05). The patient ages ranged from 20 to75 years with a median of 52 years; Table (2). There was no statistically significant difference between the positive EBV-BC patients and the healthy controls regarding the age categories (both were 66.7% and 33.3% in age <50; and >50 years old respectively). Similarly, there was no statistically significant difference between the negative EBV-BC patients and the healthy controls (50% patients, 60% controls in age <50 and 50%patients, 40% controls in age >50 years old). It was obvious in the current study that, EBV infection was predominant in the younger age group <50 years old (p- value < 0.05) in both infected patients and controls.

As detailed in Table (3); EB infected cases represented 30 (75%) of the total (40) BC patient group.

Tumor sizes in BC cases varied from <1.0 to > 3.0 cm with a median of 2 cm. Out of the thirty EB positive cases, the tumor sizes of <1cm, >1-3cm and >3cm were present in 10 (33.3%), 12 (40%), and 8 (26.7%) of positive EBV-BC cases respectively. In negative EBV-BC cases, 60% had a tumor size of >1-3 cm and 40% was >3cm in size.

Histologically, the tumors of the 30 positive EBV-BC cases fell into 2 categories: ductal; 26 (86.6%) that was more frequent than lobular carcinomas 3 (10%). There was a statistical significant difference between the positive and negative EBV-BC cases.

According to the modified Bloom Richardson grading guidelines, carcinomas were classified as grade-I where no infected cases were represented, and, grade III, (60%) were more frequent than grade I, (40%). Also, there was no statistical significant difference between the positive and negative EBV-BC cases.

The gold standard for diagnosis of EBV infection was PCR (10, 11, 12, 14). In the current study we had 20 (50%) cases

detected by IHC of EBNA-1, out of the 30 positive PCR of EBV-BC cases; Table (4). Ten and 15 cases conveyed false negative results by IHC analysis of EBNA-1 and CD21 respectively in addition to absent detection in the control groups; The IHC analysis had a sensitivity and specificity of 66.67% and 100% respectively with 100% positive predictive value and 50% negative predictive value.

ELISA assay provided a sensitivity and specificity of 83.33% and 100% respectively with 100% positive predictive value and 66.67% negative predictive value; Table (5). Therefore, five false negative cases were detected by ELISA of EBNA-1 that may be attributable to the immune-compromization of the cases since they were detected by PCR. However, negative findings need to be followed by a search for specific antibodies.

Infection with EBV virus is a frequent event. However, the reason why it has the ability to exert an oncogenic impact on some individuals while sparing others remains poorly understood. The life-long latent infection is facilitated by the virus' ability to evade host immune surveillance in part by expressing only a few factors [six nuclear antigens (EBNA-1, -2, -3a, -3b, -3c, and -LP); three latent membrane proteins (LMP-1, 2a, and -2b); and two abundant, untranslated RNAs (EBER-1 and -2)] (Sally *et al*., 2004).

Previous studies had discussed this association but without declaring the obvious oncogenic role (Mazouni *et al*., 2015). Studies revealed heterogeneous prevalence of the EBV and the BC in different localities. This association was lower in USA than Europe. In addition, these studies couldn't explain this difference apart from the viral prevalence difference and the methods of diagnosis used for detection (Huo *et al*., 2012).

This study aimed to detect whetheran association of EBV and the BC is present. Eighty subjects were enrolled in our study; 40 patients with BC and 40 age and sex matched controls. All the participants' breast and blood specimens were subjected to PCR assay, EBNA-1 immunohistochemistry and EBNA-1 IgG assessment by ELISA and we were pining to study this association in our locality.

In the prevailing study, the highest frequency was served by EBNA-1 PCR assay of Egyptian BC patients and also, in benign tissues of the controls. However, the lowest prevalence was reported by IHC study.

EBNA-1 is essential for maintenance of the viral episome and for its replication. In the current study 20 (50%) patients with BC were positively detected by IHC for EBNA-1 antigen, while, no cases were detected in the control group, (P- value; <0.05); Table (1).

By IHC study using monoclonal antibody against EBNA-1 antigen and Mayer's hematoxylin as counterstain, 26% of the obtained 92 BC samples were found to have positive EBNA-1in tumor epithelial cells by Khabaz, 2013. Also, 20% of the same antigen was detected by Brink *et al.,* 2000, while Chu *et al.,* 2001 and Herrmann and Niedobitek, 2003 failed todetect the antigen infected BC tissues. In concordance with our results, Joshi *et al*., 2009 reported 55% of EBNA-1 in BC tissues of infected patients. An Egyptian authors; Mohamed *et al*., 2012 has reported EBV infection in 35.3% of invasive breast carcinoma.

The IHC analysis had a sensitivity and specificity of 66.67% and 100% respectively with 100% positive predictive value and 50% negative predictive value. Ten and 15 cases conveyed false negative results by

IHC analysis of EBNA-1 and CD21 respectively in addition to absent detection in the control groups; which might be attributable to the absence of enzyme-substrate reaction since deionized water can sometimes contain peroxidase inhibitors that can significantly impair enzyme activity. Also, the un-appropriate pH of the substrate buffer.

The PCR method is one of the most important techniques for detection of EBV and can be used alone or with other methods.

Xue *et al.,* 2003 amplified EBV DNA in breast cancer tissues and used reverse transcriptase (rt) PCR to confirm expression of viral BART (BamH1A rightward transcription), LF3, EBNA-, BARF1, and BZLF1.

Table.1 Frequency of viral markers in positive EBV-BC patients and controls, detected by; PCR, immunohistochemistry, and ELISA technique:

Parameter	**No**	**IHC of EBNA-1 (No/%)**	**IHC of CD21 (No/%)**	**PCR of EBNA-1 gene (No/%)**	**ELISA of anti-EBNA-1 IgG (No/%)**
EBV-BC patients	40	20 (50%)	15(37.5%)	30 (75%)	25 (62.5%)
Controls	40	0	0	10 (25%)	3 (7.5%)
P-value		0.001	0.001	0.001	0.001

EBV; Epstein barr virus, BC; breast carcinoma, PCR; Polymerase Chain reaction, EBNA1; Epstein-Barr nuclear antigen-1, IHC; Immunohistochemistry, ELISA;enzyme-linked immunosorbent assay

Table.2 Frequency of EBV infection in BC cases and healthy controls detected by PCR (EBNA-1 gene) in different age groups

Age (Patient group; median; 52	**Positive EBV infection (EBNA-1 PCR)**		**Negative EBV infection**	
	Patients (30/40)	Control (15/40)	Patients (10/40)	Control (25/40)
<50	20 (66.7%) median; 40	10(66.7%)	5(50%)	15(60%)
>50	10(33.3%) Median; 64	5(33.3%)	5(50%)	10(40%)
P-value	0.01			

EBV; Epstein barr virus, BC; breast carcinoma, PCR; Polymerase Chain reaction, EBNA1; Epstein-Barr nuclear antigen-1.

Table.3 Histopathological features of PCR positive EBV-BC patients

Size of the tumor (cm); median; 2cm	**EBV infection positive** Patients 30 (75%)	**EBV infection negative** Patients 10 (25%)	**P-value**
<1	10 (33.3%)	0	0.42
>1-3	12 (40%)	6 (60%)	
>3	8 (26.7%)	4 (40%)	
Histology	**EBV infection positive** Patients 30 (75%)	**EBV infection negative** Patients 10 (25%)	
Lobular	3 (10%)	2 (20%)	0.01
Ductal	26 (86.6%)	8 (80%)	
Grade	**EBV infection positive** Patients 30 (75%)	**EBV infection negative** Patients 10 (25%)	
I	0	0	0.49
II	12(40%)	5(50%)	
III	18(60%)	5(50%)	

EBV; Epstein barr virus, BC; breast carcinoma.

Table.4 The sensitivity and specificity of IHC compared to PCR

		PCR		**Total**
		positive	**negative**	
IHC(EBNA-1)	**Negative**	10(25%)	10(25%)	20(50%)
	Positive	20(50%)	0	20(50%)
Total		30(75%)	10(25%)	40(100%)

IHC; immunohistochemistry, EBNA1; Epstein-Barr nuclear antigen-1, PCR; Polymerase Chain Reaction

Sensitivity; 66.67%, Specificity; 100 %, Positive Predictive Value: 100%, Negative Predictive Value: 50%

Table.5 The sensitivity and specificity of ELISA compared to PCR

		PCR		Total
		Positive	**Negative**	
ELISA	**Negative**	5(12.5%)	10(25%)	15(37.5%)
	Positive	25(62.5%)	0	25(62.5%)
Total		30(75%)	10(25%)	40(100%)

ELISA; Enzyme Immunosorbent Assay, ;Polymerase Chain Reaction
Sensitivity;83.33%, Specificity; 100 %, Positive Predictive Value: 100%, Negative Predictive Value: 66.67 %%

Fig.1 EBNA-1 immunohistochemistry in a case of invasive ductal carcinoma, showing granular nuclear positivity in most cells in this field (immunoperoxidase X 400).

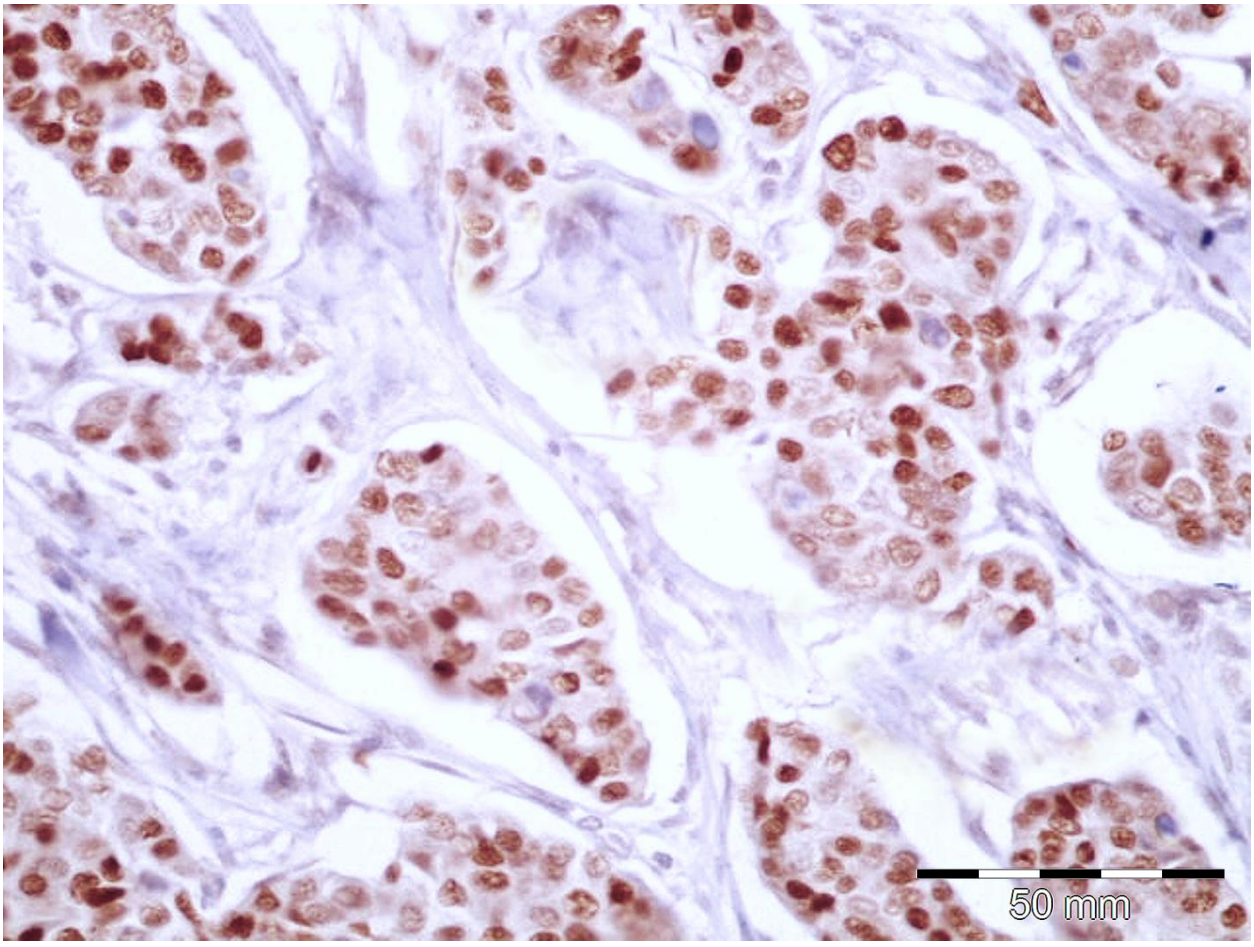
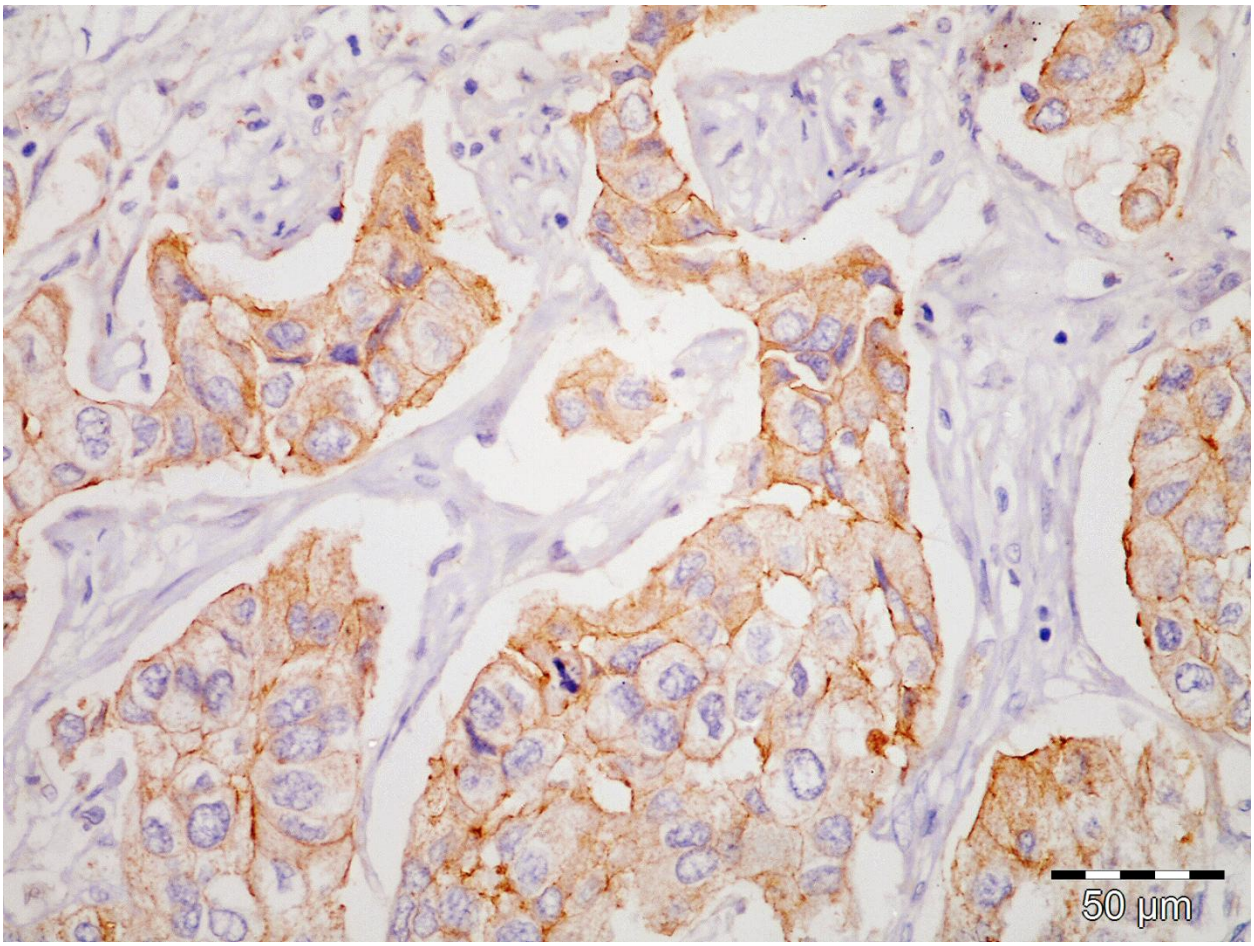


Fig.2 CD21 immunohistochemistry in a case of invasive ductal carcinoma, showing positive membranous immunostaining in more than 10% of tumor cell (immunoperoxidase X 400)



In this study, we used PCR for amplification of EBNA-1 gene. Thirty (75%) and 10 (25%) of patient group and controls were positive for EBNA-1, respectively (P- value; <0.05); Table (1).

Out of 32 studies regarding association of EBV and BC by PCR, 22 ones were positive, ranged from 2-100% for PCR of the non-healthy (tumor) samples studied, where, 10 studies were negative for the presence of the virus (Richardson *et al*., 2015). Also, a number of PCR studies failed to find EBV expression but these negative results were for healthy tissues adjacent to the EBV-positive tumors (Abdel-Rahman *et al*., 2012), indicating that EBV is likely tumor-specific.

Also, PCR of LMP1 gene, was detected by Yahia *et al.*, 2014 in 53.3% of BC patients and in 24% of the control group. It was also in concordance to Aboulkassim *et al.*, 2015. While Chu *et al*., 2001 demonstrated only 10% of BC patients for EBNA1 and EBNA-3 genome. Our PCR results showed a higher frequency of detection since we worked on the conserved region of the virus; EBNA-1 genome. The results of Labrecque *et al*., 1995; McCall *et al.*, 2000; Joshi *et al*., 2009; Zekri *et al.*, 2012 had also high detection frequencies.

Moreover, ELISA assay of EBV anti-EBNA-1 IgG showed a positive results in 62.5% (25 cases)and in only 3 cases (7.5%) of the control group. This assay provided a sensitivity and specificity positive predictive value of 83.33% and 100%, respectively with 100% and 66.67% negative predictive value; Table (5). Therefore, we had five false negative results; that may be attributable to the patient's immune-compromization. However, negative findings need to be followed by a search for specific antibodies. A significantly higher mean antibody levels were detected {50/55 tested (90.9%) cases and 27/33 tested (81.8%) controls}; in the study of Joshi *et al.,* 2009 measuring anti-EBNA-1 IgG.

The patient ages ranged from 20 to75 years with a median of 52 years; Table 2. There was no statistically significant difference between the positive EBV-BC patients and the healthy controls regarding the age categories. It was obvious that, EBV infection was predominant in the younger age group <50 years old (p- value < 0.05) in both infected patients and controls.

We found that ductal (86.6%) pathological variants of BC were more frequent than the lobular (10%); Table (3). This was in the contrary of Khabaz *et al*. (2013). Our results were nearly like that of Trabelsi *et al.,* 2008 revealed higher statistical correlation of lobular BC with EBV than ductal carcinoma. However, the frequent ductal variant was also frequent in BC patients with negative EBV detection results.

EBV was positive in grade III (60%) more than grade II (40%) with no presentation in grade I and with no statistical significance between infected and non-infected BC patients but it was frequent in the higher histopathological grades of BC. According to the modified Bloom Richardson grading guidelines of BC, Mazouni *et al*., 2015 showed no significance relation.

In conclusion, EBV was more frequent in BC cases than in the controls and in the invasive than the non-invasive histopathological grades of BC cases. The association of EBV with BC was frequent in younger age groups. Rt-PCR was more sensitive and specific than IHC and ELISA in assessment of EBV presence. A larger number of cases and healthy controls with different, ages, races and localities and with a combination of different assays is recommended for evaluating EBV association with BC.

References

Abdel-Rahman, N., Zekri, Abeer, A., Bahnassy, Waleed, S., Mohamed, *et al*. 2012. Epstein-Barr virus and breast cancer: Epidemiological and Molecular study on Egyptian and Iraqi women. *J. Egyptian National Cancer Institute,* 24: 123–131.

Aboulkassim, T., Yasmeen, A., Akil, N,. Batist, G. and Al Moustafa, A.E. 2015. Incidence of Epstein-Barr virus in Syrian women with breast cancer: A tissue microarray study. *Hum. Vaccin. Immunother.,* 1(4): 951-5.

American Cancer Society. 2010. Cancer Facts and Figures 2010. Atlanta, Ga: American Cancer Society.

Arbach, H., Viglasky, V., Lefeu, F., *et al*. 2006. Epstein Barr Virus (EBV) genome and expression in breast cancer tissues: effect of EBV infection of breast cancer cell in resistance to Paclitaxel(Taxol). *J. Virol.,* 845–853.

Brengel-Pesce, K., Morand, P., Schmuck, A., Bourgeat, M.J., Buisson, M. and Bargues, G. 2002. Routine use of real-time quantitative PCR for laboratory diagnosis of Epstein-Barr virus

infections. *J. Med. Virol*., 66(3): 360–369.

Brink, A.A., van Den Brule, A.J., van Diest, P., Meijer, C.J. 2000. Re: detection of Epstein-Barr virus in invasive breast cancers. *J. Natl. Cancer Inst.*, 92: 655–656.

Chu, P., Chang, K., Chen, Y., Chen, W., and Lawrence, M. 2001. Weiss. No Significant Association of Epstein - Barr virus Infection with Invasive Breast Carcinoma. *Am. J. Pathol.*, 159(2): 571–578.

Contesso, G., H. Mouriesse, S. Friedman, J. Genin, D. Sarrazin, and J. Rouesse. 1987. The importance of histologic grade in long-term prognosis of breast cancer: a study of 1,010 patients, uniformly treated at the Institut Gustave-Roussy. *J. Clin. Oncol.*, 5: 1378–1386.

Cox, B., Richardson, A., Graham, P., Gislefoss, R.E., Jellum, E., and Rollag, H. 2010. Breast cancer, cytomegalovirus and Epstein-Barr virus: a nested case-control study. *Br. J. Cancer*, 102(11): 1665-9.

De Paschale, M., Llerici, P. 2012. Serological diagnosis of Epstein-barr infection: Problems and solutions. *World J. Virol.*, 1(1): 31-43.

El Saghir, N.S., Khalil, M.K., Eid, T., El Kinge, A.R., Charafeddine, M., Geara, F., Seoud, M., Shamseddine, A.I. 2007. Trends in epidemiology and management of breast cancer in developing Arab countries: a literature and registry analysis. *Int. J. Surgery*, 5(4): 225–33.

Fachiroh, J.1., Paramita, D.K., Hariwiyanto, B., Harijadi, A. *et al.* 2006. Single-Assay Combination of Epstein-Barr Virus (EBV) EBNA1- and Viral Capsid Antigen-p18-Derived Synthetic Peptides for Measuring Anti-EBV Immunoglobulin G (IgG) and IgA Antibody Levels in Sera from Nasopharyngeal Carcinoma Patients: Options for Field Screening; *J. Clin. Microbiol.*, 44(4): 1459-67.

Fina, F., Romain, S., Ouafik, L., *et al*. 2001. Frequency and genome load of Epstein-Barr virus in 509 breast cancers from different geographical areas. *Br. J. Cancer*, 84: 783 – 90.

Herrmann, K., Niedobitek, G. 2003. Lack of evidence for an association of Epstein-Barr virus infection with breast carcinoma. *Breast Cancer Res.*, 5: R13–17.

Hippocrate, A., Oussaief, L., Joab, I. 2011. Possible role of EBV in breast cancer and other unusually EBV-associated cancers. *Cancer Lett.*, 305: 144–9.

Huo, Q., Zhang, N., Yang, Q. 2012. Epstein-Barr virus infection and sporadic breast cancer risk: a meta-analysis. *PLoS One*, 7(2): e31656.

Joshi, D., Quadri, M., Gangane, N., Joshi, R. and Gangane, N. 2009. Association of Epstein Barr Virus Infection (EBV) with Breast Cancer in Rural Indian Women. *Plos One*, 4(12): e8180.

Khabaz, M.N. 2013. Association of Epstein-Barr virus infection and breast carcinoma. *Arch. Med. Sci.*, 9(4): 745-51.

Labrecque, L.G., Barnes, D.M., Fentiman, I.S. and Griffin, B.E. 1995. Epstein‐Barr virus in epithelial cell tumors: a breast cancer study. *Cancer Res.*, 55: 39‐45.

Lin, J.H., Tsai, C.H., Chu, J.S., Chen, J.Y., Takada, K., *et al*. 2007. Dysregulation of HER2/HER3 signaling axis in Epstein-Barr virus-infected breast carcinoma cells. *J. Virol.*, 81: 5705–5713.

Mant, C., Hodgson, S., Hobday, R,. D'Arrigo, C. and Cason, J. 2004. A viral aetiology for breast cancer: time

to re-examine the postulate. *Inter. Virol.,* 47: 2–13.

Mazouni, C., Fina, F., Romain, S., Ouafik, L., Bonnier, P., Brandone, J.M. and Martin, P.M. 2011. Epstein‐ Barr virus as a marker of biological aggressiveness in breast cancer. *Br. J. Cancer*, 104: 332‐ 337.

Mazouni, C., Fina, F., Romain, S., Ouafik, L., Bonnier, P., Martin, P.M. 2015. Outcome of Epstein-Barr virus-associated primary breast cancer. *Mol. Clin. Oncol.,* 3(2): 295–298.

McCall, S.A., Lichy, J.H., Bijwaard, K.E., Aguilera, N.S., Chu, W.S. *et al*. 2007. Epstein-Barr virus detection in ductal carcinoma of the breast. *J. Natl. Cancer Inst.,* 93: 148-150.

Mohamed A.E., Hasen, A.M., Mohammed, G.F., and Elmaraghy, N.N. 2015. Real-Time PCR of cytomegalovirus and Epstein-Barr virus in adult Egyptian patients with systemic lupus erythematosus. *Int. J. Rheum. Dis.,* 18(4): 452-8.

Pan, L., Diss, T.C., Peng, H., Lu, Q., Wotherspoon, A.C., Thomas, J.A,. Isaacson, P.G. Epstein-Barr virus (EBV) in enteropathy associated T-cell lymphoma (EATL). *J. Pathol.,* 170: 137–43.

Richardson, A.K., Currie, M.J., Robinson, B.A., Morrin, H., Phung, Y. and Pearson, J.F. 2015. Cytomegalovirus and Epstein-Barr virus in breast cancer. *PLoS One*, 27: 10(2): e0118989.

Sally, L., Glaser, Joe, L., Hsu, and Margaret, L. 2004. Gulley. Epstein - Barr virus and Breast Cancer: State of the Evidence for Viral Carcinogenesis. *Cancer Epidemiol. Biomarkers & Prevention*, 13(5): 688-97.

Stevens, S.J.1., Verkuijlen, S.A., Middeldorp, J.M. 2005. Quantitative detection of Epstein-Barr virus DNA in clinical specimens by rapid real-time PCR targeting a highly conserved region of EBNA-1. *Methods in Mol. Biol.,* 292: 15-26.

Trabelsi, A., Rammeh, S., Stita, W,. Mokni, M., Mourou, A., *et al*. 2008. Detection of Epstein-Barr virus in breast cancers with lymphoid stroma. *Ann. Biol. Clin.*, 66: 59–62.

Xue, S.A,. Lampert, I.A., Haldane, J.S., Bridger, J.E. and Griffin BE. Epstein-Barr virus gene expression in human breast cancer: protagonist or passenger? *Br. J. Cancer*, 89: 113-9.

Yahia, Z.A., Adam, A.A., Elgizouli, M., Hussein, A., Masri, M.A. and Kamal, M. 2014. Epstein Barr virus: a prime candidate of breast cancer aetiology in Sudanese patients. *Infect. Agent Cancer*, 7; 9(1): 9.

How to cite this article:

Niveen El-Wakeel, Azza Abdel-Aziz and Hosam Roshdi. 2016. Evaluation of the Association of Epstein barrvirus with Breast Carcinoma in Egyptian Patients. *Int.J.Curr.Microbiol.App.Sci.* 5(9): 1-13. doi: http://dx.doi.org/10.20546/ijcmas.2016.509.001