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Detection of Epstein Barr Virus in Some Iraqi Women Patients with Invasive Ductal Carcinoma Using Immunohistochemistry Technique

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Abstract: Invasive ductal carcinoma (IDC) is a common breast cancer especially in women. Several factors may involve in establishment of the breast cancer in general. Variant viruses have been investigated in for their role in the establishment of breast cancer, among them is Epstein Barr virus which has been reported in the patients diagnosed with invasive ductal carcinoma. The study aimed to detect the presence of Epstein–Barr virus latent membrane protein 1 (LMP1) in a group of Iraqi women diagnosed with IDC and to correlate expression patterns with the age of the participants and with the grade and stage of cancer. A total of 30 formalin-fixed paraffin-embedded tissue of IDC biopsy samples and 29 formalin-fixed paraffin-embedded tissues of fibroadenoma as controls were collected to detect the presence of EBV LMP1 using immunohistochemistry. LMP1 expression was found in 76.67% of cases and in 58.62% of the control specimens. The expression pattern of LMP1 was determined to be statistically significant (P=0.0037) when correlated with age, grade and stage. As well as immunohistochemistry was found to be a sensitive technique in the detection of EBV. Conclusions: The study reveals that the LMP1 was over expressed in our population cohort.

Keyword: Immunohistochemistry, EBV, Invasive ductal carcinoma, FFPE.

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Introduction

Breast cancer is the most detectable malignancy in females, it is the second cause of death worldwide (1). Breast cancer is more common in the developed countries and is 100 times more common in females than in males (2). International Agency for Research on Cancer (IARC) revealed that biological factors result in 18-20% of different types of cancers (3); viruses have been recorded as an important reason for breast cancers establishment were Epstein Barr virus, Polyoma virus and mouse mammary tumor virus (MMTV) (4). EBV is classified as γ herpesvirus. EBV genome consists of a linear DNA with double strand that has 172 kb which encodes for 100 proteins (5). It is the first discovered human oncovirus (6), some researchers detected EBV in the neoplastic tissue of breast cancer (7). EBV considered as a risk factor for developing Burkitt's lymphoma, nasopharyngeal carcinoma,

post-transplant lymphoproliferative disorder, a subset of Hodgkin lymphomas, and gastric carcinomas (8). EBV infects children mostly asymptomatically, although occasionally it manifests as infectious mononucleosis (commonly referred to as mono or the kissing disease) (9). After this primary infection, EBV establishes latency a hallmark of EBV infection, leading to persistent (dormant) infections among 90% of the adult population (10).

The first report on the role of EBV in breast cancer has been described by Labrecque *et al*. (11). Meta-analysis of 30 case-control studies showed that the pooled association between EBV and risk of breast cancer is odds ratio 4.74 (12). EBV infections were found in 25% in a study conducted by Fawzy *et al*. (13) in Eygept, (14) found that 65 of 196 (33%) of cases of breast cancer had DNA of EBV when used real time PCR (11). In a mutual study conducted by Iraqi and Egyptian researchers reported that EBV-DNA was found in 90 Egyptian and Iraqi women affected with breast cancers and EBV was detected in 45% (Egyptian) and 28% (Iraqi) positive females respectively (14).

Material and Methods

Thirty invasive ductal carcinoma biopsies (as patient cases) and 29 fibroadenoma specimens (as control cases) were retrieved from the archives of histopathological laboratories of Baghdad Medical City Hospital. The samples were processed to accomplish the sectioning of the blocks, using the conventional methods. An experienced pathologist completed the histological diagnosis for the samples by hematoxylen and eosin slides. Formalin-fixed paraffin-embedded tissues (FFPE) were cut using microtome at the thickness of 4 micrometers, the films picked up with forceps and placed on a positive charged slides. Xylene used for dewaxing the sections, then rehydrated in descending concentrations of alcohol and finally rinsed in DW. The sections were immersed in 0.01 molarity of citrate buffer with pH 6.0, in a high pressure cooker for 40 minutes to begin the antigen retrieval, the sections were allowed to cool in the buffer at room temperature. A reagent of peroxidase block was applied on the slides according to the tissue size then incubated for 10 minutes at room temperature. An appropriate volume of the Epstein–Barr virus latent membrane protein 1 marker (LMP1) was added for the specimen and incubated in refrigerator overnight and followed by rinsing with phosphate buffered saline (PBS) pH 7.2 for 5 minutes. An appropriate volume of the secondary antibody was added for the specimen and incubated for 10 minutes, followed by rinsing with PBS for 5 minutes. Polyhorse radish peroxidase was added to the specimen and incubated in room temperature for 10 minutes followed by rinsing with PBS for 5 minutes. An appropriate volume of substrate 3,3'Diaminobenzidine chromogen (DAB) solution was added to the specimen and incubated for 10 minutes at room temperature in dark place followed by rinsing thrice in PBS. The slides were immersed in Mayer’s

hematoxylin bath for 15 seconds and then rinsed with tap water and dehydrated in graded alcohol, immersed in xylene then mounted with DPX to be covered with cover slip and left to dry then tested under microscope. The negative control consisted of the same section where the diluents without primary antibody were applied.

Statistical Analysis

The Statistical Analysis System- SAS program was used to detect the effect of difference factors in study parameters. T-test was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study (15).

Results

The patients' age included in this retrospective study ranged from 38 to 78 years with average of 57.4, while the age of the controls ranged from15 to 35 years with average of 28.12. Histological grading for the patients revealed that 29 (96.67%) cases were moderately differentiated (grade II) and 1(3.33%) case was poorly differentiated (grade III). Nodal involvement by cancer detected in 63.3% of IDC cases. 17 (56.7%) of the IDC cases involved left breast while 13 (43.3%) involved the right breast, for the control group 21 cases (72.41%) of left breast involvement and 8 cases (27.59%) of the right breast involvment.

Twenty three of the 30 (76.67%) IDC were positive for LMP-1 of HPV, meanwhile 17(58.62%) of the fibroadenoma was positive for the protein.

Table (1): Comparison of participants' characteristics between cases and controls for IDC.

Characteristics		IDC (N = 30)	Fibroadenoma (N = 29)	P-value	OR
Age (Year)		57.40 ± 2.23	28.12 ± 1.37	0.0001 **	1.89 (1.26-4.03)
EBV	Presence	23 (76.67%)	17 (58.62%)	0.0037 **	
	Absence	7 (23.33%)	12 (41.38%)		
Stage of Cancer	IA	1 (3.33%)			
	IIA	10 (33.3%)			
	IIIA	4 (13.33%)			
	IIB	3 (10.00%)			
	IIIB	1 (3.33%)			
	IIIC	10 (33.33%)			
	IV	1 (3.33%)			
Grade	II	29 (96.67%)		0.0001 **	
	III	1 (3.33%)			
Side of breast	Right	13 (43.33%)	8 (27.59%)	0.0084 **	1.56 (1.08-3.66)
	Left	17 (56.67%)	21 (72.41)		

IDC: Invasive ductal carcinoma, **EBV:** Epstein-Barr virus, **OR:** Odds ratios.

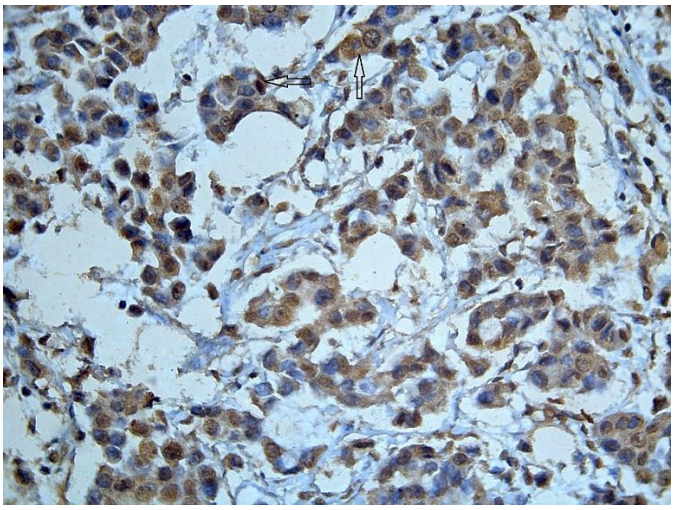

Figure (1): EBV LMP1 protein expression identified in IDC, cytoplasmic staining (arrow), Score=3 and strong intensity, IHC, high power (x40).

Discussion

International Agency for Research on Cancer (IARC) classified EBV as a group-1 carcinogen (16), despite that the associated cancers vary in their viral prevalence and in patterns of viral genes expression (17). The prevalence of EBV in nasopharyngeal carcinoma (NPCs) is 100% while for gastric carcinomas is about 10% (18). The infection with EBV represents an important step in carcinogenesis, yet epidemiological risk factors play an additional critical role in this process (19). The susceptibility to EBV driven oncogenesis include some factors as host tumor suppressor proteins among which are p53, BRCA1 and BRCA2 (20). In this study the correlation between IDC and EBV was investigated. significant positive correlation was found based on IHC. The results of variable studies shown a variable frequencies for the presence of the virus in the specimen, those differences may be attributed to the different targets that approved by the researchers to detect the virus or due to the variation in the studied groups (21). In a study by Joshi *et al*. Found that about 55% of cancerous cases revealed expression of Epstein–Barr nuclear antigen 1 (EBNA1), meanwhile no expression for the missioned gene could be obtained from the benign cases. In a study done by Xue *et al*. (19) that targeted LF3 for detection by using reverse transcriptase (RT) PCR technique and the result was the presence of the gene in 40% of the breast tumors (20). An analysis of the interaction between the host' regulator proteins with the virus' oncogene reported that oncogene and BRCA1 could be co-expressed during lytic phases of the viral replication which indicates the role viral genome in cellular transformation (22). Another study accomplished by Preciado and colleagues showed the positive percentage reaching 35% of EBV presence in breast cancer depending on IHC test and a positive 31% result when depending PCR test (23). A 45% and a 28% positive rate of

EBV-DNA in Egyptian and Iraqi malignant cases respectively yielded with In situ hybridization (ISH) method (24). In this study EBV was detected in 17 of the total 29 fibroadenoma. The role of EBV in the pathogenesis of fibroadenoma is suggested in a study performed on immunosuppressed individuals; in another study fibroadenomas were positive for EBV DNA. There is a report that missioned the presence of the virus in non- cancerous gastric epithelium such as atrophic gastric mucosa which may progress to cancer (25).

Conclusion

In the current study IHC analytical assay has been used for detection of EBV by targeting LMP1 of the virus to localize and target EBV receptors. Results have shown that EBV positivity in IDC tissues was 76.67%, while in the fibroadenoma the positivity was 58.62%. This findings suggest that EBV might have a role in the pathogenesis of breast cancer. Further research is necessary in order to determine the role of EBV in progression of breast cancer by using molecular technique.

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