The Expression of Epstein-Barr virus in Breast cancer in relation to age

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Abstract

Background: Breast cancer is the most common malignancy affecting females worldwide. The association of Epstein-Barr virus (EBV) with this cancer is a long-standing interest to this field.
Aim: to investigate the presence of EBV in breast tumor tissue in relation to age.
Patients and Methods: Paraffin-embedded tissue blocks from 45 female patients with breast tumors (ranged in age from 28 to 85 years) were retrieved. The cases were grouped into two categories: group (A): included 30 cases with breast carcinoma and group (B): included 15 cases with benign breast diseases as a control group .The expression of EBV protein was examined immunohistochemically.
Results: Twelve (40%) of the 30 breast cancer cases (group A) were reported as positive for EBV expression and significantly higher in patients less than 40 years age (P= 0.026).
Conclusion: the results of the current study might refer to the significance of EBV expression in breast tissue of young patients with breast cancer.
Key words: Age, Breast cancer, Epstein-Barr virus.

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Introduction

Younger women generally do not consider themselves to be at risk for breast cancer. However, breast cancer can strike at any age, and women of every age should be aware of their personal risk factors for breast cancer [1]. As the etiology and progression of breast cancer remains incompletely understood, novel routes of disease pathogenesis are important to consider. Viral pathogens have not been much explored, but recent interest has focused on EBV[2,3]. Epstein-Barr virus, a ubiquitous herpesvirus, is a risk factor for developing Burkitt's lymphoma, nasopharyngeal carcinoma, post-transplant lymphoproliferative disorder, a subset of Hodgkin lymphomas, and gastric carcinomas [4]. In these cancers, for which epidemiologic and molecular virological data support a causal link, EBV DNA and one or more viral gene products have been detected in the majority of tumor cells [4]. Recently, EBV was implicated as a possible contributor to a subset of breast carcinomas [2,3].The association of this herpesvirus with breast cancer have had notably inconsistent results, marked by varying EBV presence (from 0% to 50% of tumors) [5]. The aim of the present study was to determine if there is an expression of EBV protein in breast tumors and to see if it is related to the age and menopausal status of the patients

Methods:

Formalin-fixed, paraffin-embedded tissue blocks from 30 female patients with breast carcinomas, ranged in age from 28 to 85 years and to whom either mastectomy or lumpectomy was done were retrieved from the files of the Department of Pathology in Al-Kadhymia Teaching Hospital and the Hospital of Specialized Surgeries, between the years 2003 to 2005. They included 22 invasive ductal carcinomas, 4 invasive lobular carcinomas, and 4 in situ carcinomas. Among the 26 invasive carcinomas, 15 cases were classified as moderately differentiated and 11 cases as poorly differentiated carcinomas. The histopathological examination of the hematoxylin and eosin (H&E) slides prepared for each paraffin-embedded block were examined by histopathologist for histological typing, staging, and grading of the tumors. The study controls included 15 formalin-fixed, paraffin-embedded tissue blocks from patients with benign breast diseases, 6 cases with fibrocystic disease with epithelial hyperplasia and 9 with fibroadenoma.

Immunohistochemical Detection of Epstein-Barr Virus Protein (EBNA-2):

Fixed, paraffin embedded tumor tissues and control tissues were baked overnight then dipped in xyelene and ethanol (100%, 95% & 70%). After deparaffinization and rehydration, to recover tissue antigenicity, the slides were immersed in a jar containing the Antigen Retrieval Solution and placed in the autoclave, leaving the slides for 2 minutes under 121°C then the autoclave was turned off. The entire jar with slides was taken out of the autoclave and the slides were allowed to cool in the Antigen Retrieval Solution for 20 (±1) minutes at room temperature. The steps of immunostaining were followed as indicated in manufacturer protocol of The CHEMICON IHC Select® Immunophosphatase Secondary Detection System Cat.No.APR (CHEMICON INTERNATIONAL, U S A) kit. Primary monoclonal antibody in 1/100 dilution (Chemicon

International,USA) was added. Slides were incubated at 37 °C for one hour followed by the secondary antibodies reagent were applied to the specimen then the slides were placed in the humid chamber and incubated at 37°C for 30 minutes. After application of the Streptavidin Alkaline phosphatase reagent followed by Fast Red Chromogen, the sections were finally counterstained and mounted. The alkaline phosphatase activity on the chromogenic substrate results in the deposit of red insoluble precipitate which is indicative of positive reactivity (as indicated in manufacturer protocol).

Statistics: Student's *t* test and the Chi-square (χ^2) test of significance were adopted for statistical analysis. Correlation coefficient (*r*) was used as a qualitative indicator to express the relative relation between variables.

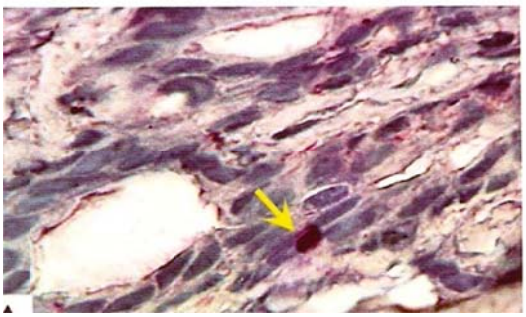
Results

Twelve of the 30 (40%) breast cancer cases (group A) were reported as positive for EBNA-2, showing nuclear immunostaining **(figure-1).** However, the percentage of positive tumor cells was low (1% or less) of the total tumor cells in any EBV positive case. All cases with benign lesions (group B) were negative for EBNA-2. However, no significant association was observed between the EBNA-2 expression and any of other clinicopathological criteria such as tumor size, invasiveness, differentiation and axillary lymph node metastasis (P> 0.05). Nevertheless, the proportion of EBV positive cases was statistically significantly higher in younger age patients with (100%, 33,3%, 27.3% and 20%) for age groups (<40, 40-49, 50-59 and >60 years) respectively (P= 0.026). Moreover, no significant association was observed in relation to the menopausal status. The results are summarized in **Table (1).**

Table 1. The association of EBV positive breast cancer lesions with age, menopausal status and clinicopathological criteria.

Variable	No. of patients	EBNA-2 +ve/total (%)	EBNA-2 no. of –ve/total (%)	2 TestX P value
Age (years)				**P=0.026**
<40	**5**	**5 (100)**	**0**	
40 - 49	**11**	**3 (27.3)**	**8 (72.7)**	
50 - 59	**9**	**3 (33.3)**	**6 (66.7)**	
≥ 60	**5**	**1 (20)**	**4 (80)**	
Menopausal Status				
Premenopausal	**23**	**11 (47.8)**	**12 (52.2)**	
Peri- or Postmenopausal	**7**	**1 (14.3)**	**6 (85.7)**	
invasiveness Tumor				**P=0.632**
Insitu	**4**	**1 (25)**	**3 (75)**	
Invasive	**26**	**11 (42.3)**	**15 (57.7)**	
Axillary LN status				**P=0.305**
Negative	**6**	**1 (16.7)**	**5 (83.3)**	
1-3 Positive	**5**	**2 (40.0)**	**3 (60.0)**	
>3 Positive	**15**	**8 (53.3)**	**7 (46.7)**	
Tumor size (cm)				**P=0.096**
< 2	**2**	**0**	**2 (100.0)**	
2 - 5	**17**	**5 (29.4)**	**12 (20.6)**	
> 5	**11**	**7 (63.6)**	**4 (36.4)**	
Tumor differentiation				**P=0.709**
moderately differentiated	**15**	**7 (46.7)**	**8 (53.3)**	
Poorly differentiated	**11**	**4 (36.4)**	**7 (63.6)**	

Figure (1): Breast cancer tissue showing immunohistochemical expression of EBV. Magnification power (x 400)



Discussion:

Findings about the EBV-breast cancer association have been variable, due in large part to technical laboratory difficulties in determining if EBV in breast tumors is in the breast cancer cells themselves, which would suggest that the virus impacts tumor development [2, 6]. One approach favored by many groups is to use immunohistochemistry for detection of the presence of EBV in tumor tissue. In the current study EBNA-2 was targeted for immunohistochemical localization of EBV, as this protein is considered to be a powerful transactivator of cellular and viral gene transcription [7] and EBV was detected in 40% of breast carcinoma cases. After reviewing the previous studies, we found that some showed consistently negative results. Those studies used immunohistochemistry or *in situ* hybridization [6, 8, 9]. In contrast, most studies showing greater than 32% positivity for EBV (2, 3,10,11,12). These contradictions reflect the different assays used, their different sensitivities, and different definitions of ''EBV positive''[6].

According to the current study, no statistically significant association was observed between EBV expression and histopathological criteria which is in agreement with other studies [12,13]. The proportion of EBV positive cases was statistically significantly higher in younger age patients with breast cancer**,** however, our sample size is small and the results cannot be generalized to normal population**.** On reviewing previous studies we found two studies on association of EBV with the risk of breast cancer in young women [14,15], their results were explained on immunological bases as immune response to EBV as a result of recent infection which would be consistent with the hypothesis that late exposure to EBV appears to be characterized by the production of proinflammatory cytokines and These proinflammatory cytokines, in particular, tumor necrosis factor-alfa and interleukin-6, stimulate aromatase function that is associated with increase in breast cancer risk [15,16]. In conclusion, our data continue to support the presence of EBV in breast cancer tissue in addition to its significant association with young age groups.

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