The Meshing between Epstein Barr Virus Nuclear Antigen-1 and P53 in Iraqi Malignant Breast Tissues

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Abstract

In Iraq, breast cancer incidence exceeds any other type of cancers and the etiology not understood well. Epstein Barr virus is a gamma herpesviruses and one of carcinogenic viruses that may implicated to breast carcinogenesis. The nuclear antigen-1 (EBNA-1) protein is the sole EBV antigen that presented in all tumors related to EBV and plays pivotal roles in carcinogenesis of the virus. Examination applied by immunohistochemistry (IHC) to detect and demonstrate the correlation between (EBNA-1) and tumor suppressor protein (P53) expression. The study includes paraffin-embedded tissue blocks of ninety 90 malignant breast tissues and thirty 30 normal breast autopsies. EBNA-1 was significantly expressed in 40/90 (44.4%) of malignant tissues while its expression in normal breast tissues was negative in all tested cases. The tumor suppressor protein P53 was showed negative expression in all normal breast tissues and positive in 27/90 (30%) in malignant breast tissues. A significant negative relationship ($r=-0.420$; $P<0.05$) revealed between EBNA-1and P53 expression. These finding reveal that EBNA-1 was evident in malignant breast tissues and demonstrate the interplay between EBV and p53 raising the possibility that viral infection may be involved in carcinogenesis process.

Keywords: *EBV, EBNA-1, P53, and Immunohistochemistry.*

Introduction

Malignancy of the breast is the top distributed cancer among females in Iraq with an estimation of 4,720 cases in 2015. The incidence of breast cancer generally exceeds any other type of cancers in Iraq (33.5% of all cancers in Iraq)[1]. The causality of breast cancer is not yet clearly understood, but its incidence related to some environmental factors like viruses such as EBV [2]. EBV belongs to the gamma herpesvirus family, affects over 90% of the world's population and one of the main viruses to be directly involved in cancer genesis[2]. EBV has been involved as a causative agent of many cancers like Hodgkin's disease, non-Hodgkin's lymphoma, Burkitt's lymphoma,, as well as nasopharyngeal carcinoma, arising in immunocompromised individuals[3]. Researches revealed a strong relationship between EBV and breast carcinoma by showing strong evidences[4]. In both primary and metastatic tumors, the constant expression of some of EBV proteins suggests that these proteins are the key player in the EBV-associated tumorigenesis[5]. The tumor suppressor gene, p53 is frequently related to many human cancers because most of the human cancers have mutated p53 [6]. Mutations in p53 happened in breast cancer and are typically associated with more aggressive tumor characteristics, but the clinicopathological and epidemiological features of p53 protein expression is still not clear yet. Some studies suggested that EBV infection induces p53 expression in away or another without causing mutations [7]. Transformed cells by EBV are delicate to apoptosis by P53 mediated pathways[7]. EBV infection of primary cells usually induces a DNA damage-signaling pathway and inhibits cellular proliferation[8]. At the same time, EBV has the capacity to cancel the effect of p53 by multiple means. EBV has many genes that blocks p53-mediated apoptosis or downregulates the expression of p53[9,10]. Epstein-Barr nuclear antigen-1 (EBNA1), demonstrated in all tumors correlated with EBV, may act as the sole protein in many tumors and supposed to be implicated in tumorgenesis[20]. The present study used

to investigate the relationship between EBV-infected patients by testing (EBNA-1) antigen expression with p53 expression in Iraqi breast cancer patients.

Materials and Method

Patients and sampling: The present study have done in a period between January 2019 and October 2019 in Baghdad. Paraffin blocks of breast biopsies were obtained from patients of breast carcinoma after mastectomy. Blocks were collected by a pathologist from Al Alweiya teaching hospital according to ethical considerations and the hospital approval. Formalin fixed, paraffin embedded tissue blocks of (90) malignant breast tissue and (30) normal breast autopsies were obtained. A faculty pathologist re-examined all slides stained with Hematoxylin and Eosin (H&E) for histopathological diagnosis of the chosen blocks.

Immunohistochemistry (IHC): Paraffin-embedded blocks sectioned using a microtome to 4 µm and placed in tissue floatation bath (40C°) and then placed on positively charged slides. The slides were set in oven at 70C° for one hour followed by a series of sequential xylene/ethanol/water washes that remove the wax and rehydrate the tissue for subsequent antibody binding. Heat-induced epitope retrieval (HIER) was applied for antigen unmasking. Heating to boiling in buffer was applied for the slides using a pressure cooker for 30 minutes. Then, the slides were transferred to PBS (phosphate buffer saline) before immunostaining. The sections quenched in a hydrogen peroxide (H_2O_2) by adding drops on the slides for 10 minutes. Protein block solution were applied to each tissue section and allowed it to remain in place for 10 minutes. Then, antibody solution was added to the slides by using micropipette 100-200µl of diluted antibody solution. The slides were incubated overnight (18-22) hour at room temperature. Streptavidin peroxidase and biotinylated Goat Anti-Mouse was added to each slide and incubated for two hours each respectively. Washing of the slides by washing buffer should be applied after each stain. Freshly prepared DAB chromogenic solution was added to the tissue sections and incubated for 10 minutes. The slides washed by distilled water and counterstained by hematoxylin. Aqueous mounting media was added to cover slips where the slides laid on. The slides left for a while to be dried and be ready for examination under the light microscope.

Results

Patients and sampling: A number of (90) malignant breast tissues and (30) normal breast autopsies were included in this study for sampling. Formalin-fixed paraffin embedded tissue blocks of breast obtained from each woman included in this study and according to ethical approval.

Epstein-Barr virus nuclear antigen expression (EBNA-1): All malignant and normal tissue blocks were tested for EBV nuclear antigen (EBNA-1) expression. From 90 breast cancer samples, 40 cases (44.4%) were positively express the antigen while all the normal control cases were show negative expression (figure1and 2). Our results show that EBNA-1 expression is significant in malignant breast tissue as compared to normal control specimens ($P<0.05$).

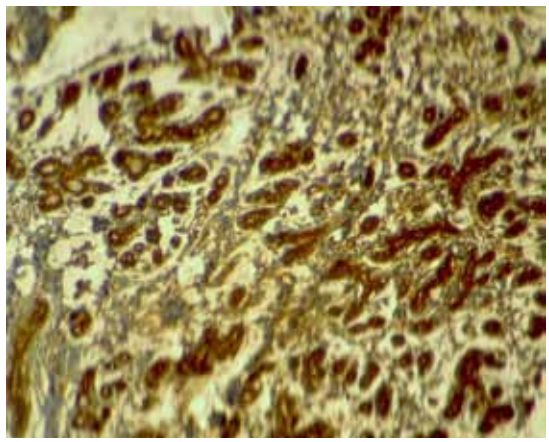

Figure 1: 40X strong EBNA-1 antigen expression of nuclear and cytoplasmic staining in breast carcinoma, brown colour is the positive result.

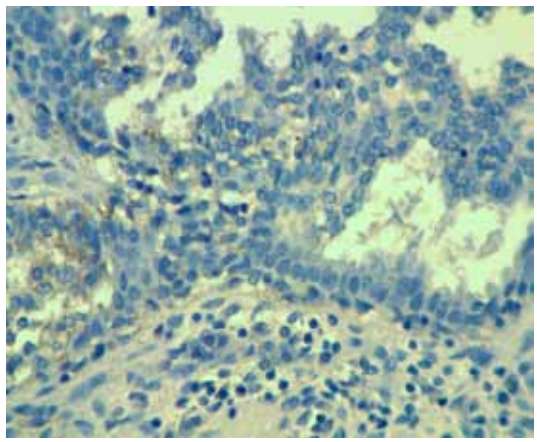

Figure 2: 10X negative EBNA-1 antigen expression in breast carcinoma. Brown colour is not present.

P53 Expression: P53 have been tested in all cases by immunohistochemistry (IHC). From (90) cases of breast carcinoma, a number of 27 (30%) cases were express P53 positively. Among them, 17 (18.8%) showed strong expression, 10 (11.1%) cases moderately express p53 (figures3, 4, and 5). According to ages, The protein expressed in 18.8% (n=17) females of age younger than or equal 50 years (≤ 50 years), While females of ages older than 50 years the results of expression 11.1% (n=10). A statistical significant (P<0.05) relation between patients ages and expression of p53. In addition, It was found 14.4% (n=13) of p53 positive expression were of the histological grade III, 12.2% (n=11) of histological grades II and 3.3% (n=3) of histological grade I. Statistical analysis was found to be statistically significant (P<0.05).

Mentioning lymph node involvement, 18.8% (n=17) of positive cases were correlated with lymph node involvement. P53 positive cases with no lymph node involvement recorded in 11.1% (n=10). No significant association have been observed (p>0.05). The tumor size also determined in the present study, It was found that the size range > 10cm was the highest (15.5%, n= 14) compared to other tumor size ranges of p53 expressed cases. Statistics of estrogen and progesterone receptors found in this study. From total number of malignant cases in the study, A percentage of (20%, n=18) that express p53 positively express estrogen negatively and only (10 %, n=9) express estrogen positively. The progesterone receptor negative cases show higher p53 expression (17.7%, n=16), whereas the positive cases seen only in (n=11). Statistical association of p53 expressed cases with hormone receptors, lymph nodes involvements and tumor size were statistically not significant (Table 1).

Table 1: Some clinic pathological factors and its relation to p53 expression in breast cancer patients

Parameter	P53 expression		
	Positive (no. of patients)	Negative (no. of patients)	P-value
Lymph node metastasis			
Node +	17	40	N.S
Node -	10	23	(P > 0.05)
Tumor size			
≤ 10 cm	13	35	N.S
> 10 cm	14	28	(P > 0.05)
Estrogen receptor			
Negative	18	42	N.S
Positive	9	21	(P > 0.05)
Progesterone receptor			
Negative	26	34	N.S
Positive	11	17	(P > 0.05)

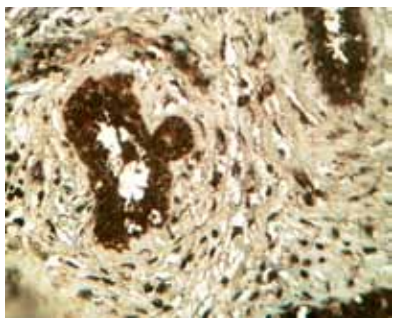

Figure 3: 40X Strong positive Cytoplasmic and nuclear expression of p53 in breast carcinoma. Dark color showing the positive result

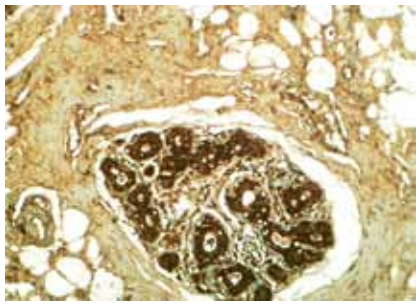

Figure 4: 40X moderate positive Cytoplasmic and or nuclear staining of P53 in breast carcinoma. Dark color showing the positive result

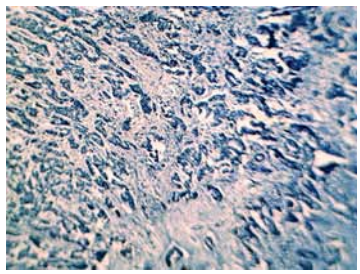

Figure 5: 10X negative p53 expression in breast carcinoma, no dark brown color appeared

The Correlation between EBNA-1 and P53 expression: Significantly negative relationship (r=-0.420; P<0.05) was recorded between (EBNA-1) antigen expression and P53 protein expression in malignant breast tissues. No other relationships noticed between EBNA-1 antigen and the clinicopathological parameters of malignant breast tissues.

Discussion

The present study revealed that EBV significantly expressed in malignant breast tissues than in normal autopsies. EBNA-1 antigen demonstrated in 44.4% of the breast cancer cases, which indicated that EBV might have a role in breast carcinogenesis. A previous studies support the present indication, serological researches on breast cancer patients on EBV relation to disease using the traditional marker of EBV revealed that EBV IgG levels score (97/208; 96%)[11]. Another study tested for both anti-EBNA-1 IgG level by ELISA technique and EBNA-1 antigen expression by immunohistochemistry technique (IHC), the results show that 90.9% of breast cancer cases were seropositive for anti-EBNA-1 IgG and EBNA-1 was positively expressed in 28/51 cases (54.9%) by IHC[12]. The nuclear antigen 1 (EBNA1) protein is the only EBV protein that presented in all tumors that are associated with EBV and plays many important roles in the latency of the virus. The cellular processes that the viral protein play is to reduce apoptosis and increase cell survival [15].

Determining the P53 expression is important for aiding in diagnosis and helping to decide the best therapeutic way in addition to predicting prognosis[13]. Although our present study found significant association of p53 expression with tumor grade, it demonstrated non-significant relationships with other tumor parameters. Previous reports indicated that, P53 expression correlated with clinical grading of the disease and age of the patients significantly. No significant statistical correlations observed with lymph node involvement, tumor size, and expression of estrogen and progesterone receptors[21,14]. In spite, these associations were not significant in statistical manners of the present study, the contrasts in the results explained to genetic, environmental, and possibly social factors [12,13].

All the previous reports of p53 expression in malignant breast tissues extended approximately from 10% to 70% [12]. During this project, p53 demonstrated in 30% of the samples. These investigations can be compared to previous researches exclusively regarding the associations with histological grading and hormonal status. The relatively high expression of p53 could be related to genetic and environmental parameters that reveals the mutation type of P53. In addition, Most of the cases at relatively advanced stages of the disease when they diagnosed for the first time and are already at higher expression of P53. That may reflect the bad prognosis in many of the present study cases [11]. P53 is a tumor suppressor gene, mutations in gene commonly occur in breast cancer. Alterations in the gene lead to change the expression of many other genes that controlled directly or indirectly by p53. The results are malfunctioning of DNA damage repair pathways, cell-cycle arrest, and apoptosis[16].

The relationship between EBNA-1 and P53 is not clear yet. A study in Brazil found that there was a correlation between EBNA-1 and p63 expression, but not between EBNA-1 and p53 [17]. Our present study revealed that there is a significant negative relationship between (EBNA-1) antigen expression and P53 in breast cancer patients meaning that increased expression of EBNA-1 lowers p53 expression. A study, dealt with a key regulator of p53 (USP7), determined that EBNA1 binding to USP7 lowers p53 levels and protect cells from apoptotic challenge [18]. A researchers in (2019) found that EBNA1 lowers p53 level in osteosarcoma cell [19].

Conclusion

The present study revealed Epstein Barr virus nuclear antigen-1was significantly expressed in malignant breast tissues and this raises the possibility that EBV may have a role in carcinogenesis of the breast. Tumor suppressor protein P53 expressed in about 30% of the malignant cases. The correlation between EBNA-1 and P53 expression was negatively significant which means that increasing the expression of EBNA-1 in malignant breast tissues may lower the tumor suppressor protein P53. This foundation may explain one the EBV roles in tumorgenesis of the breast in Iraqi patients.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

References

1. Republic of Health, Ministry of Health\ Environment. Iraqi Cancer Board. Annual Report

Iraqi Cancer Registry, 2015.

2. Pogo B, Holland J. Possibilities of a viral etiology for human breast cancer. A review. Biol Trace Elem Res. 1997. 56, 131–142.
3. Niedboitek G, Young L. Epstein-Barr virus persistence and virus-associated tumours. Lancet. 1994. 343, 333–335.
4. Yahia Z. Epstein Barr virus: a prime candidate of breast cancer aetiology in Sudanese patients. Infectious Agents and Cancer. 2014. 9:9
5. Al Moustafa A, Ahmed H, Wulf G, Sultan A. EBV-Associated Carcinomas: Presence, Role, and Prevention Strategies. Front. Oncol. 2018. 8:528.
6. Gasco M, Shami S, Crook T. The p53 pathway in breast cancer. Breast Cancer Res. 2002. 4, 70.
7. Allday M. Human B cells without neutralizing the function of p53. EMBO. J. 1995. 14,1382–1391.
8. Nikitin P. An ATM/Chk2-mediated DNA damage-responsive signaling pathway suppresses Epstein-Barr virus transformation of primary human B cells. Cell Host Microbe 2010. 8,510–522.
9. Henderson S. Induction of bcl-2 expression by Epstein-Barr virus latent membrane protein 1 protects infected B cells from programmed cell death. Cell 1991. 65,1107–1115.
10. Fries K, Miller W, Raab-Traub N. Epstein-Barr virus latent membrane protein 1 blocks p53-mediated apoptosis through the induction of the A20 gene. J Virol. 1996. 70,8653–8659.
11. Richardson A. Cytomegalovirus, Epstein-Barr virus and risk of breast cancer before age 40 years: a case-control study. Br J Cancer. 2004. 90, 2149–2152.
12. Angeloni A. Epstein-Barr virus and breast cancer: search for antibodies to the novel BFRF1 protein in sera of breast cancer patients. J Natl Cancer Inst. 2001. 93,560–561.
13. Mullauer L. Mutations in apoptosis genes: a pathogenetic factor for human disease. Mutation Res. 2001. 488,211- 31.
14. Al Joudi F, Iskandar Z, Rusli J. The expression of p53 in invasive ductal carcinoma of the breast: A study in the North-East States of Malaysia. The Medical journal of Malaysia. 2008. 63, 96-105.
15. Frappier L. Contributions of Epstein–Barr Nuclear Antigen 1 (EBNA1) to Cell Immortalization and Survival. Viruses. 2012. 4, 1537-1547.
16. Kaur R. Role of p53 Gene in Breast Cancer: Focus on Mutation Spectrum and Therapeutic Strategies. Curr Pharm Des. 2018. 24, 3566-3575.
17. Ribeiro-Silva A. Does the correlation between EBNA-1 and p63 expression in breast carcinomas provide a clue to tumorigenesis in Epstein-Barr virus-related breast malignancies?, Braz J Med Biol Res. 2004. 37, 89-95
18. Saridakis V. Structure of the p53 Binding Domain of HAUSP/USP7 Bound to Epstein-Barr Nuclear Antigen 1: Implications for EBV-Mediated Immortalization. Mol Cell. 2005. 18, 25-36.
19. Chatterjee K. The interplay between Epstein-Bar virus (EBV) with the p53 and its homologs during EBV associated malignancies, Heliyon. 2019. 14; 5(11):e02624.
20. Jiang L. EBNA1-targeted inhibitors: Novel approaches for the treatment of Epstein-Barr virus-associated cancers. Theranostics. 2018. 8: 5307-5319.
21. Hashmi A. Immunohistochemical over expression of p53 in head and neck Squamous cell carcinoma: clinical and prognostic significance. . BMC Res Notes. 2018. 11:433.