Musa Nima Mezher et al /J. Pharm. Sci. & Res. Vol. 9(7), 2017, 1173-1177

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Role of Epstein-Barr Virus (EBV) in Human Females with Breast Cancer

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Abstract

Epstein Barr Virus (EBV) contamination takesinvolved in pathogenesis of numerous forms of carcinomas, which includes gastric most cancers nasopharyngeal carcinoma, and bladder most cancers and has beenrecentlylinked with cancer of the breast. This study was designed toestimate the relationship among Epstein Barr virusencoded minorpiece of RNA (EBER) withtumors of the breast .

40patients of breast cancer had been regained from the Pathology laboratory of AL-Sadder Medical City in Najaf AL-Ashraf Governorate/Iraq. Scientificrecords were investigatedof the medical information and formalin permanent, paraffin implantedtumor tissue have been observed via Chromogenic in situ hybridization (ISH) method to the discoveryfrom the protein of virus EBER.

The manifestation of EBER in the infected tissues cancer with breast most cancers in this study became 50% (11 from 22), in which toughassociation became observed amongstthe communicationfrom EBER and sufferers with cancer of the breast. Even as not create considerable variances between ISH terms of EBER with kind of cancer, age, lymph node metastasis and grade.

Depedned on the outcomes of the presentstudy, Epstein Barr virus performs a prime part in the pathogenesis of breast most cancers.

Key word: Epstein Barr virus, Breast cancer, Epstein-Barr virus-encoded small RNA, in situ hybridization technique

INTRODUCTION

Breast cancers is the second reason of mortality in the world [1,2] and the occurrence has expanded by means of 2- fold over the last 30 years [2]. Breast cancer is the maximum frequent malignancy and the prominent reason of cancer loss amongst women in Western international countries. Even though the aetiology of breast most cancers isn't completely understood, the documentation of the reasons of breast cancer is a vital studies trouble used for the remedy techniques and development of efficacious prevention.

The prevalence of breast cancer is 23% among all cancers in the world [3], and its mortality rate is about 16% [1], so it is the utmostcommunal and fatal cancer in human females[2,4]. Risk factors of breast cancer are age, family history, menarche, delayed menopause, first pregnancy after 25 years of age, nulliparity, long-term consumption of exogenous estrogens, and obesity after menopause, and encountering ionizing ray [5].the principle applicant viruses are mouse mammary tumor virus (MMTV),human papilloma virus (HPV), bovine leukemia virus (BLV and Epstein-Barr virus (EBV)). Every of these viruses has recognized oncogenic capability and completely had been diagnosed in regular and human breast tissues (malignant).

With increasing reports of the association of EBV with epithelial cell malignancies, researchers have raised the question of whether EBV may show a role in the progress of breast cancer. Some researchers indicated that EBV could change epithelial cells, and move toward malignancy [6]. Epstein –Barr virus (EBV) has also remained found to be as an etiological reason for breast cancer [5].

Epstein Barr virus (EBV) has been involvement as a cofactor in many of human malignancies. The probability that EBV may additionally play a important role in the improvement of breast most cancers has been raised in later years. however, some of reports have proven conflicting outcomes. this can be related to the special assays employed and additionally feasible geographical versions in the incidence of this infection[7, 8]. The presnt study focused on evaluating the relationship among Epstein Barr virus encoded minor piece of RNA with tumors of the breast .

To the best of our knowledge, there are only a few studies about EBV in breast cancer in the Middle Eastern countries and few are from Iraq as well.

MATERIALS AND METHODS

Breast samples: Tumor breast tissue was collected from 40 Iraqi women, all blocks of breast tumors were confirmed by breast cancer. The patient's samples were collected from the Pathology laboratory of AL-Sadder medical city in Najaf AL-Ashraf Governorate/Iraq, for the period from September 2015 - up to March 2016. Totally patients existed women, ranging in ages from (26 to 68) years, were included in this study.

Tissue processing: (4µ thick sections) was cut onto slides for routine histopathological examination, immunohistochemistry (IHC) and in situ hybridization (ISH) the staining methodstylesusage of a diversity of dyes that must been selected for their capability to stain numerous cellular constituents of tissue and examined using light microscope. Unstained paraffin sections were used for Chromogeneic in situ hybridization analysis by using Digoxigenin-labeled oligonucleotides which target Epstein Barr Virus-encoded small RNA (EBER) and detection kit of EBER (ZytoVision GmbH. Fischkai 1D-27572 Bremerhaven. Germany)

In situ hybridization:

The 4nm thick paraffin sections from 40 breast tissue were deparaffinized by using of xylene and dehydrated using regressive concentrations of alcohol (100%, 95% and 70%) and distal water, then treated with Pepsin Solution for 20-30 minut at 37°C in a moisturecavity according to manufacture instruction (ZytoVision GmbH. Fischkai 1D-27572 Bremerhaven. Germany). Immerse slides in distilled water. Then the slides with digoxigenin-labelled probe with EBER. Denature the slides at 95°C for10 minutes on a warmdish. Transmission the slides to a moisturecavity and hybridization was then carried out for 2 hours at 37°C for RNA-targeting probes. It is vital that the cell/tissue sectionsprepare not dry out through the hybridization. The slides were soaked in Wash Buffer TBS for 5 min to remove the cover slip, and then preserved with AP-Streptavidin. One to two droplets of BCIP/NBT were positioned on tissue slice and incubate for 30 minutes at 37°C in a humidity chamber; the last was checked by seeing the slides below the microscope. Dyed precipitate will practice at the place of the probe in progressive cells. Slides remained then counterstained using eosin and slices were fixed with a DPX. Lastly Evaluation of the section material is approved out through light microscopy by a pathologist at power 400x.

Statistical analysis

The data were analyzed by using windows software packages Graphpad prism v6.Data are expressed as(mean ± standard error). t-test was used for the statically comparison between groups and to analysis the statistical differences between the groups for all measured parameter, P values of less than 0.05 were considered to be statistically significant.

RESULTS

The mean ages of the patients women with cancer of the breast was 51. 6 years when comparing with benign tumor was 54.5 years as shows in Table (1), there was significant differences (P<0.05) noticed between both groups. In the present study it was observed that breast cancer percentage was increased with the increasing age. Pathologic and medicaltopographies of patients with breast cancer and their relative to EBV whichshowed in table (2).

In situ hybridization results:

The results of ISH which demonstrated that 13 out of 22 (40%) with breast cancer cases were positive for EBER. While 23 out of 27 (60%) was not detected in healthy control group. However the statistical analysis of the distribution of positive results which demonstrated that significant differences as shown in (Table 1).

Table(2) demonstrated the correlation between expressions of EBV with dissimilar variables. The outcomespresented that there were substantial differences between ISH expression of EBV with type of cancer, age, grade and invasive of lymph node. According to SBR grading for LMP 7/40 cases (17.5%) were grade II and 17 cases turned into grade III (50%). As respects LN status, available of the 40 instances, 22 presentedpositive cases of 21/22 had N3 L.N participation and nodal metastases (55%), at the same time as 18 instances presentednegative nodal metastases (45%)(Table 3-4). As respects hormonal receptor observe; ER confirmed slight immunoreaction in 24 instances (60%), 12 instances (30%) presented slight nuclear reaction, and 2 instances (5%) showednoticeabletoughwordy immunoreaction(Table 5). negativeresponse become glaring in 2 instances (5%). As respects PR; 26 cases (65%) confirmed slight immunoreaction, eight instances (20%) and a 2 of instances about (5%) confirmed slight and marked nuclear immunoreactivity respectively(Table 6).

Table (1): Distribution of Mean age (years) Among the Studied Groups

Parameters	Benign tumor N (10)	Malignant tumor N (30)	P value
Age (years)	54.5 ± 12.4	51. 6 ± 13.2	<0.001
LMP1 (EBV) positive	1 (10%)	12 (40%)	<0.001
LMP1 (EBV) negative	9 (90%)	18 (60%)	<0.001
Total	10 (100%)	30 (100%)	<0.001

Table (2): In situ hybridization expression of negative and positive EBV and connected with grade of tumor in patients with breast cancer.

Histological (SBR) grade Grade of) tumor) *	Malignant LMP (EBV) Positive N (12)	Age (years)	Malignant LMP (EBV) Negative N(18)	Age (years)
Grade I	2 (16.6%)	40.5	4 (22.3%)	43
Grade II	2 (16.6%)	40.5	5 (27.7%)	55.2
Grade III	8 (66.6%)	59	9 (50%)	61.7
Total	12 (100%)		18 (100%)	

*Scarff-Bloom & Richardson classification

Table (3): In situ hybridization expression of positive EBV and related with type of tumor in patients with breast cancer.

Malignant tumor /LMP1 (EBV) Positive		
Type of tumor stage (TNM)	**Number (12)**	**Type of tumor Grade**
T2N3M1	2 (16.6%)	G III
T2N2M1	2 (16.6%)	G III
T2N1M1	2 (16.6%)	G III
T2NxM1	1 (8.3%)	G III
T1N1M1	1 (8.3%)	G III
T1N1M0	1 (8.3%)	G II
T1N0M1	1(8.3%)	G II
T1NxM0	1 (8.3%)	G I
T1N1Mx	1 (8.3%)	G I

Table (4): In situ hybridization manifestation of negative EBV and related with type of tumor in patients with breast cancer.

Malignant tumor /LMP1 (EBV) negative		
Type of tumor stage (TNM)	**Number (18)**	**Type of tumor Grade**
T2N3M1	3 (16.6%)	G III
T2N2M1	1 (5.5%)	G III
T1N1M0	1 (5.5%)	G III
T2N1M1	1 (5.5%)	G III
T2N1M0	1 (5.5%)	G III
T1N2M1	2 (11.11%)	G III
T1N1M0	3 (16.6%)	G II
T2N2M1	1 (5.5%)	G II
T1N0M1	1 (5.5%)	G II
T1NxM0	3 (16.6%)	G I
T1N0M1	1 (5.5%)	G I

Table (5): Hormonal receptors study of Malignante Breast tumor and Epstin Barr virus positive

Malignante Breast tumor and Epstin Barr virus positve					
	ER/PR$^+$,Her2$^+$	**ER/PR$^+$,Her2$^-$**	**ER/PR$^-$, Her2$^+$**	**ER/PR- ,Her2-**	**P value**
Age (years)	44.8 ±11.2	54 ± 13.3	53.6 ±12.3	68 ± 12.4	<0.001
LMP1 (EBV) Positve	5 (41.6%)	2 (16.6%)	4 (33.3%)	1 (8.3%)	
Grade I	1 (20%)	1 (50%)	0 (0%)	0 (0%)	
Grade II	1 (20%)	0 (0%)	1 (25%)	0 (0%)	
Grade III	3 (60%)	1 (50%)	3 (75%)	1 (100%)	

PR= progesterone receptor ER= estrogen receptor

Table (6): Hormonal receptors study of Malignante Breast tumor and Epstin Barr virus negative

Malignante Breast tumor and Epstin Barr virus negative					
	ER/PR$^+$,Her2$^+$ N(10)	**ER/PR$^+$,Her2$^-$ N (4)**	**ER/PR$^-$, Her2$^+$ N (3)**	**ER/PR- ,Her2- N (1)**	**P value**
Age (years)	53	60	58	48.5	<0.001
Type of tumor grade					
Grade I	2 (20%)	1 (25%)	1 (33.33%)	0 (0%)	
Grade II	3 (30%)	1 (25%)	1 (33.33%)	0 (0%)	
Grade III	5 (50%)	2 (50%)	1 (33.33%)	1 (0%)	

ER= estrogen receptor PR= progesterone receptor

DISCUSSION

Most cancers remains a first-rate public health project notwithstanding advancement in therapy and detection. BC is the maximum usual malignancy between women. during the last period, the EBV relationship with BC takesremainedcontinually debated notwithstanding the nicely-documented attendance of EBV genomicquantifiable in up to 51% frommalignancy. This disagreement is because of the failure of a few investigators to perceive EBV in BC [9]. This is probably due in element to epidemiological variant in EBV infections, similarvariancein the age at which the deliberate sufferers had obtained firstly EBV infection; as residents with better prevalence costs of BC resemble to people with developed chance of not on time principal EBV infection[10].

Moreover, DNA of EBVbecome no longer detected within the samples of the control group. those effects affirm that the EBV changed into controlled to tumor cells. The mainvarianceamongst instances and controls is powerfullyattributed to a role of EBV in BC. that is reinforced by numerous educations that have rummage-sale breast tissue both from numerous benign illnesses or from regular women or from usual breast tissues adjoining to the tumor as controls; such last tissues are much more prospective to carry doubtful viruses than normal tissue obtained from healthy girls. the gene products and / or genetic material of EBV have been hardly ever recognized in control tissues of the breast and were constrained to tumor epithelial [11]. even if Chu *et al.* (2001) have discovered that there are extra permeating lymphocytes in EBV- positive for BC than in EBV not associated tumors (71% in opposition to 27%), these permeating lymphocytes themselves had been EBV negative e [12].

From those results we will propose that EBV may production a role in breast most cancers oncogenesis however it's not likely to remain a firstly etiological mediator as EBV is most effective noticed in some breast most cancers cells. as a substitute, EBV frequently performances in harmony with dissimilar cofactor. It is able to adjust the conduct of previouslyaltered cells in order that they accumulate a extra violentphenotype. This concept is reinforced via the noticing that EBV-related breast cancers are extra generally violent than additional breast cancers [9] and throughmethod of the presentstudy wherein a importantrelationship has been noticed among aggressive lobular carcinoma, the histological type of the likely course of a disease or ailment., and nucleic acid of EBV detection level. similarly, EBV genome became noticed in tumors with massmore than two centemeter (T3 and T2) and in elevation histological SBR mark of invasive ductal tumors (grade III and II). The relationshipbetween the manifestation of EBV genomicquantifiableand developed BC mark has been detected through Murray *et al.* (2003). additionally they found that EBV is noticedadditionaloften in breast tumors that are hormone-receptor reduced; mortar to the aggression *of* those tumors [13]. On the other hand , no connetation was discovered in this study among DNA of EBVdiscovery and steroid receptor manifestation as the common of the BC samples studied utteredtogetherPR and ER. The idea that EBV and related cancers are negatively linked with hormones might not be correct. In research showed at some stage in the Sixties on African losses with obvious EBV related NC, It was observed that these sufferers had extreme urinary testosterone and estrogen hormoneseliminationplanes[14]. These annotations are well matched with the current discoveriesoftrainings in plantation animals, which illustration the attendance of proteins which prompt EBV transcription elements in bothendocrine and exocrine cells, comprising such cells in the lactating cow mammary gland [15].

Comprising to the deprivedprediction elements, wholly tumors caused by EBV were considerably connected with good nodal status, wherein 6/7 (87.5%) of them were connected with more than two LN association. that is in agreement with Bonnet *et al.* (1999) who identifiedalikenoticing[16]. This appointment with the invasion axillary LN indicate that the infectionvia the virus EBV may remainlinked to a great metastatic potentiality of these tumors. In the year 2001,[17], has proved that EBV protein type (EBNA-3C) metastatic suppressor protein referred to as Nm23-H1, which generally overpowers the drive of malignant cells and is discovered in all human cells (sixteen).whilst this herbal constraint on cellular movement is incapacitated with the aid of the virus, lymphatic cells and cancerous breast are allowed to metastasize, or develop. If proved, this outcome would have primary effectsconcerningtherapy and prevention of the complaint. humans with competitive styles of cancer are maximum susceptible and ought to be tested to decide the repute of preceding viral contact whilst surgeons are selecting the utmostsuitable remedy for them. It too might remain smart to carefully observer humans by a records of lively EBV contagion for initial symptoms from cancer [18].

CONCLUSION

In end, our outcomes tested the attendance of the EBV genentic material in a huge subsection of BC in Iraqi patients. The germ changed into additionalrecurrentlyIt is linked with poor predictive factors. This designates that EBV mighttooshow a position in the improvement and behavioural modification of a few violent BC. within the bright of the brand fresh procedures in giving EBV related malignancies these outcomes provide a wish that a sizeable percentage of aggressive BC may be dealt with immunotherapy or antiviral dealers.

REFERNECES

1. World Health Organization. Fact Sheet of WHO Report. The Top 10 Causes of Death in the world. [updated 2004 May 16; cited 2004 Jul 9]. Available from :http://www.WHO.org
2. Boyle P, Levin B. World Cancer Repor. ISBN-13.WHO. Availablefrom:http://www.iarc.fr/en/publications/pdfsonline/wcr/2008/index.php (2 January 2010, date last accessed).
3. Parkin, DM, Bray F, Ferlay J, Pisani P. Global cancer statistics. CA Cancer J. Clin.,55,2005, 74–108.
4. Anderson BO, Jakesz . Breast cancer issues in developing countries: an overview of the Breast Health Global Initiative. World J. Surg.32, 2008, 2578–85.
5. Robbins and Contran. Pathologic basis of disease. 8th ed.Philadelphia: Saunders; 2011. P 288-91.
6. Lawson, JS, Field, AS, Champion, S, Tran, D, Ishikura, H, Trichopoulos D. Low estrogen receptor alpha expression in normal

breast tissue underlies low breast cancer incidence in Japan. Lancet 354, 1999, 1787–88.
7. Gandhi MK, Tellam JT, Khanna R. Epstein-Barr viru associated Hodgkin's lymphoma. Br. J. Haematol.,125(3),2004,267-81.
8. Mezher,MN.A comparative study between HBV viral DNA detection and conventional serological method of diagnosis. Inter. J. pharm.Tech. Res.,9, 2016, 303-6
9. Murray PG. Epstein–Barr virus in breast cancer: artifact or etiological agent?. J. Pathol.209, 2006, 430–5.
10. Yasui Y, Potter JD, Stanford JL. Breast cancer risk and "delayed" primary Epstein–Barr virus infection. Cancer Epidemiol. Biomarkers Prev.,10,2001, 9-16.
11. Tsai JH, Tsai CH, Cheng MH, Lin SJ, Xu FL, Yang CC.Association of viral factors with non-familial breast cancer in Taiwan by comparison with non-cancerous, fibroadenoma, and thyroid tumor tissues.J. Med. Virol., 75, 2005, 276–81.
12. Chu PG, Chang KL, Chen YY, Chen WG, Weiss LM. No significant association of Epstein–Barr virus infection with invasive breast carcinoma. Am. J. Pathol.,159, 2001, 571–80.
13. Murray PG, Lissauer D, Junying J. Reactivity with A monoclonal antibody to Epstein–Barr virus (EBV) nuclear antigen 1 defines a subset of aggressive breast cancers in the absence of the EBVgenome. Cancer Res.,63,2003, 2338–43.
14. Lawson JS, Ginsburg WH and Whitaker NH. Viruses and human breast cancer. Future Microbiol.,1, 2006,33–51.
15. Broadhurst MK, Lee RSF, Hawkins S, and Wheeler TT. The p100 EBNA-2 coactivator: a highly conserved protein found in a range of exocrine and endocrine cells and tissues in cattle. Biochem.Biophys.Acta.,1681, 2005, 126–33
16. Bonnet M, Guinebretiere JM, Kremmer E. Detection of Epstein–Barr virus in invasive breast cancers. J. Natl. Cancer Inst.,91,1999, 1376–81.
17. Subramanian C, Cotter MA and Robertson ES. Epstein–Barr virus nuclear protein EBNA-3C interacts with the human metastatic suppressor Nm23-H1: a molecular link to cancer metastasis. Nat. Med.,7, 2001, 350–5.
18. Subramanian C, Knight JS and Robertson ES. The Epstein Barr nuclear antigen EBNA3C regulates transcription, cell transformation and cell migration. Front Biosci.,7, 2002, 704–16.