Detection of Herpes Simplex Virus-1 Antigen in Tissues of Breast Cancer

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Abstract

Background: Breast cancer is a common malignancy and a major cause of death in women. Many factors responsible for breast cancer such as viruses. Herpes simplex virus-1 (HSV-1), infection which has been implicated in pathogenesis of certain disease and some studies has been described the involvement of viruses in cancers.

Aim: To determine the presence of HSV-1antigen in women presented with breast cancer.

Methods: Twenty two formalin-fixed paraffin embedded breast tissue collected from the Al-Kadhimiya teaching hospital in Baghdad. Clinical data were analyzed from the medical records; in addition ten normal breast tissues used as control group. All samples were sectioned and examined by direct immunofluorescence for detection of HSV-1 antigen.

Results: Detection of HSV-1 Ag in tissues patients with breast cancer was 31.8% (7 out of 22), where statistical significant was found between expression of HSV-1 and patients with breast cancer based on statistical analysis. While no significant correlation between HSV-1 and age, grade, type of tumor and lymph node metastasis.

Conclusion: Based on the results of the current study, Herpes simplex virus-1 could be a co-factor in the oncogenesis of breast cancer or could infect cancer tissues opportunistically.

Key Word: Breast cancer, Herpes simplex virus-1, direct immunofluorescence technique. Tumorgenesis.

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Introduction

Breast cancer is the most common malignancy among women worldwide. An estimated 5-10 percent of all breast cancer is inherited, caused by mutations in the breast cancer susceptibility genes, brest cancer gene-1 (BRCA1) and brest cancer gene-2 BRCA2 [1]. Breast cancer has many known risk factors. Such as geographical, age, gender, diet, endocrine and family history of breast cancer [2].

Many agents including radiation, chemicals and viruses, have been found to induce human cancer [3]. Viral factors are the most important class of the infectious agents associated with human cancers [4]. It was estimated that 17-20 % of worldwide incidence of cancers attributable to a viral etiology [5]. However, different studies of viruses in breast cancer present conflicting results and some of these works remain in dispute. Deoxyribonucleic acid (DNA) viruses, such as specific types of human papillomaviruses (HPV), Epstein-Barr virus (EBV), human cytomegalovirus (HCMV), herpes simplex virus (HSV), and human herpes virus type 8 (HHV-8), have emerged as causal factors of some human cancers[6].

Herpesviruses comprise the largest family of viruses with oral manifestations. Eight types of herpesvirus are known to be pathogenic in human [7]. All herpisviruses

are structurally similar. Each has an icosahedra core surrounded by a lipoprotein envelope. The genome is linear double-stranded DNA, the virion doesn't contain a polymerase [8].

Herpes simplex virus-1 primarily causes mouth, throat, face, eye, and central nervous system infections, while HSV-2 primarily causes anogenital infections. However, each may cause infections in all areas [9]. In all cases HSV is never removed from the body by the immune system. Following a primary infection, the virus enters the nerves at the site of primary infection, migrates to the cell body of the neuron, and becomes latent in the ganglion [10].

Many studies of viruses in breast cancer present conflicting results and some of these works remain in dispute[11][12][13]. So this study designs to investigate the relationship between expressions of HSV-1 antigen and different parameters like: age, grade, type of tumor and lymph node metastasis in breast cancer.

Materials and Methods

Breast samples: Tumor and healthy breast tissue was collected from 32 Iraqi women. Twenty two cases were breast cancer and ten as biopsy negative. The diagnosis of breast cancer tissues were primarily based on the obtained histopathological records of samples that had been accompanied in the hospital laboratory. The patient's samples were collected during the period from 2010-2011from pathology laboratories of Al-Kadhimiya teaching hospital in Baghdad.

All patients were women, ranging in age from 27 to 70 years, were included in this study.

Practical part of this study was conducted at college of dentistry department of oral pathology.

Tissue processing: Hematoxylin and eosin stained slides were reviewed in all cases; unstained paraffin sections for detection herpes simplex virus-1 Glycoprotein C which target of HSV-1 Ag (United States biological, Cat. No. H2033-08E). By used direct immunofluorescence analysis.

Direct Immunofluorescence: US Biological herpes simplex virus-1 Glycoprotein C was used for detection of HSV-1 Ag by direct immunofluorescence assay according to manufacturer's protocol. The slides were deparaffinized and rehydrated by xylene and serially graded alcohol for 5 minutes each and then distill water. The slides were rinsed 3 times with cool phosphate buffer saline (PBS), and left to dry at room temperature, then blocked with blocking buffer (1-2% Bovine Serum Albumin) at room temperature for 2 hours. The slides were washed and left to dry at room temperature. All of the slides were treated with fluorescent-tagged primary antibody (dilution 1:10 with blocking buffer), then incubated over night in refrigerator at 4˚C.

The slides were washed, and examined by used fluorescence microscopy. The slides were considered as a positive when the specimen contained one or more cells displaying HSV-1 specific fluorescence (apple-green fluorescence) and considered negative when there is no specific fluorescence. In each run used two types of controls, Positive and negative controls. Positive control, Consisted of two patients having infection with herpes labialis, and a swab were taken from the site of infection and was put in a charged slide and the same procedure for IF was done. Negative control: Two slides were prepared as the procedure of IF to the whole samples but one slide was prepared by putting sample without using the substrate, but instead of that we used the bovine serum albumin, while the other slide

was prepared by using distilled water instead of the sample.

Statistical analysis

Fisher's exact test and student's t-test were used to obtain statistically significant differences between studied group with $p<0.05$ being considered statistically significant.

Results

The mean age of the patients with breast cancer was 46.80 years when comparing with matched group was 59.70 years. Among patients minimum age was 46 years and maximum 70 years. 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, as shows in Table (1).

Table (1): Age distribution (years) among the studied groups.

Studied groups	N	Mean	Std. Deviation	Mini.	Maxi.	Student (t-test)	
						P-value	Sig.
Healthy Control group	10	59.7	8.9	48	70	0.005	Significant
Breast cancer	22	46.8	12.1	27	70		
Total	32						

Direct immunofluorecent results for HSV-1 are summarized in table 2. Showed that positive expression of HSV-1 were detected in breast cancer tissue in 7 out of 22 cases (31.8%). While 15 out of 22 cases (68.2%) cases were negative. Herpes simplex virus-1 was not detected in the healthy control group.

Table (2): The percentage of HSV-1 in the studied groups.

Results		Studied groups		Comparison of Significance	
		Brest cancer (%)	Healthy Control	p-value	Sig.
HSV-1	Positive	7 (31.8%)	0	0.044	Significant
	Negative	15 (68.2%)	10		
	Total	22 (100 %)	10 (100%)		

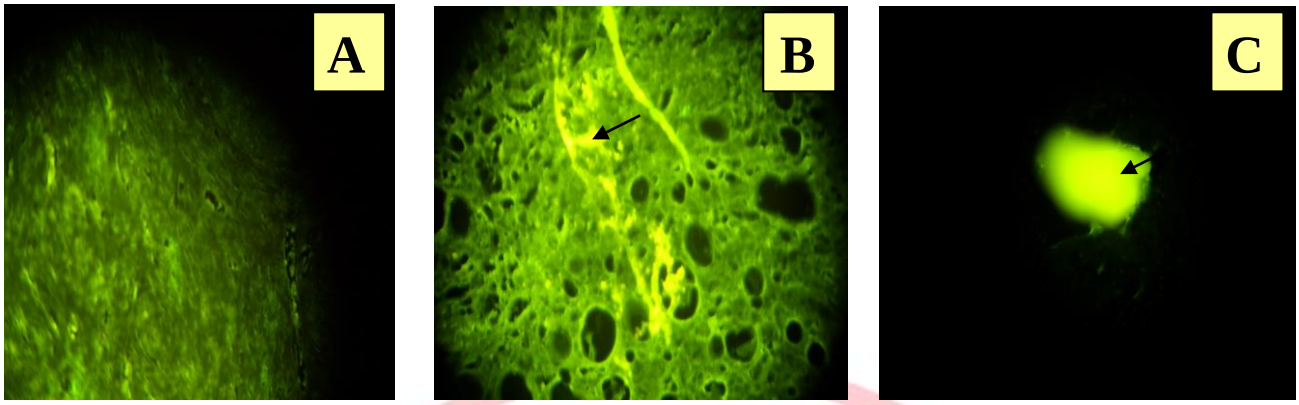

Figure (1): Direct Immunofluorescence in breast cancer section, A- Negative expression B- Low positive expression, C- High positive expression.

In the present study, as shown in table (3) there was no significant differences between expression of HSV-1 and different variable like age grade, type of tumor and lymph node metastasis.

Table (3): Direct Immunofluorescence expression of positive and negative HSV-1 and related with different perimeters profile of patients with breast cancer.

Variables		HSV-1 positive	HSV-1 negative	Comparison of Significance	
				Chi2-value	Sig.
Age	27-42	3 (42.9%)	5 (33.3%)	0.145	Not significant (p>0.05)
	43-58	4 (57.1%)	1 (6.66%)		
	59-70	0	9 (60.0%)		
Types of tumour	Invasive ductal carcinoma	3 (42.9%)	9 (60%)	0.711	Not significant (p>0.05)
	Infiltrative ducal carcinoma	3 (42.9%)	5 (33.3%)		
	Recurrent carcinoma	1 (14.3%)	1 (6.7%)		
Grade of tumour	I	1 (14.3%)	3 (20%)	0.533	Not significant (p>0.05)
	II	6 (85.7%)	10 (66.7%)		
	III	0	2 (13.3%)		
Lymph node metastasis	Invasive	3 (42.9%)	10 (66.7%)	0.290	Not significant (p>0.05)
	Non invasive	4 (57.1%)	5 (33.3%)		

Discussion.

Breast cancer is one of the most frequently diagnosed malignancies of women in many populations. Previous studies provide evidence that viruses exist in women with breast cancer and suggest viruses can be one of the risk factors for breast cancer [14,15].

The presence of HSV-1 was investigated in the present study. Seventh of 22 cases (31.8%) of breast carcinoma were positive for herpes simplex virus-1 Glycoprotein C by direct immunofluorescence. This result was

in agreement with the finding of Tsai *et al*., [16]. Who determined the presence of herpes simplex virus-1DNA by utilizing polymerase chain reaction (PCR) and Southern hybridization in patients with breast cancer and specimens from non-cancerous or other individuals with thyroid tumors or fibroadenoma. The study also agrees with the finding of Chun-Ru *et al*.[6] who indicates that DNA viruses are considered to be one of the high-risk factors closely related to human breast cancer [6].

On the molecular level, HSV-1 consists of more than 80 genes that are expressed sequentially in a strongly regulated cascade [17,18]. Apoptosis of host cells represents an important defense mechanism against viral invasion by preventing viral replication and dissemination. The extrinsic pathway of apoptosis induction is triggered by ligation of death receptors [19] or by injection of granzymes [20]. Intrinsic triggers of apoptosis such as DNA damage, oxidative stress, deprivation of growth factors, and viral infection disrupt the integrity of the mitochondrial membrane, resulting in release of cytochrome c into the cytoplasm [21].

The results of Nguyen *et al*.[22] indicate, apoptosis has been associated with herpes simplex virus 1 (HSV-1) latency and disease severity. There is an intricate balance between pro- and anti-apoptotic processes during HSV-1 infection. When anti-apoptotic pathways are suppressed, this balance is upset and the cells die by apoptosis, who referred to as HSV-1-dependent apoptosis (HDAP). It has been observed previously that HeLa cancer cells exhibit an enhanced sensitivity to HDAP. It also showed that a human mammary tumour cell line was sensitive to HDAP, whilst syngeneic normal cells were resistant. Furthermore, low-passage-number primary human mammary epithelial cells were resistant to HDAP.

When the susceptibility of human colon, brain, breast and cervical cancer cells was assessed, the only cells insensitive to HDAP were those resistant to all environmental stimuli tested. This implies that the HDAP resistance was probably due to mutations in the cellular apoptotic machinery. Thus, the susceptibility of cancer cells to HDAP requires that they possess a functional ability to undergo programmed cell death.

Regarding the HSV-1 expression the results positive rate revealed that the prevalence of HSV-1 was higher in age group 43-58(47.6%) than others as recorded. This may be related with the risk of breast cancer is higher in middle-aged and elderly women than in young women [23].This risk increases as a woman ages, rising sharply after the age of 40. In the United States, more than three-fourths of all breast cancers occur in women aged 50 or older. Women who reach menarche are at a relatively early age (12 or younger) and those who reach menopause are at a relatively late age (55 or older) are slightly more likely than other women to develop breast cancer [24].

Richardson *et al*.,(2004)[25]. Who investigated the association between cytomegalovirus (CMV) and Epstein-Barr virus (EBV) and risk of breast cancer, before age 40 years. On 208 women with breast cancer and 169 controls.

It has been hypothesised that some breast cancers might be caused by late exposure in adulthood rather than in childhood to a common virus [26].

Regarding the histological grade, the result revealed that the positive rate of HSV-1 was higher in grade II than other grade. These results were in agreement with other studies which showed high frequency of moderately differentiated breast cancer

during investigation of the relation between EBV and breast cancer[27,28].

According to the type of cancer, the present study demonstrated that most breast cancer occurred within invasive ductal carcinoma. These results are agree with the findings of other researchers [27,28]. The insignificant association correlation between HSV-1 positive rate and lymph node status. These results also could be explained due to limited sample size.

In conclusion, herpes simplex virus-1 could be a co factor in the oncogenesis of breast cancer or could infect cancer tissues opportunistically. Recommended further studies are needed with large sample size from different population to indicate the same results or study the relation with several candidate viruses such as the types of human papilloma virus (HPV), cytomegalovirus (CMV), and human herpes virus-8 (HHV-8). A more specific approach, such as combining real-time quantitative PCR to measure the amount of viral load in archival tissue samples) with laser capture microdissection (to improve localization of viral nucleic acid in benign or malignant components of a tissue sample)

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