The *In Situ* Expression of IL-6 and IL-1β in breast cancer patients

Amal H. Salman* , Mayada N. Iqbal* and Wasan A. Bakir**

* College of Medical and Health Technology , Baghdad, Iraq
** Iraqi center for cancer and medical genetic research , Al Mustansiriya University, Baghdad , Iraq

Abstract:

Breast cancer is the second most common cancer in women world. Multiple Cytokines appear to have a dominant role in human breast cancer formation. Estimation of the *in situ* expression of IL-6 and IL-1β in breast cancer patients. A sixty patients with breast cancer BC were divided into two clinical subgroups, (30) with malignant breast cancer MBC and (30) with benign breast tumor as a control group according to histological examination. In situ hybridization technique used for detection of IL-6 and IL-1β mRNA sequence in two groups. The results showed that percentages of mRNA expression of IL-6 and IL-1β were in (≥ 11-50%) for malignant breast cancer. This research also investigated that (73.3%) of benign breast tumor were expression less than (<10%) for IL-6 and IL-1 β mRNA. The ISH expression of the mean percentages of IL-6 and IL-1 β were higher levels in malignant breast cancer patients (48.13 and 56.07 ,respectively) than benign tumor (2.73 and 1.40 ,respectively), with highly significantly differences (P<0.01) of ISH expression for IL-6 and IL-1 β mRNA among two studied groups., the expression of IL-6 and IL-1 β mRNA are significantly elevated in the tissue of breast cancer patients compared with benign tumor and was found a significant correlation between the expression of IL- 6 and IL-1β mRNA in the tissue of breast cancer patients, thus the results of the present study might be explain the pathological role of these two cytokine in breast cancer.

Key words: IL-6 mRNA, IL-1β mRNA, Breast cancer, ISH.

الخلاصة

يعتبر سرطان الثدي ثاني الامراض السرطانية انتشارا عند النساء في العالم .وتلعب العديد من السايتوكينات دورا اساسيا في تكوين سرطان الثدي .الهدف من البحث هو التحري عن التعبير في الموضع لكل من انترلوكين - 6 و 1 بيتا لدى مرضى سرطان الثدي. شملت الدراسة)60(مريضة مصابة بسرطان الثدي تم تقسيمهم الى مجموعتين اعتمادا على الفحص النسيجي ، المجموعة الاولى)30(مريضة مصابة بسرطان الثدي الخبيث ، والمجموعة الثانية)30(مريضة مصابة بورم حميد .تم استخدام تقنية التعبير في الموضع للتحري عن الحامض النووي الرايبوسومي المراسل لكل من انترلوكين - 6 و 1 بيتا في المقاطع النسيجية لكلتا المجموعتين .اظهرت الدراسة ان نسبة التعبير للانترلوكين- 6 و1 بيتا هي) ≥ 11 الى 50%) عند مجموعة مريضات سرطان الثدي الخبيث بينما كانت نسبة التعبير هي) >10%) لـــــ 73.3 % من مريضات الورم الحميد لكلا من انترلوكين 6 وانترلوكين 1 بيتا . وبينت تقنية التعبير في الموضع معدل نسب مئوية عالية لأنترلوكين -6 و1 بيتا لمجموعة الورم الخبيث) 48.13 و56.07)على التوالي مقارنة بمجموعة الورم الحميد والتي شكلت النسب)2.73 و 1.40) على التوالي وهذه الاختلافات ادت الى ظهور فروقات معنوية عالية عند مستوى معنوية) >0.01) .كذلك بينت الدراسة وجود علاقة موجبة معنوية بين التعبير لأنترلوكين -6 وانترلوكين - 1 بيتا في المقاطع النسيجية لمريضات سرطان الثدي .وهكذا فأن نتائج الدراسة الحالية قد توضح الدورالامراضي لهذين النوعين من السايتوكينات في تكون سرطان الثدي وتطوره .

Introduction

Breast cancer is the most frequently diagnosed cancer and the second leading cause of death after lung cancer in women [(1)]. There is strong evidence that the tumor growth can be actively controlled by host immune system[(2)] . Cytokines are known to have both stimulatory and inhibitory effects on breast cancer growth depending on their relative concentrations and the presence of other modulating factors in the tumor microenvironment. Certain cytokines appear to prevent an effective immune response being mounted; permitting cancer growth, whereas others promote the immune system's anti-tumor capability [(3)] .Interleukin-6 (IL-6) is a cytokine with multiple biologic activities on a variety of cells. It is produced by macrophages, T cells, B cells, endothelial cells and tumor cells. IL-6 is able to promote tumor growth by upregulating antiapoptotic and angiogenic proteins in tumor cells.

1 Corresponding author : E-mail : thepharmacycollege16@yahoo.com
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It is associated with worse survival in patients with metastatic breast cancer and is correlated with the extent of disease [4]. In human breast cancer, an important role of IL-1β and IL-1RA mRNA expression was noted in various studies [5], Interleukin-1β is a highly inflammatory and prototypical multifunctional cytokine that affects nearly all cell types, often in concert with other cytokines or small mediator molecules. IL-1β elicits important proinflammatory and immunological responses, such as fever, hypotension, increasing circulating NO, recruiting neutrophils,and costimulating T cell activation by increasing IL-2R expression and inducing IL-2 production [6]. The basis of the various biologic properties of IL-1β depends on its regulatory effects on the expression of various genes and/or receptors. IL-1β induces the gene expression of the IL-1 family, other inflammatory cytokines, colony stimulating factors, and mesenchymal growth factors [7].This study aimed at estimation of the in situ expression of IL-6 and IL-1β in malignant breast cancer patients comparing to the benign breast cancer and find out the correlation between these two marker in malignant and benign patients.

Materials and Methods

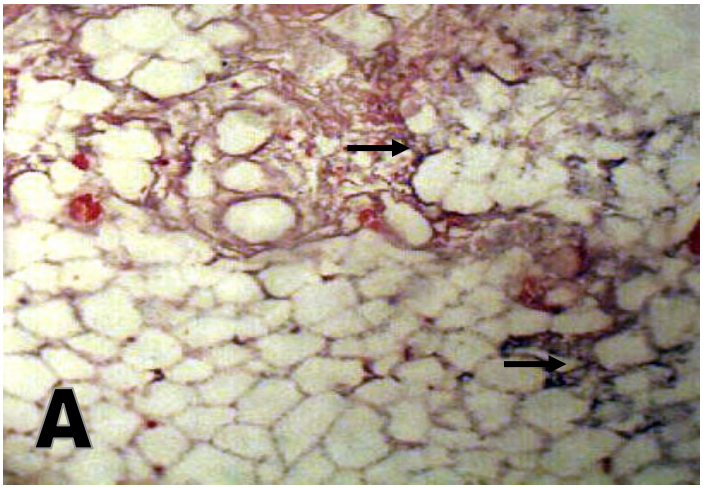
Sixty Iraqi patients with breast cancer who were admitted to AL -Yarmook and Baghdad Teaching Hospital. Patients with breast cancer (BC) were divided into two clinical subgroups according to histological examination: (30) with malignant breast cancer and (30) with benign breast tumor as a control group. Fresh samples were obtained during routine examination of surgically removed tissue, each specimen was fixed in 10% formalin then processed paraffin wax embedded section and cut into 5µm thickness, put on Fisherbrand positively charged slides for our research. ***In situ hybridization***: For in situ hybridization technique (ISH), DNA Probe Hybridization/Detection System in situ kit (Maxim Biotech, Inc., USA) was used.The probes were biotin-labeled DNA probes for human IL-6 (360 bp), and human IL-1β (556 bp), (Maxim Biotech, Inc., USA). In situ hybridization (ISH) is a technique used the high specificity of complementary nucleic acid binding to detect specific DNA or RNA sequence in the cell [8] For detection of this markers, the biotinylated DNA probe hybridize the target sequence (IL-6 and IL-1β mRNA sequence) then a streptavidin-AP (streptavidin - alkaline phosphatase) conjugate is applied followed by addition of the substrate promo-chloro-indolyl-phosphate /nitro- blutetrazolium (BCIP/NBT)which yields an intense blue-black signal appears at the specific site of the hybridized probe [9] . This directly streptavidin-AP conjugate like the biotinylated probe provides a rapid and highly sensitive detection method.Evaluation of ISH signal was done with the assistance of a histopathologist .The expression of both IL-6 and IL-1β mRNA was measured by the same scoring system, counting of the number of the positive cells in the tissue that has given a blue-black (BCIP/NBT) nuclear staining under the light microscope. The score was the average from 10 distinct high-power fields observed under ×100 magnification. The percentage of positively stained cell was calculated for each case by taking the mean of the percentages of the positively stained cell in the 10 fields. A score of 0 was given when no staining was detected, 1 if there was weak to moderate staining in less than 10% of cells, 2 if moderate to strong staining was present in 11 to 50% of cells, and 3 if strong staining in more than 50% of cells was detected [10].

Statistical Analysis

The suitable statistical methods were used in order to analyze and assess the results. Descriptive statistics results presented as percentages of frequencies ,mean, SD, SEM, minimum & maximum levels .Inferential statistics used to accept or reject the statistical hypotheses, includes: Chi-square (χ^2),T-test.Pearson Correlation (r). P - value < 0.05 and P < 0.01 were considered statistically significant. [11].

Results

The expression of IL-6 and IL-1β were detected by ISH technique. Tables **1** and **2** show the percentage of frequency scoring for IL- 6 and IL-1β mRNA expression among study groups, respectively. Chi-square test was conducted to examine the association between IL-6 and IL-1β mRNA expression in the tissue in the two groups of investigated women ,it was found that highly significant association ($p<0.01$) between them among the four scoring levels. The results showed that percentages of mRNA expression of IL-6 and IL-1β were in (≥ 11-50%) for malignant breast cancer. This research also investigated that (73.3%) of benign breast tumor were expression less than (<10%) for IL-6 and IL-1 β mRNA. On the other hand, the mean percentages of these two cytokines was significantly higher($p<0.001$) in malignant breast cancer compared with benign tumor as demonstrated in (Table 3) . The expression of IL-6 and IL-1β was heterogeneous blue-black nuclear staining in the tissue, as shown in Figure (1). In addition, this study demonstrated highly significant positive correlation ($P<0.01$)



shown in (Table 4) and Figure (2).

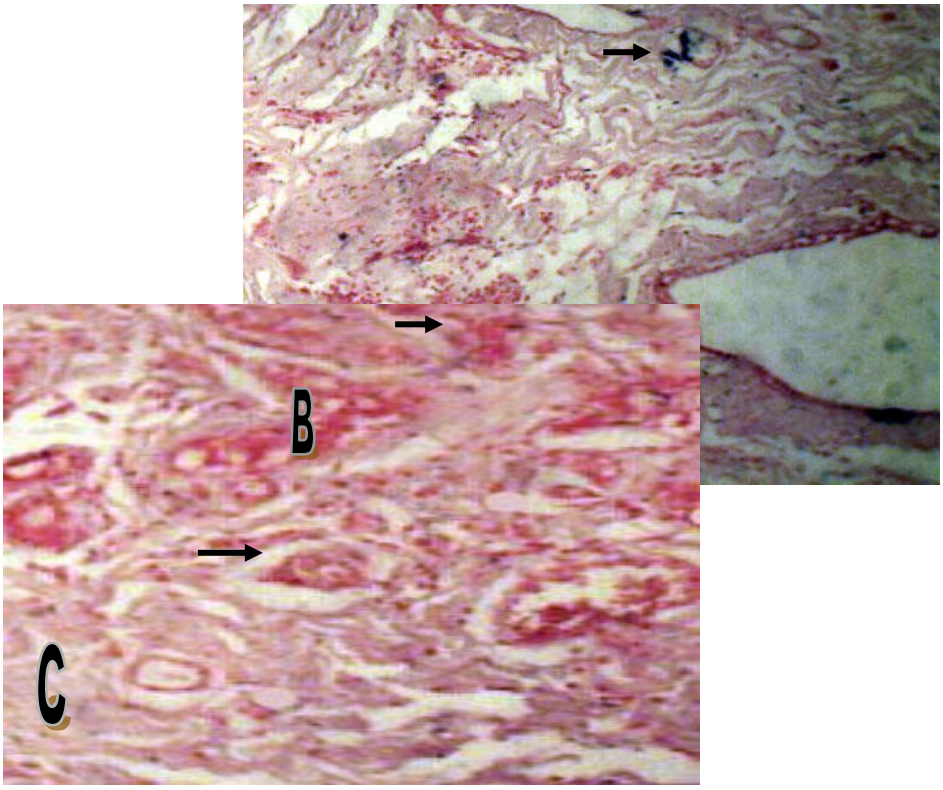


Figure (1): **Detection of IL-6and IL-1β in studied groups by in situ hybridization (ISH)**. Staining of IL-6 and IL-1β mRNA by BCIP/NBT (blue-black)counterstained with nuclear fast red. **(A)** Tissue from breast cancer patients shows positive IL-6 hybridization signals (X400)**. (B)** Tissue from breast cancer patients shows positive IL-1 β hybridization signals (X400)**. (C)** Negative control tissue.

Table (1): Distribution of ISH%IL-6 among studied groups (Malignant breast cancer & Benign breast tumor patients).

ISH%IL-6 groups		Studied groups		Total	Comparison of significant	
		Malignant BC*	Benign BT**		P-value	Sig.
0%	N	0	8	8	0.00	Highly Sig. (P<0.01)
	%	0	26.7	13.3		
< 10 %	N	0	22	22		
	%	0	73.3	36.7		
11-50 %	N	16	0	16		
	%	53.3	0	26.7		
>50%	N	14	0	14		
	%	46.7	0	23.3		
Total	N	30	30	60		
	%	100	100	100		

* = breast cancer ** = breast tumor

Table (2): Distribution of ISH%IL-1β among studied groups (Malignant breast cancer & Benign breast tumor patients).

ISH%IL-1β groups		Studied groups		Total	Comparison of significant	
		Malignant BC*	Benign BT**		P-value	Sig.
0%	N	0	8	8	0.00	Highly Sig. (P<0.01)
	%	0	26.7	13.3		
< 10 %	N	0	22	22		
	%	0	73.3	36.7		
11-50 %	N	11	0	11		
	%	36.7	0	18.3		
>50%	N	19	0	19		
	%	63.3	0	31.7		
Total	N	30	30	60		
	%	100	100	100		

* = breast cancer ** = breast tumor

Table (3): Mean of ISH%IL-6 & IL-1β levels among studied groups (Malignant breast cancer & Benign breast tumor patients)

Studied groups / Interleukins	Malignant BC* N=30	Benign BT** N=30	(T-test)	
			P-value	Sig.
ISH%IL-6				
Mean	48.13 21.03	2.73		
SD	3.84	2.49	0.00	**Highly Sig. (P<0.01)**
SEM	15 ─ 85	0.45		
Mini.─ Maxi.		0 ─ 8		
ISH%IL-1β				
Mean	**56.07**	**1.40**		
SD	**17.87**	**1.13**	**0.00**	**Highly Sig. (P<0.01)**
SEM	**3.26**	**0.21**		
Mini.─ Maxi.	**20 ─ 86**	**0 ─ 3**		

* = breast cancer ** = breast tumor

Table (4): Correlation between ISH%IL-6 level & ISH%IL-1β level among total breast cancer patients, Benign BC patients & Malignant BT patients.

Pearson Correlation	Total		Malignant B C*		Benign BT**	
	ISH%IL-6 level	ISH%IL-1β level	ISH%IL-6 level	ISH%IL-1β level	ISH%IL-6 level	ISH%IL-1β level
r	0.893		0.576		0.516	
P-value	0.00		0.001		0.004	
Sig.	Highly Sig. (P<0.01)		Highly Sig. (P<0.01)		Highly Sig. (P<0.01)	

* = breast cancer ** = breast tumor

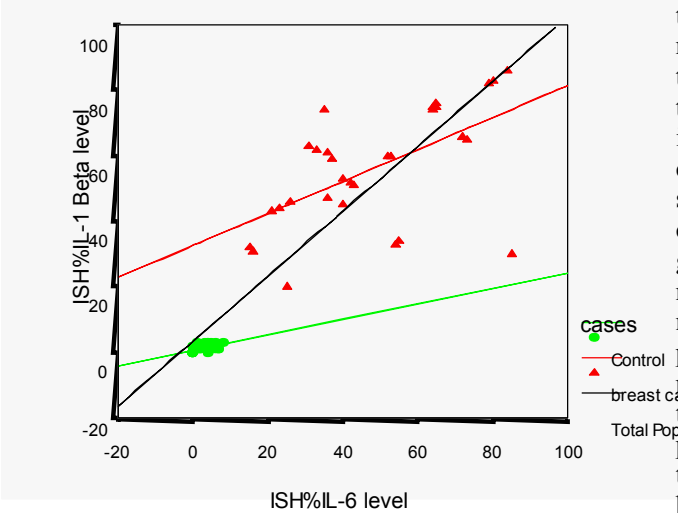


Figure (2): Correlation between ISH%IL-6 level and ISH%IL-1β level among total breast cancer patients, Benign BT patients& Malignant BC patients.

Discussion

Cytokines in general are thought to be involved in numerous physiologic and pathologic conditions. Among cytokines, IL-1β and IL-6 probably seem to play the most important role in breast carcinogenesis [12; 13] .In the present study, IL-6 and IL-1β mRNA expression was examined by in situ hybridization technique in tissue of malignant breast cancer compared with benign tumor . The IL-6 and IL-1β were expressed in a higher percentage in breast cancer tissue compared to benign tumor and we found the positive expression of IL-6 mRNA and IL-1β mRNA among malignant breast cancer were 46.7% and 63.3% were more than fifty percent (>50%),respectively . This results suggests that IL-6 and IL-1β are over expressed in breast carcinoma compared to benign tumor and might play a pathological role in malignant breast cancer. Evidence supporting this suggestion includes the fact that in human breast cancer, the elevated expression of IL-6 and IL-1β were observed in breast carcinoma tissues [5; 14; 15; 16] and in serum [17; 18] .Several studies suggest that the IL-1 system is vital in the local control of tumor growth, important in regulating ''protumorigenic'' activities within the tumor microenvironment, and contributes to angiogenesis, tumor proliferation, and tumor invasion [19; 20; 21]. Furthermore, IL-1ß and IL-6 cause tumor regression and increase median survival time in a variety of cancer patients. In contrast, elevated circulating concentrations of growth factors such as IGF-I are a surrogate risk for cancers of the breast [22; 23; 24]. It is noteworthy that IL-1 β is a prototypical proinflammatory cytokine that exerts a plethora of biological activities, including tumor regression [25]. The tumor-suppressing property of IL-1 β has been attributed mostly to its ability to prime antitumor immunity [26], but the mechanism for its direct cytostatic actions in suppressing cell cycle progression is largely unknown. The antiproliferative action of IL-1 β on human breast cancer cells is exhibited not by killing the cells but rather by preventing the ability of the late G1 progression factor, insulin-like growth factor (IGF)-I, to promote progression from late G1 into the S phase of the cell cycle [27] .This cross-talk between proinflammatory cytokine and growth factor receptors is similar in principle to that between the B cell receptor and the 2-adrenergic receptor for the neurotransmitter norepinephrine [28] and that between the IGF-I receptor and integrin-associated protein for thrombospondin-1 [29] .Moreover, the role of the IL-1 system in human breast cancer is conflicting. IL-1 has been shown to inhibit growth of breast cancer cells and to promote cellular differentiation in vitro, but it is equally known to stimulate the expression of several proteolytic enzymes in human cancer [30;31] .The consecutive degradation of extracellular matrix is a key element of local invasion and metastasis [32, 33] .In addition, they are many confounding studies about the role of IL-6 and IL-1β in tumor cell growth, but its exact role remains varied and unclear [19; 34]. It appears that the effect of IL-6 on tumor cell growth may depend on the tumor cell type, IL-6 plays a

new role in cancer biology; it promotes multidrug resistance [34] and it has been shown to be involved in intercellular signaling between mesenchyme and breast cancer epithelium.. These display an oncogenic role for IL-6; however, lacking is an understanding of the mechanisms governing IL-6 production in tumors and the biological role of this cytokine in tumorigenesis [19, 35]. The human IL-6 shows antiadhesive effects, and modulates the estrogen receptor and progesterone receptor content of these cells [35] .The elevated expression of IL-6 has been detected in multiple epithelial tumors [36] .An interesting finding , in the current study that in situ expression of IL-6 was significantly correlated ($p< 0.01$) with in situ expression of IL-1β ($r= 0.576$; $p< 0.01$) in malignant breast cancer . This results indicating that IL-6 and IL-1 β are strongly interact with each other and act synergistically, subsequently increasing their effect. This finding in agreement with that of Robison and colleagues who reported that a significant correlation between IL-6 and IL-1 immunoreactivity [13] , thus both ILs, i.e., IL-1β and IL-6 have been shown to be strongly interact and to act additively in breast carcinogenesis, subsequently inhibiting tumor growth [37] . In conclusion, in this study both IL-6 and IL-1 β mRNA showed the significant increased expression in the tissue of malignant breast cancer patients compared with benign tumor , this might be implicated in the development of malignant breast cancer. Although a significant positive correlation between these two markers in all studied group, this might reflect a close relation between these two parameters.

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