Possible Role of IL-1-α and TNF-α in Breast Cancer

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Abstract

Background: Cytokines have been used as biomarkers in research for prognosis and have been associated with symptoms and adverse outcomes in multiple conditions, including breast cancer.

Objectives: *To estimate the concentration of IL-1-α and TNF-α in serum of breast* cancer (BC) patients compared with control groups and to detect if there is association of serum levels of these interleukins with disease development.

Subjects and Methods: The levels of IL-1-α and TNF-α were measured by ELISA method in sera of 45 BC patients, 12 patients with benign breast lesions and 23 apparently healthy controls.

***Results*:** Present study was demonstrated that IL-1-α and TNF-α levels were significantly elevated in serum of BC patients as compared with controls ($p<0.001$), this elevation were significantly associated with poor prognostic factors including advanced stage and estrogen and progesterone receptors-negative status.

Conclusions: Evaluation the serum level of IL-α-1and TNF-α may be helpful as predictive non-invasive tests for tumor development in breast cancer patients.

Keywords: Breast cancer, IL-α-1, TNF-α.

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Introduction

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, and may contribute to loco-regional and/or metastatic spread, the elevation of the serum concentration of such cytokines, however, might be utilized as a marker of immune status, disease prognosis and monitoring, where as others promote the immune system's anti-tumor capability [(1)].

Interleukin 1 (IL-1) system plays an important role in human pathology and is involved in the local control of malignant disease. Since tumors can be considered 'wounds that never heal', due to their everexpanding tissue invasion and injury. Therefore, it is highly likely that proinflammatory cytokines such as IL-1 are involved in tumor growth and metastasis [(2)]. The IL-1 family of cytokines, and receptors are present within the human breast cancer (HBC) tumor microenvironment and that the IL-1 network of cytokines and receptors within the tumor microenvironment can control tumor cell subpopulation expression of other protumorigenic cytokines such as the angiogenic/growth factor, interleukin-8 and subsequently contribute to angiogenesis, tumor proliferation,and tumor invasion[(2,3)]. As well as the expression of IL-1 correlate with expression of prognostic factors such as estrogen and progesterone receotors (ER/PR) [(2)].

The multifunctional cytokine, tumour necrosis factor (TNF), is involved in the promotion of inflammatory responses and plays a

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critical role in the pathogenesis of inflammatory, autoimmune and malignant diseases [4]. It induces production of chemokines and promotes production of IL-1 and IFN-γ by lymphocytes and macrophages. Initially proposed to have anti-carcinogenic effects [5], TNF was later shown to be tumourigenic in both in vitro [6] and in vivo studies [7]. High plasma TNF levels in cancer patients are associated with a poor disease outcome [8]. TNF is also a key angiogenic molecule that may promote angiogenesis directly by stimulating endothelial cell proliferation and indirectly by modulating expression of other proangiogenic factors [9]. The current study is a trial to estimate IL-1-α and TNF-α level in the patient's sera in comparison with controls. This, however, might open a gate for entrance into the treatment of this disease.

Subjects and Methods

Subjects:

Forty five breast cancer female patients with age range from 28 to 73 years were eligible for this study. They included invasive ductal carcinoma, invasive lobular carcinoma, and in situ ductal carcinoma.The patients were admitted for surgery at Al-Kadhimia Teaching Hospital and nursing home hospital /medical city, for the period between March 2006 till March 2007. Data of estrogen and progesterone receptors status (immunohistochemically) were obtained from medical records of patients and validated by an experienced histopathologist. Controls were consisted of two groups:- A-Patient control group: - Twelve females with benign breast lesions (6 cases with fibrocystic disease and 6 with fibroadenoma) were involved in this study as a patient control group. B-Healthy control group: - A total of 23 healthy females' volunteers who have no history or clinical evidence of any breast lesions and their sex matched with BC patients were selected as a healthy control group. Venous blood samples were collected preoperative.

Methods:

The BioSource Hu IL-1α and TNF-α kits are a solid phase sandwich enzyme linked immuno sorbent assay (ELISA) (BIOSOURCE, Europe S.A., Belgium, Lot No. 053804; 054807). The absorbance of each well was read at 450 nm within 2 hours after adding the stop solution. The absorbance of the standards was plotted on graph paper against the standard concentration to construct the standard curve. The IL-1 α and TNF-α concentration for unknown samples and controls was read from standard curve.

Statistical analysis

The serums cytokines were quantitative variables, but were non-normally distributed as shown by Semirnov-Kolmogorov test, these variables are better to be described by median and the test of significance suitable for them was non-parametric tests. All the data have been analyzed statistically using Kruskall-Wallis test and MannWhitney analysis for measuring the differences between the studying groups [10].

Results

In the current study, the stages of BC for 45 patients (according to TNM system) were 23(51.11%) cases with stages (0, I and II) and 22 (48.88%) cases with stage III.

Estimation of serum level of IL-1α

Table-1 revealed a significant elevation of serum IL-1α level among BC patients (median=19.8 pg /ml) in comparison to that of control groups which include patients with benign breast lesions (BBL) (median=5.8 pg /ml) and healthy control (median=0 pg /ml) ($p<0.001$).

In addition, the median serum level of this cytokine in BC patients increased significantly with advanced stage (P<0.001) (table 2).

Estimation of serum level of TNF-α

Table-3 demonstrated a significant elevation in the level of serum TNF-1α of patients (median=46.4 pg /ml) in comparison to that of patient control (median=11.2 pg /ml) and healthy control (median=8.7 pg /ml) (p<0.001).

Also the present study showed detectable association between TNFα level and the development of disease P<0.001. Table-4 showed that the median serum level of this cytokine in BC patients increased with advanced stage.

The association of IL-1-α and TNF-α with estrogen and progesterone receptors

The results of association between IL-1α and TNFα level with ER and PR expression in breast cancer samples were shown in tables- 5 & 6. IL-1α and TNFα level was found to be inversely associated to ER and PR expression (p= <0.05).

Table 1: The difference in median levels of serum IL-1α (pg/ml) concentration among the three studied groups.

Serum IL-1	BC cases	BBL control	Healthy control	P (Kruskall-Wallis)
Minimum	1.8	1.5	0	
Maximum	53.1	9.6	3.2	
Median	19.8	5.8	0	<0.001
NO.	45	12	23	
P (Mann-Whitney)				
BC X Healthy control <0.001				
BC X BBT <0.001				

Table 2: The difference in median levels of serum IL-1α (pg/ml) according to the stage of disease

Values	Stage 0, I& II	Stage III	P (Mann-Whitney)
Minimum	1.8	4.2	
Maximum	33	53.1	
Median	6.6	27.2	<0.001
N	28	17	

N= number

Table 3: The difference in median levels of serum TNF-α (pg/ml) concentration among the three studied groups.

Serum TNF-α	BC cases	BBL control	Healthy control	P (Kruskall-Wallis)
Minimum	3.6	2.8	1.8	
Maximum	126.5	51.4	42.2	
Median	46.4	11.2	8.7	<0.001
NO.	45	12	23	
P (Mann-Whitney)				
BC X Healthy control <0.001				
BC X BBT <0.001				

Table 4: The difference in median levels of serum TNF-α (pg/ml) according to the stage of disease.

Values	Stage 0, I& II	Stage III	Mann-Whitney
Minimum	3.6	3.8	
Maximum	65	126.5	
Median	14.3	62.2	<0.001
N	28	17	

N=number

Table 5: The difference in median levels of serum IL-1α and TNF-α (pg/ml) according to the estrogen receptors.

	Estrogen receptor		
	Positive (n=21)	**Negative (n=24)**	**P**
Interleukin-1 Alfa conc.			
Range	(1.8 – 16.3)	(6 – 53.1)	
Median	5.1	23	<0.05
TNF Alfa conc.			
Range	(3.6 – 35.8)	(13 – 126.5)	
Median	11	63	<0.05

Table 6: The difference in median levels of serum IL-1α and TNF-α (pg/ml) according to the progesterone receptors.

	Progesterone receptor		
	Positive (n=26)	**Negative (n=19)**	**P**
Interleukin-1 Alfa conc.			
Range	(1.8 – 19)	(8– 53.1)	
Median	6	24.2	**<0.05**
TNF Alfa conc.			
Range	(3.6 – 41.2)	(10.4 – 126.5)	
Median	15	66	**<0.05**

Discussion

Evaluation of the role of the immune response in either the development or control of breast cancer is complex. Nevertheless, there is substantial information that in this disease, the immune response is not a host defence reaction and may even serve to facilitate cancer development. Potential mechanisms for these effects include production, by inflammatory cell infiltrates, of direct or indirect modulators of breast cell growth, e.g. cytokines [11].

Our data was in accordance with those of other authors who have demonstrated significantly higher levels of innate cells- related cytokines (IL-1 and TNF-α) in sera of patients with BC than those of control groups, moreover, there was detectable correlation between clinical stage and the serum levels of above mentioned cytokines [12-14]. In contrast to these

results, Green and coworkers in (1997) did not observe any correlation between the cytokines IL-1α, IL-1β, IL-4, IL-6, IL-8, and TNF-α, TNF-β, IL-2, IL-5, IL-7 and tumor histological grade or lymph node metastasis in breast cancer patients [15].

A major tumor-associated macrophages (TAM) derived inflammatory cytokine shown to be highly expressed in breast carcinomas is tumor necrosis factor alpha (TNF-α). It is a multifactorial cytokine, simplied by its name, TNF-α may have cytotoxic and apoptotic activities when administered to breast tumor cell lines [16].

The fact that TNF-α activities vary under different physiological conditions and in a cell-type-dependent manner contributes to a sense of ambiguity regarding its antitumor effects [16]. Indeed, recent investigations strongly suggest that the chronic expression of TNF-α in breast tumors actually supports tumor growth. The number of cells expressing TNF-α in breast carcinoma was found to be correlated with increasing tumor grade and node involvement, and TAM-derived TNF-α expression was suggested to play a role in the metastatic behavior of breast carcinomas [17]. The tumor-promoting functions of TNF-α may be mediated by its ability to induce proangiogenic functions, to promote the expression of matrix metalloproteinases (MMP) and endothelial adhesion molecules, and to cause DNA damage via reactive oxygen, the overall effect of which is promotion of tumor-related processes [11,16].

The role of other inflammatory cytokines (possibly TAM derived), IL-1 was also addressed in breast carcinoma [18]. The role of the IL-1 system in human breast cancer is conflicting. Initial analyses regarding IL-1 indicated that its levels were significantly higher in invasive carcinoma than in ductal carcinoma in situ or in benign lesions, implying that elevated levels of IL-1 are directly correlated with a more advanced disease [19].

In addition, 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 [20]. The consecutive degradation of extracellular matrix is a key element of local invasion and metastasis [21]. The mitogenic activity by IL-1 can be explained by induction of growth-related oncogene (GRO) gene expression [22] or induction of IL-8 expression via activation of the Nuclear factor κB (NFκB) and activator protein (AP)-1 signal transduction pathways [23]. The robust response of the metastatic- or mesenchymal-appearing breast carcinoma cells to either IL-1 or TNF-α may be because of elevated expression of transcription factors needed for transcription of the IL-8 gene. NF-κB, a transcription factor, which can be activated by either IL-1 or TNF-α, is an example of such a transactivator. Activated NF-κB recognizes and binds to a consensus sequence in the promoter region of the IL-8 gene. This binding is essential, but not sufficient for the induction of IL-8 expression. It is possible that the metastatic breast cell lines have factors working either coordinately or synergistically with activated NF-κB to enhance IL-8 expression [24].

Among the various prognostic factors, lack of estrogen and progesterone receptors (ER&PR) has consistently been associated with poorer prognosis [25]. Of particular note, in present study we found an inverse correlation between expression of ER&PR and cytokines (IL-1-α and

TNF-α) serum levels, which is in agreement with the findings of other studies [2, 13]. The inverse correlation between (IL-1-α and TNF-α) and ER&PR indicates that the high serum levels of these cytokines correlate with low ER&PR expression. Since low ER&PR expression is considered a prognosticator for poor disease outcome in BC, this suggests that the high IL-1 and TNF-α serum levels would predict poor outcome in BC. So, current data suggest that cytokines could be involved in the aggressiveness of ER-negative breast tumors. It was feasible that it can be used to identify patients with a poor prognosis who may benefit from more aggressive management, and this may help us to understanding the pathogenesis of this disease and ultimately may be use in the development of a new therapeutic technique.

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