

Prognostic Value of Proinflammatory Cytokines in Breast Cancer

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Abstract

Cytokines are factors that are known to have both tumor-promoting and inhibitory effects on breast cancer growth. Different cytokines play an important role in controlling the immune system. This study was carried out to investigate the alterations in serum levels of proinflammatory cytokines (IL-1 α , IL-6 and TNF- α) in breast cancer patients, and its relation with progression of this cancer. The current results revealed that there is significant elevation in median serum levels of IL-1 α , IL-6 and TNF- α , $P < 0.001$ in breast cancer patients, as compared with control groups, this elevation was significantly associated with advanced stage, $P < 0.001$. These findings suggest that serum levels of IL-1 α , IL-6 and TNF- α appears to be of some prognostic value in breast cancer.

Keywords: Breast cancer; Cytokines

Introduction

Breast Cancer (BC) is a disease affecting millions of women, as well as men all over the world. It is generally assumed that the immune response in malignant disease is impaired and thus, associated with a poor prognosis. Clinically detectable dysfunction of the immune system is generally observed in cases of disseminated disease. Therefore, research studies are focused on subclinical measurements of cell function, inflammatory mediators, cytokines and growth factors [1]. Cytokines have been investigated in numerous studies as tumor markers, a prognostic tool for staging and survival and prognostic factors of postoperative complications [2].

Cytokines are known to have both stimulatory and inhibitory effects on BC 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, whereas others promote the immune system's anti-tumor capability [3].

Interleukin-1 (IL-1) plays an important role in human pathology, and is involved in the local control of malignant disease. It is well known that proinflammatory cytokines, such as IL-1 are involved in tumor growth and metastasis [4]. The IL-1 family of cytokines and receptors are present within the BC tumor microenvironment, and can control tumor cell subpopulation expression of other pro-tumorigenic cytokines, such as the angiogenic/growth factor, IL-8 and subsequently contribute to angiogenesis, tumor proliferation and tumor invasion [5].

Interleukin-6 is a major mediator of the inflammatory response, plays a primary role in the pathophysiology of cancer. In BC patients, the extent of the increase in serum IL-6 correlates with poor disease outcome and reduced prognosis. Although, it has been argued that the cytokine may be secreted by cancer cells, the source of the IL-6 in cancer patients has not yet been determined. Cancer cells that are exposed to IL-6 or secrete the cytokine as an autocrine factor show malignant features, such as enhanced capacity to invade the extracellular matrix and increased drug resistance [6].

The multifunctional cytokine, TNF, is involved in the promotion of inflammatory responses and plays a critical role in the pathogenesis of inflammatory, autoimmune and malignant diseases [7]. It induces

production of chemokines and promotes production of IL-1 and IFN- γ by lymphocytes and macrophages [8]. Initially proposed to have anti-carcinogenic effects, TNF was later shown to be tumourigenic, in both *in vitro* studies and *in vivo* studies. It 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]. Therefore, this study was carried out to investigate the alterations in serum levels of IL-1 α , IL-6 and TNF- α in BC patients, and its relation with progression of this cancer.

Materials and Methods

Forty five breast cancer female patients, with an age range of 28-73 years were eligible for this study. The patients were admitted for surgery at Al-Kadhimiya Teaching Hospital and Nursing Home Hospital (Medical City) in Baghdad. Pathological data (histological tumor type grade, tumor stage and lymph node status) were obtained from the medical records of patients, and validated by an experienced histopathologist. Two control groups were included: 12 females with Benign Breast Lesions (BBL); six cases with fibrocystic disease and 6 with fibroadenoma, and 23 apparently healthy females had no history or clinical evidence of any breast lesions and matched by age BC patients.

Determination of serum cytokines

Five ml of serum were collected from each subject for estimating levels of IL-1 α , IL-6 and TNF- α , using Enzyme-Linked Immunosorbent Assay (ELISA) and performed as recommended in leaflet with kit, (BioSource Europe S.A. Company, Belgium).

Statistical analysis was assessed using P (Mann-Whitney test) and (Kruskal-Wallis- test). P value less than the 0.05 level of significance was considered statistically significant.

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Results

A total of newly diagnosed, 45 Iraqi females patients with BC were involved in this study, their mean age was 48.1± 5.2 years, with a range of 28-73 years compared with 12 patients with BBL; their mean age was 35.33 years with a range of 21-50 years, and 23 unrelated individuals as a healthy control, who were age matched to the patient group.

The current findings revealed a significant elevation in serum IL-1α, IL-6 and TNF-α levels among BC patients (19.8 pg/ml, 19 pg/ml, 46.4 pg /ml), respectively in comparison to that of BBL patients and healthy control (5.8 pg/ml, 6.6 pg/ml, 11.2 pg/ml), respectively, (0 pg/ml, 5.1 pg/ml, 8.7 pg/ml), respectively, (p<0.001), as shown in table 1.

Regarding the correlation between serum cytokines levels and stages of tumor, the present result observed that there is positive correlation between median serum levels of IL-1α, IL-6 and TNF-α levels, and advanced stage of tumor in BC patients, P<0.001, as clearly illustrated in table 2.

Discussion

The role of cytokines in cancer immunity and carcinogenesis has been well established. Cytokines play varied roles in cancer pathogenesis, with increasing evidence suggesting their involvement in tumor initiation, growth and metastasis [10].

The present findings are in accordance with other studies, who have demonstrated significantly higher levels of IL-1, IL-6 and TNF-α in serum of patients with BC than those of control groups [11], moreover, there was detectable correlation between clinical stage and the serum levels of IL-1, IL-6 and TNF-α [12]. Similarly, Benoy et al. [13] suggested that both an increased tumor bulk and a more malignant phenotype of the tumor may account for the gradual rise in circulating levels of cytokines. Ordemann et al. [14] and Kozłowski et al. [15] found that a high level of IL-6 was frequently observed in stage III than in other stages (I and II). In contrast, Green et al. [16] did not observe any correlation between the cytokines (IL-1, IL-6 and TNF-α) and tumor histological grade, or lymph node metastasis in BC patients.

The role of the IL-1 in BC 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

Serum IL-1	BC cases NO.=45	BBL control NO.=12	Healthy control NO.=23	P (Kruskall-Wallis)
Minimum	1.8	1.5	0	
Maximum	53.1	9.6	3.2	
Median	19.8	5.8	0	<0.001
P (Mann-Whitney)				
BC×Healthy control<0.001				
BC×BBT<0.001				
Serum IL-6				
Minimum	2.4	2	1.5	
Maximum	196.3	75.2	65	
Median	19	6.6	5.1	<0.001
P (Mann-Whitney)				
BC×Healthy control<0.001				
BC×BBT<0.001				
Serum TNF-α				
Minimum	3.6	2.8	1.8	
Maximum	126.5	51.4	42.2	
Median	46.4	11.2	8.7	<0.001
P (Mann-Whitney)				
BC×Healthy control<0.001				
BC×BBT<0.001				

Table 1: The difference in median levels of serum IL-1α, IL-6 and TNF-α (pg/ml) concentration, among the three studied groups.

Values	Stage 0, I & II NO.=28	Stage III NO.=17	Mann-Whitney
Serum IL-1α			
Minimum	1.8	4.2	
Maximum	33	53.1	
Median	6.6	27.2	<0.001
Serum IL-6			
Minimum	3.6	3.8	
Maximum	65	126.5	
Median	14.3	62.2	<0.001
Serum TNF-α			
Minimum	2.4	3.8	
Maximum	120	196.3	
Median	9.2	39	<0.001

Table 2: The difference in median levels of IL-1α, IL-6, TNF-α (pg/ml) according to the stage of disease.

advanced disease [17]. Furthermore, IL-1 has been shown to inhibit growth of BC cells, and to promote cellular differentiation *in vitro*, but it is equally known to stimulate the expression of several proteolytic enzymes in human cancer [18]. IL-6 is able to promote tumor growth by up-regulating anti apoptotic and angiogenic proteins in tumor cells. IL-6 plays a key role in regulating estrogen synthesis in normal and malignant breast tissues. The activities of estradiol 17 Beta-hydroxy steroid dehydrogenase and estrone sulfatase are all increased by IL-6 [19].

On the other hand, Leek et al. [9] reported 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. The tumor-promoting functions of TNF-α may be mediated by its ability to induce proangiogenic functions, to promote the expression of matrix metalloproteinases and endothelial adhesion molecules, and to cause DNA damage via reactive oxygen, the overall effect of which is promotion of tumor-related processes [20].

Interestingly, IL-6 and the two innate cells- related cytokines (IL-1 and TNF-α) are interrelated and may act in an additive manner, suggesting that these three cytokines form a network of related factors, that may affect tumor cell progression in a cooperative manner. In conclusion, these finding suggest that serum levels of IL-1α, IL-6 and TNF-α appears to be of some prognostic value in breast cancer.

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