Possible Role of Th-2 Cell-Related Cytokines (IL-6 and IL-10) in Breast Cancer.

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Abstract

Background: Breast cancer is a complex disease, many etiological agents are proposed to play a role in its pathogenecity, one of these factors is cytokines.

Objectives: In the present study we measured the concentration of IL-6 and IL-10 in serum of breast cancer patients and examined their association with clinicopathological variables including stages of the disease and estrogen/progesterone receptor (ER, PR) expression on tumor cells, to determine whether it associate with the disease progression.

Subjects and Methods: The study included 80 subjects, it comprised of 45 Breast cancer patients, 12 patients with benign breast lesions and 23 apparently healthy controls. ELISA method has been used for estimation the level of IL-6 and IL-10 in serum of three studied groups.

Results: There was an elevation of IL-6 and IL-10 level in the sera of BC patients with significant differences between BC and controls ($p<0.001$), also, this elevation was associated with progression of the tumor. In addition, IL-6 level was found to be inversely related to ER and PR expression ($P< 0.05$) while in regard IL-10 there was no significant differences in the median of IL-10 level between the patients who express positive and negative ER and PR.

Conclusions: These data indicated that elevated IL-6 and IL-10 serum levels are associated with BC and associate with advanced stage of disease. It was feasible that assays for serum levels of IL-6 and IL-10 can be used as predictive tests for tumor progression in BC patients.

Keywords: Breast cancer, IL-6, IL-10.

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Introduction

Both the innate and acquired arms of the immune system are believed to play crucial roles in the anti-tumor response, it is well known that the interactions of tumor cells with their microenvironment may affect tumor growth and metastasis formation, among these, cytokines were suggested to play role in breast carcinoma [1,2].

Interleukin-6 (IL-6) is a multifunctional protein with multiple biologic activities on a variety of cells.

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It is produced by macrophages, T cells (Th2), B cells, endothelial cells and tumor cells, IL-6 levels have been found to be elevated in several cancers including renal carcinoma [3], ovarian and other gynecological tumors [4], lung cancer [5], and breast cancer [6], it is able to promote tumor growth by upregulating 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-hydroxysteroid dehydrogenase and estrone sulfatase are all increased by IL-6.

Interleukin-10 is the most potent anti-inflammatory cytokine yet identified. It can also be produced by many types of tumor cells such as colon carcinoma, melanoma cells [7]

and breast cancer [8]. Thus, because of its potential 'protective' effects on tumor cells, particularly via inhibition of specific tumor-reactive cytotoxic T lymphocyte, IL-10 production and secretion may be reasonably supposed to be up-regulated in cancer patients. The current study is a trial to estimate IL-6 and IL-10 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:

IL-6 and IL-10 has been estimated by using a solid phase sandwich enzyme linked immuno sorbent assay (ELISA) (BIOSOURCE, Europe S.A., Belgium, Lot No. 053804; 053303/A).

Statistical analysis

All the data have been analyzed statistically using Kruskall-Wallis test and MannWhitney analysis for measuring the differences between the studying groups [9].

Results:

Estimation of serum level of IL-6

The level of IL-6 in sera of BC patients was significantly higher than the healthy control and patients control group (median = 19 pg /ml; 5.1 pg / ml; 6.6 pg / ml), respectively, ($p<0.001$) as shown in table 1.

The highest concentration of IL-6 was recorded in breast cancer patients with advanced stage -stage III- (median=39 pg/ml) in comparison to other stages (stage 0; stage I and stage II) (median=9.2 pg/ml), ($P < 0.001$), Table 2.

Estimation of serum level of IL-10

The level of IL-10 in sera of BC patients was significantly higher than the healthy control and patients control group (median = 41 pg /ml; 11.1 pg / ml; 6.7 pg / ml)), respectively, ($p<0.001$), as shown in Table 3.

The highest concentration of IL-10 were recorded in breast cancer patients with advanced stage -stage III- (median=59 pg/ml) in comparison to other stages (stage 0; stage I and stage II) (median=15.5 pg/ml), ($P < 0.001$), Table 4.

The association of IL-6 with estrogen and progesterone receptors

The results of association between serum IL-6 level and ER and PR expression in breast cancer samples were shown in tables- 5&6. IL-6 level was indeed found to be inversely correlated to ER and PR expression ($p= <0.05$).

The association of IL-10 with estrogen and progesterone receptors

In regard the correlation between serum IL-10 level and the expression of ER and PR, table- 5&6 revealed no significant differences in the median of IL-10 level between the patients who express positive and negative ER and PR.

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

Serum IL-6	BC cases	BBL control	Healthy control	P (Kruskall-Wallis)
Minimum	2.4	2	1.5	
Maximum	196.3	75.2	65	
Median	19	6.6	5.1	<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-6 (pg/ml) according to the stage of disease.

Values	Stage 0, I& II	Stage III	Mann-Whitney
Minimum	2.4	3.8	
Maximum	120	196.3	
Median	9.2	39	<0.001
NO.	28	17	

Table 3: The difference in median levels of serum IL-10 (pg/ml) concentration among the three studied groups.

Serum IL-10	BC cases	BBL control	Healthy control	P (Kruskall-Wallis)
Minimum	2.6	2.4	0	
Maximum	113.4	54.6	44.1	
Median	41	11.1	6.7	<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 IL-10 (pg/ml) according to the stage of disease.

Values	Stage 0, I& II	Stage III	Mann-Whitney
Minimum	2.6	3.2	
Maximum	69	113.4	
Median	15.5	59	<0.001
NO.	28	17	

Table 5: The difference in median levels of serum IL-6 and 10 (pg/ml) according to the estrogen receptors.

	Estrogen receptor		
	Positive (n=21)	**Negative (n=24)**	**P**
Interleukin-6 conc.			
Range	(2.4 – 61.8)	(13 – 196.3)	
Median	7.9	25.2	<0.05
Interleukin-10 conc.			
Range	(2.6 – 99.6)	(1.6 – 113.4)	
Median	25.8	36.5	>0.05

Table 6: The difference in median levels of serum IL-6 and10 (pg/ml) according to the progesterone receptors.

	Progesterone receptor		
	Positive (n=26)	**Negative (n=19)**	**P**
Interleukin-6 conc.			
Range	(2.4 – 74.8)	(16 – 196.3)	
Median	8.4	26.2	<0.05
Interleukin-10 conc.			
Range	(2.6 – 88.5)	(4.4 – 113.4)	
Median	29.8	33.5	>0.05

Discussion

Regarding Th2- cells- related cytokines (IL-6 and IL-10), current results were in agreement with those of other authors who have demonstrated significantly higher levels of those cytokines in sera of patients with BC than those of control groups [10,11].

Moreover, in the present study there was a positive correlation between clinical stage and the serum levels of both cytokines (IL-6 and IL-I0), this result was inagreement with findings of Ordemann and associates, in (2002), and Kozlowski *et al.,in* 2003 who found that a high levels of IL-6 and IL-10 were frequently observed in stage III than in the other two tumor stages (I and II) [12,13].

Regarding IL-10, it is produced at high concentrations by a wide number of tumor cells, including breast carcinoma, it is a dominant cytokine found in the BC cells environment [14]. Kucharzik *et al., in* (1997) have demonstrated that tumor cell derived TGF-β1 and PGE2 are major factors for IL-10 stimulation [15]. IL-10 may play an important role in tumorigenesis since it can suppress Th1 cells ability to secrete IL-2 and IFN-γ, both essential for an optimal cell-mediated anti-tumor activity [14, 16]. IL-10 does not only affect effectors cells but can lead to diminished expression of MHC molecules by the tumor cells via down regulation of Transporter Associated with Antigen Presentation (TAP1) and (TAP2) proteins of the antigen-processing machinery [17].

Both PGE-2 and IL10 have been shown to suppress antigen presentation, to suppress cytotoxic T cell (CTL) responses, and to inhibit cytokine production by T cells and APC, perhaps most importantly IL12 that plays a central role in initiation and potentiation of cellular immune responses [18, 19].

Cytokines produced by Th2 lymphocytes have been proposed to

promote cell survival by influencing the expression of proteins involved in the regulation of apoptosis. Tumor cells have been previously demonstrated to evade death signals generated by immune effectors or by therapeutic drugs through the development of effective antiapoptotic mechanism such as increased levels of caspase inhibitors or Bcl-2-family members [20].

Among the various prognostic factors, lack of estrogen and progesterone receptors has consistently been associated with poorer prognosis [21]. Of particular note, in present study we found an inverse correlation between expression of ER&PR and IL-6 serum levels, which is in agreement with the findings of other studies [22, 23]. On the other hand, we observed that IL-10 was not correlated with ER and PR status, which is in disagreed with findings of some other studies [24, 25].

The inverse correlation between IL- 6 and ER&PR indicates that the high serum levels of this cytokine 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-6 serum levels would predict poor outcome in BC.

This inverse correlation between IL-6 and ER status not only may reflect the greater aggressiveness of this subtype of breast tumors but it could also be the result of a direct regulation of cytokine expression by ER. Several reports have demonstrated a direct down regulation of cytokines by ER in different organs. This is not only the case for IL-6 [26] but also for IL-1 and TNF-α [26]. Progesterone receptors are also known to down regulate the expression of a number of cytokines, including IL-1 [27] IL-6 [28] IL-8 [29] and TNF-α [27].

Purohit *et al., in* (2002) confirmed these studies and claimed that IL-6 secretion is inhibited by estrogen synthesis in peripheral tissues, including normal and malignant breast tissues. Interestingly, they found that macrophages and lymphocytes which invade many breast tumors are important source of factors that can stimulate estrogen synthesis in malignant breast tissues which explains the high concentrations of estrogen present in breast tumors [30].

On the other hand, Chiu *et al., in* (2000), on their study on normal and transformed mammary epithelial cells reported that IL-6 secretion inhibited the growth of ER positive breast cancer cell lines. In contrast, ER negative breast cancer cell lines were resistant to IL-6 mediated growth of normal and transformed human mammary epithelial cells [31].

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