Original Article

IL-2 and IL-4 Serum Levels in Breast Cancer

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Summary:

Background: The role of cytokines in cancer immunity and carcinogenesis in general has been well established, which play an important role in the pathogenesis of many solid cancers.This study aimed to estimate serum levels of IL-2 and IL-4, and to shed light on the correlation of these interleukins with progression of breast cancer.

Patients and Methods: The study included 80 women, 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-2 and IL-4 in serum of three studied groups.

Results: This study showed elevation of IL-4 level in the sera of breast cancer patients with significant differences between breast cancer and controls ($p<0.001$), this elevation was correlated with progression of the tumor. In addition, the elevation of serum level of IL-4 was found to be inversely related to ER and PR expression ($P=<0.05$). On the other hand, there was a significant decrease in the median of serum level of IL-2 in patients as compared with control groups.

Conclusions: There was association between elevated serum level of IL-4 and breast cancer and this elevation was correlated with advanced stage of disease. In addition, there was no association between the statistical significant decrease of IL-2 serum level and the advanced stage of breast cancer.

Keywords: Breast cancer, Cytokines, ER

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Introduction:

Breast cancer (BC) is a complex disease; its etiology is multifactorial, both the innate and acquired arms of the immune system are believed to play crucial roles in the antitumor response, and the interaction between host immune system and tumor cells has been the subject of intense research over the past decades (1,2). Among the various prognostic factors, lack of estrogen receptor (ER) has been associated with poorer prognosis of BC (3). Most human breast cancers express ER, and the presence of this receptor is generally considered an indication of hormone dependence (4).In addition to ER , cytokines are now emerging as factors that are potentially involved in breast carcinogenesis (5,6). Cytokines are produced by many cell populations, but the predominant suppliers are T-helper cells and macrophages. Two functionally distinct subsets of Th cells: Th1 and Th2 secrete cytokines that promote different activities. Th1 cells produce IL-2 and IFN-γ, which activate cytotoxic lymphocytes and macrophages to stimulate cellular immunity and inflammation (7). Th2 cells secrete IL-4 and IL-5, which stimulate antibody production by B cells. It has become evident that cancer tissues also produce cytokines (5, 8).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 (9). The current study is a trial to estimate IL-2 and IL-4 level in the BC patient's sera in comparison with controls.

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Patients and Methods:

Patients

Forty five breast cancer female's patients were subjected to this study. The patients were admitted for surgery at Al-Kadhimia Teaching Hospital and nursing home hospital /medical city, during the period March 2006 - March 2007. Their ages ranged from 28-73 years, They included invasive ductal carcinoma, invasive lobular carcinoma, and in situ ductal carcinoma. Pathological data [including: histologic tumor type grade, tumor stage, lymph node status, estrogen and progesterone receptors status] were obtained from medical records of patients and validated by an experienced histopathologist. In addition twelve females with benign breast lesions (6 cases with fibrocystic disease and 6 with fibroadenoma) were available in this study as a patient control group, their ages ranged from 21-50 years. For the purpose of comparisons, 23 healthy subjects matched for age, sex and ethnic back ground (Iraqi Arabs) were selected who have no history or clinical

evidence of BC or any chronic disease and obvious abnormalities as a second control group. Venous blood samples were collected preoperative.

Methods:

Interleukin-2 and IL-4 have been estimated by using sandwich enzyme immunoassay (ELISA) technique (BIOSOURCE, Europe S.A., Belgium, Lot No. 051501/B; 060601). The procedure was done according to the manufacturer instruction as supplied with kit from BioSource Europe S.A. Company, Belgium.

Statistical analysis

The Kruskal-Wallis test was used to assess the statistical significance of difference in median between the 3 study groups. The Mann-Whitney test was used to further explore the significance of difference in median between each pair of study groups. P value less than the 0.05 level of significance was considered statistically significant (10).

Results:

Estimation of serum level of IL-2 and IL-4:-

Significant decrease was demonstrated in level of serum IL-2 in patients as compared with control groups with P<0.001, while, there was non significant differences of serum IL-2 levels in advanced stage (stage III) in comparison to other stages (stage 0; stage I and stage II), (P>0.05), as shown in tables (1&2).

In addition, table (1) revealed a significant elevation in the sera levels of IL-4 among BC patients in comparison to that of control groups which include patients with benign breast lesion and healthy subjects with P<0.001. As represented in table (2) the highest median of IL-4 serum concentration was recorded in stage III of BC patients as compared to that in patients with stages (0, I and II) .

The association of IL-2 and IL-4 with estrogen and progesterone receptors:-

In regard the correlation between serum IL-2 level and the expression of ER and PR, tables (3 and 4) revealed non significant differences in the median of IL-2 serum level between the patients who express positive and negative ER and PR, while, increased serum level of IL-4 was indeed found to be inversely correlated to ER and PR expression (p= <0.05), tables (3 and 4).

Table .1: The difference in median levels of serum IL-2 and IL-4 (U/ml) concentration among the three studied groups.

Serum IL-2	BC cases	BBL control	Healthy control	P (Kruskall-Wallis)
Minimum	0	0	0	
Maximum	7	53	15	
Median	0	10.4	3.1	<0.001
NO.	45	12	23	
P (Mann-Whitney)				
BC X Healthy control <0.001				
BC X BBT <0.001				
Serum IL-4				
Minimum	10	0	0	
Maximum	83	27	7	
Median	34	11	2.9	<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-2 and IL-4 (U/ml) according to the stage of disease.

Values of IL-2	Stage 0, I& II	Stage III	Mann-Whitney
Minimum	0	0	
Maximum	7	6	
Median	0	0	>0.05
NO.	23	22	
Values of IL-4			
Minimum	10	24	
Maximum	41	83	
Median	20	45	<0.001
NO.	23	22	

Table.3:The difference in median levels of serum IL-2 (U/ml) and IL-4 (pg/ml) according to the estrogen receptors.

	Estrogen receptor		
	Positive (n=21)	Negative (n=24)	P
Interleukin-2 conc.			
Range	(0 – 6)	(0 – 7)	
Median	0	0	>0.05
Interleukin-4 conc.			
Range	(10 – 50)	(20 – 83)	
Median	25	43	<0.05

Table.4:The difference in median levels of serum IL-2 (U/ml) and IL-4 (pg/ml) according to the progesterone receptors.

	Progesterone receptor		
	Positive (n=26)	Negative (n=19)	P
Interleukin-2 conc.			
Range	(0-6)	(0-7)	
Median	0	0	>0.05
Interleukin-4 conc.			
Range	(10 – 54)	(16 – 83)	
Median	24	45	<0.05

Discussion:

In the current study, the serum levels of Th1-cells-related cytokines (IL-2) in patients with BC were significantly lower than those in controls. In addition, patients with BC were grouped to the early and advanced stages but there was non significant difference between them. These results were in agreement with Pockaj and coworkers, in 2004 who demonstrated significant differences in levels of serum IL-2 in patients as compared with controls and revealed that the IL-2 level was not correlated with stages of BC disease (12). Regarding IL-4, present results were in agreement with those of other authors who have demonstrated significantly higher levels of Th2- cells- related cytokines in sera of patients with BC than those of control groups (11, 12). Moreover, there was a correlation between tumor stage and the elevated serum levels of IL-4, in contrast to these results, Green et al., in 1997 and Pockaj et al, in 2004 did not observe any correlation between the IL-4 and tumor stage, but the study of Green and associates analyzed only RNA levels (13, 12). According to the present data, there was a decreased in Th1/Th2 cytokines ratio in serum of BC patients. A decreased Th1/Th2 ratio has been described in a plethora of human malignancies, including lung cancer (14), breast cancer (15), urinary, bladder, renal cell, and prostate cancer (16), indicating the existence of a local and peripheral Th2-type cytokine pattern in the majority of cancer patients. 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 (17). Of particular note, in current study there was an inverse correlation between the expression of ER&PR and the elevated serum levels of IL-4, on the other hand, we observed that the decreased serum levels of IL-2 was not associated with ER and PR status, which is in disagreed with findings of some other studies (18,19). The inverse correlation between IL- 4 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-4 serum levels would predict poor outcome in BC. So, our data suggest that cytokines could be involved in the aggressiveness of ER-negative breast tumors.

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