

Serum level of Interleukin-6 in Breast Cancer Iraqi Women

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Abstract :

Objectives: The aim of this study is to estimate the roles of interleukine-6 in breast cancer Iraqi women and to assess the correlation between the serum levels of IL-6 with the progression of disease stage.

Method: Fifty patients with proved diagnosis of breast cancer by clinical examinations presented to the Al alwyah hospital for gynecological diseases and obstetrics /Breast cancer early detection clinic) in Baghdad. All were females and didn't have received any chemo/or radiological treatment .also 20 sample of apparently healthy women were involved as a control. The blood samples (5ml) were drawn from all of the studied cases in order to be used for measuring their serum level of (IL-6) by using Enzyme –Linked Immunosorbent Assay (ELISA) .

Result: Serum (IL-6) level were increased with high significant difference in Breast cancer patients and showed significant correlation in all disease stages as compared to control group with the highest level in stage I patients group.

Conclusion: serum level of (IL-6) is highly correlated with breast cancer and might have detecting and diagnostic usefulness .

Key words: Interleukine-6, Breast cancer, Iraqi women

Introduction:

Breast cancer is the malignant tumor in which normal cells in the breast begin to grow without control and no longer die most commonly form the inner lining of milk ducts or the lobules that supply the ducts with milk (1, 2).

In Iraq, breast cancer (BC) is the commonest type of female malignancy, accounting for approximately one-third of the registered female cancers according to the latest Iraqi Cancer Registry (3). As proposed by the World Health Organization (WHO), early detection and screening, especially when combined with adequate therapy, offer the most immediate hope for a reduction in breast cancer mortality (4).

Interleukins induce in vitro growth of many types of cancer such as ovarian, cervical, prostate, lung, kidney cancer and melanoma cells (5). Furthermore, their contributions to the tumor angiogenesis have been reported (6). As a growth factor IL-6 plays a significant

role in cell differentiation and is believed to be involved in tumor progression (7). Elevated IL-6 serum levels have been shown to be correlated with disease staging and unfavorable clinical outcomes in women with metastatic breast cancer (8).

Patients and Method:

Patients: Fifty patients with proved diagnosis of breast cancer by clinical examination (cytological, physical, mammogram and ultrasound) and /or histopathological examination were presented to the Al-Alwyah Hospital for gynecological diseases and obstetrics/ early detection of breast cancer clinic) in Baghdad during the period from January / 2011 to the end of August / 2011

These patients have been of different age groups, different disease stages and different geographic residencies. They were newly diagnosed breast cancer patient with different type and disease stages, pre and postoperative and their ages ranged between 24 -70 years old. They were not under any chemo/or radiological treatment

Control: Twenty apparently normal women with comparable age range of patients

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Blood Sampling: Five ml of blood were collected in plain tubes from patients and from control individuals. The samples were centrifuged at 3000 rpm for 5 minutes to separate serum. Then, after that all serum samples were stored at -20° C for two week then -80° C (according to the manufacturer instruction) .Then assay procedure was carried out by using ELISA IL-6 Kits of Ray Biotics Company (USA).

Statistical Analysis:

1- Descriptive statistics:

Summary statistic of the readings distribution (mean, SD). The comparison of significant (P-value) in any test were:

S= Significant difference (P<0.005).

HS= Highly Significant difference (P<0.001).

NS= Non Significant difference (P>0.005).

mean value was 37.38 pg/ml compared to 4.46 pg/ml for control group, with reference to the control group. The range was 16.8 - 140 pg/mL for patients compared to 2-8 pg/mL for healthy controls. Such difference was highly significant (p value ≤ 0.01), (table 1).

Interleukin-6 is found to be elevated in various inflammatory and malignant diseases including metastatic breast cancer and their levels are found to correlate with the extent of the disease (9). It is produced by some types of cancer cells and by normal stromal cells, such as fibroblasts and endothelial cells. By acting as growth factor, IL-6 is able to promote tumor cell proliferation through upregulation of anti-apoptotic and angiogenic proteins in tumor cells. Also IL-6, is a major mediator of the inflammatory response, plays a primary role in the pathophysiology of cancer (10, 11).

The study results were in agreement with a recent study in Iraq made by Ayed (12) carried out on 75 breast cancer patients and 15 healthy controls. He found that serum IL-6 level for the patients was significantly higher than normal women and strongly correlated with disease progression.

Results and Discussion:

In this study, serum IL-6 level was evaluated in 50 breast cancer patients as compared to 20 healthy controls. Considering serum IL-6 concentrations for patients, the

Table (1): IL-6 serum level in breast cancer patients between patients and controls.

Parameter	Mean $\pm$ S.E.(pg/ml)		$\geq$ P value	LSD Value
	Patients	Control		
IL-6	37.38 $\pm$ 3.59	4.46 $\pm$ 0.36	0.01	11.411**

** Highly Significant

Also in Egypt, Ahmed et al. (13) found that serum level of both IL-6 and IL-8 were found to be higher in patients than in healthy volunteers. In addition Sullivan (14) have mentioned that breast cancer patient serum and tumor IL-6 levels are clinically relevant, and therefore, should be

routinely evaluated upon diagnosis .

Statistically highly significant differences were seen in the Mean serum (IL-6) concentration of all disease stages as compared to the Mean (IL-6) serum concentration of the control group (p < 0.01).

Table(2). Comparison between stage group of breast cancer patients and control in their serum IL-6 concentration .

Parameter	Means ± SE					LSD
	Control	Stage of disease				
	(No. =20)	I (No. =5)	II (No. =24)	III (No. =18)	IV (No. =3)	
IL-6 conc.(pg/ml)	4.46 ± 0.37	45.44 ± 14.07	40.33 ± 6.64	31.64 ± 2.67	34.67 ± 2.66	22.615 **

* (P<0.01) Highly significant.

It was obvious that Mean serum (IL-6) concentration was in the highest level in stage (I) disease group of patients : 45.44(pg/ml) and the level was declined proportionally

with disease progression except in stage IV, it was 40.33(pg/ml) in stage II and 31.64(pg/ml) in stage III and 34.67 pg/ml in stage IV.

We suggest that higher mean serum (IL-6) level in early disease stage may be due to the high immune response of the body in this stage of the disease. It was reported that IL-6 antitumor activity was enhanced by induction of T cell and B cell differentiation, stimulation of cytotoxic T cells and help in producing lymphokine-activated killer cells (15).

We thought that This immune response trigger synthesis and release of this cytokine leading to augmentation of its serum level that might be utilized as a marker of immunity status and immune system activation in prognosis and monitoring of the course of cancer (16) This immune response might be attenuated with the progression of disease stage and cancer overwhelming which reflect decrease in (IL-6) synthesis.

Our hypothesis could be supported by some literatures, mentioned that IL-6 and other IL-6-type cytokines are expressed in many primary breast tumors (17,18). However, IL-6 expression is reduced in invasive breast carcinoma relative to normal mammary tissue and appears to be inversely associated with histological tumor grade (18,19).

The role of IL-6 in cancer progression is dependent on the balance of multiple pathways triggered simultaneously by the cytokine. However, concomitant stimulation of

the cells by other endogenous or exogenous factors, even at low concentrations, may tip the balance toward one biological response, e.g., proliferation and antiapoptosis, or another, e.g., growth arrest and differentiation (20). IL-6 has also been shown to influence the proliferation of normal and tumor-derived cells. IL-6 promote proliferation of hematopoietic progenitors, keratinocytes, myeloma/plasmacytoma, and Kaposi's sarcoma cells, whereas it inhibits the proliferation of M1 myeloid leukemia cells, early-stage melanoma cells, and lung and breast tumor cells. Thus, depending on the target cell, IL-6 induces various and sometimes contrasting biological responses. (10,21).

Our findings indicate higher Mean serum (IL-6) concentration in stage IV patients group: 34.67(pg/ml) as compared to stage III patients group : 31.64(pg/ml). This elevation of Mean serum IL-6 concentration may be attributed to role of IL-6 in advance stage of breast cancer and metastasis. Adler (22) Wise (23) have mentioned that Elevated IL-6 levels have been associated with advanced stage and metastasis-related morbidity.

In addition, Serum IL-6 is associated with worse survival in patients with advanced breast cancer and we suggest that at later stages of breast cancer progression IL-6 may have a net stimulatory effect on tumor growth (24).

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مستوى المصل للانترليوكين السادس لدى مريضات سرطان الثدي العراقيات

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الخلاصة:

يعد سرطان الثدي من أكثر أنواع السرطانات التي تصيب النساء في العالم ، وقد تناولت الكثير من الدراسات والبحوث العوامل المسببة لهذا المرض من أجل التوصل للتشخيص المبكر والعلاج الناجح له. تناولت هذه الدراسة بحث العلاقة بين هذا السرطان وتركيز الانترليوكين السادس كما تناولت العلاقة بين تركيز هذا الانترليوكين مع أعمار المريضات و مراحل تقدم المرض .

أجريت هذه الدراسة على 50 مريضة شُخصن بسرطان الثدي واللاتي دخلن الى مستشفى العلوية التعليمي (جميعهن من الأنثى)، بالإضافة الى مجموع 20 امرأة (سيطرة) وتضم 20 امرأة سليمة مع مراعاة فائهن العمرية ، في الفترة ما بين كانون الثاني 2011 الى آب 2011. تم سحب عينة (5 مللتر) من الدم الوريدي من 70 من الحالات الداخلة في الدراسة (المريضات والسليمات) لغرض استخدامه في الدراسة المناعية والتي يشمل قياس الانترليوكين السادس بواسطة تقنية ELISA .

بينت النتائج ارتفاع في مستوى الانترليوكين السادس حيث كانت العلاقة عالية المعنوية مقارنة بمجموعة السيطرة. كما تبين وجود علاقة معنوية بين مستوى الانترليوكين السادس في جميع مراحل المرض مقارنة بمجموعة السيطرة مع تسجيل أعلى مستوى في المراحل الاولى . بينما كانت العلاقة غير معنوية بين مستوى هذا الانترليوكين و اعمار المرضى.