Association of Interleukin 6 with Positive Immunohistochemical staining of Estrogen Receptor in Breast Cancer Patients.

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الخلاصة:

قيم مستوى تركيزالانترلوكين−6 (IL-6) في مصل الدم لمرضى سرطان الثدي و درست علاقته بالتعبير المناعي لمستقبلات الاستروجين (ER) في الخلايا السرطانية.

درست مستويات الانترلوكين−6 والتعبير المناعي لمستقبلات الاستروجين على مجموعتين الاولى مكونة من 30 امرأة مصابة بسرطان الثدي (positive breast cancer) والثانية 30 امرأة غير مصابة بسرطان الثدي كمجموعة سيطرة (negative breast cancer). أجري فحص الامتزاز المناعي المرتبط بالانزيم (ELISA) على مصل الدم للاستدلال عن الانترلوكين−6 وكذلك اخذت عينات نسيجية من ثدي المتطوعات لدراسة التعبير المناعي لمستقبلات الاستروجين باستخدام طريقة التحليل المناعي النسيجي الكيميائي.

اشارت النتائج ان هناك ارتفاع معنوي في مستوى الانترلوكين−6 في مصل الدم للسيدات المصابات بسرطان الثدي مقارنة بمجموعة السيطرة ($p<0.01$). كما وجدت النتائج ان التعبير المناعي الموجب لمستقبلات الاستروجين (ER+) في المصابات بالسرطان كانت اعلى من التعبير السالب (ER-) ($p<0.01$). واظهرت الدراسة وجود معامل ارتباط موجب ($r=249$, $p<0.05$) بين مستوى الانترلوكين−6 في مصل الدم ومستقبلات الاستروجين. و أستنتج من النتائج ان ارتفاع مستوى الانترلوكين−6 في مصل الدم متلازم مع سرطان الثدي وارتفاع مستوى الانترلوكين−6 في مرضى سرطان الثدي متلازم مع التعبير الموجب لمستقبلات الاستروجين.

Abstract

The objective of this study is the evaluation of concentration levels of interleukin-6 (IL-6) in breast cancer patients and it's relation to estrogen receptor (ER) expression on tumor cells. The expression of IL-6 and estrogen receptor , were studied on 30 patients (positive breast cancer) and 30 controls (negative breast cancer).Serum of studied groups were examined using Enzyme Linked Immuno-Sorbent Assay (ELISA) for IL-6 and breast biopsy specimens were examined using immunohistochemical staining for estrogen receptor. The serum level of IL-6 was significantly higher in patients with breast cancer compared with control ($p<0.05$). The number of positive ER expression in patients with breast cancer was higher than the negative expression ($p<0.05$). The current

study found a positive significant correlation (r = 249; $P<0.05$) between serum levels of IL-6 and estrogen receptors (ER). The results of this study suggest that elevated IL-6 serum concentration are associated with breast cancer and found that high levels of IL-6 in breast cancer patients are associated with positive estrogen receptor.

Introduction

The breast is a highly modified sweat gland that develops as an in growth from ectoderm. Anatomically, the primary secreting units consist of groups of terminal ductules with sac-like ends (alveoli), which are embedded in a fine specialized connective tissue to form the breast lobules. It is now firmly believed that breast cancer commonly starts in the epithelium which lines the terminal ductules within the lobule. Physiologically, the human female breast is under the primary control of different hormones; the role of estrogen appears to be central. Breast cancer is the second most common cancer in women world-wide [1].

Among the various prognostic factors, lack of estrogen receptor (ER) has consistently been associated with poorer prognosis[2]. Most human breast cancers express ER-α and the presence of this receptor is generally considered an indication of hormone dependence [3]. In addition to ER-α, cytokines are now emerging as factors that are potentially involved in breast carcinogenesis [4,5]. Cytokines constitute a diverse group of proteins that include haematopoietic growth factors, interferon, lymphokines and chemokines [6].

Interleukin-6 (IL-6) is a cytokine with multiple biological activities on a variety of cells. It is produced by macrophages, T, B, endothelial and tumor cells. IL-6 is an able to promote tumor growth by upregulating antiapoptotic and angiogenic proteins in tumor cells .It is associated with worse survival in patients with metastatic breast cancer and is correlated with the extent of disease[7].

Breast cancer is the most frequently diagnosed cancer and the second leading cause of death after lung cancer in women [8]. There is strong evidence that the tumor growth can be actively controlled by host immune system [9]. IL-6 is a multifunctional cytokines used in regulation of immune response and cancer cell proliferation [10].

The cytokine IL-6 is a central player in immune homeostasis and effects inflammatory reactions, acute phase response, hematopoiesis, bone metabolism[11,12,13].

Accordingly, serum IL-6 levels are currently considered a diagnostic marker for tumor progression, metastasis and prognosis in multiple cancer types (breast, prostate, lymphoma, lung, ovarian and renal cell carcinoma [14]. It is unclear whether evaluated serum levels IL-6 are a consequence of or a contributory cause to advance tumor stage[15,7].

Hence, the question as to whether the inflammatory infiltrate helps or hiders tumors is still open [16,17]. The described contrasting effects of IL-6 include either a direct enhancement of auto- and paracrine-mediated tumor growth or an anti-tumor effect by enhancement of immune response (differentiation and maturation of B-cells, T-cells, dendritic cells, macrophages) and inhibition of tumor cell proliferation. The menopause associated distributed hormonal balance in constituted by a remarkable rise of the IL-6 expression level, while a rapid decline in circulating sex hormones(estrogen, androgen) is observed [11,18,19].

Recent investigations on the long term effects of conventional hormone therapy with synthetic estrogen have demonstrated a substantially evaluated risk of thrombosis and incidence of breast, endo-metrial and ovarian cancer [20,21,22].

The aim of this study is to evaluate of serum concentration levels of IL-6 in breast cancer patients and their relation to estrogen receptor expression on tumor cells.

Materials and Methods

Patients: A total of sixty Iraqi patients who were admitted to AL-Yarmook and Baghdad Teaching Hospital. Patients ages ranged between (22-68) year, patients were divided into two clinical subgroups: (30) are the breast cancer patients and (30) patients are a control group due to histological examination.

Samples: Breast biopsies and serum were taken from each case.

Evaluation of IL-6 in serum samples using ELISA technique:

Evaluation of cytokine levels in serum by ELISA technique has two immunological steps. First step, the cytokine is captured by monoclonal antibody bound to the wells of a microtiter plate. Second step a monoclonal antibody linked to abiotinylated monoclonal antibody is added together with streptavidine-peroxidase conjugate. The solid phase antibody-antigen complex and in turn, binds the conjugate. After incubation, the wells are washed and the antigen complex bound to the well detected by addition of a chromogenic substrate. The intensity of the color developed is directly related to the specific monoclonal antibody concentration of the sample [23].

Immunohistochemical analysis (IHC) for detection of estrogen receptor(ER):

Breast biopsies were immunostaining with polyclonal antibodies to estrogen receptor by the avidin-biotin complex (DakocCrop, Denmark). The primary antibody reacts with antigen in the tissue, and then a biotin labeled secondary antibody (link antibody) binds to the primary antibody. When the conjugate is added, the biotinylated secondary anti-body will form a complex with the peroxidase-conjugated streptavidin and by adding the substrate, which contains 3,3 □-diaminobenzidine (DAB) in a chromogen solution, a brown-colored precipitate will form at the antigen site. In the peroxidase secondary detection system, the presence of a brown reaction product at the site of the

target antigen is indicative of positive reactivity. Counter stain will be pale to dark red coloration of the cell nuclei.

The use of universal DakoCytomation streptavidin- biotin system purchased from DakoCytomation (USA) Immuno-histochemistry detection kit. The rabbit anti-human antibodies against gastrin and the rabbit anti-human antibodies against somatostatin were from DakoCrop (Denmark).

Counting the number of positive cells which gave brown cytoplasmic staining system under light microscope. The extent of the IHC signal was determined in 10 fields (X100magnification). The percentage of positively stained cell was calculated for each case by taking the mean of the percentages of the positively stained cell in the 10 fields. ER expression was considered positive when at least 10% of invasive tumoural cells exhibited nuclear staining, regardless of intensity [24].

Statistical analysis: Student test (t-test) was used for the quantitative data. The relationship between the factors was measured qualitatively by using the correlation coefficient(r). The lowest level of significance was when the probability ($p<0.05$) and the highly significance was ($p<0.01$) [25]. $P>0.05$ = no significant difference; $P<0.05$ = a significant difference.

Results

Table-1 shows that there are statistically significant increase in serum levels of Il-6 (97.3 ± 3.70) in breast cancer patients compared with controls (3.5±0.48)**.**

The results indicate that there are a highly significant difference ($P<0.01$) between positive and negative expression of estrogen receptors (ER) in patients with breast cancer. ER expression in patients with breast cancer was (17.4± 0.32) whereas the expression in control group was (6.1± 0.17), these results were shown in table-2.

There was (80) % of ER positive and (20) % of ER negative in breast cancer patients as shown as in table-2.

In patients with breast cancer, the current study found a positive significant correlation (r =249 ; $P<0.05$) between serum levels of IL-6 and estrogen receptors (ER) (table-3).

The expression of ER was heterogeneous dark brown nuclear staining in the tissue, as shown in Figure-1.

Groups	No.	Mean±Std. Error	Comparison of significant	
			P-value	Sig.
Control	30	3.5± 0.48	0.000	Highly Sig. (P<0.01)
Breast cancer patients	30	97.3± 3.70		
Total	60			

Table-1: Mean distribution of serum IL-6 level (Pg/ml) among studied group.

Estrogen Receptors Status	No.	(%)	Mean± Std. Error	Comparison of significant	
				P-value	Sig.
Positive	24	(80%)	17.4±0.32	0.000	Highly Sig. (P<0.01)
Negative	6	(20%)	6.1±0.17		
Total	30	(100%)			

Table-2: Expression of estrogen receptors in patients with breast cancer by (IHC).

Variable	groups	Correlation Coefficient r =	*P* value
IL-6 and estrogen receptors	Control	0.516	>0.05
	Breast cancer	0.249	<0.05

Table-3: Pearson correlation (r) between IL-6 and estrogen receptors in studied groups.

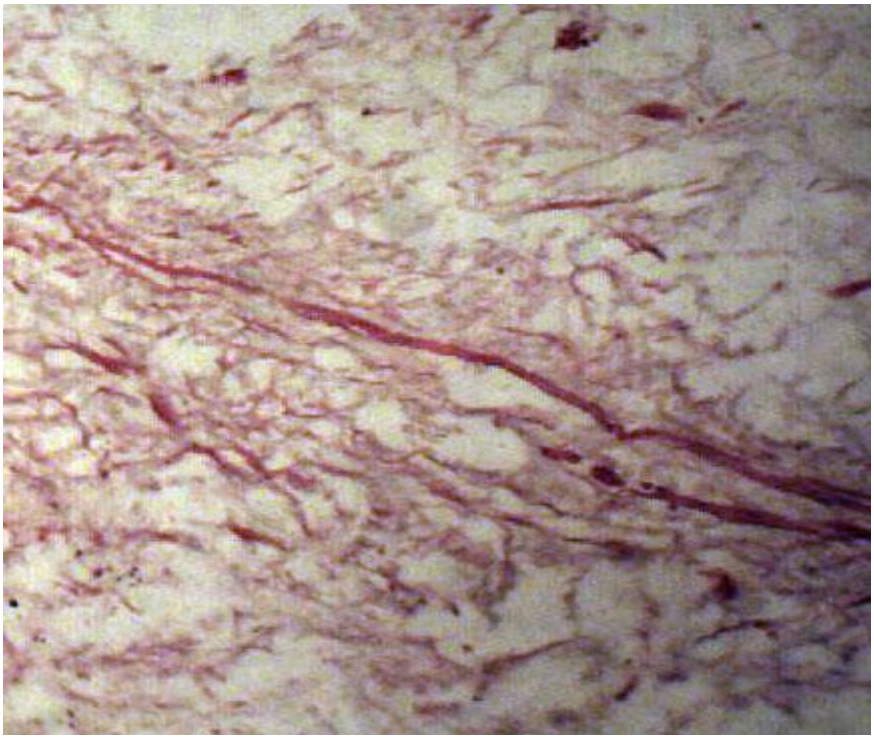

Figure-1: Immunohistochemical staining IL-6 and ER proteins in studied groups. Staining by DAB chromogen (dark brown) counterstained with nuclear fast red. Tissue from breast cancer shows positive estrogen receptor immunostaining (X400).

Discussion

The present study illustrated that the mean levels of IL-6 among normal females are (3.5pg/ml) and highly increased among cancer cases, with a mean of (97.3 pg./ml). Statistically, these data revealed a highly significant values ($p<0.01$) are shown in (Table 1). Our data in accordance to Benoy et al. [26] which level was significantly higher among patients with breast cancer compared to healthy controls. Also, Asgeirsson et al. [27] in his study on sixty breast cancer patients found an increase in serum levels of IL-6 in (27 %) of cases compared to (2 %) in controls. So, the authors implicate IL-6 as a possible factor in breast cancer progression and metastasis. Jablnka and pietruska, [28] found an increased capacity of unstimulated cells from the patients to produce IL-6.

Based on IHC analysis the results of this study show 80% for positive (ER) and 20% for negative (ER) in breast cancer patients [29].

In addition, the current study showed the positive expression of estrogen receptor (ER) in patients with breast cancer was (17.4± 0.32) whereas the negative expression was**(** 6.1± 0.17) with highly significant difference ($p<0.01$).

Most human breast cancers express ER-α, and the presence of this receptor is generally considered an indication of hormone dependence [3]. In addition to ER-α, cytokines are now emerging as factors that are potentially involved in breast carcinogenesis [4,5].

Another study by Chiu et al [30] show on normal and transformed mammary epithelial cells reported that IL-6 secretion inhibited the growth of estrogen receptor positive (ER+) breast cancer cell lines. In contrast, (ER-) breast cancer cell lines were resistant to IL-6 mediated growth of normal and transformed human mammary epithelial cells. Purohit et al., 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 [31].

Appoint to be noted in the present study was found a positive significant correlation (r = 249 ; $P<0.05$) between serum levels of IL-6 and estrogen receptors (ER) in patients with breast cancer .This results indicating decreasing expression of ER associated with increasing expression of IL-6 in patients with breast cancer .previous study showed that IL-6 cytokine involved in different physiologic and pathophysiologic processes such as inflammation, bone metabolism, synthesis of C-reactive protein, and carcinogenesis ,IL-6 has also been shown to inhibit the growth of various breast cancer cell lines, shows antiadhesive effects ,and modulates the estrogen receptor and progesterone receptor content of these cells [32,33].

In summary, the present study indicates that elevated IL-6 serum concentrations are associated with breast cancer and found that over expression of IL-6 in estrogen receptor positive in patients with breast cancer. This can be possibly used to diagnose women with breast cancer and to identify patients with a poor prognosis who may benefit from more aggressive management.

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