Correlation of interleukin 6 (IL-6) with estrogen and progesterone receptor expression in breast cancer patients

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Summary:

Background: Multifunctional cytokines play important and only partially defined roles in mammary tumor development and progression. Normal human mammary epithelial cells constitutively produce interleukin 6(IL-6) and a non-secreted form of tumor necrosis factor. Transformation of mammary epithelial cells by different oncogenesis is frequently associated with alterations of cytokine/ growth factor production and responsiveness.

Methods: We measured levels of 1L-6 in 84 females with breast cancer and examined their correlation with clinicopathological variables including stages of the disease and estrogen and progesterone receptor expression on tumor cell.

Results: Our results revealed significantly higher serum IL-6 in breast cancer patients (12.98 pg/ml) compared to a healthy group (2.05pg/ml) (P<0.05) and a direct association with different clinical stages. And we found that expression of this cytokine was inversely associated with estrogen and progesterone receptor cells.

Conclusion: Serum levels of IL-6 are highly elevated in breast cancer patients and correlate with tumor progression. Assay for serum levels IL-6 can be used as predictive non-invasive tests for tumor progression in breast cancer patients; also our data suggest that cytokine could be involved in the aggressiveness of ER-PR negative breast tumors. Expression of interleukin 6(IL-6) correlates with estrogen and progesterone receptor in breast cancer patients.

Keyword: Interleukin 6, breast cancer, estrogen-progesterone receptor

Introduction:

Breast cancer represents the leading cause of cancer death among women in developed countries (1). Among the various prognostic factors, lack of estrogen receptor (ER) has consistently been associated with poorer prognosis (2). Most human breast cancer expresses ER-a, and the presence of this receptor is generally considered on indication of hormone dependence (3). In addition to ER-a, cytokines are now emerging as factors that are potentially involved in breast carcinogens (4, 5). Cytokines constitute a diverse group of proteins that include heamatopoietic factors, interferons, lymphokines and chemokines (6). Multifunctional cytokines play important and only partially defined roles in mammary tumor development and progression. Normal human mammary epithelial cells (MECs) constitutively produce interleukin 6 (IL-6) and a nonsecreted form of tumor necrosis factor. MEC transformation by oncogenesis frequently associated with alterations of cytokine/ growth factor production and responsiveness. This seems particularly true in the case of IL-6(7). Interleukin 6 (IL-6) is a multifunctional regulator of immune responses, haemopoesis and acute phase reactions, as well as of cell growth and is also involved in a variety of disease, including malignancies(plasmacytama/myeloma, leukuemia/lymphoma, melanoma, kaposis sarcoma, renal cell carcinoma, salivary gland carcinoma (9). Production of IL-6 iS up-regulated by cytokines such as IL-1 and tumor necrosis factor (TNF) (10) as well as by certain oncogenes including K-ras.

In addition, it has been shown that the production of IL-6 in modulated by hormones, such as steroids. These observations suggest that different pathways, could lead to the altered expression of this cytokine (11). The aim of this study was to evaluate the serum level of IL-6 in breast cancer patients at different clinical stage in relation to estrogen and progesterone receptor expression on tumor cells.

Patients and methods:

84 female patients with breast cancer were included in the study. Clinical staging was established on the basis of the American joint Committee on cancer (12). Samples were examined after mastectomy before any adjuvant therapy was given. The results were compared to 20 controls subject with benign tumor. Samples were collected from patients at Al-Kindy Hospital and the Hospital of Nuclear Medicine and Radiotherapy in Baghdad, during the period from (December 2007-Februray 2008). The presence of estrogen and progesterone receptor were determined according to manufacture's instructions of ER/RP kit procedure (BioGenx). And enzyme linked immunosorbent assay (ELISA) was used for determination of serum IL-6 level according to the specifications of the manufacturer (MBTECH). Monoclonal antibody specific for IL-6 cytokine was fixed to the surface of a microtiter plate. The standard sample and standards were applied. A second antibody which was biotinylated was used to detect the antigen,, streptavidin alkaline phosphates was then added. Addition of phosphate substrate (sigma) for the enzyme allowed detection of the bonded antigen via a colorimetric reaction.

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Statistical method: Statistical analysis of the present study was conducted using variance (ANOVA). Lowest significant difference (LSD) to exact test and correlation were used to asses the significance between the studied groups (P=0.02).

Results:

The clinical stages and the mean value of interlukin-6 are summarized in(table 1).The mean age of the study group was 47 years (range 30-69 years) 8 cases were found to be stage I;18 cases were stage II ;20 cases were stage III;and 38 cases were stage IV.Serum levels of IL-6 were assessed in patients with breast cancer and 20 healthy control .Stage I patients has the lowest mean value of IL-6 (12.98pg/ml). The highest level was present in stageIV patients with a mean value of (44.11 pg/ml). There was a significant increase in level of IL-6 (P<0.05) in stages I, II, III and IV when compared with healthy controls and there is a significant correlation between serum IL-6 levels and different clinical stages of breast cancer (P<0.05).

Table (1) Serum IL-6 levels (pg/ml) in breast caner patients at different clinical stages.

Clinical stage	Number	IL-6 level (pg/ml)
StageI	8	12.98
Satge II	18	26.81
Stage III	20	32.09
Stage IV	38	44.11
Control	20	2.05
Total	104	12.12

In table 2, 3 patients were grouped according to IL-6 negativity and IL-6 positivity. Interestingly, among the 56 patients with breast cancer and who were ER positive, only 10 cases were IL-6 positive, while among the 28 patients with breast cancer and who were ER negative, 20 were IL-6 positive, that showed a significant direct correlation between the IL-6 and ER negative, Figure (1, 2) A significant directly correlation was also found between PR and IL-6 expression (p=0.02), among the (54) PR positive carcinoma 8 cases were IL-6 positive, while among the (30) PR negative tumors 42 were IL-6 positive.

Table 2: Association between IL-6, estrogen and progesterone receptor expression in breast cancer patients.

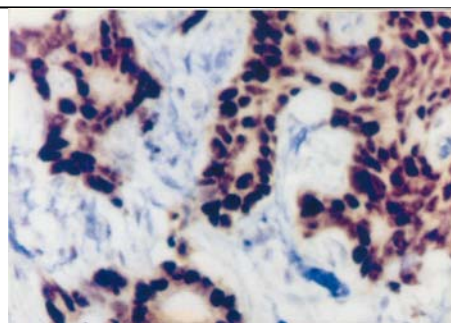| Receptor expression	No. of patients %	Negative for IL-6	Positive for IL-6	P. value
Estrogen (Total)	84			
Positive	56 (66.6%)	46(82,14)	10(7.85)	NS
Negative	28 (33.3%)	8(28.97)	20(71.42)	0.02
Progesterone(Total)	84			
Positive	54 (64.28%)	46(89.18)	8(4.81)	NS
Negative	30 (35.71%)	6(20)	24(80)	0.02

Figure 1: Invasive Ductal Carcinoma, Moderately Differentiated, Showing PR-Positivity (X400)
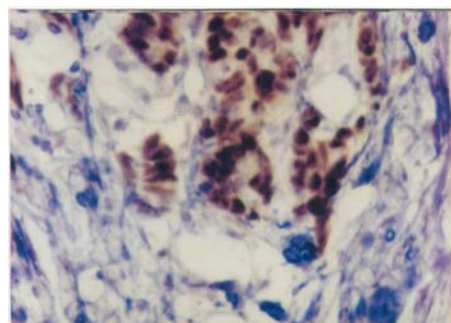

Figure 2: Invasive Ductal Carcinoma, Moderately Differentiated, Showing ER-Positivity (X400)

Discussion:

It is well known that the interactions of tumor cells with their microenvironment may affect tumor growth and metastasis formation. Among these, inflammatory cells and cytokines were recently suggested to play a key role in breast carcinoma (4). IL-6 is a multifunctional cytokine used in regulation of immune response and cancer cell proliferation. Significantly higher serum levels were detected in breast cancer patients compared to a normal healthy control group with direct association to different clinical stages. These results were in agreement with result reported by Zakizewska et al (13). In this study, 1L-6 expressions was directly correlated with expression of ER and PR that represent important mammary differentiation markers which is in agreement the findings of other studies (14, 15, and 16). The potential interaction between cytokines and steroid hormones has already been suggested by studies of breast cyst fluid (17). It was observed that IL-1 and IL-6 do increase estrogen synthesis by stimulating aromatase and estradiol-dehydrogenase activities in breast cancer cells. In addition, it has been reported that ER-positive human breast cancer cells express the IL-6 receptor and that their proliferation is inhibited by addition of IL-6(18, 19). Various cytokines, such as interleukin 1(IL-1), IL-6, IL-11 and tumor necrosis factor, which are released from carcinoma cells and/or inflammatory cell were also demonstrated to be capable of significant induction of aromatase expression in breast cancers. Several studies have reported that IL-1 or IL-6 levels correlated inversely with ER levels (20). This inverse correlation between cytokines and ER status

might not only reflect the greater aggressiveness of this subtype of breast tumors but it could also be the results of a direct regulation of cytokine expression by ER. Several reports have demonstrated a direct downregukation of cytokines by ER in different organs (21). Chiu et al (22) on their study on normal and transformed mammary epithelial cells reported that IL-6 secretion inhibit the growth of estrogen receptor positive ER+ breast cancer cells 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 (23) confirmed these studies and claimed that IL-6 secretion is inhibited by estrogen synthesis in peripheral tissues, including normal and malignant breast tissue. 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.

Conclusions:
Serum levels of IL-6 are significantly elevated in the sera of patients with cancer breast and this elevation is associated directly with progression and stages of the disease. So concentration of IL-6 in the sera of breast cancer patients can be used as non-invasive predictive indications of tumor progression and could be used in the follow-up of breast cancer patients. Also our data suggested that cytokines could be involved in the aggressiveness of ER/PR negative breast tumors.

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