

Serum Level of Interleukin 6 & Tumor Necrosis Factor in Iraqi Breast Cancer patients

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Abstract:

Interleukin 6 (IL-6) and tumor necrosis factor α (TNF- α) may be used as a prognostic value in breast cancer. Serum levels of IL-6, TNF - α were elevated significantly, among the 40 sera samples of female breast cancer patients compared to the 20 healthy control samples ($P < 0.05$).

The level of IL-6 and TNF- α were significantly higher in metastasis breast cancer patients than those with local site ($P < 0.05$), Furthermore, these results showed a positive correlation between IL-6 and TNF - α levels; the coefficient of correlation (r) was 0.98 which was statistically significant ($P < 0.05$). These results indicate that serum IL-6 and TNF - α level may be used in the assessment of breast cancer patients.

Introduction:

Breast cancer is the second most common cause of death following bronchogenic carcinoma ⁽¹⁾. Tumor markers may help in the initial assessment of the extent of the disease and resistance to chemotherapy. Interleukin 6 (IL-6) is a proinflammatory cytokine, which is produced by a number of immune system cells; fibroblasts, macrophages, T and B Lymphocytes, endothelial cells, keratinocytes and tumor cells ^(1, 2). IL-6 induces synthesis of acute phase response proteins in hepatocyte and terminal differentiation of B cells to antibody producing plasma cells ⁽¹⁾. Furthermore it may play a role in the proliferation and metastasis of cancer by up regulating antiapoptotic and angiogenic proteins in tumor cells ⁽²⁾. Elevated levels of IL-6 were observed in breast cancer patients suggesting that IL-6 produced from breast cancer cells are more resistant to chemotherapeutic agents and yield poor survivals ^(2, 3).

Tumor necrotic factor α (TNF α) is a multifunctional cytokine, which induces acute response. It activates endothelial cells and promotes neutrophil migration; it induces production of endothelial growth factors and stimulates alkaline phosphatase activity in osteoblasts ⁽⁴⁾. It is used as an antitumor agent, because of its ability to induce apoptosis ^(5, 6). The elevated levels of TNF α were observed in breast cancer patients may indicate its prognostic significance for advanced cancer patients, reflecting the severity of staging for invasive breast cancer ^(7, 8).

The aim of this study was to evaluate serum levels of IL-6 and TNF- α in sera of breast cancer patients at different clinical stages among Iraqi patients.

Materials & Methods:

Patients: Forty female patients were included in this study; the mean age group was 45 year ranging from 28-65 years old. Clinical staging were established on the basis of the American joint Committee on cancer (9). They were classified in to four stages each stage included 10 cases.

Samples: The blood samples of Breast cancer patients **were** collected at Hospital of nuclear medicine and radiotherapy in Baghdad, (10. Nov. to 12 Dec. 2005) before giving any adjuvant

therapy .The control group included 20 healthy female blood samples. Serum separated and preserved at -20c, to be used for examinations.

Enzyme linked immunosorbent assay for IL-6 and TNF α detection.

Two enzyme linked immunosorbent assays (ELISA) sandwich type were used, one for IL-6 detection, the second for TNF α detection. Both were manufactured by MAB Tech AB in Sweden. The instructions of the procedure for each kit were followed, as it was recommended by the manufacturer.

Statistical Analysis: The lowest significant (LSD) Correlation, mean value, and standard deviations were used to assess the significance between the groups⁽¹⁰⁾.

Results:

Serum levels of IL-6 and TNF α were assessed in 40 female patients, with breast cancer and 20 healthy female controls. The clinical stages and the mean values for IL-6 and TNF α were summarized in table (1), in which the highest IL-6 level was detected in stage (IV) 40 ± 5.2 pg/ml, while the lowest was at stage (I) 15.3 ± 6.1 pg/ml, compared to 5.1 ± 4.9 pg/ml in the controls. Similar results were shown in TNF α levels, that the highest level was in stage (IV) 66 ± 6.1 pg/ml, and the lowest was at stage (I) 27 ± 5.1 pg/ml , compared to 4.5 ± 4.8 pg/ml in the controls. There was a clear positive correlation between the level of IL-6 and TNF α value, r value was 0.098. Furthermore there was a significant difference between all the groups when compared to the controls. ($P < 0.05$).

Table (2) shows serum IL-6 and TNF α levels in breast cancer patients with different metastasis sites , the mean value of Il-6 serum level in local recurrences , liver , bone and numerous materials was 25.4 ± 0.3 pg/ml , 60 ± 5.1 pg/ml , 50 ± 3.2 pg/ml , 67 ± 5.3 pg/ml respectively. The statistical analysis showed significant differences in IL-6 level in patients with different metastasis sites, LSD test showed significant increase in the level of IL-6 in patients with liver, bone and numerous metastasis, when compared with other patients ($P < 0.05$) , however there were no significant differences in the level of Il-6 in the patients with bone , local , liver , numerous sites when compared to each other.

The results obtained for TNF α level in patients with local , bone and numerous metastasis were ; 41 ± 0.3 pg/ml , 51 ± 2.1 pg / ml , 30 ± 4.2 pg/ml and 65 ± 1.2 pg/ml respectively. LSD test showed significant increase in the level of TNF α in patients with bone and numerous metastasis, when compared with other patients ($P < 0.05$). However, there was no significant difference in the level of TNF α between different patients group.

Table (1) Serum IL-6 and TNF- α levels in breast cancer patients at different clinical stages presented mean \pm SD pg / ml

Clinical stage	No, samples	IL-6 mean \pm SD pg / ml	TNF- α mean \pm SD pg / ml
I	10	15.3 \pm 6.1	27.5 \pm 5.1
II	10	30 \pm 7.1	50 \pm 6.2
III	10	34 \pm 10.1	49 \pm 5.2
IV	10	40 \pm 5.2	66 \pm 6.1
Control	20	5.1 \pm 4.9	4.5 \pm 4.8
*LSD	P<0.05 (S)**		
*** r	0.098 (IL-6 & TNF α)		

*LSD = Least Significant Value

** S = Significant between the groups

*** r = Correlation coefficient

Table (2) Serum IL-6 and TNF α levels in breast cancer patients with different metastasis sites

Site of Metastasis	No. samples	IL-6 mean \pm SD pg / ml	TNF α mean \pm SD pg / ml
Local	17	25.4 \pm 0.3	41 \pm 0.3
Liver	4	60 \pm 5.1	51 \pm 2.1
Bone	8	50 \pm 3.2	30.1 \pm 4.2
Numerous	11	67 \pm 5.3	65 \pm 1.2
Total	40		

Discussion:

In this study a significant high serum level of IL-6 was detected in Iraqi breast cancer patients, compared with the normal control group with direct association to clinical stages (table 1). Similar results were found by other researchers ^(1,3).

Conze and his colleagues in 2001 indicated that, the high level of IL-6 is produced by multi drug resistant breast cancer cells, and the autocrine production of IL-6 by tumor cells is an important factor in determining the susceptibility or resistance of these cells to drug treatment ⁽²⁾.

On the other hand, serum IL-6 level was significantly higher in metastasis breast cancer patients than those with local recurrences (table 2). Similar results presented by Beny and his Colleagues in 2002, who showed a 10 times increase in IL-6 level in metastasis breast cancer patients compared with local site disease ⁽¹¹⁾.

TNF is a powerful stimulating cytokine, which influences various types of cells ⁽⁷⁾, Sheen - Chen and his colleagues in 1997 have indicated that evaluation of TNF α level may be a valuable parameter for reflecting the severity of staging for invasive breast cancer ⁽⁸⁾. In this study the level of TNF α is correlated significantly with disease stage, when compare to healthy controls (table 1). Others indicated that about 21.2 % of breast cancer patients who responded positively to chemotherapy had considerable decrease on the TNF α level, when compared to their initial levels ⁽⁷⁾.

In table (2); the highest level of TNF α was among patients with numerous metastasis sites 65 ± 1.2 pg/ml, compared to local site and other metastasis sites. These differences were statistically significant ($P < 0.05$) and are similar to results presented by others^(7, 8).

In this study, there was a clear positive correlation between IL-6 and TNF α levels (table 1), this may explain the role of these two cytokines in tumor growth, that the prediction of survival for patients with metastatic breast cancer is often inaccurate, and may be indicated by biological parameters, among these parameters the level of IL-6 and TNF α .

The level of IL-6, which is secreted from cells of the immune system, might be used as a marker of immune system activation and disease progression⁽⁷⁾, as it stimulates cancer growth and contributes to local recurrence as well as metastasis^(1, 2, 3, 7).

Many factors may influence the cytokine production and its level in patients' serum, which may reflect a relationship between these substances and the disease progression or even the response to chemotherapy^(12, 13).

Our results suggest that IL-6 and TNF α levels are discrimination factors and can be used as a prognostic value in breast cancer, however more studies are required to investigate the role of IL-6 and TNF α as a predictive value for response to chemotherapy.

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