Determination of Serum IL-8 Level in Women with Breast Cancer and Their Correlation With Disease Progression

Zahraa Abdul Muhsin Mohammed

Microbiology Department, College of Medicine, Al-Mustansiriya University.

Abstract :

Background: Interleukin-8 (IL-8) is a pleiotropic cytokine with a range wide of physiologic and pathophysiology activities, in addition to its role as an immunomodulatory cytokine. IL-8 is thought to function as a growth and differentiation factor in human cancer, by modulating metastasis and angiogenesis.

Objectives: Determination of serum level of IL-8 in women with breast cancer, bengin tumor and healthy women and to assess, whether it correlates with the disease progression.

Subjects and Methods: In the current study, the IL-8 levels were measured in 41 women with breast cancer, 21 with bengin tumor and 20 healthy women. Serum concentrations of human IL-8 were determined by enzyme – linked immunosorbant assay (ELISA)

Results: The levels of serum IL-8 were increased significantly in breast cancer patients compared with bengin tumor and healthy women ($P<0.05$).

Higher serum concentrations of IL-8 were seen in stage III of the disease compared to values obtained from stage II patients ($P>0.05$).

Conclusions: The present study indicates that elevated IL-8 serum concentrations are strongly associated with breast cancer and correlated with clinical stage of disease.

These results suggest that serum IL-8 measurements may be useful in estimation of disease progression in women with breast cancer.

Keywords: breast cancer, interleukin -8, benign tumor

Introduction:

Breast cancer represents the leading cause of cancer death among women in developed countries (1).

In Iraq, breast cancer is the commonest type of female malignancy, accounting for approximately one-third of the registered female cancers according to the latest Iraqi cancer registry 2).

This shows that the breast is the leading cancer site among the Iraqi population in general, surpassing even bronchogenic cancer (3). The treatment of breast cancer has advanced greatly from simple surgery to surgery combined with chemotherapy, radiotherapy and endocrine therapy. Despite adequate surgical intervention and adju-
……………………………………………………………..

Corresponding Address:
Zahraa Abdul Muhsin Mohammed
Microbiology Department, College of Medicine, Al-Mustansiriya University.
Email: zahraamicro@yahoo.com

vant treatment a substantial number of patients still die of recurrence and metastasis (4).

The biology of breast cancer is complex, involving oncogenesis, evasion of host immune defense mechanisms, angiogenesis, invasion and metastasis (5).

Recent efforts have focused on dissecting the role and prognostic value of tumor cell derived cytokines in human breast cancer (3).

Cytokines, cell communication mediators, play important roles in a wide range of physiological processes, such as cell growth, differentiation, apoptosis, wound healing and homeostasis (6).

A number of autocrine and paracrine cytokine loops influence tumor development. Cytokines not only provide defense against cancer cells, but also promote cancer cell growth at every stage of cancer development. Alteration of cytokines level is associated with cancer progression, response to chemotherapy and metastatic status (7).

Several reports have elaborated on the involvement of che-

mokines in tumor growth, invasion and metastasis. More specifically, interleukin -8 (IL-8) which is a member of the CXC chemokine family related proinflammatory cytokines (8). It is of particular interest as it is expressed by many types of tumors including prostate, colon, lung, ovary and melanoma cancer (9). Interleukin -8 binds with high specifity to the CXR1 and with less specifity to CXR2 both receptors are expressed on stromal cells and tumor cells, both receptors are expressed on stromal cells and tumor cells (10).

So the principal objective of this study was to determine the concentration of IL-8 in blood serum of breast cancer, bengin breast tumor and healthy women and their correlation with clinical stage of breast cancer

Subjects and Methods:

A.Subject:

Analysis was performed in 41 breast cancer women diagnosed in Al-Kadhymia Teaching Hospital. Age of the patients ranged from 30 to 72 years.

Clinical diagnosis was routinely confirmed by the histopathological examination of the tumor tissue samples. Examined patients were in clinical stage (22 II & 19 III) according to TNM classification. None of the patients suffered from infectious, allergic, autoimmune or other systemic disease.

The control groups included: twenty-one women with suspicious breast mass who were found subsequently to have a negative breast biopsy (bengin tumor)and twenty healthy women. The present study was performed during the period from January /2011 to the end of October/2011.

B. Blood collection and IL-8 measurement:

Blood samples were collected before treatment initiation and immediately centrifuged after collecting at 500xg for 10 minutes. Serum sample were stored at (-20 ℃). Interleukin-8 concentrations were determined in serum samples by enzyme-linked immunosorbant assay (ELISA) kit, according to the instructions provided by manufacturer (BioSource Europe S.A.). The Biosource IL-8-EASIA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiterplate.

The assay uses monoclonal antibodies (MAbs) directed against distinct epitopes of IL-8 .Calibrators and samples react with the capture monoclonal antibody (MAb 1) coated on microtiter well and with a monoclonal antibody (MAb 2) labeled with horseradish peroxidase (HRP) .After an incubation period allowing the formation of a sandwich: coated MAb1-human IL-8-MAb2-HRP, the microtiterplate is washed to remove unbound enzyme labeled antibody. Bound enzyme labeled antibody is measured through a chromogenic reaction. Chromogenic solution (TMB) is added and incubated.

The reaction is stopped with the addition of Stop Solution and the microtiterplate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is proportional to the IL-8 concentration.

C.Statistical analysis:

Statistical analysis was performed using t-test compare among patients & control groups. A value of ($P<0.05$) was considered statistically significant.
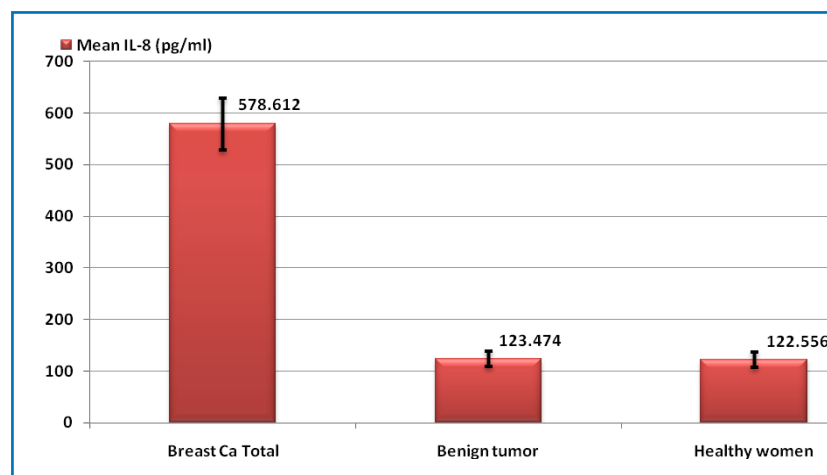
Results:

Figure-1 represented the comparative analysis of IL-8 levels in patients with breast cancer, bengin tumor and healthy volunteers. Breast cancer patients have the following value of IL-8 (mean ±SD) (578.612±49.618 pg/ml) compared with control subjects bengin tumor (123.474±14.852 pg/ml) and healthy women (122.556±15.138 pg/ml).

The differences in serum IL-8 between the controls and patients with breast cancer is significant ($P<0.05$).

The distribution of IL-8 serum concentration according to clinical stages of breast cancer is shown in figure-2.

Higher serum concentration of IL-8 were seen in stage III of the disease compared to value obtained from stage II patients, but no significant differences were seen ($P>0.05$).

Figure -1- Serum level of IL-8 (pg/ml) in breast cancer & control groups.
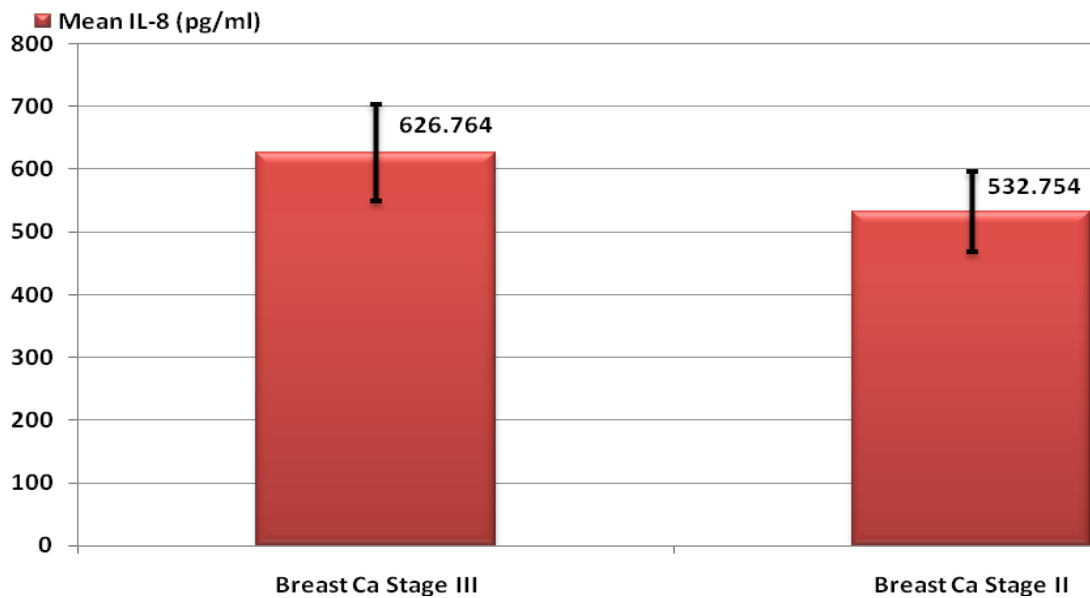
Figure -2- Serum level of IL-8 (pg/ml) according to clinical stage.

Discussion:

According to our results, breast cancer patients exhibited significantly higher serum concentrations of IL-8 compared to bengin tumor and healthy comparison subjects(Figure 1).Interleukin -8 is an important factor involved in human breast cancer progression (11).

Increased expression of IL-8 and /or its receptors has been characterized in cancer cells; endothelial cell, infiltrating neutrphils and tumor –associated macrophages suggesting that IL-8 may function as a significant regulatory factor within the tumor microenvironment (12).

All breast cancer cells expressed the IL-8 receptors, CXR1 and CXR2, whereas only 50% of the bengin breast tissue samples expressed either CXR1 or CXR2 (13, 14).

It was reported that interleukins stimulate cancer cell growth and contribute to locoregional relapse as well as metastasis (15).

Permanent synthesis and release of these cytokines lead to increased serum cytokines concentration and act as marker of immunity status and immne system activation for prognosis and monitoring the course of cancer progression (5).

In addition to adversely affecting disease progression in persons with cancer, elevated level of proinflammtory cytokines have been associated with distressing symptoms in individuals with breast cancer (16).

Cytokines may play a role in pathophysiology of neuropsychiatric of the immune and neuro-endocrine system proinflemmatory cytokines have been associated with de-

pression in persons with cancer during treatment and in fatique in survivors of breast cancer (17). IL-8 also has mitogenic, angiogenic and motogenic properties in different cancer cells models (8).

Additionally IL-8 was primary know to be chemotactic for neutrophils. Inflammatory infiltrates have been associated with enhanced tumor growth and worse survival; this might be attributed to the release of angiogenic growth factors by neutrophils and macrophages (18).

In this study, it was found higher serum concentrations of IL-8 in stage III of the disease compared to values obtained from stage II patients (P>0.05) (Figure-2). This finding in our study confirmed by kozlowski etal (5) study who found no significant differences between two stages of disease. IL-8 has been shown to be up-regulated in several human cancers including breast cancer (19).

Higher serum levels of IL-8 in breast cancer patients have been associated with poor prognosis, increased tumor burden and decreased post relapse survival (20). The frequency of increased results and absolute value of IL-8 levels showed tendency to significant increase with the stage of disease (21).

In conclusion, our study suggests that IL-8 measurement can be used to diagnose women with breast cancer and to identify patients with a poor prognosis who may benefit from more aggressive management.

References:

1. Chavey, C ; Bibeae, F; Bourgade, G.S; Lazennec, G.(2007) Ostrogen receptor negative breast cancer exhibit high cytokine content. Breast Cancer Research. 9(1): R15.
2. Iraqi Cancer Board. Results of the Iraqi cancer Registry. (2004). Baghdad, Iraqi Cancer Registry center. Ministry of Health.
3. Alwan, N. A. (2010). Breast cancer: demographic characteristic and clinical pathological presentation of patients in Iraq. EMHJ. 16(11): 1159-65.
4. Yao, C; Lin, Y; Chua,I; Bi, J; Zhu, YF; Wang, S.M. (2007). Interleukin -8 modulates growth and invasiveness of estrogen receptor negative breast cancer cell. Int. J. cancer, 121: 1949-57.
5. Kozlowski, L; ZakrZewska, I; Tokajuk,P; Wajtukiew, MZ. (2003). .Concentration of interleukin-6 (1L-6), Interlakin-8 (IL-8) and interleukin -10 (1L-10) in blood serum of breast cancer patients. Annals Academic Medical Bialostocensis, 48:82-84.
6. Lin, Y; Huong, R; Chen, L; Li,S; Jordan, C.(2004). Identification of interleukin -8 as estrogen – regulated factor involved in breast cancer invasion and angiogenesis by protein arrays. Int. J. cancer, 109:507-15.
7. Green, S and Chambon, P. (1986). A superfamily of potentially oncogenic hormone receptors. Nature. 324:615-7.
8. Benoy, I.H; Sargado, R; Damg V.P; Gybers, K. (2004). Increased serum IL-8 in patients with early and metastatic breast cancer correlates with carly dissemination and survival. Clin-cancer Research, 10 (21):7157-62.
9. Freund, A; Jolival, L.; Durand. S; Nathalieok,A (2004) Mechanisms underlying different expression of interleukin -8 in breast cancer cells. Oncogene, 23(36):6105-14.
10. Rossi, D. and Zlotnik, A. (2000). The biology of chemokine and their receptors. Ann. Rev. Immunol. 18:217-42.
11. Pan, Q; Kieer, C.G; Irani, J; Mesri, E.A; Dick, R.D. (2002). Copper deficiency induced by tetrathiomolybdate suppresses tumor growth and angiogenesis. Cancer Research, 62(48):85-89.
12. Waugh, D.J and Wilson, C. (2008). The interleukin-8 pathway in cancer. Clin. Cancer. Research, 14(21):6735-41.
13. Miller, L.J; Kurtzman, SH; Wang, Y. (1998). Expression of interleukin -8 receptors on tumor cells and vascular endothelial cells in human breast cancer tissue Anti cancer Research, 18:77-81.
14. Ahmed, O; Adel ,A.M; Diab, D.R; Gobran. N (2006) Prognostic value of serum level determination in diagnosis of bengin and malignant tumor Pol. Merkur Lekarski, 13(76):302-4.
15. Premkumar, V.G; Yavaraj,S;Vjayasarathy, K; Gangadran DG and Sachanadam, P.(2007). Serum cytokines levels of interleukin 113, 6, 8 tumor nerosisfactor in breast cancer patients treated with Tamoxifen and supplemented with co-enzyme Q10, Riboflavin & Niacin. Basic clinical pharmacology and Toxicology, 100:387-91.
16. Musselmon, D; Miller. A; Porter A; Manatanga A. (2001). Higher than normal plasma interleukin-6 in cancers patients with depression. Preliminary findings. American Journal psychiatry, 158(8):1252-57.
17. Lyon, DE. (2008). Cytokines comparisons between women with breast cancer and women with a negative breast biopsy. Nur. Res, 57(1):51-58.
18. Chen J.J; Yoa P.L; Yuan A. (2003). UP-regulation of tumor interleukin -8 expression by infiltrating macrophays, its correlation with tumor angiogenesis of patients survival in non small lung cancer. Clin cancer Res, 9:927-37.
19. Green, A.R; Green M; while, V. (1997) Expression of cytokines messenger RNA in normal and neoplastic human breast tissue identification of IL-8 as a potential regulatory factor in breast tumor. Int. J. cancer, 72:937-41.
20. Bendrick, C; and Dobrosin C (2009). Estradiol increases IL-8 secretion of normal human breast tissue and breast cancer in vivo. Journal of immunology, 182(1):1371-78.
21. Zakrzewska, I; Kozlowski,.L; Wajlukiewic,M. (2002) Valus of interleukin-8 determination in diagnosis of bengin and malignant tumor. Pol Merkur Lekarski, 13(76):302-4.

تحديد مستوى الانترلوكين8- في مصول النساء المصابة بسرطان الثدي وعلاقته بتقدم المرض.

زهراء عبد المحسن محمد

فرع الاحياء المجهرية, كلية الطب, الجامعة المستنصرية

الخلاصة:

المقدمة: يعتبر الانترلوكين 8- متعدد الانتحاء وذو مدى واسع من النشاطات الفيزيائية وفسيولوجية الامراض بالاضافة الى دوره في التحوير المناعي، كما يعتقد ان وظيفته كعامل للنمو والتمايز بتغيير عملية الانتشار وتكوين الاوعية في السرطان .

الهدف: تحديد مستوى الانتركولين 8- في مصول النساء المصابة بسرطان الثدي، الورم الحميد والنساء الاصحاء وتقييم علاقته بتقدم المرض.

طريقة البحث: في الدراسة الحالية تم قياس الانترلوكين8- في واحد واربعين عينة من النساء المصابة بسرطان الثدي واحدى وعشرين من النساء المصابة بالورم الحميد وعشرين من النساء الاصحاء. وقد تم تحديد تركيز الانترلوكين 8- باستخدام تقنية الاليزا (ELISA).

النتائج: اظهرت النتائج وجود زيادة معنوية في النساء المصابة بسرطان الثدي مقارنة مع النساء المصابة بالورم الحميد والاصحاء، كما لوحظ زيادة تركيز الانترلوكين8- في المرحلة الثالثة للمرض مقارنة مع القيمة المستحصلة في المرحلة الثانية للمرض

الاستنتاج: تشير الدراسة الحالية الى ان ارتفاع مستوى الانترلوكين 8- يقترن بقوة مع سرطان الثدي. كما انه يرتبط مع المرحلة السريرية للمرض. تقترح هذه النتائج بأن قياس الانترلوكين8- ذو فائدة في تقدير تقدم المرض في النساء المصابة بسرطان الثدي.