Original Article

The Role of Interleukin-10 in Women with Metastatic Invasive Ductal Carcinoma

Amina N. Althwani* **BSc, MSc, PHD**
Mohammed A. Najm ** **BSc, MSc**

Summary:

Background: Breast cancer is the malignant tumor that forms from the uncontrolled growth of abnormal breast cells. It usually affects tissues involved in milk production (Ductal and lobular tissues). It is the most common malignancy in women and it remains one of the greatest health threats facing women around the world as we enter the 21st century**.**

Objectives: To estimate the role of IL-10 in the progression of invasive ductal carcinoma.

Patients and Method: Seventy three metastatic invasive ductal carcinoma Iraqi women were admitted to Nuclear Medicine Hospital in Baghdad and 15 samples of apparently healthy women were involved as a control. The blood samples (2 mL) were drown from all studied cases in order to be used for measuring their serum level of IL-10 by using Enzyme-linked Immune Sorbent Assay technique.

Result: The results have shown that the high significant relation was that of elevated IL-10 levels, which increased in 98.6% of patients.

Conclusion: The result indicates that the highl significant elevation of serum IL-10 concentration are strongly associated with metastatic invasive ductal carcinoma, this may be helpful in adding further vision in the diagnosis of women with this type of cancer.

Key words: interleukin-10, metastatic invasive ductal carcinoma

Fac Med Baghdad 2011; Vol. 53, No. 3
Received Jan. 2011
Accepted June 2011

Introduction:

Breast cancer is one of the most common cancers in women in the developed countries of the world and is the cause of death in approximately 20% of all females who die from cancer in these countries. In the US, breast cancer rates are among the highest in the world, annual rate is 104.2 per 100,000 adjusted to the World Standard Population (1). In 1998, 178,000 new cases of breast cancer were diagnosed in US and approximately 40,000 Americans die from breast cancer every year (2). Asia has the lowest breast cancer incidence and mortality rates in the world. The rates have been increasing over the last 20 years in all age groups in all countries of the world (3).

In Iraq, the numbers of breast cancer cases were steadily rising after the 1991 war (4, 5), the most frequent histological types of breast cancer in Iraq are infiltrative ductal carcinoma, which represent 77.2% from the total cases diagnosed (6).

Recently, the contribution of interleukins, pleiotropic cytokines, in cancer progression has been demonstrated. Macrophages, monocytes and lymphocytes as well as cancer cells have been documented to produce and secrete (IL-10) (7). These cytokine in an autocrine or paracrine manner induce in Beside melanoma cells (8).vitro growth of ovarian, cervical, prostate, lung and In the context of breast cancer risk, IL-10 may

act as a two-edged sword: on one hand, elevated IL-10 levels could facilitate development of cancer by supporting tumor escape from the immune response, and on the other hand; its anti-angiogenic effects are supposed to prevent or reduce tumor growth and metastasis (9).

However, there are also other types of interleukins, which involved either in the stimulation of breast carcinoma progression; such as Interleukin-2 (IL-2), or act as inhibitory factor that inhibits the growth of breast cancer cells and represent a potential pathway to affect cell death in breast cancer; such as Interleukin-4 (IL-4) (10, 11).

Material, method and patients:

The study was done in period between October, 2009 and middle of April, 2010.

Patients study group:

The study was conducted on fifteen blood samples of apparently healthy women as a control group to be compared with seventy three metastatic invasive ductal carcinoma patients (all were women), who were examined and diagnosed by the oncologist at the Hospital of Radiology and Nuclear Medicine in Baghdad. Two ml of blood were taken from each individual and left to clot at room temperature, then

* *Institute of Genetic and Biotechnology/ Baghdad University*
** *Alfaluja General Hospital kidney cancer*

centrifuged and serum was collected in aliquots to stored in (-18C) until needed for IL-10 estimation.

Kit and reagents: Human interleukin-10 (IL10) ELISA kit (BioLegend, Netherlands).

Interpretation of results: The results were calculated by interpretation from a standard curve that is performed in the same way as that of sample. The average absorbance for each sample on the vertical axis was located and the corresponding IL-10 concentration on the horizontal axis was read. Statistical analysis was done using SPSS version computer software (Statistical Package for Social Sciences). T test was used to analyze the data, and the calculation of mean difference, P value<0.01 level of significance was considered statistically highly significant.

Results:

Serum IL-10 has been evaluated in 73 breast cancer patients and 15 healthy controls that have been included in this study. Regarding patients ages, the mean age was 47.1 years old. It was obvious that the distribution of the patients was predominantly seen between 50-59 years age groups ($\chi 2 = 7.033$, $p < 0.001$) as shown in figure-1.

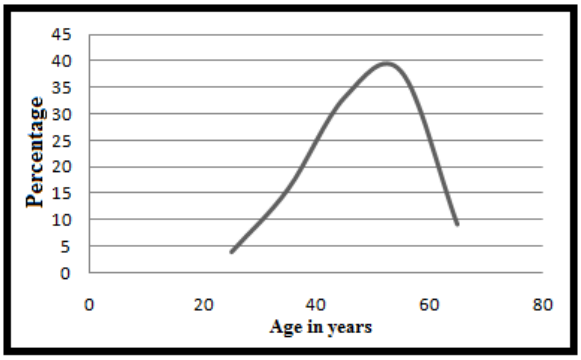

Figure (1): The distribution of patients according to the age group.

The ages of patients were studied in correlation with their serum IL-10 concentrations; the result showed that there were no any significant correlations, (Table - 1).

The result has clarified that serum IL-10 concentration increased in 72 from 73 patients (98.6%), the range and median serum IL-10 concentrations for breast cancer patients have been 7.8 - 58.9 (pg/mL) and 27.54(pg/mL) respectively compared to 4 - 8.3 (pg/mL) and 5.74 (pg/mL) for healthy controls, this considered statistically highly significant (P<0.01), (Table-2).

Table-1: Correlation between patients' ages and their serum IL-10 concentrations.

Parameters	Age groups				P value	LSD Value
	Least than 40 year	40-49 year	50-59 year	More than 59 year		
IL-10	26.96 ± 2.71	27.50 ± 1.74	28.41 ± 2.04	24.71 ± 4.89	0.867	8.076 ns

ns = Not significant

Table-2: Comparison between serum IL-10 levels of metastatic invasive ductal carcinoma patients and healthy control in P, median and range values.

Parameter		Control N=15	Breast cancer patients N=73
IL-10 concentration (pg/ml)	Median	5.74 ± 0.37	27.54 ± 1.16
	P value		0.0001
	Range	4 - 8.3	7.8 - 58.9
	Percentage of elevated values	0	98.6%
	Number of patients with extremely elevated values	0	3
	Number of patients with extremely lowered values	0	1

Furthermore; the result has revealed that there is no any significant correlation between serum IL-10 concentrations in breast cancer patients and the increase in tumor size (T) or the increase in the number of lymph nodes involved (N), (Table-3):

Table-3: correlation of IL-10 levels with tumor size and number of lymph nodes involved.

Tumor size grade (T)	Number of patients	Average Serum IL-10 concentration		Lymph node involved Grade (N)	Number of patients	Average Serum IL-10 concentration
T1	19	26.8		N1	24	28.3
T2	18	28.1		N2	28	26.1
T3	21	27.1		N3	21	28.6
T4	15	28.4		P Value = 0.918 (n.s)		
P Value = 0.962 (n.s)						

Discussion:

Overwhelmingly, breast cancer incidence and death rates generally increase with age. During 2002- 2006, 95% of new cases and 97% of breast cancer deaths occurred in women aged 40 and older (12). In agreement with the results of the present study, a recent study in Iraq showed that the incidence of breast cancer has significantly occurred after the age of 40 (13, 14), also a group of researchers both in USA and Australia have found that the incidence of breast cancer increasing sharply after the age of 40 (15, 16).

A cohort study has shown that breast cancer incidence followed the age trend reported in national data, with increasing incidence each 5-years age group up to 75–79 years and then somewhat lower incidence at age 80–84 years. (17). In contrast to the results obtained in this study, another study has found that during 2002-2006, women aged 75-79 had the highest incidence rate, 441.9 cases per 100,000. (12), these findings may be explained by the fact that the increasing life expectancies in western countries have resulted in an increase in the proportion of the population that is elderly. For instance, the percentage of the US population that was aged 75 or more years has increased from 2.6 percent in 1950 to 5.9 percent in 2000 (18). The present data confirmed that there is no correlation between the increasing ages of patients and their serum IL-10 concentrations; because the serum IL-10 levels in patients with the same age group were extremely different, for example; five patients whose age was 60 years, showed highly varied serum levels of IL-10 (21.8, 32, 34, 42.6 and 58.5 pg/mL), and vice versa, there were patients from different age groups but had the same IL-10 concentrations, for example; two patients gave 23 (pg/mL) serum IL-10 concentrations; their ages were 37 and 47 years and also other two patients had 20 (pg/mL) serum IL-10 concentrations; with 41 and 56 years old. Although the relationship between IL-10 and cancer has been studied extensively, the ultimate role of IL-10 in tumor biology remains enigmatic. The significance of IL-10 production within the tumor microenvironment, which can be sustained by malignant cells and tumor-infiltrating macrophages (TIM) and lymphocytes [including natural killer (NK) and T cells], is debated (19). In agreement with the present result, Kozłowski *et al*. (2003)(20) in his study which carried out on 45 breast cancer patients and 25 healthy controls in Białystok/Poland in a trial to assess the role of IL-10 in breast cancer, has found that the range and average serum IL-10 concentration for patients were 5.6 – 37 (pg/mL) and 24.7(pg/mL) compared to 3.9 – 8.8 (pg/mL) and 5.7(pg/mL) for healthy control. Serum IL-10 levels have extremely varied among patients; three patients revealed very high IL-10 concentrations (52.5, 58.5 and 58.9 pg/mL) compared to healthy controls, conversely, one patient showed very low serum IL-10 concentrations (7.8 pg/mL) which appeared to be within the range of serum IL-10 for healthy controls, (Table 1). The remaining patients have shown different serum IL-10 concentration which appeared mostly to be near the average range of IL-0 level which considered statistically highly significant compared to IL-10 levels for healthy controls as mentioned in table (1). While serum IL-10 levels in present data revealed highly significant elevation in patients with metastatic invasive ductal carcinoma compared to healthy control group, its values hadn't been influenced by the increase in the tumor size (T)(p value = **0.962**) or the increase in the number of positive lymph nodes (N)(P value = **0.918**), so we didn't need to involve a non metastatic invasive ductal carcinoma patients as a third group in this study (we didn't need to make further comparison between serum IL-10 levels in metastatic and non metastatic breast cancer patients), because the current result led us to an idea, which is that metastasis may be the only factor that is responsible for increasing serum IL-10 concentration in metastatic invasive ductal carcinoma patients while other factors such as tumor size or number of lymph node do not affect the IL-10 levels. There is not a fully clear idea about whether the increasing serum IL-10 concentrations in breast cancer patients reflect good or poor prognosis because of the huge number of controversial findings about the real role of IL-10 in cancer microenvironments. Some researchers were confirmed that IL-10 can favor tumor growth *in vitro* by stimulating cell proliferation and inhibiting cell apoptosis, high systemic levels of IL-10 correlate with poor survival of some cancer patients (21, 22), while others provided opposite findings (higher IL-10 associated with better survival) were observed the cytokine levels in tumor samples (23), and the data from studies correlating IL-10 polymorphism and cancer risk/prognosis are controversial (22). IL-10 can also inhibit tumor-induced angiogenesis and enhance the production of

tumor-toxic molecules [e.g., nitric oxide (NO)], which leads to tumor regression in some preclinical models (24).

References:

1- Lavigne, J.A.; Helzlsoure, K.J. and Huang, H.Y. (1997). An association between the allele coding for a low activity variant of catechol-O-methyltransferase and the risk for breast cancer. Cancer Res. 57: 5493-5497.

2- Black, M.H.; Magklara, A.; and Obiezu, C.(2000). Expession of a prostate associated protein, human glandular KalliKrein (hK_2) in breast tumors and in normal breast secretions. British Journal of Cancer, 82:361-367.

3- Osborne, CK. (1998). Steroid hormone receptors in breast cancer management. Breast Cancer Res. Treat., 51: 227-238.

4- Jaffer, S.G. (1999). Cytogenetic study of Breast cancer in Iraq. M.S.c thesis submitted to the College of Science, Baghdad University

5- Jasim, S.L. (2004). Genetic polymorphism of breast tumor using polymerase chain reaction based techniques. Ph.D thesis submitted to the College of Science, Baghdad University.

6- Registration of cancer council in Iraq. (2000); Ministry of Health, Iraq/Baghdad.

7- Moore, K.W.; de Waal Malefyt, R.; Coffman, R.L. And O'Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol, 19.683-765.

8- Giri, D.; Ozen, M. and Ittmann, M. (2001). Interleukin-6 is an autocrine growth factor in human prostate cancer. Am J Pathol.159.2159-2165.

9- Langsenlehner, U.; Krippl, P.; Renner, W.; Yazdani-Biuki, B.; Eder, T.; Ko¨ppel, H.; Wascher, T. C.; Paulweber, B and Samonigg, H (2005). Interleukin-10 promoter polymorphism is associated with decreased breast cancer risk. Breast Cancer Research and Treatment.90. 113–115

10-Gooch, J.L.; Lee, A.V. and Yee,D.(1998). Interleukin 4 Inhibits Growth and Induces Apoptosis in Human Breast Cancer Cells. CANCER RESEARCH. 158. 4199-4205.

11-García-Tuñón, I.; Ricote, M.; Ruiz, A.; Fraile, B.; Paniagua,R. and Royuela,M.(2004). Interleukin-2 and its receptor complex (α, β and γ chains) in in situ and infiltrative human breast cancer: an immunohistochemical comparative study. Breast Cancer Res.6(1):1-7

12-American Cancer Society (ACS). (2010). Cancer Facts & Figures 2010. Atlanta, Georgia. American Cancer Society. Inc

13- Mahdi, M.G. (2009). A Molecular Study of Loss of Heterozygosity in Tissue Samples Isolated from Breast Cancer Patients in Relation with Their Sex Hormones Status.M.SC. Thesis. Genetic Engineering and Biotechnology Institute, Baghdad University.

14-Karim, M. N. (2010). Assessment of Multidrug Resistance Genes and Apoptotic Genes by Multiplex PCR and Estimation of Suppresser and Apoptotic Protein by Immunihistochemistry and Electrochemical Biosensor in Breast Cancer Patients. PH.D. thesis. College of Medicine, Alnahrain University

15-Wu, S. C.; Hotes, J.; Fulton, J. P.; Chen, V. W.; Howe , H. L. and Correa, C . (2002). Cancer in North America, 1995-1999. Volume III: Combined Cancer Incidence Rates. North American Association of Central Cancer Registries. Springfield. IL, U.S.A; p.1-6.

16-Edwards, B. K. ; Howe, H. L. ; Ries, L. A. ; Thun, M. J. ; Rosenberg, H. M. ; Yancik, R. ; Wingo, P. A. ; Jemal, A. and Feigal, E. G. (2002). Annual report to the nation on the status of cancer, 1973-1999, featuring implications of age and aging on U.S. cancer burden. Cancer. 94. 2766-2792.

17-Sweeney, C.; Cindy, K. B.; Kristin, E. A.; DeAnn, L.; and Aaron, R. (2004). Risk Factors for Breast Cancer in Elderly Women. Am. J. of Epid. Vol. 160.

18-Ries, L. A. G.; Eisner, M. P. and Kosary, C. L. (2003). SEER cancer statistics review, 1975–2000. J. Nat. Can. Inst. Vol.6

19-Vicari, A. P. and Trinchieri, G. (2004) Interleukin-10 in viral diseases and cancer: exiting the labyrinth? Immunol. Rev.202. 223–236.

20-Kozłowski, L.; Zakrzewska, I.; Tokajuk, P. and Wojtukiewicz, M.Z.(2003). Concentration of interleukin-6 (Il-6), interleukin-8 (Il-8) and interleukin-10 (Il-10) in blood serum of breast cancer patients. Annals' Academiae Medicae Bialostocensis. 48, 82-84

21-Sredni, B.; Weil, M.; Khomenok, G.; Lebenthal, I.; Teitz, S.; Mardor, Y.; Ram, Z.; Orenstein, A.; Kershenovich, A. and Michowiz, S. (2004) Ammoniumtrichloro (dioxoethylene-o,o') tellurate (AS101) sensitizes tumors to chemotherapy by inhibiting the tumor interleukin 10 autocrine loop. Cancer Res. 64. 1843–1852.

22-Lech-Maranda, E.; Baseggio, L.; Bienvenu, J.; Charlot, C.; Berger, F.; Rigal, D.; Warzocha, K.; Coiffier, B. and Salles, G. (2004). Interleukin-10 gene promoter polymorphisms influence the clinical outcome of diffuse large B-cell lymphoma. Blood.103. 3529–3534.

23-Lu, C.; Soria, J. C.; Tang, X.; Xu, X. C.; Wang, L. and Mao, L. (2004) Prognostic factors in resected stage I non-small-cell lung cancer: a multivariate analysis of six molecular markers. J. Clin. Oncol. 22.4575–4583.

24- Asadullah, K.; Sterry, W. and Volk, H. D. (2003) Interleukin-10 therapy-review of a new approach. Pharmacol. Rev. 55. 241–269