Immunological Study on Breast Cancer in Hilla Province

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Abstract

Background: Cancer is one of the leading causes of death in the general population. Breast cancer is the top**.** In Iraq, where the population was exposed to high levels of depleted uranium following the first and second Gulf Wars, breast cancer became the most common tumor type in females.[1]Interleukin-10 (IL-10) and Interleukin 12 (IL-12) are the main immunological interplayers against cancer, and their impairment can be observed in many cancers, including breast cancer. Over the last ten years, there has been a three-fold increase in the incidence of breast cancer, with most of this increase being attributed to a particularly aggressive type of the cancer.

Objectives: The high incidence rate of breast cancer in the last few years in Iraq inspired us to create an investigation about the immunological bases of breast cancer in a series of local Iraqi women to see whether any differences in this immunological profile could be found in a population exposed to depleted uranium.

Methods: A Case-control study was designed and applied. A total of 40 subjects were chosen for the immunological study. Blood samples were obtained at Hilla General Teaching Hospital, Breast Cancer Centre (Pathology Department). The ELISA method was used for the interleukins and β2-microglobulin detection.

Results: In this study, the association between the levels of the IL-10 , IL-12 and β2microglobuline and breast cancer was evaluated. The data showed significant increase in these cytokines levels among breast cancer patients and healthy controls.

Conclusions: The conclusions that can be extrapolated by this study are that, there was a significant increase in the levels of the IL-10 , IL-12 and β2-microglobuline among breast cancer patients and healthy controls.

Keywords: Breast cancer, interleukins, Iraq.

الخلاصة

المقدمة: مرض السرطان هو من الأسباب الرئيسية للوفاة في الكثير من دول العالم و سرطان الثدي يأتي على راس القائمة في العراق, وبسبب التعرض لليورانيوم المخصب أثناء حرب الخليج, أصبح سرطان الثدي العامل الأول للوفاة عند النساء.[1] الانترلوكين العاشر والثاني عشر(IL-10 & IL-12) يلعبان دورا رئيسيا في الجهاز المناعي وقد لوحظ الكثير من اختلال التوازن لهذين العاملين المناعيين في الكثير من حالات السرطان بما في ذلك سرطان الثدي0في السنوات العشرة السابقة, كان هناك ثلاثة أضعاف زيادة في حالات سرطان الثدي وغالبية الحالات كانت ذات صفات غير اعتيادية0

الأهداف: هذه النتائج حثتنا لعمل دراسة حول الأساس المناعي لسرطان الثدي لمجموعة من النساء العراقيات المتعرضات لليورانيوم المخصب

طرق العمل: في هذه الدراسة تم استخدام نمط الحالة-والحالة المسيطرة, تم اخذ أربعين عينة دم من المشاركين في الدراسة في مركز سرطان الثدي لمستشفى الحلة التعليمي, قسم الأمراض. استخدمت طريقة ال "ELISA " في التحليل المناعي.

النتائج: في هذه الدراسة وجد بان هنالك علاقة مهمة بين سرطان الثدي و مستوى الانترلوكينات في الجسم.

Introduction

Breast cancer is the most prevalent malignancy in women and its incidence is increasing worldwide. In the western hemisphere the lifetime risk of developing breast cancer is more than 10%. In Iraq, where the population was exposed to high levels of depleted uranium following the first and second Gulf Wars, breast cancer become the most common tumor type in females. Al-Dujaily,*et.al.*2008 indicated that, over the last ten years, there has been a three-fold increase in the incidence of breast cancer, with most of this increase being attributed to a particularly aggressive type of the cancer.[1]

Cancer immunology is the study of interactions between the immune system and cancer cells (also called tumors or malignancies). It is also a growing field of research that aims to discover innovative cancer immuno-therapies to treat and retard progression of this disease.[2]

Cytokines play a role in human breast cancer carcinogenesis. In fact many cytokines have been found expressed by cancer cells or produced in the microenvironment of the primary or metastatic tumor. Among interleukins, IL-10, IL-12 mainly interfere with cell-mediated immunity response. In breast cancer, IL-10 has been associated with immune suppression at the tumor site. While the principal role of IL-12 in breast cancer is that, breast cancer induces a local IL-12-dependent type I immune response likely directed towards tumour-associated antigens. IL-10 is mainly produced by activated macrophages and inhibits them by negative feed-back. IL-10 is also secreted by many cancer cells and at a higher rate by metastatic cancer cells. In breast cancer, IL-10 has been associated with immune suppression at the tumour site and increased tumorigenicity. Increased concentrations of IL-10 are frequently detected in the serum of patients with cancer. IL-10 is produced by Th2 cells, some activated B lymphocytes or macrophages and by many cancer cells. IL-10 can be secreted at a higher rate by metastatic cancer cells than by lymphocytes. It down regulates the inflammatory response of cell-mediated immunity. IL-10 also inhibits antigen presentation, IL-12 production and induction of T helper type 1 responses in vivo(Skewing of the immune system towards a Th2 cytokine profile). [3]

IL-12 plays a key role in the transition between cell-mediated immunity and adaptive immunity. IL-12 stimulates NK cells and T cells to produce IFN-γ, which activates macrophages to kill phagocytosed foreign substances including microbes. It also increases cytolytic activity by stimulating CD8 cells. It is predicted that, breast cancer induces a local IL-12-dependent type I immune response likely directed towards tumour-associated antigens.[4]

Beta 2 microglobulin is a part of the HLA-A, -B, -C antigens expressed on nearly all cells that have a nucleus and to that extent is not a cause of worry; however, when the serum levels of this molecule is too high then it is most likely reflects a serious underlying condition. The tumor expression of beta-2-microglobulin could serve as a marker of tumor biologic behavior .[5]

Alwan, 2010 predicted that, in addition to being the most important cancer in Iraq, there are other features that justify increasing efforts for early diagnosis of breast cancer including the tendency for this disease to affect younger women, the obvious rise in incidence rates and the prevalence of advanced stages at presentation associated with more aggressive

tumour behavior resulting in greater fatality rates. [6]

During the past few years, the incidence of breast cancer in Iraq is dramatically increased. Moreover, little is known about the potential relationship between breast cancer and the immune components represented by IL-10, IL-12 and beta 2 microglobulin ; neither the effect on the risk nor the possible involvement of these parameters in the pathogenesis of breast cancer in the population have been studied. Therefore, our work adopts this task and regards it as its priority. In this study we aim to report our observations about whether there is any impairment in the regulation of the immune system represented by IL-10, IL-12 and β2-microglobulin were associated with breast cancer among the study population.

Patients and methods

Study population: A Case-control study design was applied. A total of 40 subjects were chosen for the immunological study. The primary sets of subjects were drowning from those attending the breast cancer center in Hilla general teaching hospital during February 2010 – January 2011. All patients and controls that were involved in this study, lived in Hilla city and its peripheries where depleted uranium containing weapons were drawn.[1]

Four groups were recruited; 20 breast cancer patients group, 5 benign breast tumor patients, 10 healthy controls & 5 first degree relatives to a known case of breast cancer patients. Both of the breast cancer & the benign breast tumor patients were clinically, radiologically & histopathologically confirmed as having breast cancer & benign breast tumor, respectively, while healthy control & first degree relatives to the breast cancer patients were clinically & radiologically confirmed as having no breast problems. All cases & controls were of Iraqi origin resident in Al-Hilla city, Iraq.

Specimens Collection: Blood samples from forty subjects were obtained at Hilla General Teaching Hospital, Breast Cancer Centre, and Pathology Department. Forty blood samples were collected in duplicate sample for each patient. For breast cancer patients, only those who didn't take chemotherapy were included in the study. The samples were transferred to the laboratory as soon as possible, for sera separation.

Detection of Interleukins: The ELISA (Enzyme-Linked-Immuno-Sorbent Assays) me'thod was used for the interleukins detection according to the manufacturer's instructions supplied by BioSource Company.

Detection Of β2-Microglobulin: According to the manufacture company (DRG). The β2-microglo-bulin EIL test is an enzyme immunoassay for the measurement of β2-microglobulin in serum. The enzyme immunoassay allows the quantitative determination of β2-microglobulin from serum.

Results

In this case-control study we evaluated the association between the levels of the IL-10, IL-12 and β2microglobuline and breast cancer in the study population. The data showed significant increase in these cytokines levels between breast cancer patients and healthy controls.

IL-10 was demonstrated to have higher levels in patients with breast cancer than in other groups in the study. In the reverse, IL-12 in sera of patients with breast cancer was lower than others. Results about the levels of

interleukins IL-10 and IL-12 are presented in figures (1) and (2).

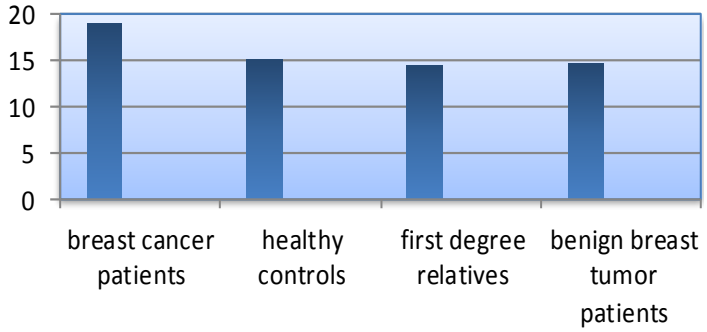

Figure 1. IL-10 Levels

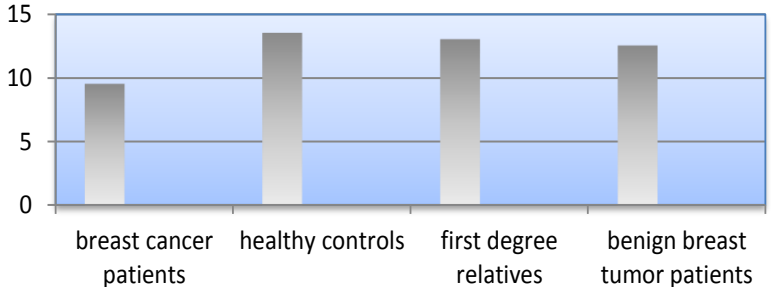

Figure 2. IL-12 Levels

Human β2-microglobulin is part of the HLA-A, -B, -C antigens expressed on the surface of most nucleated cells; a component of the complex which binds antigens for presentation. In the present work we noticed that, β2microglobuline high levels were associated with breast cancer, and to lower extent, with benign breast tumor patients but not with healthy controls or first degree relatives. Results about β2microglo-buline are showed in figure (3).

Discussion

Breast cancer is the most common malignancy threatening the health and life of women and it's incidence has increased in recent years in both developed and developing countries. Biologic mechanisms leading to the development of breast cancer are not clearly understood, but the role of cytokines in cancer immunity and carcinogenesis has been well established. In the present study, 40 sera were subjected to testing for IL-10, IL-12, and β2-microglobulin levels.

Kong, *et. al.* 2010 demonstrated that, IL-10 is an important immunoregulatory cytokine. As an immune response modulator, IL-10 can both stimulate and suppress the immune response. Numerous studies have shown that IL-10 may be involved in the pathogenesis of cancer, but the results were inconsistent.

The relative increment of IL-10 levels among breast cancer patients detected in the present study came in accordance with Kong,*et.al.*2010, who predicted that, increased serum IL-10 levels could facilitate development of cancer by suppressing expression of MHC class I and II antigens and preventing tumor antigen presentation to CD8-cytotoxic T lymphocytes, we are in agreement with this fact, which may give a reasonable explanation to these findings about the elevated level of IL-10 in sera of breast cancer patients, in comparison with healthy

control, first degree relatives, and patient with benign tumors.[7]

Although the relationship between interleukin-10 (IL-10) and cancer has been studied extensively, the ultimate role of IL-10 in tumor biology remains enigmatic. Mocellin, *et. al.* 2005 demonstrated that, the significance of IL-10 production within the tumor microenvironment, which can be sustained by malignant cells and tumor-infiltrating macrophages and lymphocytes [including natural killer (NK) and T cells], is debated.

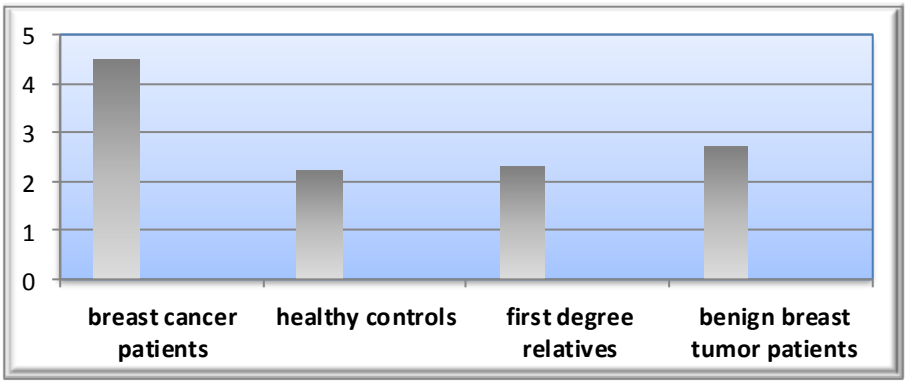

Figure 3. β2-microglobulin levels

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: As a result of its ability to inhibit several key phenomena underlying an adaptive immune response, several authors sustain the teleological hypothesis that IL-10 is an immunosuppressive molecule secreted by tumors (or tumor-infiltrating immune cells) to allow malignant cells to escape from immune surveillance. This, together with the IL-10 property of stimulating B cell function, led to the classification of IL-10 among Th2 cytokines with the physiological role to terminate T cell-mediated immunity and start a humoral immune response .[8]

Several factors influence tumor fate, among them the immune responses against tumor antigens. The results regarding the association between an elevated IL-10 level and breast cancer declared in this work were also confirmed by Bolpetti, *et. al.* 2010 who postulated that, tumor cells display several mechanisms of immune evasion. Among them, the expression of suppressor cytokines like IL-10 has been described in many malignancies. Tumor cells do not express or respond to IL-10, but recruit leukocytes which, within the tumor environment, produce this cytokine. Macrophages associated to tumors environment play a role in tumor growth. As it was demonstrated, these macrophages express IL-10, so a hypothesis is raised that IL-10 may be involved in the mechanism by which this macrophage population facilitates tumor growth.[9]

In this study, the selection of IL-10 and IL-12 was based upon the fact that, these two interleukins are good reflectors of the immune status, by other words whether the shift is toward Th1 or Th2 cytokines. This fact was used by Kallio,*et.al.*2001 so that, the utility of the balance between interleukin-10 and interleukin -12 can be used as reliable markers for identifying infections in cancer patients on admission. Thus, IL-10 can be used as a screening method for identifying infections in cancer patients and the

ratio of IL-10 to IL-12 for confirming the diagnosis.[10]

Likewise, The relatively high levels of β2-microglogulin in association with breast cancer, and to lesser extent with benign breast tumor patients of this study was explained by Kimber, *et.al.* 2010 as that, β2-microglogulin is expressed on blood cells and subsequently released into the serum. Beta2-microglobuin is used as an adjunct test for active disease, cell turnover, and tumor presence.[11] These results of β2-microglogulin were also consistent with Giglio, *et.al.* 1989, who found a significantly positive association between tumor beta 2-M expression and the degree of lymphocytic infiltration in the tumor tissue. Beta 2-M serum levels were determined by an enzyme-linked immunosorbent assay in samples from women with breast cancer. No significant correlations were found between tumor beta 2-M expression and several histologic attributes such as type, histologic and nuclear grades, vascular invasion, and lymphocytic infiltration. Beta 2-M was not associated with markers of disease extension such as TNM, staging and axillary lymph node involvement or with estrogen, progesterone, and glucocorticoid receptor levels.[5]

Conclusions and Recommendations

The conclusions that can be extrapolated by this study are that there was a significant difference in the level of the IL-10, IL-12 and β2-microglobuline between breast cancer patients and healthy controls. Furthermore, The result obtained from this work about the expanding burden of breast cancer in the middle east region in general, and Iraq in particular, justifies the demand for establishing comprehensive national cancer control programs for early detection of breast cancer, on an immunological base, as a major approach to control the disease.

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