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Association of Serum Leptin and C-Reactive Protein in Women with Breast Cancer

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Abstract:

This study was conducted to evaluate the association of serum leptin and hs C-reactive protein with breast cancer. Two groups were included in the study. The first group included 45 newly diagnosed women with breast cancer. The second group included 42 women with benign breast lump as a control group. Blood samples (5 mL) were taken from the patient and the control groups and analyzed for serum leptin and hs C-reactive protein. Serum CA15-3 was also measured in breast cancer patients. The epidermal growth factor receptor 2 (HER2/neu), estrogen and progesterone receptors were determined in breast cancer patients by using immune-chemical method. Serum leptin was significantly higher ($p \leq 0.05$) in breast cancer patients than that in the control group; however, no significant difference was noticed between the two groups for serum hs C-reactive protein. No significant difference was noticed between HER2/neu positive or negative in breast cancer patients for serum leptin or hs C-reactive protein. However, serum CA15-3 in HER2/neu positive patients was significantly higher ($p \leq 0.05$) than that in HER2/neu negative patients. No significant difference was noticed between positive and negative estrogen breast cancer patients for serum leptin, hs C-reactive protein or CA15-3. In addition, no significant difference was noticed between positive and negative progesterone for serum leptin, hs C-reactive protein or CA15-3. A strong significant positive correlation was noticed between serum leptin and BMI in the control group; however, no significant correlation was noticed between serum leptin and BMI in the breast cancer patients. In conclusion, serum leptin may be used as a prognostic factor for breast cancer. Serum C-reactive protein in HER2/neu positive breast cancer patients is higher than in HER2/neu negative patients.

Key words: Leptin, hs C-reactive protein, HER2/neu, CA15-3 breast cancer.

علاقة مصل دم اللبتين وبروتين التفاعل نوع C في النساء المصابات بسرطان الثدي

الخلاصة:

أجريت هذه الدراسة لتقييم علاقة مصل دم اللبتين والبروتين التفاعل عالي الحسلسية نوع Cبسرطان الثدي. شملت الدراسة على مجموعتين، احتوت المجموعة الاولى على 45 امرأة مصابة ومشخصة حديثا بسرطان الثدي. وشملت المجموعة الثانية على 42 امرأة مشخصة بكتلة حميدة في الثدي كمجموعة سيطرة. اخذت عينات الدم (5 مل) من المريضات ومن مجموعة السيطرة وتم قياس مصل دم اللبتين وبروتين التفاعل عالي الحساسية نوع C. وكذلك تم قياس مصل دم CA15-3 في المريضات المصابات بسرطان الثدي. وتم تحديد المستقبل الثاني لعامل نمو البشرة البشري (HER2/neu) ومستقبلات الاستروجين والبروجستيرون في المريضات المصابات بسرطان الثدي باستعمال طرق كميائية مناعية. وكان مصل دم اللبتين في المريضات المصابات بسرطان الثدي اعلى معنويا ($p \leq 0.05$) منه في مجموعة السيطرة. من ناحية اخرى لم يكن هناك فرق معنوي بين المجموعتين لمصل دم بروتين التفاعل عالي الحساسية نوعC . ولم يكن هناك فرق هناك فرق معنوي بين مصل دم اللبتين وبروتين التفاعل عالي الحساسية بين مريضات HER2/neu الموجب والسالب. من ناحية اخرى كان مصل دم CA15-3 في مريضات HER2/neu الموجب اعلى منه معنويا في المرضى السالب ($p \leq 0.05$). ولم يكن هناك فرق معنوي لمصل دم كل من اللبتين وبروتين التفاعل عالي الحساسية و CA15-3 بين مريضات الاستروجين الموجب والسالب. كذلك لم يكن هناك فرق معنوي لمصل دم كل من اللبتين وبروتين التفاعل عالي الحساسية وCA15-3 بين مريضات البروجستيرون الموجب والسالب. وكان هناك علاقة طردية قوية بين مصل دم اللبتين ومؤشركتلة الجسم في مجموعة السيطرة. من ناحية اخرى لم يكن هناك علاقة معنوية بين

مصل دم اللبتين ومؤشر كتلة الجسم في المريضات المصابات بسرطان الثدي. وكان الاستنتاج ان مصل دم اللبتين من الممكن ان يستعمل كعامل تشخيصي لمرض سرطان الثدي. ان مصل دم بروتين التفاعل عالي الحساسية في مرضى سرطان الثدي نوع HER2/neu الموجب اعلى منه في المرضى السالب.
الكلمات الرئيسية: اللبتين، بروتين التفاعل عالي الحساسية، HER2/neu ، سرطان الثدي.

Introduction:

Breast cancer is the most common type of cancer and the most common cause of cancer death among women [1]. Female breast cancer also accounts for about 26% of new cases of cancer and 15% of cancer death [2]. Breast cancer has become a major threat to female health in Iraq, where it is the leading cause of death after cardiovascular diseases among women, with a cancer-related mortality rate of 23% [3]. Around the world, there are large variations of breast cancer in incidence, mortality, and survival among different countries. Several factors including age, ethnicity, diet, and life-styles underlie these variations [1].

Leptin is required for normal mammary gland development and lactation. However, it might also contribute to mammary tumorigenesis [4]. There are several reports of the presence of leptin receptor (Ob-R) in breast tumor [5]. High serum levels of leptin were a risk factor for breast cancer [6]. In addition, both leptin and its receptor were overexpressed in breast cancer, especially in high grade tumor but were absent in normal breast epithelial tissues [7].

C-reactive protein (CRP) acts as a classical acute-phase protein displaying rapid its concentration in response to acute inflammation, infection, and tissue damage [8]. C-reactive protein was elevated in cancer including breast cancer [9]. C-reactive protein is positively correlated with leptin level [10]. Independent effect of C-reactive protein and alterations in the level of leptin was accompanied by an increase in breast cancer risk incidence [11].

HER2 is so named because it has a similar structure to human epidermal growth factor receptor, or HER1. Neu is so named because it was derived from a rodent glioblastoma cell line, a type of neural tumor. It is associated with a more aggressive disease, higher recurrence rate, and increased mortality [12]. CA 15-3, for carcinoma antigen 15-3, is a tumor marker for many types of cancer, most notably breast cancer [13].

A significant correlation between serum leptin and estrogen and progesterone status was noted [14]. In addition, leptin can transactivate the epidermal growth factor receptor 2 (HER2/neu) through both epidermal growth factor receptor and Janus-activated kinase 2 activation [15].

The aim of this study was to evaluate the association of serum leptin and hs C-reactive protein with breast cancer. The connection of serum leptin with HER2/neu or hormonal status in breast cancer patients was also studied.

Patients and methods

This study was conducted at Ninevah Medical Center and Al-Jamhorry Teaching Hospital, Mosul, Iraq, during the period from September 2013 to February 2014. Two groups were included in the study. The first group included 45 newly diagnosed women with breast cancer with age range 24-70 years (mean ± SD: 33.6 ± 11 years). The second group included 42 women with benign breast lump (control group) with age range 17-69 years (mean ± SD: 47.7 ± 13 years).

Blood samples (5 mL) were taken from the patient and control groups and analyzed for serum leptin (DRG Liptin ELISA Kit, USA) [16], and hs C-reactive protein measured by enzyme linked immunosorbent assay (ELISA) technique, using (ACCU-BIND hs CRP ELISA kit, USA) [17]. Serum CA15-3 was also measured in breast cancer patients by immunochemical method by using monoclonal antibodies against CA15-3 (Minividas, USA, Kids by Biomerieux company, France). HER2/neu, estrogen

and progesterone receptors were determined in breast cancer patient by immunochemical method (Dako company, Denmark).

Patients with any disease other than breast cancer, or taking any medication were excluded from the study. Data are presented by mean ± SD and were analyzed by using unpaired t-test. Chi square was used for categorical data. Correlation coefficient was used to determine the relationship between serum leptin and age or BMI. *P* values less than 0.05 were considered significant. Statistical analysis was performed using SPSS package version 17.

Results

Table 1 shows no significant difference in age; however, body mass index (BMI) in the breast cancer group was significantly ($p \leq 0.05$) higher than the control group. Married females were more dominant in both groups. Most of the patients in both groups were presented with breast lump and only 4.8% of benign breast lump patients had lymph involvement, while more than 73.3% of the breast cancer patients ($p \leq 0.05$) had lymph involvement**.** No significant difference was noticed between right and left tumor side in the patient and control groups.

Table 1. Characteristics of patients and subjects

Variables	Benign breast Patients (controls) (n=42)	Breast cancer patients (n=45)
Age (years)	33.73 ± 10.82	48.26 ± 13.06
BMI	27.77 ± 4.701	31.49 ± 4.928*
Marital status		
Single	9 (21.4%)	4 (8.9%)
Married	33 (78.6%)	41 (91.1%)
Presentation at examination		
Lump	35 (83.3%)†	43 (95.6%)†
Nipple discharge	6 (14.3%)	2 (4.4%)
Mastalgia	1 (2.4%)	0 (0%)
Tumor site		
Right	25 (59.5%)	24 (53.3%)
Left	17 (40.5%)	21 (46.7%)
Lymph node involvement		
Yes	2 (4.8%)†	33 (73.3%)†
No	40 (95.2%)	12 26.7%)

BMI: body mass index, SD: standard deviation

* $p \leq 0.05$ between groups, † $p \leq 0.001$ within the group

Table 2 shows that serum leptin and hs C-reactive protein were significantly higher ($p \leq 0.05$) in breast cancer patients than the control group.

Table-2: Serum leptin and hs C-reactive protein in breast cancer patients and controls

Variables	Controls (n=42)	Patients (n=45)
Serum liptin	17.59 ± 12.13	37.69 ± 20.1*
Serum C-reactive protein	8.34 ± 6.80	83.47 ± 48.56*

*p≤ 0.05

Table-3: Serum leptin, hs C-reactive protein, and CA15-3 in breast cancer patients

Parameters	HER2/neu positive (n=25)	HER2/neu negative (n=20)
Serum leptin	39.55 ± 19.30	32.76 ± 17.85
Serum hs C-reactive protein	77.26 ± 40.58)	88.96 ± 57.67*
CA15-3	46.8 ± 15.47	40.2 ± 9.07*

* $p \leq 0.05$

Table-3 shows no significant difference between HER2/neu positive or negative patients for serum leptin. However, serum hs C-reactive protein and CA 15-3 in HER 2/neu positive patients were significantly higher ($p \leq 0.05$) than in HER 2/neu negative patients.

No significant difference was noticed between estrogen positive or negative patients for serum leptin, hs C-reactive protein or CA 15-3. In addition, no significant difference was noticed between progesterone positive or negative patients for serum leptin, C-reactive protein or CA 15-3. A significant positive correlation was noticed between serum leptin and BMI in the control; however, no significant correlation was noticed between serum leptin and BMI in the breast cancer patients. (Data not shown).

Discussion

The association of serum leptin and breast cancer was documented by many studies [4,6]. Serum leptin was involved with different aspect of tumor pathology of the breast, such as cell growth, angiogenesis, and metastasis [18]. The significant correlation between serum leptin, and estrogen and progesterone status was noticed [14]. Leptin also can transactivate HER 2/neu through both epidermal growth factor receptor and Janus-activated Kinase 2 activation [15].

Serum leptin, in the present breast cancer patients, was significantly higher than the control group. This study was consistent with other workers [19, 20]. However, few studies did not find significant changes in serum leptin in breast cancer patients compared with healthy controls [14]. High level of serum leptin can promote the proliferation and progression of various types of cancer including breast cancer [21].

In this study, hs C-reactive protein was significantly higher in breast cancer than controls. Other studies demonstrated a higher cancer risk in people with elevated C-reactive protein [22, 23]. The association between elevated C-reactive protein levels and poor breast cancer prognosis are tumor behavior [11] and inflammatory pathway [24].

The paired organ in human body may demonstrate asymmetry in cancer incidence and progression [25]. In this study, the incidence of right sided breast cancer was not significantly different from the left sided. The present results are inconsistent with the established and well recognizable data published from western world [26, 27]. In Pakistani women left sided breast cancer was higher than the right side [28]. The reason of cancer laterality may suggest underlying genetic factors [29]. Few studies have revealed a possible correlation with relatively larger left breast size, unilateral lactation [30], detection biased due to predominant right handedness [31] or

denser left breast [32], or birth place but not race [33].

This work revealed that 73.3% of breast cancer patients had lymph node involvement which could be related to low education among Iraqi patients, in addition to the embarrassment and shyness of the patients. The early detection and treatment of the disease in western countries could be due to educational programs and developed medical facilities.

The studied patients with positive HER_2/neu showed significant elevated serum CA15-3, compared with negative HER_2/neu patients. The elevated serum CA15-3 can be a prognostic indicator to advanced stage and recurrence of the cancer [34].

No significant difference was noticed between HER_2/neu positive and negative of the present patients for serum leptin. These results are in agreement with other associates [14]. No significant difference was also noticed, in this study, between estrogen/progesterone positive receptors and negative for serum leptin. Aliustaoglu et al. [14] showed a significant difference for serum leptin and expression of estrogen and progesterone. However, these authors did not find any difference of serum leptin in HER_2/neu positive and negative patients.

Leptin may transactivate HER2/neu through epidermal growth factor receptor which can cause the growth of breast cancer cells with HER_2 overexpression [15]. Leptin may manipulate breast cancer development in relation to estrogen receptor status and aromatase activity, suggesting functional crosstalk between leptin and estrogen signaling [19]. In addition, leptin has been shown to up-regulate the expression of vascular endothelial growth factor (VEGF) and VEGF receptor type 2 (VEGFR2) and it is known that VEGF plays an important role in tumor angiogenesis, thereby promoting tumor growth and metastases [18].

This study showed insignificant correlation between leptin and BMI in cancer patient. Ozet et al. [35] described higher serum leptin levels in patients with breast cancer but serum leptin did not correlate with BMI. However, higher serum leptin levels in patients with breast cancer was correlated with BMI [36,37]. The weak association between leptin and BMI in breast cancer patients may be due to the stress of surgery on appetite and changes in lifestyle after diagnosis. Appetite changes from psychological effects on discovery of the disease may also contribute.

In conclusion, serum leptin may be used as a prognostic factor for breast cancer. Serum C-reactive protein was significantly higher in HER2/neu positive than HER/neu negative breast cancer patients.

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