Journal of BUON **15:** 369-372, 2010

ORIGINAL ARTICLE

Tissue leptin levels in patients with breast cancer

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Summary

Purpose: *High leptin serum levels, overexpression of leptin and its two main receptor isoforms, OBR-L and OBR-S, have been documented in breast cancer patients. In the present study, the relationship between tissue leptin levels and breast cancer was evaluated.*

Methods: *Thirty-three normal breast tissue samples and 33 breast cancer tissue samples from 33 patients with breast cancer were evaluated. The association of tissue leptin levels and important prognostic factors related to breast cancer was analyzed.*

Results: *Mean tissue leptin levels in breast cancer tissue samples (5.02±1.06 pg/ml) were significantly higher than those found in normal breast tissue (2.02±0.83 pg/ml; p=0.01). No correlation was found in tissue leptin levels and menopausal status, hormone receptor and HER-2/neu status, lymph node involvement, and histopathologic features.*

Conclusion: *High leptin levels were significantly higher in breast cancer tissue compared with normal tissue. No special correlation was found between tissue leptin levels and different clinicopathological characteristics.*

Key words: breast cancer, cancer tissue, leptin, normal breast tissue, prognostic factor

Introduction

Leptin is a peptide and an adipocyte-derived hormone; it is composed of 167 amino acids with a molecular mass of 16 kDa and plays a role in the control of the body weight [1-3]. Plasma leptin levels positively correlate with the body mass index (BMI). The main role of leptin is to control satiety and energy expenditure, acting as a neurohormone-regulating energy balance and food intake in the hypothalamus [4-6]. In addition, new biological functions of leptin, such as antiapoptotic and angiogenic activity, have also been recently reported [7,8].

Obesity and obesity-related status such as type 2 diabetes mellitus and insulin resistance (IR) are associated with high levels of leptin. The relationship among obesity, IR and breast cancer has been well documented. Obesity increases postmenopausal breast cancer risk by 30-50% [9-11]. There are regulatory dysfunctions in metabolic, neuroendocrine and other systems in cancer patients. On the other hand, few studies report on the role of leptin in these kinds of dysfunctions in cancer patients [12]. Stattin et al. indicated that leptin may promote tumor growth by stimulating angiogenesis in prostate cancer [13].

In breast tissue, leptin is required for normal mammary gland development and lactation, but it might also promote breast tumorigenesis [14,15]. The association of leptin and breast cancer has been previously reported. Tessitore and Vizio showed that the plasma leptin levels and mRNA expression in adipose tissue of breast cancer patients were significantly higher than those in a healthy control group and it was thought that plasma leptin levels could be used as a prognostic marker in breast cancer [16-18]. Furthermore, other studies indicated that leptin and leptin receptor that were overexpressed in breast cancer patients predicted poor prognosis [19-22].

In this study, we analyzed the relationship of leptin levels between normal breast tissue and breast cancer tissue. The association of tissue leptin with the clinicopathological factors of breast cancer was also evaluated.

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Received 01-08-2009; Accepted 03-10-2009

Methods

Thirty-three consecutive patients with breast cancer who had no metastatic disease were studied. Thirty-three breast cancer tissue samples and 33 normal breast tissue samples of the same patients (control group) were analyzed. Menopausal status, type of operation, tumor size, tumor stage, hormone receptors status, HER-2/neu status, lymph node involvement and hormonal therapy were recorded. Breast cancer was staged according to the American Joint Committee for Cancer staging system [23]. We calculated BMI according to Quetelet's formula as the ratio of body weight to body height squared (kg/m^2). Fasting blood glucose was obtained early in the morning and the serum was immediately separated by centrifugation and stored at -20º C until further analysis. The status of estrogen receptors (ER) and progesterone receptors (PR) was determined by immunohistochemical staining and HER-2/neu receptor staining was analyzed by the Standard Hercept Test procedure (Dako 5204) [24].

All biopsy specimens were immediately frozen and stored at – 80º C until tissue homogenization. The frozen specimens were homogenized in ice-cold homogenization buffer (50 mM HEPES, 0.2% Triton X-100, 1 mM ethylenediaminetetraacetic acid [EDTA] and 0.1 mM phenylmethylsulfonyl fluoride [PMSF], pH 7.4) at 13500 rpm in Ultraturrax T25 (Janke & Kunkel, IKA® Labortechnic, Staufen, Germany) using a method described previously [25]. The homogenates were centrifuged at 2900 rpm for 15 min and the supernatants were stored at -80º C until analysis. Samples were coded to ensure anonymity and all analyses were performed in a blinded fashion. Before performing the assays, samples were brought to room temperature (18-25º C) and mixed gently. Tissue content of leptin in biopsy specimens were measured by enzyme-linked immunosorbent assay (ELISA) method using the ELX 800 (BIO-TEK Instruments Inc., Winooski, VT, USA) with commercially available kit, and normalized to the total protein content of the tissue homogenates. Tissue concentrations of leptin were measured with a Human Leptin ELISA Kit from Ray Biotech, Inc (Norcross, GA, USA). Written informed consent was obtained from each subject included in the study.

Statistical methods

Statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA) software. Descriptives of the parameters were quoted as mean ± SD and 95% confidence intervals (95% CI). Normality of the data was tested using Kolmogorov-Smirnov test. Parameters normally distributed were compared with Student's t-test. Mann Whitney U test was applied for non-normally distributed data. P-values less than or equal to 0.05 were considered statistically significant.

Results

Clinical and laboratory characteristics of breast cancer patients are shown in Table 1. Significant correlation (p=0.01) between serum fasting glucose and tissue leptin levels was detected in breast cancer patients (Table 2).

The summary of tumor size, tumor stage, type of surgery, hormonal therapy, lymph node involvement as well as ER, PR, and HER-2/neu receptor status is listed in Table 3. The status of ER, PR, HER-2/neu receptors and lymph node involvement did not affect the breast cancer tissue leptin levels. Also, there was no relationship between tumor stage, tumor size, CEA, CA 15-3 and the levels of tissue leptin (p >0.05; Table 2).

Leptin levels in tumor tissue were significantly higher compared with the controls (5.02±1.06 vs. 2.03±0.83; p=0.01; Figure 1). No correlation between BMI and breast cancer tissue leptin levels was found (p >0.05).

In the subset analysis according to menopausal status, the tissue leptin levels were almost similar in postmenopausal (2.13±1.14 pg/ml) and premenopausal (2.89±1.19 pg/ml) women (p=0.84).

Discussion

The association of obesity with the risk of breast cancer development has been previously reported in epidemiological studies which suggested that obesity increased the postmenopausal breast cancer risk by 30-50% [9-11,26,27]. Recent reports have indicated that

Table 1. Characteristics of breast cancer patients

Characteristics	*Patients n=33*
Age, years, mean ±SD (range)	50.2±13.7 (31-78)
Menopausal status, n (%)	
Premenopausal	13 (39.3)
Postmenopausal	20 (60.7)
Height (cm ±SD)	167.9±4.7
Weight (kg ±SD)	71.9±8.1
BMI (kg/m^2 ±SD)	25.6±2.8
Fasting blood glucose (mg/dl ±SD)	128.1±7.2
CEA (ng/ml ±SD)	0.66±0.2
CA 15-3 (U/ml ±SD)	42.3±13

BMI: body mass index, SD: standard deviation

Table 2. Correlation between tissue leptin levels and clinicopathological variables in patients with breast cancer

Variables	*Leptin (pg/mL) p-value*
Fasting blood glucose (mg/dL)	0.01
Estrogen receptor	0.23
Progesterone receptor	0.12
HER-2/neu expression	0.34
Tumor size	0.16
Tumor stage	0.67
Lymph node involvement	0.09
CEA (ng/ml)	0.45
CA 15-3 (U/ml)	0.44

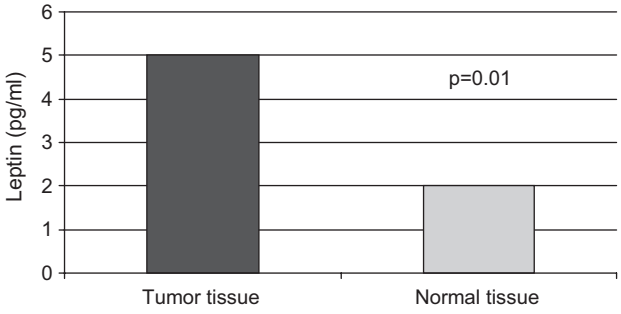

Figure 1. Mean tissue leptin levels in patient and controls.

leptin expression is elevated in overweight and obese subjects. Leptin is an adipocyte-derived hormone, involved in the regulation of body weight and sexual maturation. It might be involved in the development and/or progression of different malignancies such as breast, colorectal and prostate cancer [13,16,18]. Leptin can stimulate cell growth, counteract apoptosis, and induce migration and expression of matrix degrading enzymes and angiogenic factors in different cancers [1-3,7,8,15]. The circulating leptin is an essential factor regulating fat metabolism and it might be involved in the development of breast cancer. Moreover, serum leptin levels have been shown to be significantly increased in breast cancer patients compared to controls [18,28].

Table 3. Clinicopathological characteristics of breast cancer patients

Characteristics	*Breast cancer tissue n (%)*
Type of surgery	All cases had MRM
Tumor size (cm)	
≤ 2	16 (48.1)
2-5	14 (44.4)
>5	3 (7.5)
Lymph node involvement	21 (63.6)
Stage	
I	3 (9.1)
II	20 (60.6)
III	10 (30.3)
Estrogen receptor	
Positive	14 (42.4)
Negative	19 (57.6)
Progesterone receptor	
Positive	13 (39.3)
Negative	20 (60.7)
HER2/neu	
Positive	15 (45.4)
Negative	18 (54.6)
Hormonal therapy	26 (78.7)
Tamoxifen	11 (42.3)
Aromatase inhibitor	15 (57.7)

MRM: modified radical mastectomy

Leptin expression and its two main receptor isoforms, OBR-L and OBR-S, have been previously investigated in breast cancer patients. Relevant studies suggested that leptin and OBR are overexpressed in breast cancer and OBR-S is an independent prognostic factor [19-22]. In our study, we evaluated leptin levels in breast cancer and normal breast tissue samples. We found that tissue leptin levels in breast cancer patients were significantly higher compared to controls. For that reason, tissue leptin levels might be considered as a possible prognostic factor for breast cancer patients. No significant differences in tissue leptin levels between pre and postmenopausal women were detected in our study. Yet, previous reports found that the serum leptin levels were higher in postmenopausal breast cancer patients than in controls [18,28]. This difference could be related to the small sample size of our study.

High glucose levels stimulate the proliferation of cultured breast cells [29], and the plasma leptin concentration is positively correlated with fasting glucose and plays an important role in glucose metabolism, while elevated leptin levels have been associated with an increased risk for type II diabetes mellitus [30,31]. We also detected increased tissue leptin levels along with increased fasting serum glucose in breast cancer patients.

Those increased tissue leptin levels with increased fasting glucose were compatible with the relevant literature and might be related to fasting insulin levels.

In our study ER, PR, HER-2/neu receptors and lymph node involvement status did not correlate with the levels of tissue leptin. Moreover, no relationship could be detected among tissue leptin levels and tumor size and stage. Miyoshi et al. suggested that leptin mRNA levels were significantly higher in ER-positive than in ER-negative breast tumors, but without correlation among tumor size, menopausal status, histologic grade and lymph node involvement [21]. On the other hand, Ishikawa et al. showed that the expression levels of both leptin and OBR tended to increase with increas-

ing tumor size, although this relation was not significant [19].

In conclusion, we found that tissue leptin levels were significantly higher in breast cancer tissue compared with normal breast tissue. Leptin may have a promoting influence on carcinogenesis in general and breast cancer in particular. Our results need to be confirmed by a prospective study including analysis of leptin and its receptors in blood and tissue samples of breast cancer.

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