ORIGINAL ARTICLE

Association of serum soluble leptin receptor and leptin levels with breast cancer

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Background: Leptin plays a key role in the regulation of energy expenditure and is known to circulate in both free and bound forms. Soluble leptin receptor (sOB-R) is a unique circulating form of leptin receptor that can bind to leptin. Leptin and leptin receptor have been implicated in processes leading to breast cancer initiation and progression. Our study was aimed to investigate the relationship between serum levels of sOB-R and leptin with breast cancer. **Materials and Methods:** Serum leptin and sOB-R levels were measured by enzyme-linked immunosorbent assay in 100 women with breast cancer cases compared with 100 age and body mass index (BMI)-matched controls without cancer. Lipid profiles were measured by enzymatic method. **Results:** The median serum levels of sOB-R in controls were significantly higher than that in breast cancer cases (odds ratio [OR], 1.98; 95% confidence interval [CI] = 0.77-188.2) versus (OR, 0.140; 95% CI = 0.09-98.1). Conversely, the median serum level of leptin in breast cancer cases was significantly higher than that in controls (OR, 67.90; 95% CI = 2.77-129.9) vs. (OR, 28.30; 95% CI = 0.60-113.1). Breast cancer was significantly associated with higher serum level of leptin (OR = 1.027, 95% CI = 1.017-1.038). Conversely, breast cancer was correlated with lower serum level of sOB-R (OR = 0.983, 95% CI = 0.969-0.997). Moreover, free leptin index (FLI) (leptin/sOB-R ratio) was associated with breast cancer (OR = 1.028, 95% CI = 1.015-1.042). The serum sOB-R level was negatively associated with leptin, BMI, and high density lipoprotein (r = −0.238, −0.186, and −0.168, respectively). **Conclusion:** Our results suggested that FLI and serum leptin level rather than serum level of sOB-R was associated with the breast cancer.

Key words: Breast cancer, free leptin index, leptin, soluble leptin receptor

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INTRODUCTION

Leptin (LEP), the obese (ob) gene product[1] is thought to play a key role in the regulation of energy expenditure and body fat homeostasis.[2] leptin exerts its pleiotropic actions directly through distinct receptors (ob-R) encoded by the diabetes (db) gene.[3] In humans, the circulating leptin level is increased in obesity, and is positively correlated with the total body fat mass, suggesting that a hallmark of obesity is not leptin deficiency, but leptin resistance.[4] It has been reported that leptin stimulate the proliferation of various cell types and is considered to be a new growth factor. Moreover, hyperleptinemia is a common feature of obese women who have a risk of breast cancer higher than those with normal weight.[5]

Leptin receptor was identified as a member of the cytokine family of receptors. The leptin receptor gene was found to encode at least five alternatively spliced forms, ob-Ra, ob-Rb, ob-Rc, ob-Rd, and ob-Re.[6] Besides membrane-bound isoforms of the leptin receptor with varying cytoplasmic length, a soluble form of the leptin receptor (sOB-R) has been demonstrated.[7] sOB-R consists entirely of the extracellular ligand-binding domain and lacks the transmembrane residues and intracellular domain responsible for signal transduction. In their study Sinha *et al*.[8] they found the existence of circulating leptin-binding proteins and reported that the greater part of leptin circulated in the bound form in lean subjects, whereas in obese subjects, the greater part of leptin circulated as the free form. Landt *et al*.[9] have reported that only free leptin was detectable in cerebrospinal fluid (CSF), suggesting that it was the biologically active form. Lammert *et al*.[10] observed that leptin-binding activity was correlated with levels of the sOB-Rand that sOB-R was the major leptin-binding protein in the circulating human blood.

Leptin receptor has been found in the most tissue; particularly in the central nervous system, pancreas, kidney, liver, skeletal muscle, adrenal, and hematopoietic structure.[11,12] It has been reported that serum sOB-R level is low in obese individuals.[13,14] Conflicting to the

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serum levels of leptin, the sOB-R level increased after weight loss by a low-calorie diet[11] or stomach surgery.[15,16]

In breast cancer tissue, it was shown that leptin and leptin receptor are both expressed and that they act to favor cancer proliferation and metastasis.[17,18] Controversial results have been reported regarding the detection of leptin levels in breast cancer patients.[19,20] However, the most reports indicate that higher leptin serum levels are associated with advanced stage breast cancer.[21,22] There are, however, few studies in Iranian healthy controls or breast cancer cases concerning to the leptin receptor. Therefore, the aim of this study was to investigate changes in sBO-R in breast cancer cases compared with controls, and to evaluate the relationship between sOB-R level, leptin, lipid profile, and breast cancer.

MATERIALS AND METHODS

Subjects

This study consists of two groups. One group was composed of 100 unrelated women with confirmed breast cancer. The diagnosis of cancer was confirmed by histopathology analyses. Clinical information such as stage of the breast cancer, menopausal status at the time of onset, hormonal receptor status (estrogen receptor [ER], progesterone receptor), tumor size, and body mass index (BMI) was obtained from the hospital records. Tumor size was measured by the bidimensional product of the horizontal and vertical dimensions. The second group was composed of 100 unrelated age and BMI-matched women without any personal or family history of breast cancer or other malignancies to serve as controls. Control subjects were selected randomly among the people whom admitted to the same hospital during the same period. All patients and subjects enrolled in the study informed about the study and consent was taken. This study was approved by the Clinical Research Ethics Committee of Ahvaz Jundishapur University of Medical Sciences. Women with suspected breast cancer without histological confirmation and those that refused sample donation were excluded from the study.

Measurements

Body mass index was calculated as body weight (in kg) divided by square height (m^2). Based on hospital records body weight and height of all the participant were measured by standard methods, while they wearing light clothing and not wearing shoes. A volume of 5 mL of blood samples were collected into without EDTA-treated tubes from all the participants. Sera were obtained from blood samples by processing of clotting and centrifugation. The serum samples were stored at 2-8°C for not more than 24 h prior to lipid profile determination. A serum aliquot was stored frozen at −70°C for serum leptin and sOB-R measurement.

Laboratory techniques

Total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were determined using commercially available kits (Pars Azmoon Inc., Tehran, Iran). Serum leptin concentration was measured by enzyme-linked immunosorbent assay (ELISA) using a commercially available human leptin ELISA kit (Mediagnost, Reutlingen/Germany, E07). The inter- and intra-assay coefficients of variation were 6.8 and 2.55%, respectively. sOB-R concentration was measured by ELISA using a commercially available human sOB-R ELISA kit (Boster Biological technology, LTD C. No. EK0439) composed of two monoclonal antibodies raised against the extracellular domain of sOB-R. Briefly, we diluted samples 1:2 with dilution buffer prior to use. Then, 100 µL human leptin receptor standards and diluted samples were pipette into 96-well microtiter plates coated with antileptin receptor monoclonal antibody. After incubation at 37°C for 90 min, the wells were washed 3 times and incubated at 37°C for 30 min with the monoclonal antibody labeled with horseradish peroxidase. The wells were again washed 5 times and incubated at 37°C in dark for 15-20 min with tetramethylbenzidine (TMB) regent. Then, 100 µL TMB stop solution was added to each well to stop the reaction, and finally, the absorbance at 450 nm is measured on a microtiter plate reader. The intensity of the color formed is directly proportional to the concentration of sOB-R in the sample. A set of standards is used to plot a standard curve from which the amount of sOB-R in patient samples and controls can be directly read.

Statistical analyses

All the statistical analyses were performed using SPSS software for Windows version 15.0 (SPSS, Inc., Chicago IL, USA). The median, range, and 95% confidence interval (CI) for median were calculated. We used nonparametric test for the analysis, because the data were not normally distributed even after logarithmic transformation. Thus, a significant difference among the serum levels of leptin, sOB-R, anthropometric measurements and lipid profiles in breast cancer cases and control subjects were assessed by Mann–Whitney U-test (two-tailed). The relationships between variables were evaluated using Spearman's correlation. The association between breast cancer and serum levels of leptin, sOB-R, anthropometric measurements and lipid profiles were determined as ORs and 95% CIs according to the unconditional logistic regression analysis. $P < 0.05$ considered as statistically significant.

RESULTS

Baseline characteristics

Table 1 represents median, range, and 95% CI for age, anthropometric variables, sOB-R, leptin and lipid

Table 1: Medians, ranges and 95% CI of age, anthropometric, LEP, sOB-R, and lipid profiles in breast cancer cases and control subjects

Variables	Controls (n = 100)		Breast cancer cases (n = 100)		*P* value
	Median (range)	95% CI	Median (range)	95% CI	
Age (years)	48.5 (33.0-63.0)	34.5-61.0	48.0 (27.0-73.0)	28.5-72.4	0.522
BMI (kg/m^2)	28.0 (17.0-39.30)	20.1-38.2	26.9 (18.0-37.4)	19.3-36.8	0.075
Total cholesterol (mg/dL)	202.0 (114.0-445.0)	119.1-37.4	193.50 (103.0-310.0)	127.7-28.9	0.204
LDL-C (mg/dL)	93.5 (69.0-125.0)	71.5-122.9	89.5 (48.0-132.0)	51.0-126.9	0.585
HDL-C (mg/dL)	46.0 (20.0-74.0)	30.5-71.4	56.5 (30.0-74.0)	32.0-72.0	<0.001
TG (mg/dL)	120.0 (50.0-404.0)	51.0-334.9	89.5 (52.0-327.0)	56.0-289.1	0.012
LEP (ng/mL)	28.30 (0.5-126.0)	0.60-113.1	67.90 (2.20-132.4)	2.77-129.9	<0.001
sOB-R (ng/mL)	1.98 (0.77-191.63)	0.77-188.2	0.140 (0.09-148.05)	0.09-98.1	<0.001
LEP/sOB-R ratio	10.43 (0.01-145.0)	0.01-97.9	139.96 (0.20-1442.2)	0.44-128.8	<0.001

P values derived by Mann–Whitney U-test (two-tailed). BMI = Body mass index; HDL-C = High density lipoprotein cholesterol; LDL-C = Low density lipoprotein cholesterol; sOB-R = Soluble leptin receptor; CI = Confidence interval; LEP = Leptin; TG = Triglyceride

profiles. Leptin, HDL, and leptin/sOB-R levels were significant higher, sOB-R and TG levels were significant lower in breast cancer cases compared with the controls ($P < 0.02$ and $P < 0.001$, respectively). The levels of total cholesterol and LDL-C were not significantly difference between the two groups ($P > 0.05$, respectively).There were not significantly differences of age, and BMI in breast cancer cases compared with the controls ($P > 0.05$, respectively).

Correlations between leptin, soluble leptin receptor, anthropometric and lipid profiles

When we analyzed the whole study subjects combined [Table 2], the serum sOB-R level was negatively correlated with BMI, leptin and leptin/sOB-R ratio ($P < 0.05$ and $P < 0.001$, respectively). On the other hand, the serum leptin level was positively correlated with BMI, and leptin/sOB-R ratio ($P < 0.05$ and $P < 0.001$, respectively). In addition, serum leptin level was negatively correlated with TG and sOB-R ($P < 0.05$ and $P < 0.001$, respectively).

Correlations between leptin, soluble leptin receptor, and other measured variables and breast cancer

Unconditional logistic regression models were used ($\alpha = 0.05$, $\beta = 0.1$) to investigate the association between the risk of breast cancer and BMI, serum levels of leptin, sOB-R and lipid profiles. Results revealed that the high serum level of leptin, leptin/sOB-R ratio, and HDL associated with breast cancer, and their corresponding odds ratios (ORs) being 1.027(95% CI = 1.017-1.033, $P < 0.001$); 1.028 (95% CI = 1.015-1.042, $P < 0.001$), and 1.063 (1.033-1.093, $P < 0.001$), respectively. An inverse association between serum level of sOB-R and breast cancer was observed (OR = 0.983, 95% CI = 0.969-0.997, $P = 0.015$). However, BMI was not associated to breast cancer [OR = 0.945, 95% CI = 0.886-1.0.09, $P = 0.090$; Table 3]. There were no significant association between any of the other variables including age, total cholesterol, LDL, and TG and breast cancer.

Table 2: Correlation coefficients of age, anthropometric, LEP, sOB-R, and lipid profiles in study subjects

Variables	LEP	sOB-R	LEP/sOB-R
Age (years)	−0.032	−0.044	−0.143
BMI (kg/m^2)	0.173*	−0.186*	−0.016
Total cholesterol (mg/dL)	0.035	−0.044	−0.028
LDL-C (mg/dL)	−0.11	0.138	−0.279
HDL-C (mg/dL)	0.159	−0.168	0.298**
TG (mg/dL)	−0.188*	0.07	−0.168**
LEP (ng/mL)	1	−0.238**	0.590**
sOB-R (ng/mL)	−0.238**	1	−0.208*
LEP/sOB-R ratio	0.590**	−0.238**	1

*$P < 0.05$; **$P < 0.001$. LEP = Leptin; sOB-R = Soluble leptin receptor; BMI = Body mass index; HDL-C = High density lipoprotein cholesterol; LDL-C = Low density lipoprotein cholesterol; TG = Triglyceride

Table 3: Association between breast cancer and serum levels of LEP, sOB-R and lipid profiles based on a case and control analysis

Variables	β	OR	95% CI	Wald	*P* value
Age	−0.01	0.99	0.961–1.02	0.417	0.518
BMI	−0.056	0.945	0.886–1.009	2.881	0.09
Total cholesterol	−0.005	0.995	0.989–1.001	2.509	0.113
LDL-C	−0.005	0.995	0.978–1.013	0.304	0.582
HDL-C	0.061	1.063	1.033–1.093	17.95	<0.001
TG	−0.004	0.996	0.991–1.00	3.159	0.076
LEP	0.027	1.027	1.017–1.038	26.62	<0.001
sOB-R	−0.018	0.983	0.969–0.997	5.9	0.015
LEP/sOB-R ratio	0.028	1.028	1.015–1.042	16.97	<0.001

P value derived by unconditional logistic regression analysis. CI = Confidence interval; BMI = Body mass index; HDL-C = High density lipoprotein cholesterol; LDL-C = Low density lipoprotein cholesterol; OR = Odds ratio; TG = Triglyceride; LEP = Leptin; sOB-R = Soluble leptin receptor

DISCUSSION

In this study, we observed a significant positive association between free leptin index (FLI) and serum leptin levels with breast cancer risk. In addition, higher serum sOB-R concentrations were found in healthy control subjects than the breast cancer cases. Contrary, the serum level of leptin in breast cancer cases was significantly higher than those

in healthy control subjects. We demonstrated relationships between serum level of sOB-R and BMI, serum leptin and leptin/sOB-R ratio.

We found that the serum levels of leptin in breast cancer patients were significantly higher than those controls and leptin increased risk for breast cancer. These results were concordant with the findings of Rahmati-Yamchi *et al*.[23] and Niu *et al*.[24] Llanos *et al*.[25] and Macciò *et al*.[26] have reported that leptin increased risk for breast cancer in postmenopausal women, but had no relationship with onset of premenopausal breast cancer. In their study Dieudonne *et al*.[27] they found that MCF-7 cells expressed leptin receptor and leptin could influence the growth of human mammary cancer MCF-7 cells. Okumura *et al*.[28] investigated the effects of leptin on the MCF-7 line of human mammary cancer by evaluating cell doubling-up time, DNA replication, levels of proteins associated to cell cycle and expression of protein kinase C isozyme, and reported that hyperleptinemia increased breast cancer cell proliferation through accelerated cell cycle progression. On the other hand, Yuan *et al*. have reported that leptin stimulates the growth of breast cancer in the nude mice and promotes the proliferation and migration of MCF-7 human breast cancer cells through the extracellular-signal regulated kinase pathway.[18]

Findings of several studies indicate that leptin is involved with different aspects of tumor pathology such as cell growth, angiogenesis and metastasis.[29-31] A study of Italian subjects documented that blood leptin levels in postmenopausal patients with ER + breast cancer significantly correlated with pathological staging.[32] Similarly, a number of investigators observed higher blood leptin concentrations in breast cancer patients than in controls.[33,17] In a study conducted by Garofalo *et al*.,[34] 92% of primary breast cancer cases and 83% of lymph node metastasis showed overexpression of leptin and Ob-R, respectively in breast tumor tissues. In the same manner, Jardé *et al*.[35] have reported significant overexpression leptin and Ob-R in primary and metastatic breast cancer relative to noncancer tissues. They also observed that leptin positively correlated with Ob-R in primary tumors and that the expression of both proteins was more abundant in high-grade tumors. In another study, Mahabir *et al*.[4] detected 85% and 75% overexpression of leptin and Ob-R respectively in primary breast cancer cases, with the expression of leptin significantly correlated with that of Ob-R. In addition, Ob-R expression in cancer tissue was positively correlated with ER status and tumor size.

Leptin provides its central and peripheral effects through binding to its receptor located on the cell surface.[4] Several isoforms of long- and short-forms of leptin receptors are expressed in humans. The long form of leptin receptor with the full length of intracellular domain is expressed primarily in the hypothalamus, and the short forms of leptin receptor (oB-Rs) are typical for peripheral tissues. sOB-R is an exceptional form, which includes exclusively of extracellular domain of membrane-bound leptin receptors.[36] The function of sOB-R is not completely understood, but believed to delays the clearance of leptin from the circulation and thus, increased leptin levels and bioavailability and as a consequence, potentiates its effect.[37] On the other hand, the plasma levels of sOB-Rs correlate with density of the leptin receptors on cell membranes.[38] One-way of characterizing the balance between leptin and sOB-R is the FLI, which is determined by calculating the ratio between the concentrations of leptin and sOB-R.[39] In obese children the levels of leptin are higher and the levels of sOB-R are lower than in nonobese children.[40]

Although originally, free leptin levels could be measured only by a gel filtration chromatography method, Magni *et al*.[13] reported that the ratio of circulating leptin to sOB-R (leptin/sOB-R) was strongly related to the percentage of body fat, and this ratio was thought to be an index of free leptin. In this study, the leptin/sOB-R ratio was significantly higher in breast cancer cases. Moreover, to the best of our knowledge, this is the first report demonstrated leptin/sOB-R ratio was positively correlated with leptin and HDL, and negatively correlated with TG, and sOB-R levels in a sample of Iranian subjects with breast cancer and controls. Sinha *et al*.[8] confirmed the existence of leptin-binding proteins and reported that in lean subjects the greater part of leptin circulated in the bound form, whereas in obese subjects, the greater part of leptin circulated as the free form. Landt *et al*.[9] have reported that only free leptin was detectable in CSF, suggesting that it was the biologically active form. Lammert *et al*.[10] observed that leptin-binding activity was correlated with levels of the sOB-R and that sOB-R was the major leptin-binding protein in the circulating human blood.

To the best of our knowledge, ours is the first study to provide information about the association of serum levels of sOB-R and leptin with breast cancer cases in a sample of Iranian subjects. Due to the limitations inherent in a case–control study and low sample size, this study cannot elucidate the mechanism or determine the direction of causality, further prospective studies with larger sample size are necessary to clarify the impact of FLI and serum level of leptin on breast cancer risk.

CONCLUSION

It is speculated that high FLI and serum level of leptin rather than low serum level of sOB-R was associated with the breast cancer in a sample of Iranian population.

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AUTHORS' CONTRIBUTION

Ghorban Mohammadzadeh coordinated the study, carried out the design, analyzed the data and prepared the manuscript. Mohammad-Ali Ghaffari, provided assistance in the design of the study, coordinated all the experiments and participated in manuscript preparation. Ahmad Bafandeh, carried out the design, participated in most of the laboratory experiments and blood sampling. Seyed-Mohammad Hosseini provided assistance for all experiments and participated in the patient's selection for the study. All authors have read and approved the content of the manuscript.

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