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Association of Serum Adiponectin and Leptin Levels with Breast Cancer in Iraqi Women

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Abstract Obesity has been associated with an increased risk of cancer especially postmenopausal breast cancer. Adipokines have been hypothesized to underlie this association. Adiponectin and leptin are adipocyte-secreted hormones that opposite each other not only in their biological activities but also in their effects on breast neoplastic cells. in addition, Leptin receptor, found in both membrane-bound isoforms and a soluble form (sOB-R), sOB-R bind to leptin in circulation and correlate with its activity. The aim of this study was to investigate the relationship between serum levels of adiponectin, leptin and sOB-R with breast cancer. Methods: In a case-control study Serum adiponectin, leptin and sOB-R levels were measured by enzyme-linked Immunosorbent assay in 48 women with histologically confirmed breast cancer and compared with 41 age and BMI matched women without breast cancer as control groups. Control groups were divided into; Control group 1 (C1) contains 26 healthy women and control group 2(C2) contains 15 women with breast benign diseases. Results: The mean serum levels of adiponectin in both controls (C1 and C2) were significantly higher than that in breast cancer cases (25.08±5.59and 22.49±3.49) versus (15.84±6.21) respectively. Conversely, the mean serum levels of leptin in both controls (C1 and C2) were significantly lower than that in breast cancer cases (27.98±6.85 and 34.50±10.59) versus (48.97±24.56) respectively. Higher circulating levels of sOB-R (140.21±74.20) were significantly associated with an increased risk of breast cancer as compared to controls (47.13±38.30 and 27.68±23.58) for C1 and C2 respectively. In addition, the association of BMI with breast cancer was non-significant. Conclusion: These data suggest that dysregulation in hormones (adipokines) secreted by adipose tissue may be associated with breast cancer independently of BMI. Further prospective studies examining the role of adipokines in the etiology of breast cancer are warranted.

Keywords: *breast cancer, obesity, adiponectin, leptin, soluble leptin receptor*

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1. Introduction

Breast cancer (BC) is the most common cancer in women worldwide [1]. According to the latest Iraqi Cancer Registry, BC is the commonest type of female malignancy, accounting for approximately one-third of the registered female cancers [2].

Obesity has been consistently associated with an increased risk of postmenopausal BC; in addition, obesity in women diagnosed with BC is associated with greater tumor burden and with higher-grade tumors lead to overall poorer prognosis and/or increased mortality for both premenopausal and postmenopausal women [3,4,5,6]. In Iraq, a survey held by the Ministry of Health reported that the prevalence of overweigh and obesity (47%-67%) among females and males respectively [7]. Another study carried out in Basrah over the period from May 2003 to April 2010 found that; obesity and overweight was seen in 55.1% of population (54.7% women, 45.3 % men), prevalence of overweight alone was 31.3% (50.2% men and 30.9% women) and obesity was 23.8% (61.1% of women, 18.6% men), Obesity was more prevalent in women than in man [8].

The implications of obesity and cancer association for cancer prevention and the hormonal mechanisms by which obesity may affect BC risk are not fully understood. Several possible mechanisms for this association have been hypothesized, including increased estrogen production in non-ovarian tissues, increased insulin and IGF and changes in circulating adipokines concentrations [9,10,11].

Adipocytes within a context of obesity, by the action of "adipokines", participate in a highly complex cross talk with tumour cells to promote tumour progression [12]. Animal studies, microarray analysis, and in vitro tumor studies provide evidence that adipose tissue and these adipokines can directly influence tumor growth [13,14]. Some of the most compelling evidence comes from a study demonstrate that, tumors formed from human BC cells injected into adipocytes of mice grew three times larger than tumors from human BC cells injected into the fibroblasts of mice [14].

Two adipokines, leptin (Lp) and adiponectin (ApN) have been studied for their influence on the BC risk and tumour biology, their biological activities as their effects on breast neoplastic cells are largely in opposition to each other [12], for this reason, the two adipocytokines are combined to calculate the Lp: ApN ratio. It was observed that people with an increased Lp: ApN ratio have a higher risk developing BC [15]. The higher the Lp: ApN ratio the greater is the tumor size, indicating a positive correlation [10].

Adiponectin (ApN) has in contrast to Leptin, anti-diabetic, anti-inflammatory, anti- atherogenetic effects as well as anti-neoplastic effect [16]. ApN level and body mass index are inversely correlated. normal weight people have a physiologically increased serum ApN level in contrast, in obese individuals ApN biosynthesis is down regulated, which leads to hypoadiponectinemia, not only BMI, but also the risk for BC is inversely correlated with ApN level in serum [17,18].

Leptin is an anorexigenic peptide that plays a key role in energy homeostasis and appetite control [19]. In addition, Leptin has been shown to have mitogenic effects on epithelial cells and to promote cellular proliferation, migration, and invasion in breast cancer cell lines [20,21]. In humans, the circulating Lp level is increased in obesity, suggesting that a hallmark of obesity is not Lp deficiency, but Lp resistance [22,23].

Lp exerts its pleiotropic actions directly through distinct receptors (ob-R) encoded by the diabetes (db) gene and was identified as a member of the cytokine family of receptors, The Lp receptor gene was found to encode at least five alternatively spliced forms, ob-Ra, ob-Rb, ob-Rc, ob-Rd, and ob-Re [22,24]. Besides membrane-bound isoforms of the Lp receptor with varying cytoplasmic length, a soluble form of the Lp receptor (sOB-R) has been demonstrated [25]. sOB-R, a special Lp receptor with the extracellular domain only, is formed by ectodomain shedding of Lp receptors on the cell surface [25]. Lammert et al. observed that Lp-binding activity was correlated with levels of the sOB-R and that sOB-R was the major Lp-binding protein in the circulating human blood [26]. The function of sOB-R is not entirely clear but believed to delay the clearance of Lp from the circulation and, thus, increase its availability [27]. In addition, there is evidence suggesting that sOB-R not only alters the clearance of Lp but also potentiates Lp action [28].

2. Subjects and Methods

2.1. Subjects

This case –control study consists of three groups. First group was composed of 48 unrelated women with histopathologically confirmed breast cancer that had not undergone any previous treatment. In addition to two control groups that include; 26 apparently healthy women as control group 1(C1) and 15 women with breast benign (fibroadenoma) as control group 2 (C2), the diagnosis of fibroadenoma was confirmed by both mammography and/or histopathology analyses, both matched for BMI and age of breast cancer patient and without any personal history of breast cancer or other malignancies. Control subjects were selected randomly among women admitted to the Women's Health Center for early detection of breast cancer/ Baghdad during the same period. All patients and subjects enrolled in the study informed about the study and consent was taken. The study was approved by the Clinical Research Ethics Committee of Pharmacy College University of Baghdad.

Clinical information was obtained from the hospital record for all patients. The dataset contains information on the following: Type of cancer, Age and menopausal status at diagnosis, body mass index, Chemotherapy status, hormonal receptor status (estrogen receptor, progesterone receptor), Tumor type, size, grade and Lymph Node Status.

All Participants completed interview-administered questionnaires regarding demographic and behavioral factors, including smoking history, recreational physical activity, medical, reproductive, and family history. Subjects with a history of smoking, previous cancer and endocrine-related illnesses or had taken exogenous hormones in the three months preceding blood collection were excluded from the study.

2.2. Measurements

Body mass index was calculated as body weight (in kg) divided by square height (m^2). all the participant were measured by standard methods, while they wearing light clothing and not wearing shoes. Waist and hip circumference was determined by measuring tap with cm scale. Waist to hip ratio (WHR) determined by dividing waist circumference (in cm) on hip circumference (in cm) [29].

2.3. Laboratory Evaluation

Specialized laboratory staff who did not participate in the study did Blood specimen collection from each woman. A volume of 5 mL of blood samples were collected into gel-containing tubes and Sera were obtained by processing of clotting and centrifugation. The serum samples were stored frozen at −70 ℃ for serum adiponectin, leptin and sOB-R measurement.

Serum adiponectin, leptin and sOB-R concentrations were measured by enzyme-linked immunosorbent assay (ELISA) using a commercially available kits, human adiponectin ELISA kit (CUSABIO®/China, E07270h), human leptin ELISA kit (CUSABIO® / China, E04649h) and human sOB-R ELISA kit (CUSABIO®/China, E04647h) respectively.

The technique used depends on a quantitative Sandwich-Assay using two specific and high affinity antibodies, the microtiter plate provided in the kit has been pre-coated with an antibody specific to substance to measure. Briefly, after dilution according to manufacturing manual samples pipetted into the microtiter plate wells and incubated for 2 hours at 37 ℃, after removing any unbound substances, a biotin-conjugated polyclonal antibody specific for measured substance is added to the wells and incubated for 1 hour at 37 ℃. After washing, strepto-avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated again for 1 hour at 37 ℃. Following a wash to remove any unbound avidin-enzyme reagent, a TMB (3, 3’5, 5' tetra methyl-benzidine) substrate solution is added to the wells and color develops in proportion to the amount of substance bound in the initial step. The

enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm [26,30,31].

2.4. Statistical Analysis

Statistical software (SPSS version 21, Chicago, IL, USA) was used for data input and analysis. Continuous variables were presented as mean ± standard deviation of mean (SD). One-way analysis of variance (ANOVA) was used to examine the degree of difference among studied groups, followed by Fisher least significant difference (LSD) to identify significantly different means. Pearson's correlation coefficient (r) was used to test the statistical correlations between studied parameters. The association between breast cancer and serum levels of adiponectin, leptin, sOB-R, Lp/ApN ratio, Lp/sOBR ratio, and anthropometric measurements were determined as ORs and 95% CIs according to the conditional logistic regression analysis. In all data present in this study, findings with P value less than 0.05 were considered statically significant.

3. Results

3.1. Baseline Characteristics

Table 1 represents mean ± standard deviation (SD) for age, anthropometric variables, adiponectin, leptin, Lp/ApN ratio,sOB-R and leptin/sOB-R ratio.

Table 1. Mean±SD for age, anthropometric, ApN, Lp, Lp/ApN, sOB-R, and Lp/ sOB-R in the studied groups

variable	Healthy control (C1) N=26	Breast cancer (BC) N=48	P1 value	breast benign (C2) N=15	P2 value
Age (yrs.)	47± 10.28	50.4±12.23	0.216	36.6±8.33	<0.001
BMI (kg/m^2)	29 + 4.28	30± 5.02	0.785	29.9+4.39	0.644
WHR	0.82 ± 0. 078	0.86 ±0.071	<0.01	0.81 ± 0.052	<0.01
Adiponectin (µg/ml)	25.08 ±5.59	15.84 ±6.21	<0.001	22.49 ±3.49	<0.001
Leptin (ng/ml)	27.98 ±6.85	48.97 ±24.56	<0.001	34.50 ±10.59	0.012
Lp /ApN ratio	1.16 ±0.39	3.26 ±2.09	<0.001	1.58 ±0.54	<0.001
sOB-R (ng/ml)	47.13 ±38.30	140.21 ±74.20	<0.001	27.68 ±23.58	<0.001
Lp /sOB-R ratio	1.83 ±1.30	0.96 ±1.98	0.04	2.26 ±1.52	0.016

P1: level of significance between C1and BC; P2: level of significance between C2 and BC; N: number of subject; BMI= body mass index, WHR= waist hip ratio; Lp/ApN: leptin to adiponectin ratio; sOB-R: soluble leptin receptor; Lp/ sOB-R: leptin to soluble leptin receptor ratio

As summarized in Table 1, the results of the study showed no significant difference (P > 0.05) regarding the mean age ± SD between BC group (50.4 ±12.2) and the healthy (C1) controls (47 ±10.28), however, the breast benign group (C2) have significantly different mean age (36.6 ±8.33), (p<0.001) as compared to BC group. BMI was comparable in all three groups, however; WHR was significantly higher in BC group (p<0.01) as compared to C1 and C2 groups.

Adiponectin was significantly lower in BC cases compared with the controls (P< 0.001), Leptin was significantly higher in BC as compared to C1 and C2 groups (p< 0.001, p< 0.02) respectively, Lp /ApN ratio and sOB-R levels were significantly higher in breast cancer cases when compared with C1 and C2 groups (P< 0.001). Contrary, Leptin/sOB-R ratio was significantly lower in breast cancer cases compared with the C1 and C2 (P< 0.05, P< 0.02) respectively.

3.2. Correlations Studies

3.2.1. Correlation between Adiponectin, Leptin, Soluble Leptin Receptor and Anthropometric among All Studied Groups Combined

Table 2. Correlations between adiponectin, leptin, Lp/ApN ratio, sOBR, Lp/sOBR ratio and anthropometric

variable	ApN	Lp	Lp/ApN	sOBR	Lp/sOBR
age	-0.068	0.204	0.132	0.192	-0.011
BMI	0.083	0.494**	0.329**	-0.044	0.219*
WHR	-0.178	0.540**	0.449**	0.216*	0.118
ApN	1	-0.137	-0.512**	-0.519**	0.125
Lp	-0.137	1	0.838**	0.321**	0.001
Lp/ApN	-0.512**	0.838**	1	0.529**	-0.180
sOBR	-0.519**	0.321**	0.529**	1	-0.590**
Lp/sOBR	0.125	0.001	-0.180	-0.590**	1

*P < 0.05; **P < 0.01.ApN =adiponectin; Lp =Leptin; sOB-R =Soluble leptin receptor; BMI = Body mass; WHR= waist to hip ratio

As shown in Table 2, when the whole study subjects was analyzed, the serum adiponectin level was negatively correlated with Lp/ApN ratio and sOB-R level (P< 0.01),this was in contrast to leptin which correlated positively with Lp/ApN ratio and sOBR(P <0.01). In addition, leptin and Lp/ApN ratio also correlated positively with both BMI and WHR (P< 0.01) while Lp/sOBR ratio correlated positively with BMI (P< 0.05). sOB-R correlated positively with WHR and Lp/ApN ratio (P< 0.05 and P< 0.01, respectively) while there was a negative correlation between sOB-R and Lp/sOB-R ratio (P< 0.01).

3.2.2. Association of Adiponectin, Leptin and Soluble Leptin Receptor and Breast Cancer

Conditional logistic regression models were used to investigate the association between the risk of breast cancer and the markers measured in the study. Results revealed that the high serum level of leptin (OR =1.098, 95% CI= [1.033-1.168]; P < 0.01), sOB-R (OR =1.024, 95% CI= [1.009-1.040]; P < 0.01) were associated with breast cancer. An inverse association between serum level of adiponectin and breast cancer was observed (OR =0.0791, 95% CI= [0.693-0.903]; P < 0.01 as showed in Table 3. There were no significant association between any of the other variables including age, BMI, WHR, Lp/ApN ratio and Leptin/sOB-R ratio and breast cancer.

Table 3. Association of serum levels of adiponectin, leptin and soluble leptin receptor and breast cancer based on a case and control analysis

variable	β	OR	95%CI	Wald	P value
Leptin	0.094	1.098	1.033-1.168	8.918	0.003
sOBR	0.O24	1.024	1.009-1.040	9.393	0.002
Adiponectin	-0.234	0.791	0.693-0.903	12.019	0.001

P value derived by conditional logistic regression analysis. CI = Confidence interval; sOB-R =Soluble leptin receptor; OR = Odds ratio

4. Discussion

As obesity has been associated with the development of breast cancer, adipocytokines, a group of polypeptide growth factors and cytokines, which are produced exclusively by adipose tissue, such as adiponectin and leptin, may underlie the association between obesity and breast cancer risk [10].

In this study, we found that the serum levels of leptin, soluble leptin receptor and Lp/ApN ratio were significantly higher in breast cancer patients than controls. Contrary significantly lower serum levels of adiponectin and Lp/sOBR ratio were found in breast cancer cases than control subjects. Rrelationships were demonstrated between serum levels of leptin, Lp/ApN and Lp/sOBR ratio with BMI. In addition, serum levels of ApN, Lp, Lp/ApN ratio, Lp/sOBR ratio and WHR ratio were correlated with sOB-R serum levels as shown in Table 2. There was non-significant difference regarding the mean age of BC cases and the first control group, healthy control (C1); however, the difference was significant as compared to the second control, breast benign control (C2), since benign breast diseases usually more common among younger age women.

Adiponectin serum levels decrease with increasing body fatness while leptin levels increase. Functionally, they appear to oppose each other's actions. In addition, sOB-R, although the function of sOB-R is not entirely clear, but believed to modulate leptin effect by increasing its availability [27]. Moreover, there is evidence suggesting that sOB-R can also potentiates leptin action [28]. Altered levels of adipokines or their cognate receptors in cancers can ultimately lead to an imbalance in downstream molecular pathways. The role of these adipocytokines, adiponectin and leptin, in addition to sOBR in Iraqi breast cancer patients remains to be determined. It is possible that the levels of adiponectin and leptin receptors, as well as the balance of serum adiponectin and leptin, are critical factors in mammary tumorigenesis.

The result of the current study suggest that serum adiponectin levels are inversely associated with breast cancer; this was consistent with many other studies [32]-[36]. Miyoshi et al [32] was the first to found a strong inverse association between adiponectin and breast cancer risk among both pre- and post-menopausal women. This was supported by other studies [35,36]. However, other groups reported that low adiponectin levels are associated with breast cancer only in post-menopausal women [33,34,37]. Adiponectin concentration has a significant negative correlation with estradiol in postmenopausal but not in premenopausal women [38]. That might help to explicate the divergence, but the mechanism of adiponectin on postmenopausal women and premenopausal women still need to be discussed.

One possible mechanism explaining associations between obesity and cancer other than estrogen is hyperinsulinemia or insulin resistance. It has been noted that adiponectin stimulates the sensitivity of peripheral tissue to insulin; decreased levels of adiponectin are associated with increased serum insulin levels, which accompany insulin resistance [39]. Insulin enhances the activity of insulin-like growth factor-1 (IGF-1), and high levels of circulating IGF-1 are correlated with risk of development of breast cancer [40]. However, Mantzoros et al [33] reported that associations between serum adiponectin and breast cancer risk were independent of possible effects of major components of the IGF system, leptin, and body mass index.

Adiponectin can blocks proliferation of BC cells [41]. In vitro assays have shown that a number of different BC cell lines express one or both of the ApN receptors and show reduced growth and/or increased apoptosis in response to ApN [42]. Furthermore, the low serum adiponectin levels were found to be significantly associated with large tumor size (>2 cm) and high histological grade (2+3), indicating that tumors with high proliferation activity are more likely to develop under the low adiponectin condition [32]. It is well established that obesity is associated with poor prognosis [3,4,5,6]. This association might be partly explained by the low serum adiponectin levels seen in obese breast cancer patients.

Our study was conducted among 48 breast cancer patients, 26 healthy controls and 15 breast benign controls, The result of our study suggest that the circulating leptin level varies among these different population groups from low to high: healthy people<breast benign diseases patients<breast cancer patients. This observation was consistent with a recent meta-analysis that included 23 studies (2058 breast cancer patients, 2078 healthy controls and 285 breast benign controls) suggests that leptin might play a role in the formation and development of breast carcinoma and has potential for development as a diagnostic tool [43].

The association of higher serum levels of leptin with BC risk were concordant with the findings of many other studies [44,45,46]. Llanos et al [47] and Macci ò et al [48] have reported that leptin increased risk for breast cancer in postmenopausal women, but had no relationship with onset of premenopausal breast cancer.

Findings of several studies indicate that leptin is involved with different aspects of tumor pathology such as cell growth, angiogenesis and metastasis [49,50,51]. Ishikawa et al [52] showed that, leptin might play a role in the carcinogenesis and metastasis of breast cancer, possibly in an autocrine manner. Garofalo et al [53] reported that high leptin level in obese breast cancer patients might contribute to the development of antiestrogen resistance. Animal studies also support a role for leptin in mammary tumor development as evidenced by the fact that mice deficient in leptin Lep^{ob} Lep^{ob}, or with non-functioning leptin receptors,, $Lepr^{db}$ $Lepr^{db}$ [54], did not develop transgene-induced mammary tumors. Moreover, results from animal studies suggested that leptin receptor antagonists might be a new option for breast cancer treatment [55].

The leptin-mediated proliferation involves a possible interaction between leptin and oestrogen systems to promote breast carcinogenesis [56], as leptin can exhibits oestrogen-producing activity by enhancing aromatase mRNA expression, aromatase protein content and its enzymatic activity and enhances the sensitivity of breast cells to oestrogens via up-regulation of ERα in MCF-7 breast cancer cells [57,58]. In addition, the effect of both leptin and oestradiol on ZR-75 cell proliferation is higher than leptin or oestradiol alone [59]. However, numerous in vitro studies carried out with breast cancer cell lines have found a proliferative effect of leptin in ER-positive cell

lines [20,21,60,62], as well as in ER-negative cell lines [49]. These data suggest that leptin-induced cell proliferation cannot be explained only by oestrogen-dependent mechanisms.

In vitro studies also support the role of leptin and leptin receptor in BC cell proliferation [21,63,64]. Dieudonne et al. [21] found that MCF-7 cells expressed leptin receptor and leptin could influence the growth of human mammary cancer MCF-7 cells. Okumura et al [63] investigated the effects of leptin on the MCF-7 line of human mammary cancer and reported that hyperleptinemia increased breast cancer cell proliferation through accelerated cell cycle progression. On the other hand, Yuan et al [64] 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.

The soluble leptin receptor is involved in modulating leptin activity [26]. In our study we found that high sOB-R levels were strongly associated with a risk of BC, suggesting the overexpression of the leptin receptor in serum could cause an increase of leptin bioactivity or a reduction of leptin clearance due to association with the high molecular binding protein.

Jardé et al [56] had reported significant overexpression of leptin and Ob-R in primary and metastatic breast cancer relative to non-cancer 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. Garofalo et al [65] found that, 92% of primary breast cancer cases and 83% of lymph node metastasis showed overexpression of leptin and leptin receptor (Ob-R), respectively in breast tumor tissues. This was consistent with the finding of Mahabir et al [23] 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.

Magni et al [66] reported that the ratio of circulating leptin to sOB-R was strongly related to the percentage of body fat. In this study, the leptin/sOB-R ratio was significantly lower in breast cancer cases and it was positively correlated with BMI. Moreover, sOB-R rather than leptin/sOB-R ratio was positively correlated with WHR, leptin and Lp/ApN and negatively correlated with adiponectin and Lp/sOB-R ratio levels. This may indicate an interplay between dysregulated adipocytokines level and their receptors as response to central adiposity.

To the best of our knowledge, ours is the first study to provide information about the association of serum levels of adiponectin, leptin and sOB-R with breast cancer cases in a sample of Iraqi 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 these biomarkers on breast cancer risk.

5. Conclusion

High serum level of leptin and soluble leptin receptor and Low serum level of adiponectin were associated with the breast cancer independent of BMI in a sample of Iraqi population.

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