

Association of serum leptin with serum estradiol in relation to breast carcinogenesis: a comparative case-control study between pre- and postmenopausal women

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Background/aim: The aims of this study were to observe the interhormonal relationship of serum leptin with serum estradiol and to compare pre- and postmenopausal women for possible implications in breast carcinogenesis.

Materials and methods: This comparative case-control study encompasses 175 breast cancer patients and 175 age-, sex-, and BMI-matched healthy controls. For comparison, the subjects were divided into pre- and postmenopausal groups according to their menstrual history. Blood samples were analyzed for serum leptin and serum estradiol by the ELISA method.

Results: Higher mean values of serum estrogen and leptin were observed in breast cancer cases among both the pre- and postmenopausal groups. While estimating the association of serum leptin with serum estradiol, a weak positive ($r = 0.186$) but statistically significant ($P = 0.022$) correlation was found among the premenopausal subjects, while a moderate positive association ($r = 0.556$) was found among the postmenopausal subjects, which had high statistical significance ($P = 0.001$). Furthermore, correlation coefficients (r) of the pre- and postmenopausal groups were compared using Fisher r -to- z transformation and found to have a statistically significant difference ($z = 4.03$, $P = 0.0001$).

Conclusion: The findings of this study suggest the mitogenic effects of leptin on mammary tissue via augmenting peripheral estrogen production more significantly among postmenopausal subjects. Thus, the therapeutic modulation of leptin may be a potential adjuvant treatment for breast cancer patients with high levels of leptin along with high estrogen concentration as a consequence.

Key words: Breast cancer, leptin, estradiol, menopause

1. Introduction

Adipose tissue, which is an active endocrine organ, plays an important role in carcinogenesis via releasing proteins having endocrine functions like leptin, adiponectin, interleukin-6, and tumor necrosis factor- α (1). In addition to synthesis of adipocytokines, adipose tissue also contains a set of enzymes that can metabolize and produce steroid hormones (2). The extent of its importance is indicated by the almost complete contribution of estrogen in postmenopausal women and half of the testosterone in premenopausal women by adipose tissue alone. Steroidogenic enzymes that are expressed in adipose tissue include hydroxysteroid dehydrogenases, hydroxylases, reductase, glucuronosyltransferase, and, importantly, aromatase, which convert androstenedione and testosterone to estrone and estradiol, respectively (3).

Leptin, an adipocytokine, is a 16-kDa-peptide hormone. It is mainly present in adipose tissue and blood, while its receptors are abundant in peripheral tissues (4). Leptin has been linked with various cancers including endometrial, gastric, prostate, and breast cancers. Expression of leptin and its receptors were found in normal mammary tissue as well as breast cancer tissue; both leptin and its receptors have been found to be elevated in cancer tissue when compared with normal tissue (5). Circulating leptin concentration is associated with the incidence of breast carcinoma, and induces breast cancer carcinogenesis and invasion. Leptin exerts its growth effect on breast cancer cell lines through direct activation of multiple signaling cascades (6); however, increased expression of aromatase enzyme by leptin in the adipose tissue may indirectly promote carcinogenesis by augmenting estrogen biosynthesis (7).

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The growing evidence for leptin being involved in cell growth and proliferation of breast cancer opens up many arguments about its possible mechanisms. The aim of the present study was to observe the concentrations of serum leptin and serum estradiol among diagnosed breast cancer patients and healthy controls. Furthermore, this study presents a novel comparison of the interhormonal relationship of serum leptin with serum estrogen between pre- and postmenopausal subjects.

2. Materials and methods

This comparative case-control study was conducted by collaboration between the Department of Surgery, Civil Hospital, Karachi, and the Department of Biochemistry, University of Karachi. A total of 175 newly diagnosed breast cancer patients presenting to the Breast Clinic, Civil Hospital, Karachi, were enrolled after their informed consent was obtained, while 175 healthy controls not having any disease were recruited from various screening camps. For comparison, the subjects were divided into pre- and postmenopausal groups according to their menstrual history.

Sample size was estimated by using the 36.4% frequency of breast cancer reported in a study conducted by Bhurgri et al. (8) in Karachi, Pakistan. Ethical approval was acquired from the Research and Ethics Committee, Board of Advanced Studies and Research, University of Karachi, vide letter no: BASR – 0716 / Sc. Those subjects who had received any treatment for breast cancer, including breast surgery, chemotherapy, or radiotherapy, were excluded from the study. Subjects who were on hormone replacement therapy or contraceptive pills, antiestrogen drugs, or antidiabetic or insulin treatments were also excluded.

The informed consent form was administered and the objectives and benefits of the study were explained to the subjects in the local language to get approval for their participation. After an overnight fast, blood samples were obtained by vein puncture under fully aseptic measures. For premenopausal women, blood samples were collected in the first week (early) of the follicular phase of the menstrual cycle to lower inconsistency among subjects. Serum was separated after clotting, centrifuged, and stored at -30°C . Serum leptin and estrogen were analyzed using an ELISA kit manufactured by DIASource, Belgium. Intra- and interassay CV for leptin was 13.3 and 10.2, respectively, while its detection limit was 0.04 ng/mL.

The data were later entered and analyzed using IBM SPSS 20 (9) statistical software and OpenEpi (v.3) open source calculator (10). Student's t-test was used to compare mean values, while association between variables was estimated by Pearson's correlation. Significance of difference between correlation coefficients was estimated

via Fisher r-to-z transformation by using the online statistical computation website VassarStats (11).

3. Results

This study encompasses 175 patients and 175 age-, sex-, and BMI-matched healthy controls. To determine the differences between patients and controls in both pre- and postmenopausal women discretely, subjects were grouped according to their menopausal status.

Comparisons of parameters were performed among patients and controls grouped according to menopausal status in the Table. There were 152 premenopausal subjects, while there were 198 postmenopausal subjects. No significant difference was observed for age or BMI between the patients and controls in both groups independently, while a significantly younger age at menarche was found among the patients compared to the controls in both pre- and postmenopausal groups. Significantly higher mean values were observed for serum leptin in the breast cancer patients among both the pre- and postmenopausal groups. Similarly, higher serum estrogen levels were found in both groups, but the higher level attained statistical significance in the postmenopausal group only.

Considering the subjects overall, no association ($r = 0.029$) was found between serum estrogen and serum leptin. However, after stratification according to menopausal status, we found a weak positive association ($r = 0.186$) among the premenopausal subjects that attained statistical significance ($P = 0.022$) (Figure 1), while a moderate positive association ($r = 0.556$) with high statistical significance ($P = 0.001$) was found among the postmenopausal subjects (Figure 2). Serum estrogen as a dependent variable could be predicted based on serum leptin levels among the postmenopausal subjects only, with a moderate value of coefficient of determination ($R^2 = 0.309$). Furthermore, correlation coefficients (r) of the pre- and postmenopausal groups were compared by using Fisher r-to-z transformation and found to have a statistically significant difference ($z = 4.03$, $P = 0.0001$).

4. Discussion

The rising incidence of breast cancer and related deaths in the Asian population (12) has aroused public concern, requiring more intense measures. Reiterating gene-environment interaction, numerous studies have shown a prevalence of metabolic dysregulation among breast cancer cases. Elevated fasting blood glucose (13), elevated leptin (14), and obesity (15) have been associated with increased risk of breast cancer, particularly among postmenopausal women. Adipose tissue supports carcinogenesis by secreting growth factors and cytokines (16,17) that can possibly be modulated as future preventive and therapeutic measures.

Table. Characteristics of pre- and postmenopausal breast cancer patients and healthy controls.

No.	Parameters	Premenopausal			Postmenopausal		
		Patients n = 69 (mean ± SEM)	Controls n = 83 (mean ± SEM)	P-value	Patients n = 106 (mean ± SEM)	Controls n = 92 (mean ± SEM)	P-value
1	Age (years)	34.30 ± 0.49	34.62 ± 0.49	0.647	53.86 ± 0.47	53.45 ± 0.55	0.573
2	Abdominal circumference (cm)	73.74 ± 1.91	75.88 ± 1.03	0.305	74.37 ± 1.18	75.06 ± 1.29	0.695
3	BMI (kg/m ²)	22.36 ± 0.53	21.49 ± 0.39	0.185	21.12 ± 0.39	21.70 ± 0.41	0.313
4	Age at menarche (years)	11.89 ± 0.24*	12.91 ± 0.17	0.001	11.92 ± 0.16**	12.93 ± 0.19	0.000
5	Age at menopause ^a	-	-	-	45.33 ± 0.59	43.84 ± 0.47	0.055
6	Serum estrogen (E2) (pg/mL)	111.44 ± 6.81	106.58 ± 5.22	0.567	41.83 ± 1.59**	25.05 ± 1.90	0.001
7	Serum leptin (ng/mL)	22.59 ± 1.17**	14.28 ± 1.157	0.001	25.73 ± 1.16*	20.74 ± 1.125	0.002

The Table shows the comparison of age, body mass index (BMI), serum estrogen, and serum leptin of cases with controls grouped according to menopausal status into pre- and postmenopausal women. Significance of difference was determined by an independent t-test between cases and controls. * P-value < 0.05 statistically significant.; ** P-value < 0.001 statistically significant.

This study demonstrates higher mean values of leptin and estrogen among breast cancer patients in both the pre- and postmenopausal groups. This supports earlier studies that showed an association of higher leptin levels (4,5,18) and higher estrogen levels (19,20) with breast cancer. However, various other studies have found no association between elevated leptin and breast cancer risk (21,23).

Determination of association between leptin and estrogen reveals dissimilar findings in the pre- and postmenopausal groups. For the premenopausal group, a weak positive correlation was found, while a moderate positive association with high significance was found for the postmenopausal group. A statistically significant (P < 0.001) greater difference between correlation coefficients was observed in the postmenopausal group. This association may be explained by enhanced aromatase activity via leptin converting androgens into estrogens, particularly in the postmenopausal state, in the presence of low estrogen production by the ovaries. The aromatase enzyme that is expressed chiefly in the granulosa cells of the ovaries for converting androgens into estrogens is also expressed in adipose, liver, and other tissues (23). In postmenopausal women, the primary site of aromatase activity and estrogen biosynthesis shifts from the ovaries to

adipose tissue, and thus contributes to peripheral estrogen biosynthesis, which has been suggested by a positive correlation of estrogen with BMI after menopause (19).

Studies conducted by Magoffin et al. (24) and Kitawaki et al. (25) have previously demonstrated that leptin increases aromatase activity in adipose stromal cells and granulosa cells, respectively. Other studies conducted by Maggioni et al. (26) and Catalano et al. (27) showed that leptin increased mRNA expression, content, and activity of the aromatase enzyme in a breast cancer cell line. Hence, leptin may act in an autocrine, paracrine, or endocrine fashion, and exerts its mitogenic effect on mammary tissue profoundly by increasing estradiol production.

However, this study has inherent limitations, including those accompanying the cross-sectional nature of the study design. The patients and controls were age-, sex-, and BMI-matched; however, the study was not adjusted for ethnicity and other risk factors. Similar future studies are advocated that should be population-based, multicentric, and prospective to determine any causal relationships.

In conclusion, the findings of this study suggest that the proliferative effects of leptin on mammary tissue via augmenting peripheral estrogen production are more significant among postmenopausal subjects. Implicitly,

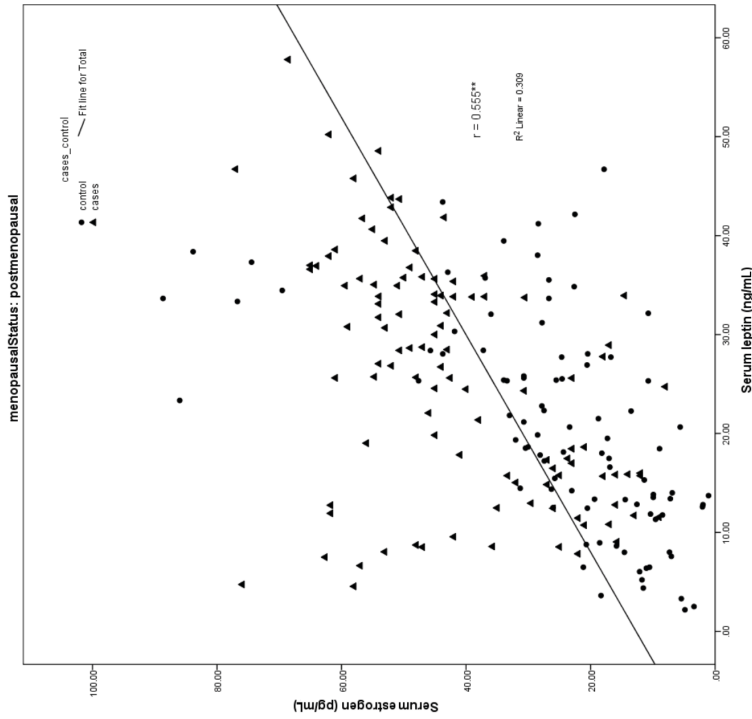


Figure 2. Association of serum leptin with serum estradiol in postmenopausal subjects. Scatterplots illustrate the association of serum leptin with serum estrogen. Distribution of data points for patients and controls is represented by (▲) and (●), respectively. The strength of association and relationship between parameters are described by the linear trend line, values of correlation coefficient (r), and coefficient of determination (R²).

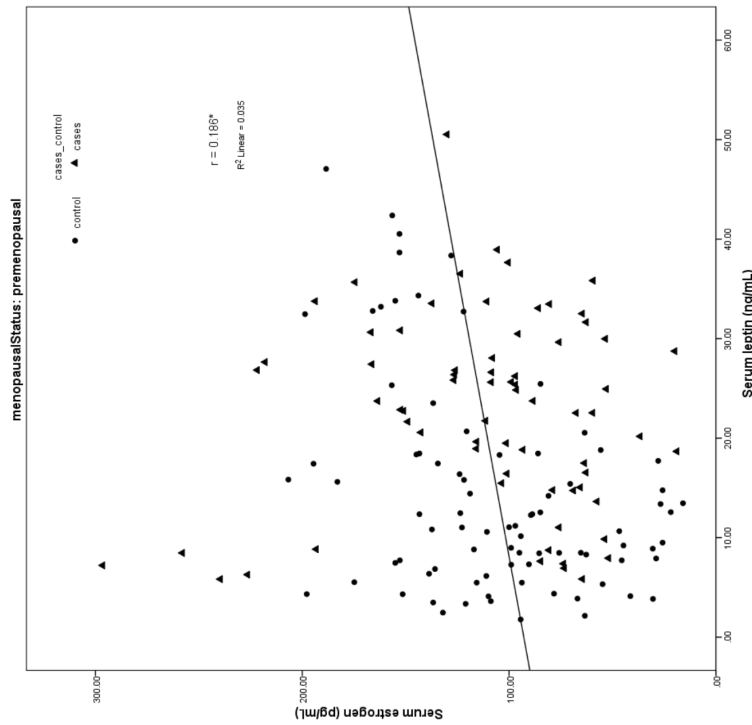


Figure 1. Association of serum leptin with serum estradiol in premenopausal subjects.

the therapeutic modulation of leptin may be advocated to disrupt the leptin–aromatase–estrogen axis, particularly among postmenopausal women. This proposition may provide a target for potential adjuvant treatment of breast cancer in patients with high levels of leptin along with high estrogen concentrations.

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