CLINICAL STUDY

Correlation between leptin level and hypertension in normal and obese pre- and postmenopausal women

Kawaljit Kaur Khokhar¹, Sharda Sidhu¹ and Gurcharan Kaur²Departments of ¹Human Genetics and ²Biotechnology, Guru Nanak Dev University, Amritsar 143005, Punjab, India

(Correspondence should be addressed to G Kaur; Email: kgurcharan.neuro@yahoo.com)

Abstract

Objective: The present study was aimed at evaluating the correlation between leptin and hypertension in normal and obese hypertensive pre- (Pre-M) and postmenopausal (Post-M) women of Jalandhar city (Punjab, India).

Design: For the present study, 78 Pre-M and Post-M women were recruited in four categories as follows: i) normal normotensive, ii) obese normotensive, iii) normal hypertensive, and iv) obese hypertensive. Body mass index was considered as the index of obesity. Guidelines given by JNC-VII were followed for the assessment of hypertension. Leptin was assayed by sandwich ELISA, and estradiol (E₂) was assayed by competitive ELISA.

Results: Leptin level was found to be significantly higher in normal Pre-M women ($P < 0.02$) than that of normal Post-M women. Obese subjects had significantly higher leptin level ($P < 0.001$) than the normal women. In the case of hypertensive subjects, leptin level was significantly higher than that of normotensive counterparts. E₂ level was found significantly lower in Post-M women ($P < 0.001$) than that of Pre-M women as well as in hypertensive women than that of normotensive subjects. A positive correlation was observed between blood pressure (BP) and leptin, but significant association was observed in hypertensive normal and obese Pre-M and Post-M women only.

Conclusion: It is concluded from the present findings that leptin contributes to the regulation of BP in hypertensive normal as well as in the obese Pre-M and Post-M women. So, leptin may be a regulator of BP in hypertensive women independent of the degree of obesity and the menopausal status.

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Introduction

Leptin is a key neuroendocrine hormone, which plays an important role in regulating food intake, metabolism, and fat accumulation, and it may also affect blood pressure (BP) and contribute to hypertension through sympathetic activation in the vasculature or at the renal level (1–3). Leptin's role in causing hypertension has been almost unapproached until leptin was discovered in 1994. Cross-sectional investigation in human subjects shows increasing leptin concentrations with rise in BP in both the normotensive (4) and hypertensive range (5, 6). The high risk of developing cardiovascular diseases in obese subjects has also drawn attention to study the neurohormonal effects of leptin on cardiac functioning and remodeling. A relationship between the circulating leptin level with hypertension and associated cardiovascular morbidity has been reported recently (3, 7, 8). Kramer *et al.* (9) also observed increased leptin levels among hypertensive elderly subjects and reported that higher leptin level increases 70% chances of risks for subsequent hypertension.

Leptin level has been shown to be higher in premenopausal (Pre-M) women than that of

postmenopausal (Post-M) women suggesting a stimulatory effect of estrogen and/or progesterone and an inhibitory action of androgens on leptin levels (10). Obese individuals for some unknown reasons become resistant to the appetite and weight-reducing effects of leptin. So, the level of leptin increases in obese subjects (11). The association between hypertension and obesity suggests that adipose tissue may play an important role in the regulation of BP (12).

The present study was designed to compare the serum leptin levels in normal and obese Pre-M and Post-M women of Jalandhar city (Punjab, India), and further the possible correlation between leptin level and BP was evaluated to find out whether serum leptin is a predictor of BP in the hypertensive women.

Subjects and methods

For the present cross-sectional study, data were collected from 78 educated working females in the age group of 30–60 years from Jalandhar city (Punjab, India). The study was approved by the Guru Nanak Dev University ethical review committee. The written

consent was obtained from the subjects after explaining the objectives as well as the methodology of the study. Each subject was personally interviewed to evaluate whether she corresponded to the study's criteria for menopausal status, hormone therapy, and other medication. Subjects who reported that they did not have their menses for at least 1 year were considered as Post-M (13) and were included in the study. Women who had regular menstrual periods at the time of examination were considered as Pre-M. Subjects undergoing hormone therapy were excluded from the study. Anthropometric measurements like height, weight, and skin folds (biceps, triceps, suprailiac, and subscapular) were taken with standard methods (14). Body mass index (BMI) was calculated as per criteria given by World Health Organization (WHO) and International Obesity Task Force (IOTF) (15). Subjects with BMI ≥ 25 kg/m² were considered as obese, and subjects with BMI 18.5–22.9 kg/m² were considered as normal (15). BP was measured using auscultatory technique with mercury sphygmomanometer as in normal clinical practice. The subjects were seated with arm supported at heart level, and BP was calculated twice with an interval of 5 min (16). The average systolic BP (SBP) and diastolic BP (DBP) measurements were calculated for each subject. Hypertension status of study sample was assessed using standard criteria formulated by JNC-VII (17). Subjects with SBP ≥ 140 mmHg and DBP ≥ 90 mmHg were considered as hypertensive. Fat mass (FM) was calculated from percent body fat according to the method devised by Durnin & Womersley (1974) (18).

Study design

Subjects were divided into two groups; Pre-M (39) and Post-M (39). They were further categorized as normal normotensive (9), obese normotensive (10), normal hypertensive (10), and obese hypertensive (10). Fasting blood samples were collected; serum was separated and stored in aliquots in the freezer. Leptin was estimated by sandwich ELISA, using Leptin ELISA Kit manufactured by Bio-Line, S.A., Brussels (Belgium). The intra-assay and inter-assay coefficients of variation were 3.6 and 5.2% respectively. Estradiol (E₂) was estimated by competitive ELISA using E₂ Kit manufactured by Adaltis Italia (Cassalechio di Reno, Italy). The intra-assay coefficient of variation was 4.8–7.2%, and inter-assay coefficient of variation was 5.4–9.6%. We assayed levels of leptin and E₂ in normal and obese Pre-M and Post-M hypertensive women and compared these values with their normotensive counterparts. Additionally, the influence of BMI, FM, and BP on leptin levels was evaluated.

Leptin assay

Leptin level was assayed by solid-phase sandwich enzyme immunoassay. The assay used MABs immobilized on

Table 1 Anthropometric variables of pre- (Pre-M) and postmenopausal (Post-M) women of studied group. The values are presented as mean $\pm$ s.d.

Variables	Pre-M				Post-M			
	Normal normotensive	Obese normotensive	Normal hypertensive	Obese hypertensive	Normal normotensive	Obese normotensive	Normal hypertensive	Obese hypertensive
<i>n</i>	9	10	10	10	9	10	10	10
Age (years)	43.99 $\pm$ 1.54	43.77 $\pm$ 5.45	44.25 $\pm$ 1.09	44.41 $\pm$ 2.13	52.78 $\pm$ 4.92 ^d	52.43 $\pm$ 1.75 ^d	52.50 $\pm$ 2.54 ^d	52.40 $\pm$ 1.60 ^d
Height (cm)	157.63 $\pm$ 4.99	152.64 $\pm$ 5.49	161.43 $\pm$ 6.29	155.64 $\pm$ 5.62	156.52 $\pm$ 2.46	154.94 $\pm$ 6.73	155.30 $\pm$ 4.39	156.11 $\pm$ 6.62
Weight (kg)	49.61 $\pm$ 2.95	67.80 $\pm$ 8.69 ^d	51.40 $\pm$ 2.91	77.13 $\pm$ 11.44 ^d	51.10 $\pm$ 3.86	73.50 $\pm$ 5.21 ^d	51.15 $\pm$ 2.13	74.00 $\pm$ 12.01 ^d
Body mass index (kg/m ²)	20.05 $\pm$ 1.52	29.01 $\pm$ 2.57 ^d	19.81 $\pm$ 1.33	31.84 $\pm$ 3.41 ^d	20.95 $\pm$ 1.39	30.74 $\pm$ 3.01 ^d	21.23 $\pm$ 0.75	30.41 $\pm$ 4.18 ^d
Fat mass (kg)	15.34 $\pm$ 2.97	28.33 $\pm$ 5.61 ^d	15.40 $\pm$ 2.56	31.79 $\pm$ 5.41 ^d	17.63 $\pm$ 3.34	30.64 $\pm$ 4.12 ^d	17.34 $\pm$ 3.37 ^c	32.25 $\pm$ 7.45 ^d

Data were analyzed using *t*-test where ^d is the comparison between Pre-M normal and obese normotensive and normal and obese hypertensive; ^c is the comparison between Pre-M and Post-M normal normotensive and Pre-M and Post-M normal hypertensive; ^d is the comparison between Pre-M and Post-M obese normotensive and Pre-M and Post-M obese hypertensive, and values indicated with those superscripts are significant at a level of *P* < 0.001.

microtiter plates to which the standards and the samples reacted. After an incubation period, the bound enzyme-labeled antibody was measured through a chromogen reaction. Absorbance was read at 450 nm, and leptin concentration was determined.

E₂ assay

E₂ was estimated by competitive type immunoassay where HRP-labeled E₂ competed with E₂ present in the patient sample for a fixed and limited number of antibody sites immobilized on the wall of the microstrip. After incubation, the absorbance of the solution was read at 450 nm.

Statistical analysis

Data were maintained on a questionnaire proforma and then managed on an excel spreadsheet. Data analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) for Windows. Results were presented as mean $\pm$ s.d. The differences in anthropometric, physiological, and biochemical variables between Pre-M and Post-M women were calculated using *t*-test. We used ANOVA method to analyze the comparison between the groups. Pearson's correlation was computed to observe the correlation of leptin with the different variables. Forward stepwise multiple linear regression analysis was performed to analyze the independent effects of predictor variables.

Results

Table 1 shows anthropometric characteristics of Pre-M and Post-M women. Pre-M women were of lower age than Post-M women, whereas both the groups were matching for height, weight, BMI, and FM in all the categories. Table 2 presents the physiological and biochemical variables of the studied groups. The mean value of SBP was found significantly higher in normotensive normal ($P < 0.02$) and obese ($P < 0.05$) as well as hypertensive normal ($P < 0.001$) Post-M women than that of Pre-M counterparts. Similarly, the mean value of DBP was found significantly higher in the hypertensive normal ($P < 0.001$) and obese ($P < 0.001$) Post-M subjects than that of Pre-M counterparts. Leptin level was found significantly higher in the obese Pre-M and Post-M women ($P < 0.001$) than that of the normal subjects. Furthermore, both normal and obese hypertensive Pre-M women showed significantly higher leptin level than normotensive counterparts ($P < 0.01$ and $P < 0.001$ respectively). Similar trend in the leptin level was observed in the Post-M women. Normal and obese hypertensive Post-M women had higher leptin levels than normotensive counterparts ($P < 0.01$ and $P < 0.05$ respectively). On comparison between Pre-M and Post-M women, the leptin level was found higher in the

Table 2 Physiological and biochemical variables of Pre-M and Post-M women of studied group. The values are presented as mean $\pm$ s.d.

Variables	Pre-M			Post-M		
	Normal normotensive	Obese normotensive	Normal hypertensive	Obese normotensive	Normal hypertensive	Obese hypertensive
<i>n</i>	9	10	10	10	10	10
SBP (mmHg)	104.55 $\pm$ 8.28	117.60 $\pm$ 10.40 ^c	145.10 $\pm$ 3.95 ^D	128.0 $\pm$ 8.74 ^{b, a''}	154.70 $\pm$ 6.44 ^{D, d''}	168.46 $\pm$ 9.21 ^{c, D'}
DBP (mmHg)	74.88 $\pm$ 6.97	80.00 $\pm$ 5.27	95.30 $\pm$ 4.99 ^D	80.40 $\pm$ 8.41	104.70 $\pm$ 3.59 ^{D, d''}	104.73 $\pm$ 6.48 ^{c, d''}
Leptin (ng/ml)	12.37 $\pm$ 3.71	40.93 $\pm$ 17.31 ^d	17.66 $\pm$ 3.52 ^C	40.40 $\pm$ 19.60 ^{d'}	11.94 $\pm$ 4.96 ^{c', C}	57.52 $\pm$ 16.66 ^{d', A'}
Estradiol (pg/ml)	137.17 $\pm$ 34.21	89.35 $\pm$ 4.12 ^a	129.92 $\pm$ 42.37	12.60 $\pm$ 7.51 ^{d, d''}	14.58 $\pm$ 3.81 ^{e, D}	13.23 $\pm$ 5.45 ^{a''}

Data were analyzed using *t*-test where ^{a, c, and d} are the comparison between Pre-M normal and obese normotensive and normal and obese hypertensive; ^{b, c', and d'} are the comparison between Pre-M and Post-M normal normotensive and Pre-M normal hypertensive; ^{a'', c'', and d''} are the comparison between Pre-M and Post-M obese normotensive and Pre-M obese hypertensive; ^{C and D} are the comparison between Pre-M normal normotensive and hypertensive and Post-M normal normotensive and hypertensive; ^{A, C', and D'} are the comparison between Pre-M obese normotensive and hypertensive and Post-M obese normotensive and hypertensive where ^a and ^A = $P < 0.05$; ^b and ^B = $P < 0.02$; ^c and ^C = $P < 0.01$; ^d and ^D = $P < 0.001$.

Table 3 Pearson's correlation of leptin with anthropometric, physiological, and biochemical variables in normotensive subjects.

Variables	Pre-M				Post-M			
	Normal		Obese		Normal		Obese	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
Age (years)	0.024	0.950	−0.330	0.352	−0.081	0.823	0.158	0.698
Height (cm)	−0.463	0.209	0.293	0.413	0.201	0.571	−0.208	0.592
Weight (kg)	0.679	0.044*	0.755	0.011*	0.905	0.000 [‡]	0.567	0.087
BMI (kg/m ²)	0.918	0.000 [‡]	0.870	0.000 [‡]	0.965	0.000 [‡]	0.882	0.000 [‡]
Fat mass (kg)	0.844	0.004 [†]	0.855	0.001 [‡]	0.970	0.001 [‡]	0.794	0.006 [†]
SBP (mmHg)	0.460	0.213	0.522	0.139	0.575	0.105	0.320	0.367
DBP (mmHg)	0.458	0.215	0.529	0.116	0.493	0.178	0.307	0.380
Estradiol (pg/ml)	0.871	0.002 [†]	0.429	0.216	0.737	0.023*	0.025	0.943

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure. * $P \leq 0.05$, $^{\dagger}P \leq 0.01$, $^{\ddagger}P \leq 0.001$.

normotensive as well as hypertensive Pre-M subjects than that of Post-M counterparts. E_2 level was significantly higher in the Pre-M women than that of the Post-M women ($P < 0.001$). In Pre-M group, E_2 level was significantly higher in the normal subjects than that of the obese women in both normotensive ($P < 0.001$) and hypertensive categories ($P < 0.05$).

We performed ANOVA to compare non-obese (BMI < 23.0) and obese (BMI ≥ 23.0) normotensive and hypertensive Pre-M and Post-M women. The models were highly significant among both Pre-M and Post-M groups. The *post hoc* analysis revealed significant differences for serum leptin and SBP levels between normotensive and hypertensive Pre-M (leptin, $F = 55.76$, $P < 0.001$; SBP, $F = 60.76$, $P < 0.001$) as well as Post-M women (leptin, $F = 30.82$, $P < 0.001$; SBP, $F = 93.78$, $P < 0.001$). Pearson's correlation revealed significant correlation of leptin with BMI and FM in all categories and BP in hypertensive subjects only (Tables 3 and 4). We, additionally, performed a forward stepwise multiple regression analysis using leptin as a dependent variable to evaluate the independent influence of BP on serum leptin concentration in the Pre-M and Post-M hypertensive subjects. We entered age, weight, height, BMI, FM, E_2 , SBP, and DBP in stepwise multiple linear regression models. The analysis revealed that there

was significant influence of SBP on leptin levels, even after controlling FM and BMI in hypertensive subjects ($\beta = 0.206$, $t = 4.99$, $P < 0.001$). No correlation was observed between E_2 level and hypertension in both the Pre-M and Post-M subjects.

Discussion

Higher leptin level in hypertensive than that of normotensive women shown in the present data clearly suggests a direct correlation between leptin level and hypertension among the Pre-M and Post-M subjects. These data are supported by the previous reports, but these studies have been carried out either in the obese Pre-M and Post-M subject (3) or in the lean Pre-M and Post-M women (19). Similarly, Haque *et al.* (20) reported correlation between leptin and BP in adult females, but this study did not take into account the menopausal status of the women. Furthermore, the present data clearly indicate that leptin level in the normotensive as well as hypertensive Post-M women is significantly lower than that of the Pre-M subject. Since these Pre-M and Post-M subjects had matching values for BMI and FM, so the decline in leptin levels may be attributed to the menopausal status. Recent

Table 4 Pearson's correlation of leptin with anthropometric, physiological, and biochemical variables in hypertensive subjects.

Variables	Pre-M				Post-M			
	Normal		Obese		Normal		Obese	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
Age (years)	0.754	0.012*	0.084	0.804	−0.155	0.670	−0.026	0.942
Height (cm)	−0.500	0.141	0.651	0.033*	−0.077	0.831	−0.111	0.761
Weight (kg)	0.144	0.690	0.841	0.000 [†]	0.656	0.039*	0.700	0.024*
BMI (kg/m ²)	0.718	0.019*	0.723	0.011 [†]	0.956	0.000 [‡]	0.870	0.000 [‡]
Fat mass (kg)	0.663	0.034*	0.837	0.000 [‡]	0.760	0.010 [†]	0.896	0.000 [‡]
SBP (mmHg)	0.708	0.020 [†]	0.944	0.000 [‡]	0.945	0.000 [‡]	0.763	0.010 [†]
DBP (mmHg)	0.829	0.003 [†]	0.789	0.007 [‡]	0.869	0.000 [‡]	0.881	0.000 [‡]
Estradiol (pg/ml)	0.487	0.602	0.242	0.747	0.876	0.000 [‡]	0.258	0.472

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure. * $P \leq 0.05$, $^{\dagger}P \leq 0.01$, $^{\ddagger}P \leq 0.001$.

studies (6, 21) reported that leptin levels are associated with increased risk for hypertension in subjects who were adjusted for age, BMI, insulin, and creatinine, etc. Chronic hyperleptinemia has been shown to enhance sympathetic nervous activity and reduces nitric oxide-dependent vasodilation and natriuresis (22).

The previous studies have reported that leptin stimulates renin–angiotensin (23, 24) and sympathetic system (12), which may affect BP level in humans. Leptin also stimulates natriuresis. So, it is possible that a blunted effect of leptin may predispose to hypertension in humans (25, 26). Scientists have observed the relationship between serum leptin and heart beat in hypertensive subjects (27, 28). Role of leptin in causing cardiovascular diseases and hypertension has also been reported by the previous studies (29–31). Recently, Patel *et al.* (7) also reported positive association between leptin and DBP. The relationship between leptin and hypertension was also studied by Sabatier *et al.* (8), and their results illustrated that leptin-mediated actions may be diminished not only by reducing leptin-producing adipose tissue, but also by increasing physical activity in individuals with high levels of adiposity. Another recent study (9) has reported increased leptin levels among hypertensive elderly subjects.

Fall in E_2 level followed by decreased leptin level in the normotensive as well as hypertensive Post-M women as compared to the Pre-M subjects suggests that E_2 may be involved in controlling leptin level, and fall in E_2 level at menopause may cause drop in the level of leptin in Post-M women. A link has been suggested between E_2 and leptin levels in women (32). A significant increase in the serum leptin concentrations on the 10th, 17th, and 24th day during the menstrual cycle suggested a possible role for estrogen in the stimulation of leptin secretion (33). However, there are contradictory findings regarding the relationship between E_2 and leptin concentration. Di Carlo *et al.* (34) observed an increased serum leptin level in the untreated Post-M women as compared to the Pre-M subjects. The relationship between leptin and E_2 in obese subjects has not been established. So, the possible stimulatory action of E_2 on leptin needs to be confirmed by further studies.

The major finding of the present study is the correlation between leptin and BP in hypertensive Pre-M and Post-M women. The present study is supported by the findings of Kennedy *et al.* (35) who demonstrated the relationship between elevated SBP and DBP and plasma leptin levels in hypertensive men, whereas Suter *et al.* (27) reported a significant relationship between SBP and plasma leptin levels in hypertensive women but not in hypertensive men. Correlation of leptin with BMI and FM has also been reported earlier (10, 36, 37).

Based on the significant correlation observed between BP and leptin level in the hypertensive subjects and not in normotensive subjects, it is suggested that leptin may be a regulator of BP in hypertensive subjects only. The association between leptin and BP seems to be

independent of some potentially important variables such as BMI, FM, and menopausal status. These findings may further contribute to understanding the association between leptin levels and hypertension, a major factor that causes cardiovascular complications.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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