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Relationship between serum leptin and type 2 diabetes mellitus and their association with obesity and menopausal status

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ABSTRACT

The present study was aimed to evaluate the role of leptin in the etiology of type 2 diabetes mellitus in women with respect to obesity and their menopausal status. 595 pre and postmenopausal women were recruited for the study and out of them, 40 pre- and 40 postmenopausal women were selected for biochemical analysis. Each group was divided into four categories as; non-obese control, obese control, non-obese diabetic, and obese diabetic with 10 subjects in each group. BMI was calculated and diabetic status was determined following WHO guidelines. Leptin, estradiol and insulin levels were assessed and Lipid estimation was performed as per standardized methods. Degree of dyslipidemia was higher in obese as compared to non-obese, diabetic as compared to control, and Post-M women as compared to Pre-M women. Obese subjects had significantly higher leptin and insulin levels as compared to non-obese subjects. On the other hand, diabetic Pre- and Post-M subjects had lower leptin and insulin levels as compared to their control counterparts. Higher degree of dyslipidemia and reduced leptin level coupled with reduced insulin level observed in diabetic pre- and postmenopausal women as compared to their control counterparts suggests some link between leptin, dyslipidemia and insulin and its possible association with diabetes.

Key words: Dyslipidemia, Type 2 Diabetes, Leptin, Menopause

INTRODUCTION

Type 2 diabetes mellitus (T2DM), commonly known as an obesity related metabolic disorder, is rapidly emerging as a global health care problem that threatens to reach pandemic levels in a short span of time. Shaw *et al.* [1] have estimated that the world prevalence of diabetes will be affecting 439 million adults by 2030. In India, considered to be the *Diabetic capital of the World*, the situation is all the more critical. Furthermore, the prevalence has been observed higher among women, especially the postmenopausal women [2].

Among the various factors implicated in the etiology of this disease, the role of leptin- *the obesity gene product*, is increasingly being recognized. Obesity, a state of hyperleptinemia, confers a minimum three to ten fold higher risk of T2DM [3,4]. These findings draw attention to the possible role of leptin in the etiology of T2DM. Furthermore, obesity is also associated with insulin resistance and hyperinsulinemia. It has been reported that Insulin and leptin share a common central signaling pathway [5].

Clinical, *in vitro* and animal studies showed that leptin and insulin are highly correlated with each other. Leptin has been shown to improve insulin sensitivity and glucose metabolism in leptin treated rats [6] and a similar response has been reported in human subjects [4].

Menopause, a milestone in a women's life has also been reported to influence the levels of insulin and leptin. Rosenbaum *et al* [7] reported that the leptin concentration was 33% less in postmenopausal than in premenopausal women. Similarly, in our recent study [8], we also observed lower leptin levels in postmenopausal women although the differences were significant in case of non-obese subjects only. The fluctuations in leptin levels during the menstrual cycle observed by Mannucci *et al*.[9] also support the role of estradiol in the regulation of leptin secretions. Menopause, accompanied by dyslipidemia, hyperinsulinaemia and hyperleptinaemia has been reported in literature [10].

The menopausal status is also known to further enhance the incidence of obesity and T2DM. Estradiol, the main female hormone which acts as a shield in Pre-M women is also held responsible for the higher occurrence of diabetes among Post-M women [3,11]. Although many previous studies have reported association between obesity and T2DM but the relationship between obesity hormone 'leptin' and T2DM is still controversial [12,13]. The regulation of leptin by estradiol in human has also been a topic of debate.

In our previous findings we observed the association between leptin and hypertension. It was further observed that non-obese postmenopausal women had lower levels of leptin as compared to their premenopausal counterparts. Higher leptin levels among obese pre- and postmenopausal subjects pointed towards leptin resistance irrespective of the menopausal status [8]. The present study was planned to investigate the possible association of leptin with T2DM. Since T2DM is also associated with obesity and dyslipidemia, and higher degree of obesity and dyslipidemia has been reported in post-menopausal women [10,14], it was further planned to find out if relationship between leptin and T2DM affects differently in non-obese and obese pre- and postmenopausal women.

MATERIALS AND METHODS

For the present cross-sectional study, a total of 595 subjects (330-Pre-M; 265-Post-M) in the age group 30-60 years working in various hospitals and educational institutes of Jalandhar (Punjab, India) were recruited for anthropometric measurements and biochemical studies. The written consent was obtained from the subjects. The study was approved by the Guru Nanak Dev University Ethical Review Committee. The menopausal status was assessed as per WHO [15] guidelines. Anthropometric measurements like weight and height were taken with standard methods [16]. BMI was calculated and the subjects were divided into non-obese and obese categories [17]. Status of diabetes was assessed as per WHO recommendations [18]. Blood samples were collected, serum was separated and stored in the freezer till biochemical analysis.

Study Design

Pre-M and Post-M women were further categorized as; non-obese control, obese control, non-obese diabetic, and obese diabetic with 10 subjects in each group. The term 'control' here refers to 'non-diabetic'. Lipid profile was assessed by kit manufactured by Crest Biosystems, Goa, (India). Total serum Cholesterol (TC), triglycerides (TG) and lipoproteins; heavy density lipoproteins (HDL-C) and low density lipoproteins (LDL-C) were estimated. The absorbance in each case was measured with semi-autoanalyser RA-50 (Bayer India Limited). TC was determined by enzymatic (CHOD-PAP) colorimetric method [19] and TG by enzymatic (GPO-PAP) method [20]. HDL-C was estimated by precipitation method [21] and LDL-C by Friedewald formula [22]. Leptin and insulin were estimated by sandwich ELISA, using Leptin ELISA and INS-ELISA Kits, respectively manufactured by Bio-Line, S.A., Brussels (Belgium). The intra-assay and inter-assay coefficients of variation in case of leptin were 3.6 and 5.2%, respectively, and in case of insulin these values were 3.0-5.3% and 4.5-9.5%, respectively. Estradiol was estimated by competitive ELISA using Estradiol Kit manufactured by Adaltis Italia (Italy). The intra-assay coefficient of variation was 4.8–7.2%, and inter-assay coefficient of variation was 5.4–9.6%.

Statistical Analysis

Data was maintained on excel spread sheet. Analysis was performed using SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA)) version 16 for windows. Results were presented as mean± S.D. The differences in anthropometric, physiological, and biochemical variables between Pre-M and Post-M women were calculated with't-test'. ANOVA method was used to analyze the comparison between the groups. Pearson's

correlation was computed to observe the correlation of leptin with different variables. Forward stepwise multiple linear regression analysis was performed to analyze the independent effects of predictor variables.

RESULTS

The prevalence of T2DM in the pooled sample (595 subjects) was observed as 11.26% (N=67). It was further observed that the prevalence of T2DM was 6.36% higher among obese subjects as compared to their non-obese counterparts (4.02% more in obese Pre-M and 7.14% more in obese Post-M women). As compared to Pre-M subjects, the occurrence of diabetes was 6.24% higher in Post-M women (2.24% in non-obese Post-M and 6.36% in obese Post-M women) (data not shown). **Table 1** presents the anthropometric and physiological variables of Pre- and Post-M women groups enrolled for the biochemical study. Post-M women were significantly of higher age ($p<0.001$) but almost matching for weight, height and BMI to Pre-M women in all the categories. Obese Pre- and Post-M women had higher mean values of weight ($p<0.001$), BMI ($p<0.001$) and blood glucose as compared to their non-obese counterparts. Furthermore, the level of glucose was significantly higher in diabetic Pre- and Post-M subjects as compared to their control counterparts ($p<0.001$).

Table I Anthropometric and Physiological variables of pre- (Pre-M) and postmenopausal (Post-M) women of studied group

Variable	Pre-M				Post-M			
	Non-Obese Control	Obese Control	Non-Obese Diabetic	Obese Diabetic	Non-Obese Control	Obese Control	Non-Obese Diabetic	Obese Diabetic
Age (Yrs.)	43.99±1.54*	43.77±5.45*	42.29±2.95	45.40±2.02^{b}	52.78±4.92*$^{d'}$	52.43±1.75*$^{d'}$	55.80±1.81$^{d'}$	54.61±3.49$^{d'}$
Height (cm)	157.63±4.99	152.64±5.49	160.55±4.85	153.17±5.92^{c}	156.52±2.46	154.94±6.73	156.32±3.43	153.42-6.33
Weight (kg)	49.61±2.95	67.80±8.69 ^{d}	50.70±1.90	76.50±6.63^{d}	51.10±3.86	73.50±5.21 $^{d'}$	50.40±3.09	69.21-8.71$^{d'}$
BMI (kg/m^2)	20.05±1.52*	29.01±2.57*	19.73±1.13	32.86±4.34^{d}	20.95±1.39*	30.74±3.01* $^{d'}$	20.69±1.54	29.44±3.09$^{d'}$
Glucose (mg/dl)	91.33±9.14	98.00±10.57	193.30±17.95^{D}	196.20±13.59$^{D'}$	114.67±11.92	121.70±11.02	195.20±14.5^{D}	215.27±16.84$^{D'}$

The values are mean±S.D. Data were analyzed using t-test where symbols (b), (c), and (d) represent the comparison between Pre-M non-obese and obese control as well as diabetic subjects; (d') is the comparison between Post-M non-obese and obese control as well as diabetic women; (d'') is the comparison between Pre-M and Post-M non-obese control and diabetic subjects; (d''') represents the comparison between Pre-M and Post-M obese control and diabetic subjects; (D) is the comparison between Pre-M non-obese control and diabetic as well as Post-M non-obese control and diabetic subjects, whereas (D'), is the comparison between Pre-M obese control and diabetic as well as Post-M obese control and diabetic women where 'a' =P<0.05; 'b' =P<0.02; 'c' =P<0.01; 'd' and 'D'=P<0.001

*= These mean values of age and BMI are of control pre- and postmenopausal women. 'Control' here means non-diabetic. These are also normotensive and these values have been used to compare with hypertensive subjects in our another finding (Khokhar et al.,2010)

Table 2 depicts the biochemical variables of the same Pre- and Post-M groups. Leptin levels were significantly higher in obese control as well as diabetic Pre- and Post-M women as compared to their non-obese counterparts ($p<0.001$). Whereas, diabetic subjects had lower leptin levels as compared to their control counterparts in both the groups. In comparison to Pre-M, Post-M women also had lower leptin levels but the differences were statistically significant in case of non-obese control ($p<0.02$) and diabetic subjects ($p<0.001$).

Higher mean values of insulin were observed in obese control and diabetic subjects as compared to non-obese women in both the groups and the differences were statistically significant except in case of diabetic Post-M women. Lower insulin levels were observed in diabetic non-obese and obese women as compared to controls in both the groups but the differences were not statistically significant. On comparison between Pre- and Post-M women, slightly higher levels of insulin were observed in Post-M women and the differences were not statistically significant.

Estradiol levels were significantly lower in obese control as well as diabetic Pre- and Post-M women as compared to their non-obese counterparts. Diabetic subjects had still lower estradiol levels as compared to controls in both the groups. In comparison to Pre- M women, estradiol levels were significantly lower ($p<0.001$) in non-obese and obese control as well as diabetic Post-M women.

Higher mean values of non-friendly TC, TG, LDL-C and lower mean value of friendly HDL-C was observed in obese control and diabetic subjects as compared to their non-obese counterparts in both Pre- and Post-M groups. Diabetic non-obese and obese subjects had still

Table II Biochemical variables of pre- (Pre-M) and postmenopausal (Post-M) women of studied group

Variable	Pre-M				Post-M			
	Non-Obese Control	Obese Control	Non-Obese Diabetic	Obese Diabetic	Non-Obese Control	Obese Control	Non-Obese Diabetic	Obese Diabetic
Leptin (ng/ml)	12.37±3.71*	40.93±17.31*	10.74±5.21	31.52±3.28^{d}	6.69±4.75*$^{b'}$	40.40±19.60* $^{d'}$	4.75±1.43$^{d''}$	29.49±7.79$^{d'}$
Insulin(μIU/ml)	11.44±7.63	19.54±7.86^{a}	9.44±5.52	16.66±8.78^{a}	13.94±5.20	20.69±5.99$^{a'}$	12.96±5.33	20.13±11.78
Estradiol(pg/ml)	137.17±34.21*	89.35±4.12*^{d}	96.70±20.18^{C}	72.66±28.18^{a}	22.67±6.21*$^{d''}$	12.60±7.51* $^{d', d'''}$	20.20±4.11$^{d''}$	11.65±4.03 $^{d',d'''}$
TC (mg/dl)	152.59±20.76	201.57±30.28^{d}	178.82±15.18^{C}	201.03±20.65^{a}	168.21±17.50$^{a''}$	205.70±33.88$^{b'}$	203.06±24.82$^{a''C}$	215.63±60.62
TG (mg/dl)	90.12±17.98	131.20±49.70^{a}	114.60±19.53^{C}	148.54±30.32$^{bC'}$	107.09±29.26	142.67±18.20$^{b'}$	132.93±23.74$^{a''B}$	156.17±48.64
LDL-C (mg/dl)	73.17±12.13	135.89±25.54^{d}	76.90±16.76	129.70±26.46^{c}	104.82±20.69$^{c''}$	146.71±32.75$^{b'}$	142.56±17.69$^{c''C}$	149.40±28.75
HDL-C (mg/dl)	62.03±13.44	43.15±9.71^{b}	45.02±8.61^{C}	40.38±7.47	46.37±5.02 $^{d''}$	40.48±10.62	32.60±4.99$^{c''D}$	33.76±6.14$^{a''}$

TC-total cholesterol, TG-triglycerides, LDL-C-low density lipoproteins cholesterol, HDL-C-high density lipoproteins cholesterol

Digits in parentheses are the number of subjects. The values are mean±S.D. Data were analyzed using t-test where symbols (a), (b), (c), and (d) represent the comparison between Pre-M non-obese and obese control as well as diabetic subjects; symbols (a'), (b') and (d') represent the comparison between Post-M non-obese and obese control as well as diabetic subjects; (a''), (b''), (c''), and (d'') symbolize the comparison between Pre-M and Post-M non-obese control as well as diabetic; (d''') and (a''') stand for the comparison between Pre-M and Post-M obese control and diabetic; symbols (B), (C) and (D) signify the comparison between Pre-M non-obese control and diabetic and Post-M non-obese control and diabetic; (C') is the comparison between Pre-M obese control and diabetic where, 'a' =P<0.05; 'b' and 'B' =P<0.02; 'c' and 'C' =P<0.01; 'd' and 'D'=P<0.001

*= These mean values of leptin and estradiol are of control pre- and postmenopausal women. 'Control' here means non-diabetic. These are also normotensive and these values have been used to compare with hypertensive subjects in our another finding (Khokhar et al.,2010)

higher mean values of TC, TG, LDL-C and lower mean value of HDL-C as compared to controls in both Pre- and Post-M groups but significant differences were observed in non-obese subjects.

As compared to their Pre-M counterparts, non-obese control and diabetic Post-M women had significantly higher mean values of TC (p<0.05 in both), LDL-C (p< 0.01 in both) and lower mean value of HDL-C (p<0.001 and p<0.01 in non-obese control and diabetic, respectively). Obese control and diabetic Post-M subjects also had higher mean values of TC, TG, LDL-C and lower levels of HDL-C as compared to their Pre-M counterparts but the differences were statistically significant in case of HDL-C (p<0.05) in obese diabetic subjects only.

ANOVA was performed to compare non-obese and obese control and diabetic Pre-M and Post-M women. The models proved to be highly significant in both Pre-M and Post-M groups. The analysis revealed significant differences for serum leptin and insulin levels in non-obese and obese control as well as diabetic Pre-M women (leptin, F=26.01, P<0.001; Insulin, F=3.72, P<0.001). In post-M women, significant differences in non-obese and obese control as well as diabetic subjects were observed in case of leptin only (F=22.81, P<0.001). On comparison between Pre-M and Post-M women for leptin and insulin levels in different categories, the model again proved highly significant (Leptin, F=20.96, p<0.001; Insulin, F=3.09, p<0.01). Pearson's correlation in Tables 3 and 4 reveal significant correlation of leptin with BMI, insulin and lipid profile in control and diabetic Pre- and Post-M women. We additionally performed a forward step wise multiple regression analysis to evaluate the influence of insulin on leptin. The analysis revealed that there was significant influence of insulin on leptin levels even after controlling for their BMIs in control (β= 2.16, t=7.53, p<0.001) as well as diabetic (β= 0.851, t= 4.69, p<0.001) subjects when Pre- and Post-M groups were combined together.

DISCUSSION

Although, Pre-M and Post-M women chosen for the biochemical study were matched for their BMI values (Table I), the degree of dyslipidemia and reduction in leptin level was more pronounced in Post-M women. In one of our previous study [23], we observed that the prevalence of overweight and obesity was significantly higher in Post-M women as compared to their Pre-M counterparts.

It was observed that leptin level in non-obese and obese diabetic Pre-M women was lower than their control counterparts, whereas, in Post-M women, this decrease was significantly higher than Pre-M women. Overall, the degree of dyslipidemia was higher in Post-M women as compared to Pre-M women.

The current data suggests that reduced leptin levels in diabetic subjects might have led to hyperlipidemia and hyperglycemia in diabetic Pre- and Post-M subjects as compared to control women. Further higher degree of dyslipidemia in diabetic Post-M women as compared to diabetic Pre-M women may be associated with the menopausal status of the former. The association between lipid profile and leptin has been reported in literature. In animal studies, leptin deficiency has been observed associated with hyperglycemia, insulin resistance and hyperlipidemia and leptin administration improved hyperglycemia, insulin resistance and levels of LDL-C, HDL-C and hypertriglyceridemia [24]. The underlying mechanisms are still not clear, but these findings reflect the possible association of leptin with lipid profile and playing a role in the etiology of T2DM.

Insulin level is considered as the marker to assess diabetes. In present studies, we observed the correlation between leptin and insulin (Table III and IV) and the association between leptin and insulin sensitivity has been reported in literature also [25].

Table III Pearson's correlation of Leptin with Anthropometric and Biochemical variables in Control subjects

Variable	Premenopausal women		Postmenopausal women	
	Non-Obese	Obese	Non-Obese	Obese
Age	**0.024**	**-0.330**	**-0.081**	**0.158**
BMI	0.918***	0.870***	0.965***	0.882***
Insulin	0.840**	0.939***	0.946***	0.803**
TC	0.835***	0.852***	0764*	0.852***
TG	0.539	0.607	0.559	0.707*
LDL-C	0.876***	0.703*	0.621	0.793*
HDL-C	-0.767*	-0.735*	-0.243	-0.623

BMI-Body mass index, TC-total cholesterol, TG-triglycerides, LDL-C-low density lipoproteins cholesterol, HDL-C-high density lipoproteins cholesterol

****=p<0.001; **=P<0.01, *=p<0.05*

Lower leptin and insulin levels observed in diabetic pre- and postmenopausal women as compared to control subjects in the present study further suggests the association between the two and the possible role in the occurrence of diabetes among Pre-M- and Post-M women. The data is supported by the previous findings, but these studies have either been carried out only in obese women or in obese men and women or in non-obese women only [11,12]. None of these studies took into account the menopausal status of the women in one study.

Table IV Pearson's correlation of Leptin with Anthropometric and Biochemical variables in Diabetic subjects

Variable	Premenopausal women		Postmenopausal women	
	Non-Obese	Obese	Non-Obese	Obese
Age	**0.209**	**0.663**	**0.529**	**0.444**
BMI	0.892***	0.925***	0.754*	0.843***
Insulin	0.803*	0.953***	0.749*	0.797*
TC	0.729*	0.937***	0.773*	0.661*
TG	0.871**	0.831**	0.831**	0.700*
LDL-C	0.853***	0.760*	737**	667*
HDL-C	-0.793**	-0.678*	-0.792*	-0.593

BMI-Body mass index, TC-total cholesterol, TG-triglycerides, LDL-C-low density lipoproteins cholesterol, HDL-C-high density lipoproteins cholesterol

****=p<0.001; **=P<0.01, *=p<0.05*

There are conflicting reports in literature on the role of leptin in causing diabetes. The differences among different reports could possible be due to different anthropometric variables studied and the gender of the diabetic subjects. Present findings are supported by a recent study conducted by Ghafoor *et al*. [26] reporting lower leptin levels in diabetic subjects as compared to controls who were matched for their BMIs. A possible explanation of lower leptin levels in diabetic subjects in the present study may be due to the altered body fat distribution or to relative insulin deficiency in diabetes. Subjects with diabetes have increased visceral fat and less subcutaneous fat and furthermore, visceral fat produces less leptin than subcutaneous fat [27]. Subjects with diabetes, therefore, would be expected to have lower circulating leptin than BMI matched controls as observed in present and other studies [11,12]. Another

possibility of lower leptin levels in diabetic subjects can be attributed to the insulin level as the latter was also found to be lower in diabetic subjects as compared to controls. Previous studies have reported that insulin is an important stimulator of leptin production. A decrease in the number of functional insulin-producing beta-cells occurs in diabetic subjects. As a result, insufficient insulin is produced by beta-cells, lowering insulin level in T2DM subjects which further contributes to the pathophysiology of T2DM [28]. Although these two hormones, and the receptors on which they act, are unrelated and structurally distinct, they exert overlapping effects in the arcuate nucleus, a key hypothalamic area involved in energy homeostasis. Defects in either insulin or leptin signaling in the brain result in hyperphagia, disordered glucose homeostasis, and reproductive dysfunction [29].

On comparison between Pre-M and Post-M women, lower leptin levels and higher insulin levels were observed in control as well as diabetic Post-M women as compared to their Pre-M counterparts. As the Pre- and Post-M women were matched for their BMIs in all the categories while choosing for the study, the present difference in their leptin and insulin levels may be attributed to their menopausal status. In the present study, lower estradiol levels were observed in Post-M women as compared to their Pre-M counterparts, as expected. A link has been suggested between estradiol and leptin levels in women [7,8]. A significant difference in fasting serum leptin levels was noted between non-obese pre- and postmenopausal women groups ($P<0.001$), with the premenopausal group having a significantly higher mean serum leptin level as compared with post menopausal group [30]. Estrogen increases *in vivo* leptin production in rats and humans [31]. Similarly, a recent study [32] conducted on Tunisian women has also reported higher leptin levels in premenopausal women as compared to their postmenopausal counterparts and suggested that menopausal status is a predictor of leptin level whereas, others did not find such an association [33,34,35]. So, the relationship between estradiol and leptin has not been established and needs further exploration. Higher insulin levels among Post-M women may be due to insulin resistance in these subjects. The effect of estradiol on insulin regulation is also well known [36]. A significant decline in insulin resistance after estrogen therapy in Post-M women has also been reported [37].

Higher degree of dyslipidemia observed in Post-M women as compared to their Pre-M counterparts may be attributed to the decline in their estradiol levels. Further lower estradiol levels in diabetic Pre-M and Post-M women as compared to their control counterparts in the present study suggest the protective role of estradiol in non-diabetic subjects. Based on the current observations, It may be suggested that fall in estradiol level in diabetic subjects as compared to controls might have led to the deterioration of lipid profile. Menopause, which is associated with significant decline in estradiol level may be associated with alteration in leptin level and worsening of lipid profile in Post-M women. Postmenopausal women are older than premenopausal women. Inverse relationship between leptin and aging has also been reported in literature [38].

Although obesity and menopause is observed to be associated with higher prevalence of T2DM but the association among leptin, insulin, estradiol and lipid profile in diabetic subjects needs further investigation which may help the clinicians to suggest the safe and effective preventive measures for reducing the incidence of T2DM in Post-M women. To the best of our knowledge, this is a first study of its kind where leptin's role in diabetes has been studied with respect to the menopausal status of the women.

Limitation of the study

We feel that we made so many sub groups which led to little sample size in each group. This may be considered as the limitation of this study.

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