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Association of Serum Leptin with Beta Cell Dysfunction and Insulin Resistance among Different Subgroups of Prediabetes

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Abstract

Background: Accumulating evidence indicates that various adipokines released from adipose tissue have been involved in abnormal insulin signalling in obesity and type 2 diabetes. However, it is not entirely clear whether these alterations in serum adipocyte concentrations are already present in the prediabetic state. The present study was designed to test the hypothesis that elevated levels of serum lepin an index of insulin sensitivity is independently associated with insulinemic indices among different subgroups of prediabetes.

Materials: Under an observational cross-sectional design a total of 116 Control subjects (M/F, 58/58) and 99 prediabetic subjects (55/44) consisting of 49 Impaired Fasting Glucose (IFG) and 50 Impaired Glucose Tolerance (IGT) were investigated. Serum glucose was measured by glucose-oxidase method. Serum insulin and leptin were measured by ELISA techniques. Insulin secretory function (HOMA%B), insulin sensitivity (HOMA%S) and Homeostasis Model Assessment-insulin resistance (HOMA-IR) were calculated from Homeostasis Model Assessment (HOMA).

Results: Compared to the Control, IFG and IGT subjects had significantly higher levels of serum leptin (ng/mL) ($p<0.001$) and HOMA-IR ($p=0.001$) respectively. However, compared to their Control counterparts, IFG and IGT subjects had significantly lower levels of HOMA%B ($p=0.001$) and HOMA%S ($p=0.010$). On binary logistic regression analysis, serum leptin [OR (95% CI): 1.074 (1.019-1.131), $p=0.007$] and reduced HOMA%S [OR (95% CI): 0.972 (0.950-0.995), $p=0.015$] were found to be significant determinants of IGT group after adjusting the effects of WC and TG. In the same analysis, serum leptin [1.109 (1.054-1.167), $p<0.001$] and reduced HOMA%B [0.966 (0.951-0.981), $p<0.001$] were found to be significant predictors of IFG group after adjusting the effects of WC and TG.

Conclusion: Elevated levels of serum leptin may have an association with the state of IFG and IGT of pre-diabetes and this association, in turn, is mediated by insulin secretory dysfunction and reduced insulin sensitivity during this disorder.

Keywords: Leptin; Prediabetes; IFG; IGT; HOMA%B; HOMA%S; HOMA-IR

Introduction

Prediabetes the prior state of type 2 diabetes mellitus is a hyperglycemic condition that is characterized by Impaired Fasting Glycaemia (IFG), Impaired Glucose Tolerance (IGT) and combination of both defects (IFG-IGT) [1]. Prior clinical and epidemiological studies reveal both insulin resistance and insulin secretory dysfunction considered as the pathophysiologic determinants of hyperglycemia among prediabetes [2,3]. Obesity in association with insulin resistance and the components of metabolic syndrome manifests as the causative factors of prediabetes [4].

Adipose tissue in addition to its pathological and physiological action of the body it provides a link between obesity and insulin resistance where it acts as an active endocrine and paracrine organ, secreting a large number of biologically active compounds that involved in the metabolic homeostasis, collectively called adipokines [5]. Leptin, a 167 amino acid containing adipokine derived from adipocyte, which under normal physiological conditions regulate feeding behavior, energy expenditure, adipose tissue mass, facilitate glucose utilization and alters insulin secretion [6]. It plays a functional role in glucose homeostasis through its effects on the synthesis of insulin which also the risk factors for the development of prediabetes. Leptin inhibits the synthesis of insulin by inhibiting the pre-proinsulin mRNA expression in β cells. By activation of ATP-sensitive potassium channels leptin reduces the secretion of insulin from pancreatic β cells leading to the development of type 2 DM.

The exact mechanism whereby elevated levels of serum leptin predispose to prediabetes is still unknown. During obesity, lipotoxicity occurs in the extrahepatic tissues which in turn suppress the responsiveness of non-adipose tissues to the insulin thereby inhibiting the glucose uptake and its subsequent disposal into the hepatocytes. It was suggested that insulin resistance to leptin in the β-cells during glucose intolerant state, might prevent the inhibitory effect of leptin on insulin secretion resulting in hyperinsulinemia, which might exhaust pancreatic β-cells leading to the development of hyperglycemia.

Wang et al. [7] on 574 Chinese subjects found a relationship of plasma leptin with insulin resistance and prediabetes independent of adiposity demonstrating plasma leptin levels as a useful biomarker for screening of prediabetes in this population. However, it remains controversial in the relationship between hyperleptinemia and hyperinsulinemia independent of body fat. When adipose tissue mass increases insulin stimulates the production of leptin; while leptin, *via* its negative feedback, decreases the secretion of insulin, leading to glucose uptake for energy utilization or storage [8,9]. To date, very few data have been examined to see the association of serum leptin with insulinemic indices among different subgroups of prediabetes and to our knowledge, there is no available data in Bangladeshi population to see the relationship of serum leptin with the pathophysiological determinants of different categories of prediabetes.

Materials and Methods

The study subjects were recruited from the OPD of Bangladesh Institute of Health Sciences General Hospital, Dhaka, Bangladesh where the participants came to diagnose their metabolic evaluation over a period of one year (April 2012 to June 2013). Ethical approval was approved from the local ethics committee of the Diabetic Association of Bangladesh (DAB) (BADAS, Ref no: BADAS-ERC/13/00106). After taking a brief history, the preliminary selection was done, and the purpose of the study was explained in details to each subject and their verbal consent was taken. Blood samples were collected from 215 (two hundred and fifteen) consecutive subjects consisting of 116 Control (M/F, 58/58), 49 Impaired Fasting Glycemia (IFG) (33/16) and 50 Impaired Glucose Tolerance (IGT) (22/28) respectively. The confirmation of normoglycemia, IFG, IGT, and Diabetes were based on 2006 WHO recommendations for the diagnostic criteria for diabetes and intermediate hyperglycemia [10]. Subjects who had any acute and chronic illness of hepatic, cardiac and renal dysfunction, alcoholism, malignancy, treated with glucose and lipid lowering drugs, and pregnant subjects were excluded.

A predesigned case record form was used to record relevant clinical, medical, demographic and socio-economic data such as age, sex, educational status, and occupational status from the consenting subjects. Anthropometric and clinical assessments included measurements of waist and hip circumference, weight, height and systolic and diastolic blood pressure were recorded by standard procedures. Body Mass Index (BMI) was calculated by dividing weight (kg) by height (m) squared. After 8-10 hours overnight fasting, a total of ~6.00 mL venous blood was drawn from the participants under aseptic conditions for measurements of serum glucose, lipid profile (cholesterol, triglyceride, and HDL-cholesterol), liver enzyme (alanine aminotransferase), creatinine, insulin, and leptin respectively. For measurement of OGTT, study subjects allowed to drink glucose solution (75 g of anhydrous glucose are dissolved in 250 to 300 mL of water) and to avoid the test result interruption subjects were requested to be in a resting condition until the second blood was drawn.

After 2 hours of glucose intake, the second draw of a blood sample (~3.00 mL venous blood) was taken for measurement of postprandial serum glucose and insulin. Blood samples were maintained at 40 c until separation by centrifugation and serum were frozen at -30°C within an hour of sample collection. For measurement of HbA1c, ~2 mL whole blood was taken in EDTA vial. Fasting and postprandial serum glucose was measured by glucose oxidase (GOD/PAP method/GLUC Flex reagent cartridge, Cat No: DF 40), total cholesterol (enzymatic endpoint method (CHOD-PAP)/CHOL Flex reagent cartridge, Cat No: DF 27), triglyceride (GPO-PAP method/TGL Flex reagent cartridge, Cat No: DF 69A), and HDL-cholesterol (AHDL method/HDL Flex reagent cartridge, Cat No: DF 488), creatinine, and alanine aminotransferase (enzymatic colorimetric method) and the test analysis was done using a conventional automated analyzer (Dimension RxL clinical chemistry system, Siemens Healthcare Diagnostics Inc. USA). LDL-cholesterol was calculated by the Friedwald's formula [11] and HbA_{1c} was measured by the HPLC technique using VARIANT Hemoglobin A_{1c} Recorder Pack kit (1000 tests), Bio- Rad Laboratories, USA (Bio-Rad variant and TURBO, USA). Serum insulin (Cat No: EIA-2935) and leptin (Cat No: EIA-2395) were measured by enzyme-linked immune assay using commercial ELISA kits (DRG International, Inc., USA) with the precision of coefficient of variation (%CV) for serum insulin was 6.3% (intra-assay's) and 8.1% (inter-assay's) and for serum leptin the corresponding values were 5.2% and 6.8% respectively. From the values of fasting serum glucose and fasting serum insulin, the insulin secretory function (HOMA%B) and insulin sensitivity (HOMA%S) was calculated using the HOMA2 Calculator. Homeostatic model assessment of insulin resistance (HOMA-IR) was quantified according to the formula: HOMA-IR=(fasting insulin (μIU/mL)×fasting glucose (mmol/L))/22.5 [12]. Body fat mass was measured by the Omron Body Fat Monitor (HBF-302, Kyoto, Japan). Height (cm), weight (kg), age (yrs) and sex of subjects were set to the monitor. Then the subjects held the monitor by both hands with upper limbs horizontal in standing position. The machine was then put on and body fat mass (%) was recorded from the monitor.

Data were analyzed by statistical package for the social sciences (SPSS) for Windows version 17.0 (SPSS Inc., Chicago, IL, USA). The normally distributed variables were expressed as mean ± SD and an absolute value of the number (percentages). Analysis of variance (ANOVA) was done to compare the anthropometric, clinical and biochemical characteristics of the study subjects.

Binary logistic regression analysis was used to predict the determinants of IFG and IGT state considering group (Control as a reference in both cases of IFG and IGT) as a dependent variable and waist circumference, serum triglyceride, serum leptin, HOMA%S and HOMA%B as independent variables. Bivariate Pearson's correlation and regression curve analysis were performed to see the relationship of serum leptin with significant variables of insulin resistance among the prediabetes. Multiple linear regression analysis was done to see the independent association between insulinemic indices and serum leptin with IFG and IGT subjects considering HOMA%B and HOMA-IR as the dependent variable and waist circumference, serum triglyceride and serum leptin as the independent variables. Receiver Operating Characteristic (ROC) curve analysis was used to assess the ability of serum leptin in the alteration of glucose metabolism among prediabetes by calculating the maximum area under the curve and their 95% Confidence Intervals (C.I) with highest sensitivity and specificity. A p value less than 0.05 was considered statistically significant.

Results

Clinical and metabolic characteristics of the study subjects

The anthropometric, clinical and biochemical characteristics of the Control, IFG and IGT subjects are shown in Table 1 and the serum leptin with insulinemic indices are shown in Figure 1.

Variables	Control subjects (n=215)	IFG subjects (n=49)	IGT subjects (n=116)	p-value
Gender (n, %)				
Males/ Females	58 (50)/ 58 (50)	33 (67.3)/ 16 (32.7)	22 (44)/ 28 (56)	0.048
Age (years)	44.77 ± 9.09	48.37 ± 8.45	42.40 ± 9.18	0.004
BMI (kg/m^2)	25.57 ± 2.87	25.20 ± 3.29	26.50 ± 3.12	0.084
WC (cm)	89.97 ± 7.86	89.04 ± 8.56	90.76 ± 7.55	0.561
HC (cm)	98.44 ± 8.68	94.94 ± 8.19	96.36 ± 8.03	0.04
WHR	0.91 ± 0.05	0.93 ± 0.04	0.94 ± 0.04	0.002
%BF	29.24 ± 5.18	29.45 ± 6.53	33.48 ± 9.94	0.001
SBP (mmHg)	114.48 ± 20.11	113.47 ± 11.82	115.90 ± 12.40	0.772
DBP (mmHg)	77.33 ± 13.69	73.37 ± 9.09	77.30 ± 9.96	0.13
FSG (mmol/l)	5.41 ± 0.35	6.24 ± 0.27	5.53 ± 0.31	<0.001
PPSG (mmol/l)	6.37 ± 0.92	6.80 ± 0.91	8.96 ± 1.05	<0.001
HbA1C (%)	5.46 ± 0.45	5.78 ± 0.45	5.83 ± 0.63	<0.001
TC (mg/dl)	198.60 ± 40.84	204.22 ± 44.60	190.26 ± 33.09	0.218
TG (mg/dl)	150.35 ± 66.95	177.51 ± 107.28	164.14 ± 69.54	0.117
HDL-c (mg/dl)	39.41 ± 8.65	38.29 ± 6.20	38.86 ± 7.56	0.695
LDL-c (mg/dl)	127.70 ± 39.38	131.27 ± 36.74	118.82 ± 31.04	0.216
ALT (U/L)	37.98 ± 35.96	30.16 ± 12.94	33.52 ± 17.14	0.246
Serum leptin (ng/ml)	9.72 ± 6.85	15.74 ± 9.08	13.38 ± 6.45	<0.001
FSI (mIU/ml)	14.84 ± 5.29	16.53 ± 7.75	16.38 ± 7.92	0.201
PPSI (mIU/ml)	42.18 ± 20.20	51.38 ± 39.96	65.45 ± 26.83	<0.001
HOMA%B	125.97 ± 31.30	104.0 ± 33.38	125.02 ± 41.50	0.001
HOMA%S	60.49 ± 29.48	53.56 ± 24.95	47.62 ± 12.23	0.01
HOMA-IR	1.92 ± 0.68	2.23 ± 0.96	2.39 ± 0.84	0.001

Results are given as mean ± standard deviation or number of subjects (%); Level of significance was calculated by ANOVA; n=number of subjects; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; WC, waist circumference; HC, hip circumference; WHR, waist to hip ratio; %BF, percent body fat; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; FSG, fasting serum glucose; PPSG, postprandial serum glucose; HbA1C, glycated hemoglobin; TC, total cholesterol; TG, triglycerides; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; ALT, alanine transaminase; FSI, fasting serum insulin; PPSI, postprandial serum insulin; HOMA%B, insulin secretion assessed by homeostasis model assessment; HOMA%S, insulin sensitivity assessed by homeostasis model assessment; HOMA-IR, homeostasis model assessment insulin resistance.

Table 1: Anthropometric, clinical and biochemical characteristics of the study subjects.

Compared to their Control counterparts, IFG and IGT subjects had significantly higher levels of age (p=0.004), HC (p=0.040), WHR (p=0.002), %BF (p=0.001), FSG (p<0.001), PPSG (p<0.001), HbA1c (p<0.001), serum leptin (p<0.001), PPSI (p<0.001) and HOMA-IR (p=0.001) respectively.

However, compared to their Control counterparts, IFG and IGT subjects had significantly lower levels of HOMA%B (p=0.001) and HOMA%S (p=0.010) respectively.

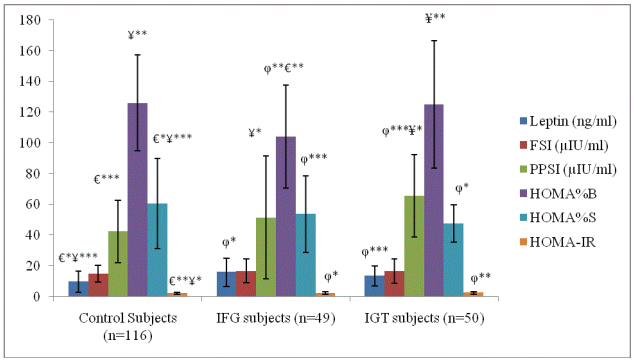

Figure 1: Serum leptin, fasting serum insulin, postprandial serum insulin, HOMA%B, HOMA%S and HOMA-IR levels among the Control, IFG and IGT subjects. Data are presented as mean ± SD; n=number of subjects; the level of significance at p<0.05 calculated by ANOVA. $^{\varphi*}$p<0.05, $^{€*}$p<0.05, $^{¥*}$p<0.05 considered statistically significant when compared with Control, IFG and IGT group respectively.

Association of serum leptin, HOMA%B and HOMA%S with the state of IFG and IGT

To evaluate the contribution of serum leptin and insulinemic indices with IFG and IGT group taking Control as a reference after adjusting the effects of pertinent variables are presented in Table 2.

Variables	Coefficient	S. E.	p-value	Odds Ratio	95% C.I.	
					Lower Bound	Upper Bound
IFG group as dependent variable; R^2=0.244						
WC (cm)	0.014	0.025	0.58	1.014	0.965	1.065
TG (mg/dl)	0.007	0.003	0.011	1.007	1.002	1.013
Serum leptin (ng/ml)	0.104	0.026	<0.001	1.109	1.054	1.167
HOMA%B	-0.035	0.008	<0.001	0.966	0.951	0.981
Constant	-0.633	2.135	0.767	0.531	-	-
IGT group as dependent variable; R^2=0.110						
WC (cm)	0.005	0.024	0.82	1.005	0.96	1.053
TG (mg/dl)	0.001	0.003	0.745	1.001	0.996	1.006
Serum leptin (ng/ml)	0.071	0.027	0.007	1.074	1.019	1.131
HOMA%S	-0.028	0.012	0.015	0.972	0.95	0.995
Constant	-0.79	2.445	0.747	0.454	-	-

Dependent variable: IFG and IGT considering the Control as a reference; Adjusted R^2=0.244 and 0.110; the level of significance at p<0.05; S.E, standard error; C.I, confidence interval; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; WC, waist circumference; TG, triglycerides; HOMA%B, insulin secretion assessed by the homeostasis model assessment; HOMA%S, insulin sensitivity assessed by the homeostasis model assessment.

Table 2: Binary logistic regression analysis taking group (IFG and IGT where Control as reference) as dependant variable after adjusting the effects of major confounders.

In binary logistic regression analysis, serum leptin (OR: 1.109, 95% CI: 1.054-1.167, p<0.001) and HOMA%B (OR: 0.966, 95% CI: 0.951-0.981, p<0.001) were found to be significant determinants of IFG subjects whereas, serum leptin (OR: 1.074, 95% CI 1.019-1.131, p=0.007) and HOMA%S (OR: 0.972, 95% CI: 0.950-0.995, p=0.015) were found to be significant determinants of IGT subjects after adjusting the effects of WC and TG respectively.

Correlation of serum leptin with significant variables causing alteration of insulin action in IFG and IGT subjects

The relationship of serum leptin with significant variables is shown in Table 3 and with insulinemic indices are presented in Figure 2.

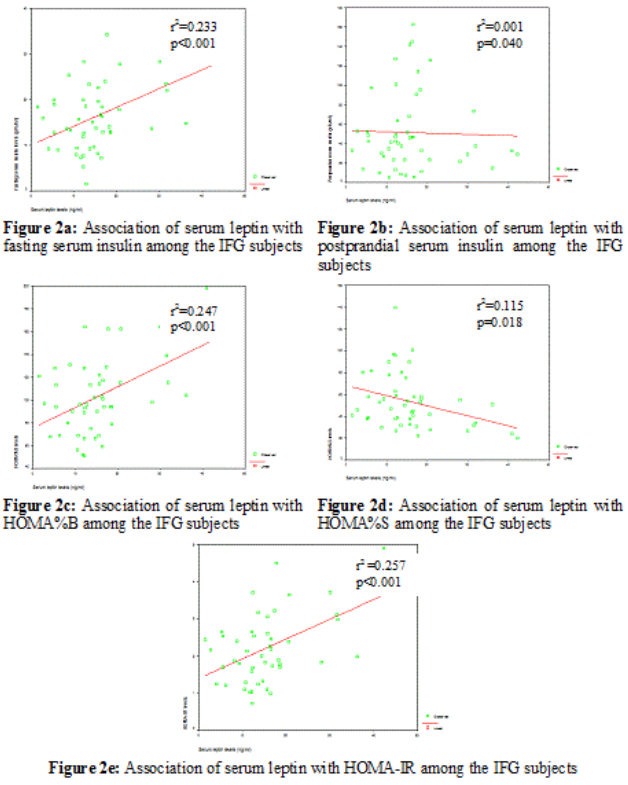

Figure 2a: Association of serum leptin with fasting serum insulin among the IFG subjects

Figure 2b: Association of serum leptin with postprandial serum insulin among the IFG subjects

Figure 2c: Association of serum leptin with HOMA%B among the IFG subjects

Figure 2d: Association of serum leptin with HOMA%S among the IFG subjects

Figure 2e: Association of serum leptin with HOMA-IR among the IFG subjects

Figure 2: (a) Association of serum leptin with fasting serum insulin among the IFG subjects. (b) Association of serum leptin with postprandial serum insulin among the IFG subjects. (c) Association of serum leptin with HOMA%B among the IFG subjects. (d) Association of serum leptin with HOMA%S among the IFG subjects, (e) Association of serum leptin with HOMA-IR among the IFG subjects. (f) Association of serum leptin with fasting serum insulin among the total subjects. (g) Association of serum leptin with HOMA-IR among the total subjects. (h) Association of serum leptin with HOMA%S among the total subjects.

In bivariate Pearson's correlation analysis and regression curve analysis, serum leptin showed significant positive correlation with age (r=0.348, p=0.014), BMI (r=0.316, p=0.027), TC (r=0.415, p=0.003) TG, (r=0.301, p=0.036), FSI (r=0.483, p<0.001), and HOMA-IR (r=0.507, p<0.001) whereas, serum leptin showed significant negative correlation with HOMA%B (r=-0.497, p<0.001) and HOMA%S (r=-0.339, p=0.018) in IFG subjects. However, in IGT subjects, serum leptin did not show any significant correlation with variables of insulin action. In addition, serum leptin in Control subjects showed significant positive correlation with FSG (r=0.308, p<0.001), TG (r=0.153, p=0.025), FSI (r=0.219, p=0.001), and HOMA-IR (r=0.272, p<0.001) as well as significant negative correlation with HOMA%S (r=-0.189, p=0.006) respectively.

Variables	Control subjects (n=215)		IFG subjects (n=49)		IGT subjects (n=116)	
	r-value	p-value	r- value	p-value	r-value	p-value
Age (years)	-0.07	0.308	0.348	0.014	-0.022	0.882
%BF	0.118	0.085	0.258	0.073	0.005	0.975
BMI (kg/m^2)	0.081	0.234	0.316	0.027	-0.189	0.189
FSG (mmol/L)	0.308	<0.001	-0.099	0.498	-0.156	0.278
TC (mg/dL)	0.085	0.212	0.415	0.003	0.069	0.632
TG (mg/dL)	0.153	0.025	0.301	0.036	-0.052	0.717
FSI (μIU/mL)	0.219	0.001	0.483	<0.001	-0.059	0.684
HOMA%B	0.03	0.659	0.497	<0.001	0.068	0.637
HOMA%S	-0.189	0.006	-0.339	0.018	-0.007	0.963
HOMA-IR	0.272	<0.001	0.507	<0.001	-0.002	0.988

Results are given by Pearson's correlation coefficient r and statistical significance p<0.05; IFG: Impaired Fasting Glucose; IGT: Impaired Glucose Tolerance; %BF: Percent Body Fat; BMI: Body Mass Index; FSG: Fasting Serum Glucose; TC: Total Cholesterol; TG: Triglycerides; FSI: Fasting Serum Insulin; HOMA%B: insulin secretion assessed by the Homeostasis Model Assessment; HOMA%S: insulin sensitivity assessed by the Homeostasis Model Assessment; HOMA-IR: Homeostasis Model Assessment Insulin Resistance.

Table 3: Correlation of serum leptin with some significant variables among the study subjects.

Independent association of HOMA%B and HOMA-IR with serum leptin levels in IFG and IGT subjects

To explore the association of HOMA%B and HOMA-IR with IFG and IGT subjects after adjusting the effects of pertinent variables considering HOMA%B and HOMA-IR as dependent variables and WC and TG as independent variables are demonstrated in Table 4. In multiple linear regression analysis, only IFG subjects showed a significant positive association of HOMA%B with serum leptin (β=0.280, p=0.004) as well as significant negative association with TG (β=-0.280, p=0.035) after adjusting the effect of WC.

The cutoff values of serum leptin from the ROC curves for the prediction of alteration of insulinemic indices among the study subjects

The ROC curve analysis was performed to determine the cutoff values with different percentiles of serum leptin to maximize sensitivity and specificity in identifying the deterioration of insulin action among the Control and prediabetes and shown in Table 5 and Figure 3.

Variables	Coefficients (β)	t-value	p-value	95% C.I.	
				Lower Bound	Upper Bound
IFG subjects (dependent variable HOMA%B; R^2=0.358)					
Constant	-	0.265	0.792	-73.096	95.213
WC (cm)	0.160	1.296	0.202	-0.344	1.582
TG (mg/dL)	-0.280	2.179	0.035	0.006	0.166
Serum leptin (ng/mL)	0.391	3.074	0.004	0.489	2.354
IGT subjects (dependent variable HOMA-IR; R^2=0.078)					
Constant		0.236	0.815	-2.738	3.465
WC (cm)	0.228	1.596	0.117	-0.007	0.058
TG (mg/dL)	-0.161	-1.137	0.261	-0.005	0.002
Serum leptin (ng/mL)	0.023	0.158	0.875	-0.035	0.041

Dependent variable: HOMA%B and HOMA-IR; Adjusted R^2=0.358 and 0.078; the level of significance at p<0.05; C.I: Confidence Interval; IFG: Impaired Fasting Glucose; IGT: Impaired Glucose Tolerance; WC: Waist Circumference; TG: Triglycerides; HOMA%B: insulin secretion assessed by the Homeostasis Model Assessment; HOMA-IR: Homeostasis Model Assessment Insulin Resistance.

Table 4: Multiple linear regression analysis to explore the association of serum leptin with insulinemic profile among the study subjects after adjusting the effects of major confounders.

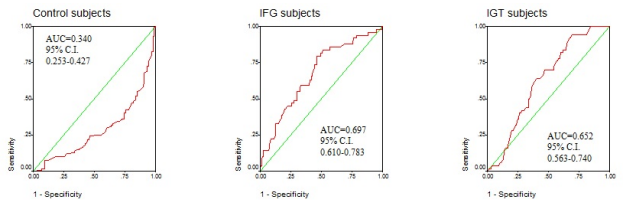

Figure 3: Leptin receiver operating characteristics curves for Control, IFG and IGT subjects.

In Control, IFG and IGT subjects, the cutoff values of serum leptin were 7.44, 10.84 and 8.45 ng/mL at 50th, 25th and 25th percentile with the highest AUC of 0.340 (95% C.I. 0.253-0.427; OR, 4.556), 0.697 (95% C.I. 0.610-0.783; OR, 5.433) and 0.652 (95% C.I. 0.563-0.740; OR, 3.764) respectively. The sensitivity, specificity, PPV and NPV of Control subjects were 50.0%, 82.0%, 41.41% and 86.56% respectively.

The corresponding values for IFG were 75.5%, 36.2%, 46.83% and 86.04% for IGT were 76.0%, 45.7%, 41.75% and 84.0% respectively.

Variables	Control subjects			IFG subjects			IGT subjects		
	Percentile			Percentile			Percentile		
	10	25	50	10	25	50	10	25	50
Cutoff values	3.05	4.91	7.44	5.43	10.84	14.39	6.21	8.45	12.76
AUC	0.405	0.453	0.34	0.598	0.697	0.624	0.66	0.652	0.625
p-value	0.035	0.005	<0.001	0.011	<0.001	0.003	<0.001	<0.001	0.002
95% C.I.	0.36-0.545	0.31-0.49	0.25-0.42	0.50-0.68	0.61-0.78	0.52-0.72	0.57-0.74	0.56-0.74	0.53-0.72
Sensitivity (%)	90.5	75	50	87.8	75.5	49	88	76	50
Specificity (%)	10	94	82	68.1	36.2	24.1	56	45.7	25
PPV (%)	32.25	35.07	41.41	35.24	46.83	46.15	40.36	41.75	46.29
NPV (%)	100	90.62	86.56	86.04	86.04	77.87	89.47	84	77.67
Odds ratio	1.476	5.222	4.556	3.357	5.433	3.017	5.754	3.764	3
95% C.I.	1.32-1.64	1.51-18.05	2.03-10.22	1.31-8.58	2.55-11.53	1.49-6.09	2.27-14.56	1.78-7.92	1.49-6.01

IFG, impaired fasting glucose; IGT, impaired glucose tolerance; AUC, the area under the ROC curve; ROC, receiver operating characteristic; the level of significance at p<0.05; C.I, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Table 5: The cutoff values of serum leptin from the ROC curves for the prediction of alteration of insulinemic indices among the study subjects.

Discussion

Leptin-insulin signalling via hypothalamic action, therefore, regulates the adipose tissue mass; however, hyperleptinemia due to impaired insulin action increases the central obesity that contributes to the risk factor of insulin resistant syndrome among prediabetes. Data examining the association of serum leptin as an index of insulin sensitivity among type 2 diabetes is well studied. Nevertheless, this association among different subgroups of prediabetes with concomitant insulin pathophysiology has not been investigated thoroughly. In this context, the present study was assessed to investigate the association of serum leptin with different levels of glycemic tolerance and to explore whether this association is mediated by insulinemic indices during this disorder.

Our observation revealed that circulating levels of serum leptin was significantly higher in IFG and IGT subjects compared to the Control which is consistent with a cross-sectional study by Al-Daghri et al. [13] who found a higher level of serum leptin among Saudi diabetics and prediabetics. In accordance, a cohort study of 574 Chinese lean subjects also revealed a significantly higher level of plasma leptin in prediabetic subjects compared to the NGT [7]. The possible explanation of elevated levels of serum leptin among the glucose intolerant subjects is due to impaired insulin action by altering the adiposity.

The results obtained from our binary logistic regression analysis we found serum leptin, HOMA%B and triglycerides were found to be significant determinants of IFG subjects after adjusting the confounding variables of WC. In the same analysis, we also found serum leptin and HOMA%S were found to be significant predictors of IGT subjects after adjusting the pertinent variables of WC and triglycerides respectively. In fact, the adipo-insular axis of insulin and leptin where they act as bidirectional hormonal feedback loop demonstrated that insulin resistance resulting from hyperinsulinemia reported inducing hyperleptinemia through increased adiposity [14-16] by altering glucose and lipid metabolism. Martins et al. [17] reported hyperinsulinemia and insulin resistance strongly influenced hyperleptinemia with the conclusion that elevated levels of serum leptin is the best predictor of insulin resistance with the consequence of CVD, metabolic syndrome and type 2 diabetes in both sexes of non-diabetic individuals. Fischer et al. [18] found a higher fasting leptin levels among those type 2 diabetic subjects who had lower tertiles of insulin sensitivity with the observation that a strong correlation exists between insulin resistance and leptin concentrations in insulin-resistant type 2 diabetic men is believed to be mediated by insulin. A number of studies have been studied extensively and reviewed to see the association of serum leptin with insulin resistance among different study subjects [19,20] while other studies reveal, no correlation exists between leptin and insulin resistance [13] among prediabetes. Two possible mechanisms can be postulated underlying no association of leptin with insulin resistance: i) Hyperleptinemia linked with hyperinsulinemia mediated insulin resistance *via* adiposity ii) Due to the wide distribution of visceral adipose tissue among the abnormal glucose tolerant subjects there is a limited production of leptin.

Our study results demonstrated a significant positive correlation of serum leptin with fasting serum insulin, HOMA%B and HOMA-IR as well as a significant negative correlation with HOMA%S among the IFG subjects. Tamer et al. [21] in their hospital based type 2 diabetic patients also demonstrated a significant positive correlation of serum leptin with fasting serum insulin. It appears that increased leptin concentrations have a potential role in the development of insulin resistance thereby ensuing altered glucose metabolism among prediabetes. Li et al. [22] in a cross-sectional study of 783 rural

residents found that higher serum concentrations of leptin were inversely associated with β-cell function. It has been postulated that insulin plays an important role in the secretion of leptin from adipose tissue enhancing the regulation of adipose tissue mass. During obesity impaired insulin signaling due to hyperleptinemia results insulin resistance induced by hyperinsulinemia among the prediabetes.

In our settings, we found a significant positive association of HOMA%B with serum leptin and triglycerides after adjusting the effect of WC among the IFG subjects. Studies have demonstrated that amelioration of leptin receptor within the hypothalamus results in defective leptin transport across the Blood Brain Barrier (BBB) with the consequence of impaired leptin signaling that leads to the excessive accumulation of triglycerides within the adipocytes as well as other peripheral tissues [23]. The excessive deposition of lipid molecules within the non-adipose tissues referred to as 'ectopic fat' that disrupts the normal insulin signal transduction pathway.

In our multiple regression analysis, we didn't find any significant association of HOMA-IR with serum leptin among the IGT subjects which is in line with a nested case-control study of India and found no significant association between circulating serum leptin levels and type 2 diabetes where insulin resistance is common [24]. In other words, an elevated level of serum leptin is a hallmark feature of insulin resistant syndrome. It has no direct association with insulin resistance; however, central obesity has a linking role of hyperleptinemia with insulin resistance *via* interactions of adipose tissue derived other adipocytokines. Moreover, reduced leptin sensitivity due to abnormalities of leptin receptor in the hypothalamus enhances the accumulation of excessive triglycerides within the adipocytes as well as other non-adipose tissues (kidney, muscle, liver, and pancreas) attenuating insulin sensitivity and secretion [25]. As a result, hyperleptinemia induces hyperinsulinemia (an index of insulin resistance) among the prediabetes [7].

The present study also aimed to determine the cutoff value of serum leptin among the prediabetes which can be used as a diagnostic tool for screening of pre-diabetes. To best of our knowledge, this is the first study to find the cut off values of serum leptin among Bangladeshi prediabetes. The cutoff value of serum leptin in Control, IFG and IGT subjects were 7.44, 10.84 and 8.45 ng/mL at 50th, 25th and 25th percentile respectively. The sensitivity, specificity, PPV and NPV of Control subjects were 50.0%, 82.0%, 41.41% and 86.56% respectively. The corresponding values for IFG were 75.5%, 36.2%, 46.83% and 86.04% for IGT were 76.0%, 45.7%, 41.75% and 84.0% respectively. In accordance with our results, Askari et al. [26] performed the variation of serum leptin with the similar BMI and showed a fasting leptin cutoff of 15 ng/mL among the non-diabetic subjects for the detection of insulin sensitivity (sensitivity 72.7%, specificity 56.3%, and PPV 69.6%). Esteghamati et al. [27] in their population-based study showed leptin cutoff value for the prediction of metabolic syndrome based on modified International Diabetes Federation (IDF) and adult treatment panel III (ATPIII) criteria. The study revealed leptin cutoff value for men are 3.6 ng/mL (PPV, 56.5%; NPV, 72.7%) followed by IDF criteria and 4.1 ng/mL (PPV, 49.6%; NPV, 78.1%) for ATPIII criteria. The corresponding values for women are 11.0 ng/mL (PPV, 53.8%; NPV, 73.0% for IDF criteria and PPV, 60.1%; NPV, 64.9% for ATPIII criteria). Gijón-Conde et al. [28] in their cross-sectional study reported a higher leptin value in female compared to their male counterparts for the prediction of cardiometabolic abnormalities with the cutoff of male was 6.45 ng/mL (AUC, 0.716; sensitivity, 71.4%; specificity, 60.2%) and for female was 23.75 ng/mL (AUC, 0.722; sensitivity, 72.3%; specificity, 58.7%).

Our study has several limitations. The sample size was relatively small with a cross-sectional design therefore; it was not possible to identify the cause and effect associations between serum leptin and insulinemic indices among various subgroups of pre-diabetes. Prospective studies with large sample size having both sexes in different subgroups of pre-diabetes are needed to understand the role of serum leptin with the alteration of insulin action during this disorder. Though we used anthropometric measures (WC, HC, %BF, WHR and BMI) as index of obesity while, computed tomography and magnetic resonance imaging considered as gold standard technique for the measurement of visceral and subcutaneous fat, however, due to cost, technical complexity, and other resource limited settings it was not performed in our study. Other measures of insulin resistance (ISI, hyperglycemic clamp, etc.) could not be done due to logistic limitations to understand the role of serum leptin based on specific insulin pathophysiology among the prediabetes.

Conclusion

Our study shows the existence of a significant positive correlation of serum leptin with insulinemic indices (FSI, HOMA%B, and HOMA-IR) as well as it shows a significant negative correlation with HOMA %S among the IFG subjects. Significant high levels of serum leptin were found among IFG and IGT subjects compared to the Control. Though, leptin is a good biomarker of obesity, it is not directly related with insulin resistance which revealed the fact that an association could exist between serum leptin levels and insulin resistance with the causation of anthropometric indexes of obesity and dyslipidemia among the IGT subjects, however; in IFG subjects, hyperleptinemia seemed to be associated with insulin secretory dysfunction independent of dyslipidemia and central obesity. The present study also revealed increased levels of serum leptin and reduced insulin sensitivity were found to be significant predictors of IGT state independent of changes in central obesity and dyslipidemia. In addition, serum leptin and insulin secretory dysfunction were found to be significant determinants of IFG state independent of changes in central obesity and dyslipidemia.

Author Contributions Statement

Data collection, the precision of lab test analysis, statistical analysis of data and manuscript draft: IAH; Laboratory test analyzed and interpreted the data: MRS; Statistical analysis and interpretation: FJ; Manuscript draft and review: RZ; Experiment design, critical review of the manuscript, lab facilities: LA. All of the authors read and approved the final version of the manuscript.

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Declaration of Conflicting Interests

The authors declare that they have no conflict of interest associated with this paper.

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