Serum Leptin and Earlier Stages of Nephropathy among Type2 Diabetic

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Abstract:

Objective: *To evaluate the relationship of Serum Leptin and Early Nephropathy among uncontrolled type 2 Diabetes Mellitus patients of Bangladesh Institute of Research and Rehabilitation in Diabetes Endocrinology and Metabolism (BIRDEM), Dhaka and Endocrinology department of Sylhet MAG Osmani Medical College (SOMC) & Hospital Sylhet, Bangladesh.*

Method: *This study was carried out from January 2013 to December 2015 among 100 type 2 diabetic patients from the outpatient department (OPD) BIRDEM and SOMC hospital.*

Results: *16 out of 30 controls and 65 out of 100 people with diabetes have a family history of diabetes. Early retinopathy and neuropathy were observed in 45.5% and 36.5% diabetic subjects. Mean±SD of serum urea in control subjects was 28.65±6.27; in diabetic subjects was 30.21±7.67. Serum creatinine in controls was 1.12±0.24, in diabetic subjects was 1.13±0.25. Serum leptin levels in control subjects {1.65 (0.05-8.66)} was lower than diabetic counterpart {1.21 (0.11-13.3)}. Leptin levels in male controls {(1.29 (0.05-2.49)} was significantly (P 0.000) lower than the female controls {4.03 (0.05-8.66)}.*

Conclusion: *It was evident that there was very little or no association between serum leptin level and the indices of renal function. No changes of circulating serum Leptin concentration in the earlier stages of Diabetic Nephropathy were found.*

Keywords: *Diabetes Mellitus, Obesity, Serum Leptin, Renal Function, Nephropathy.*

Introduction

Leptin the product of ob gene secreted by mature adipocytes from white adipose tissues and is supposed to transmit a satiety signal into the central nervous system. It was observed that plasma leptin levels is strongly correlated with BMI and other indices of adiposity like Waist Hip (W/H) ratio, percent body fat and total fat mass and also the fasting Insulin levels in both Rodents and human[1-5]. Diabetes Mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defect in insulin secretion, insulin action or both. The chronic hyperglycemia of Diabetes is associated with long term damages, dysfunction and failures of various organs specially the heart, blood vessels, kidneys, eyes and nerves. Obesity is a disorder that imposes a great burden to human life with considerable morbidities and mortalities. Obese state is associated with dyslipidemia, hyperinsulinemia, insulin resistance and glucose intolerance [6-8]. The risk of development of type2 Diabetes becomes greater with increasing

obesity and insulin resistance. So that increasing adipose tissue mass could lead to insulin resistance, hyperinsulinemia, hyperleptinemia and Diabetes Mellitus[1]. Serum Leptin level was also found to be affected by disorders of renal function. Kidneys are among the organs expressing highest leptin receptor and is thought to be the major organ of leptin degradation and clearance[9]. Patients with end stage renal disease (ESRD) and patients with ESRD undergoing Dialysis is associated with raised serum Leptin levels [10--12]. Trials suggested that total body mass, distribution of body fat, serum insulin and the status of renal function are the important determinants of serum leptin concentration. It is documented that advanced renal diseases in the setting of diabetes Mellitus is associated with impairment of Lepiin degradation and clearance by the kidneys, so that serum leptin levels is raised along with various other hormones like parathyroid hormone (PTH), renin, aldosterone, pro-insulin, Insulin, glucagon and motilon [10,11]. But serum leptin levels have been found to have no correlation with the indices of renal function, such as serum urea, creatinine and BUN.

There is no conclusive data regarding serum Leptin concentration and earlier stages of renal diseases with or without diabetes mellitus. Few investigators examine the effect of earlier stages of renal diseases on serum leptin concentration in the setting of type2 diabetes with documentation that Leptin levels are elevated in these patients in the stage of microalbuminuria and macroalbuminuria suggesting that renal leptin degradation and clearance is already impaired in the early stages of renal diseases[13].

But many others documented that there are no differences in serum leptin levels with or without diabetic nephropathy in their earlier stages[14] and documented no differences in serum Leptin concentration in Diabetic nephropathy to that of age and BMI matched control subjects[13,,14]. Diabetic nephropathy alters renal metabolism of Leptin. However alteration of Leptin metabolism that results in measurably serum concentration differences are only limited to patients with a very late renal disease. Many investigators examine the effect of renal replacement therapy in the form of hemodialysis (HD) and peritoneal dialysis (PD) and observed that serum leptin levels increases by many folds in patients receiving regular hemodialysis or treating exclusively by peritonial dialysis [7,12]. HD is known to activate the expression of cytokines, such as TNFα and interleukin-1 which have been reported to stimulate leptin production. But it was believed that hyperleptinemia in dialysis patients is not a sequele to HD or PD, rather due to the impairment of renal leptin degradation in patients with end stage renal diseases (ESRD).

Impaired renal leptin clearance is unlikely to be the sole mechanism for leptin accumulation in ESRD, as because the net renal extraction of leptin is only 12% of circulating leptin in normal humans and 20% in rats[16, 17]. Hormonal substances accumulate in ESRD patient, like insulin and GH may be a stimuli for leptin production. Presumably persistent elevation of leptin could inhibit food intake leading to malnutrition in the already catabolic uremic state. If future studies characterize leptin metabolism in ESRD patient as maladaptive, then interventions to prevent or reduce leptin accumulation would be desirable. Chronic therapy with recombinant human erythropoietin has also been suggested to decrease serum leptin levels in patients with ESRD[18, 19]. In this study our main goal is to evaluate the relationship of serum leptin and earlier stages of nephropathy in the setting of uncontrolled type2 Diabetes mellitus in a group of Bangladeshi population.

Objective:

To assess the relationship of serum leptin and early nephropathy in patients with uncontrolled type 2 Diabetes Mellitus in a group of Bangladeshi population.

Methodology

Types of study:
This was a case and control study.

Place and duration of study:
The study was conducted with the Biomedical Research Group (BMRG) in the Research Division of BIRDEM, Dhaka, in collaboration with the Endocrinology department of SOMC Hospital, Sylhet, Bangladesh during the period of January 2013 to December 2015.

Study population: A total of 100 type 2 diabetic subjects, 30-50 years of age, irrespective of glycemic status, duration of diabetes, BMI and sex were recruited from the outpatient department (OPD) of BIRDEM hospital, Dhaka and SOMC hospital, Sylhet , Bangladesh. Prior to recruitment, diabetes mellitus was confirmed according to current American Diabetic Association (ADA) criteria for the diagnosis and classification of diabetes mellitus. Control subjects (n=30) were selected from friends of the patients within 5 years of age band without diabetes or Impaired Glucose Regulation (IFG, IGT) determined according to ADA criteria[20] and having no clinical thyroid diseases or evident systemic diseases documented on clinical evaluation. Informed written consent was taken from all recruited diabetic and control subjects for the purpose of the study.

Exclusion criteria:
1. Type 2 diabetes with acute metabolic de-compensation.
2. Type 2 diabetes with clinically detectable thyroid diseases.
.3 Type 2 Diabetes with clinically diagnosed other acute or chronic systemic diseases.
4. Diabetic subjects with overt nephropathy in which serum creatinine > 2mg/dl
5. Pregnancy and postmenopausal woman.

Method:
Controls and diabetic subjects were assigned for the study according to selection criteria and as per availability and was given an appointment to come in a particular date. They were requested to fast overnight for at least eight hours and in the subsequent morning 16 ml of venous blood was drawn from the antecubital vein by using 25 cc disposable plastic syringe with 18G needle for the estimation of fasting serum leptin, insulin, C-peptide, glucose, HbAlc, serum urea, creatinine, One ml of collected venous blood was taken in an anticoagulant containing vial for estimation of HbAlc. Remaining 15ml of blood was kept in 3 separate plain test tubes in equal amounts (5ml in each) to centrifuge immediately. Blood sample contained in the test tube was centrifuged for 15 minutes at a rate of 4000 rpm. A total of 200 µl of serum was collected in appropriately labeled eppendorf in duplicate with the help of micropipette for each of the biochemical parameters. Then the serum sample was preserved immediately at -30° C for analysis. Urine sample was collected in an appropriately scheduled date and preserved in an eppendorf tube accordingly and refrigerated in the same way at -30^{O} C.

History and clinical examination:
Detailed socio-demographic and clinical data were recorded in a pre-designed case record form. These include age, sex, residing area, occupation, socioeconomic status, dietary habit, exercise, alcohol and

smoking habit, duration of diabetes, associated diseases like hypertension, obesity, dyslipidemia, coronary artery disease, cerebrovascular diseases, peripheral vascular diseases and crystal deposition diseases. Family history of these diseases were also been noted. Classical and non-classical features of diabetes mellitus and any adverse outcome of diabetes on life style was noted by taking history from the diabetic subjects.

Height, weight, BMI, waist circumference, hip circumference, waist hip ratio (WHR), waist height ratio (W/Ht) of all the controls and diabetic subjects were recorded. Systolic and diastolic blood pressure of all patients and control subjects was recorded. Blood pressure was measured by using mercury sphygmomanometer after at least 5 minutes of recumbence in a calm and quiet environment. Systolic blood pressure <130 mm Hg and the diastolic blood pressure <80 mm Hg was taken as the cut-off value for categorizing the normal and the abnormal values among diabetic population [207]. Diabetic neuropathy was tested by appropriate clinical test. Autonomic function test were done by documenting the heart rate variability and blood pressure response on standing. Motor neuropathy was tested by eliciting jerks and reflexes by the percussion hammer. Retinopathy of all diabetic subjects was screened by routine dilated fundoscopy of the BIRDEM Opthalmology out patient department. For the documentation of nephropathy, urine albumin in mg /l and urine creatinine in g/l was estimated to calculate the albumin creatinine ratio (ACR).

Statistical analysis:

All the data were expressed as mean standard deviation, median (range) and/or number (%) as appropriate. Statistical analysis was done by using SPSS 7.5 packages for windows. Appropriate statistical test of significance like unpaired t test, one way analysis of variance (ANOVA) and Mann-Whitney test was used as necessary. $P < 0.05$ was taken as minimum level of significance. Tabulation and/or drawing, either in a graph or diagram, were utilized as necessary for data presentation.

Results:

Figure-1: Gender distribution of the study group.

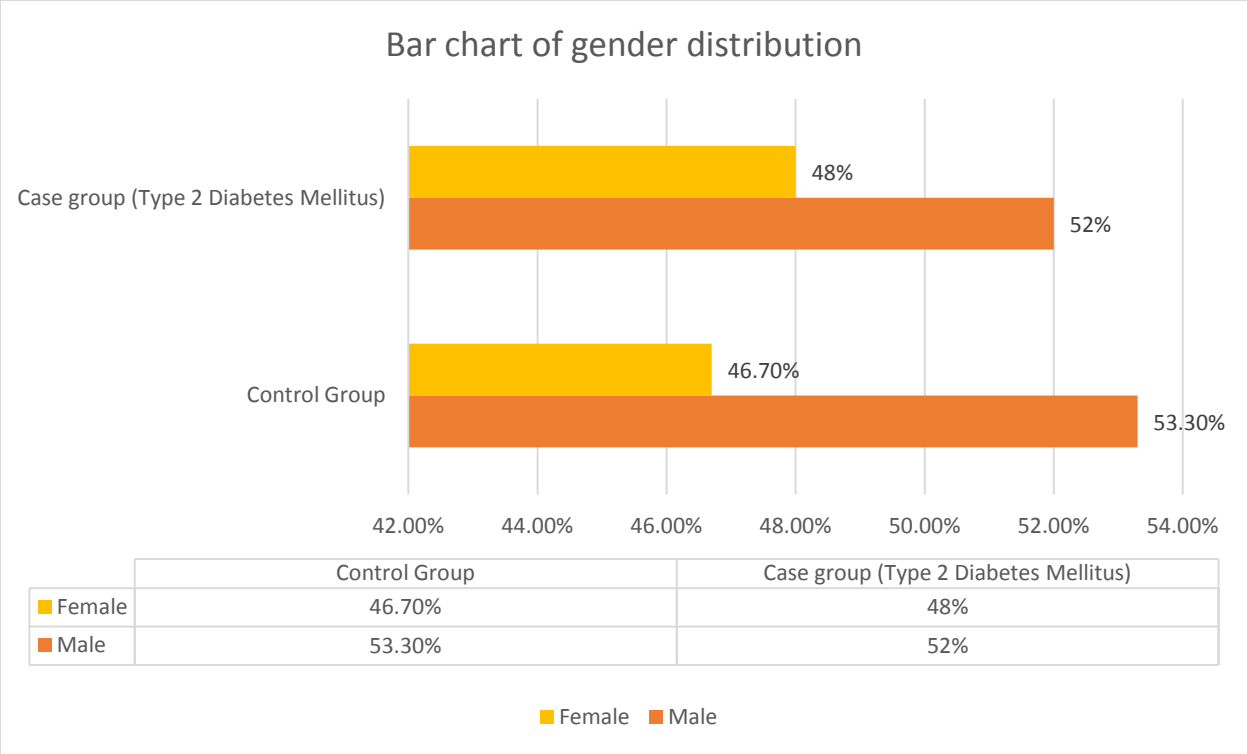

Figure-1 shows the gender distribution of the study group both in controls and type-2 Diabetic subjects Male patients was found to be higher in both the groups which ia 53.3% and 52% diabetic and control subjects respectively. The figure is given below describes it in detail:

Table-1: Socio-Demographic status of the study groups

Groups	**Age mean ± SD**	**Annual Income**	**Family Member**	**SBP mean ± SD**	**DBP mean ± SD**	**Duration of DM, years**
Controls (n =30)	39.53± 5.24	120000 (30000-220000)	6±1	120±23	80±7	-
DM (n=100)	39.24± 5.79	100000 (20000-200000)	6± 2	124 ±17	80±10	0.02 (0.01- 6)
t/p value						
Cont vs DM	-.248/ 0.804	1114/0.32*	1.1770/.241	1.177 0/ .241	1.101/ 0.273	

(t/p value was calculated by student ' t' test , *U/p value was calculated using Mann Whitney U test)
In table-1 shows demographic status of the study group where mean ±SD of age of the control and diabetic subjects were 39.5±5.2 and 39.2±5.8 respectively. Duration of diabetes is one month to six years. Systolic and diastolic blood pressure of the control and diabetic subjects were almost similar and it was within normal range. The following table is given below in detail:

Table-2: Clinical status of the study group

Clinical history		Controls		Type-2 Diabetes mellitus	
		Number	Percentage	Number	Percentage
Sex	Male	16	53.3	52	52
	female	14	46.7	48	48
Type of work	Sedentary	27	90	84	84
	Physical work	3	10	16	16
Exercise	Regular	11	36.7	23	23
	Irregular	19	63.3	57	57
	No Exercise	0	0	20	20
Smoking	Smoker	2	6.7	20	20
	Non Smoker	27	90	70	70
	Past Smoker	1	3.3	10	10
FH diabetes	Present	16	53.3	65	65
	Absent	14	46.7	23	23
FH HTN	Present	15	50	48	48
	Absent	15	50	36	36
FH obesity	Present	14	44	46	46
	Absent	16	55.2	54	54
FH CAD	Present	8	26.7	24	24
	Absent	22	73.3	52	52
FH CVD	Present	7	23.3	27	27
	Absent	23	73.3	50	50
H/O CAD	Present	1	3.3	38	38
H/O CVD	Present	0	0	06	6
Retinopathy	Present	0	0	35	35
Neuropathy	Present	0	0	35	35
Nephropathy	Present	0	0	25	25
Anti DM drugs	Present	0	0	24	24
Typical Symptoms	Present	0	0	37	37
Atypical Symptoms	Present	0	0	63	63

In table-2 shows clinical status of the study group where 16 out of 30 controls and 65 out of 100 diabetics have family history of diabetes. Family history of hypertension was found in 15 out of 30 and 48 out of 100 controls and diabetic subjects respectively. Family history of obesity was found in 44% controls and 46% diabetic subjects. Around 25% of both controls and diabetic subjects have family history of coronary artery diseases (CAD) and cerebrovascular diseases (CVD). Early retinopathy and neuropathy were observed in 45.5% and 36.5% diabetic subjects. Nephropathy was documented in 25 diabetic subjects.

Table-3: Indices of renal function in the study subjects

Parameters	Controls	DM	P value

	MC	FC	TC	MD	FD	TD	MC vs MD	Fc vs FD	TC vs TD	MD vs FD
S Urea	30.38± 5.52	26.69± 6.69	28.65 ± 6.27	31.93 ±7.85	28.34 ±7.07	30.21 ±7.67	1.00	1.00	1.012/ 0.313	0.083
S Creat	1.24± 0.27	0.99± 0.11	1.12± 0.24	1.26± 0.21	0.99 ±0.22	1.13± 0.25	1.00	1.00	0.115/ 0.909	0.000
Urine*	15..93 (1.68-28.7)	3.0(2.01-16.26)	11.48 (1.68-28.7)	3.40 (0.96-419)	6.16(1.0-812)	4.18 (0.96-814)	107 /0.278	54/ 0.186	467/ 0.97	6.0/ 0.199
ACR*	19.88 (1.0-36.79)	8.77 (1.91-15.38)	14.47 (1.0-36.79)	8.79 (1.93-331.0)	15.26 (1.90-3585)	11.13 (1.92-3585)	107/ 0.554	54/ 0.242	390/ 0.72	5.0/ 0.136

(MC=Male control, FC= Female Control, TC= Total Control, MD=Male Diabetic, FD= Female Diabetic, TD=Total Diabetic) **Serum Albumin and ACR was expressed as Median (Range)

In table-3 shows indices of renal function in the study subjects where mean± SD of serum urea in control subjects was 28.65±6,27 and in diabetic subjects was 30.21±7.67. Serum creatinine in controls was 1.12±0.24 and in diabetic subjects was 1.13±0.25. Urine albumin (mgm/l) in controls was {11.48 (168-28.7)} and in diabetic subject was {4.18 (0.96-814)}, (p= 0.970). ACR (mgm/gm creatinine) in control subjects was {14.47 (1-36.79)} and in diabetic subjects was {11.13 (1.92-3585)}, (p= 0.720). None of the indices of renal function was statistically significant among diabetic and control subjects.

Table-4: Leptin level among diabetic and control subjects

Parameters	Controls			DM			P value			
	**MC	**FC	**TC	**MD	**FD	**TD	MC vs MD	Fc vs FD	Tc vs TD	MD vs FD
**Leptin	1.29 (0.05-2.49)	4.03 (0.05-8.66)	1.65 (0.05-8.66)	0.71 (.11-2.420)	2.74 (.25-13.28)	1.21 (0.11-13.3)	278/ 0.046	241/ 0.110	1206/ 0.10	296.5 /0.000

**(MC=Male control, FC= Female Control, TC= Total Control, MD=Male Diabetic, FD= Female Diabetic, TD=Total Diabetic)
**Serum Leptin level was expressed as Median (Range)

In table-4 shows leptin level among diabetic and control subjects where Serum leptin levels in control subjects {1.65 (0.05-8.66)} was found lower than their diabetic counterpart {1.21 (0.11-13.3)}, but it was not significant: (P=0.1000). But the leptin levels in male controls {1.29 (0.05-2.49)} was found significantly (P 0.000) lower than the female controls {4.03 (0.05-8.66)}. Similar observation was also noted in comparison between male diabetic {0.71 (0.11-2.42)}: and female diabetic subjects {2.74 (0.25-13.28)}; (p= 0.0001).

Table-5: Glycemic Status, Serum Insulin, C-Peptide and Leptin levels with indices of renal function in diabetic subjects according to BMI.

Groups	FPG mg/dl	HbA1c %	S Urea	S Creat	U Album	ACR	Serum Insulin	Serum C peptide	Leptin
BMI A N=55	11.77± 5.18	10.35± 2.63	30.59 ±8.20	1.12± 0.24	3.4 (0.96-208)	8.22 (2.35-2341)	6.0 (1.9-38.0)	0.74 (0.06-3.62)	0.80 (0.11-0.83)
BMI B N=35	9.66±3.79	8.83± 2.01	30.19 ±7.21	1.16± 0.30	4.0 (1.62-419)	10.71 (1.92-3585)	8.6 (2.3-48.9)	0.76 (0.12-5.11)	1.8/ (0.25-13.3)
BMI C N=10	9.96±3.30	9.10± 2.01	28.17 ±6.40	1.08± 0.14	15.41 (2-814)	45.59 (3.22-3280)	14.9 (4.9-21.5)	0.94 (0.20-2.13)	4.5 (1.26-7.3)
P value					U/p value				
A Vs B	0.108	.013	1.000	1.000	76/0.13	640/0.89	710/ 0.036	811/ 0.21	524/0.000
A vs C	0.761	.399	1.000	1.000	137/0.02	146/0.085	140/ 0.014	219/ 0.30	49/0.000
B vs C	1.000	1000	1.000	1.000	121.5/0.21	92/0.088	120 /0.13	172/ 0.93	93/0.024

(BMI A=BMI upto 25). (BMI B=BMI 25.1-30). (BMI C=BMI>30)
(P value was calculated by ANOVA Bonferrony, U/p value was calculated using Mann-Whitney U test)

In table-5 shows the glycemic status, fasting serum Insulin, C-peptide and Leptin levels with the indices of renal function in diabetic subjects according to BMI. Among the indices of renal functions urinary albumin levels in BMI C category was significantly (p=0.02) higher compared to BMI A category. ACR of the diabetic subjects was increased with increasing BMI but no significant difference was observed among the three BMI categories. Serum urea and serum creatinine levels in diabetic subjects showed no differences in BMI groups. Marked increase in serum Insulin and leptin concentration with increasing BMI was also noted among the diabetic subjects.

Figure 2 and Figure 3:
Showed the relationship of BMI with serum leptin of the diabetic and control subjects. Here figure-2 showed a strong positive correlation between BMI with serum leptin in diabetic patients.

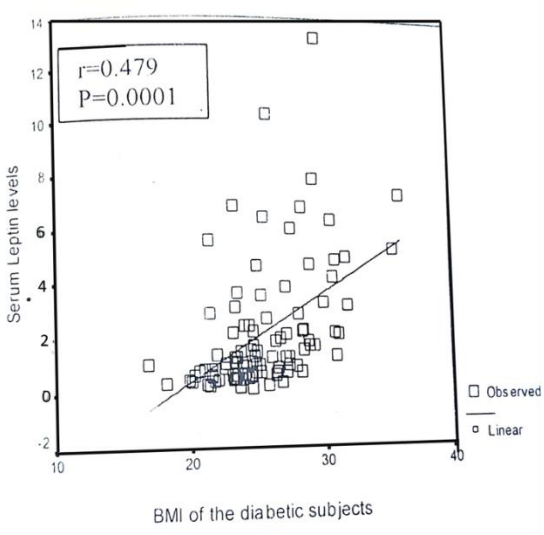

Figure-2: Relationship of BMI with Serum leptin of the diabetic subject

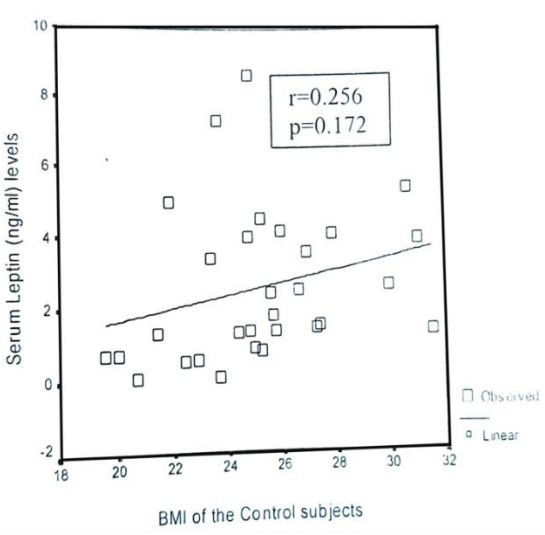

Figure-3: Relationship of BMI with Serum leptin of the control subject

Table-6: Glycemic Status, Serum Insulin, C-Peptide and Leptin levels with indices of renal function in diabetic subjects according to HbA1c.

Groups	FPG	HbA1c	S Urea	S Creatinine	S Insulin	Serum CPeptide	Serum Leptin	Urine Albumin	ACR
Group A N=13	5.97 ±1.8	6± 0.56	27.15± 6.80	1.08± 0.24	5.9(3.5 -12.5)	0.88(0.1 1-5.1)	2.26(0.4 4-6.8)	3.1(1-814)	5.22(2.4-10049)
Group B N=15	7.7 ±1.9	7.37± 0.32	26.24± 7.35	1.16± 0.30	8.0(3.2 -16.3)	0.71(0.1 2-2.1)	3.08(0.7 0-7.9)	4(1-232)	7.2(2.5-3585)
Group C N=72	12.39 ±4.5	10.84± 1.89	31. 58 ±7.52	1.15± 0.26	7.9(19 -48.9)	0.74(0.0 6-3.6)	0.98(0.1 1-13)	6.16(!.0 -379)	12.41(1.9-2341)
P value					U/p Value				
A vs B	0.749	0.09	1.00	1.00	75/ 0.30	88.5/ 0.67	79/ 0.30	81/ 0.66	50/ 0.57
A vs C	0.000	0.000	0.15	1.00	343/ 0.13	392/ 0.35	291.5/ 0.03	325/ 0.29	251/ 0.63
B vs C	0.000	0.000	0.103	0.81	513/ 0.76	469/ 0.42	253.5/ 0.001	468/ 0.67	396/ 0.93

(Group A=HbA1c < 7%, Group B=HbA1c 7%-8%, Group C=HbA1c > 8%)

(P value was calculated using one way analysis of variance, U/p value was calculated using Non-parametric Mann Whitney U test.)

Table 6: Showed that The indices of renal function ware categorized on the basis of HbA1c. It was found that the higher the HbA1c; there there was significantly higher serum leptin

concentration. But this was not observed in case of serum Insulin , C-peptide or in the case of indices of renal function

Figure 4 and Figure 5:

Showing the relationship of serum Insulin and C-peptide with circulating leptin concentration among the Diabetic subjects. It was found that both Insulin and C-peptide were positively correlated with Serum leptin among the diabetic subjects. Similar observation was also noticed in control subjects also.

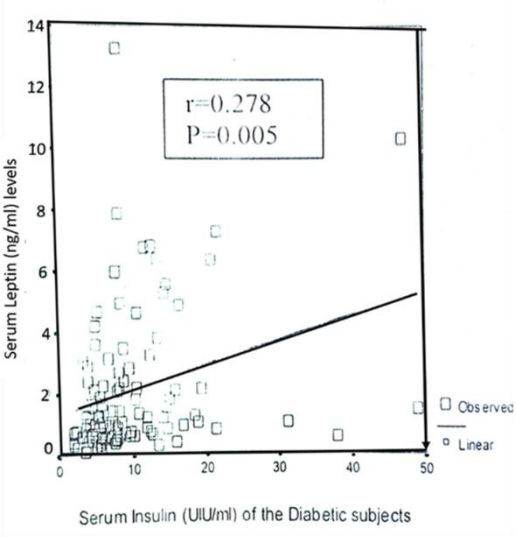

Figure-4: Relationship of insulin with serum leptin of the diabetic subjects

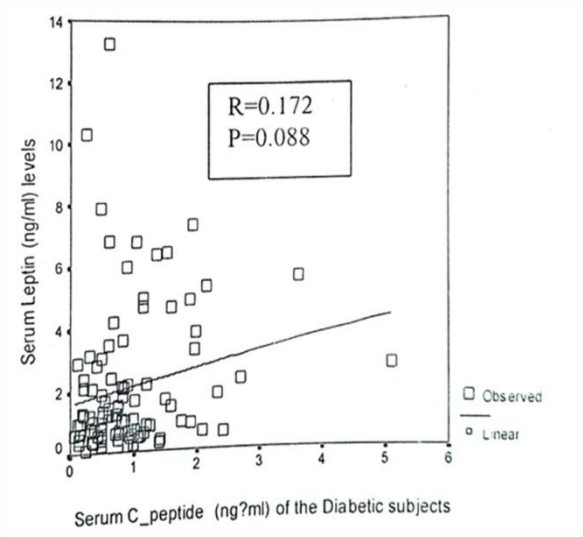

Figure-5: Relationship of serum C peptide with serum leptin of the diabetic subjects

Figure 4: demonstrated that serum insulin . and leptin have strong positive correlation with each other among the diabetic subjects

Figure 5: demonstrated that serum C peptide and. leptin were positively correlated with each other in diabetic subjects

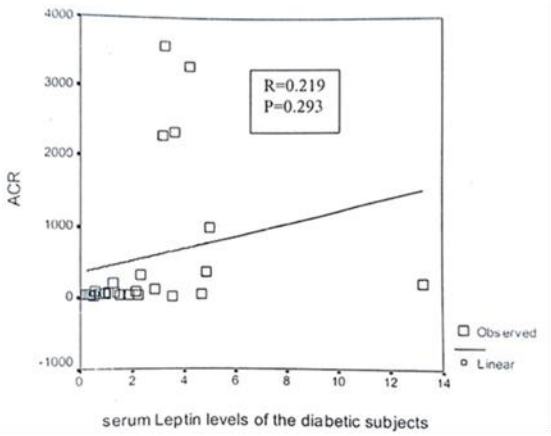

Figure-6: Relationship of serum leptin with ACR of the diabetic subjects with ACR>30mg/g creatinine

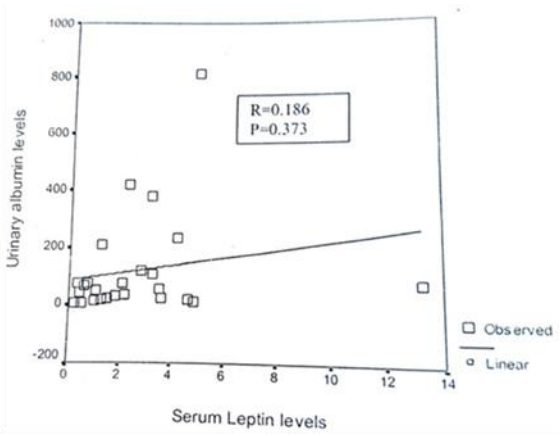

Figure-7: Relationship of serum leptin with Urinary albumin of the diabetic subjects with ACR > 30mg/g creatinine

Figure 6 and figure 7: Showed the relationship serum leptin with ACR and urine albumin among the diabetic subjects when ACR is above 30mg/g creatinine Our study demonstrates no correlation between ACR and urine Albumin with circulating leptin concentration

Discussion:

Type 2 diabetes subjects remain mostly asymptomatic and on presentation this group of patients has one or more microvascular end point like nephropathy, retinopathy and neuropathy[21]. Relationship of serum leptin and incipient nephropathy among type 2 diabetic subjects was analyzed. Renal function of the diabetic and control subjects was evaluated by measurement of serum urea, serum creatinine, urinary albumin and albumin creatinine ratio (ACR). It was found similar in both the groups. However, when a cut-off value (ACR 30mg/gm creatinine) was used to distinguish microalbuminuria from normoalbuminuria, 25 diabetic patients were found to have microalbuminuria. When the indices of renal function were classified according to HbA1c and BMI Urinery albumin and ACR increased with increasing BMI and HbA1c. Serum leptin levels among the diabetic subjects having miroalbuminuria were found similar to that of normoalbuminuria.

Previous studies done in abroad among human subjects demonstrated that circulating serum leptin concentration are strongly correlated with BMI, percent body fat, total fat mass and other indices of obesity [1].The present study also attempted to document the baseline serum leptin concentration among the healthy population, variation of serum leptin in different glycemic status and BMI groups among type 2

diabetic subjects, variation of sexes and the impact of early nephropathy on circulating leptin concentration.

Serum leptin level was found to be positively correlated with BMI in both controls and diabetic subjects and the diabetic subjects have shown strong positive correlation at p=0.0001 levels (fig:2 and 3). Female subjects have shown significantly higher values of serum leptin (3-4 times) (table-4) than their male counterpart irrespective of glycemic status and BMI (table 5, 6). Similar degrees of hyperleptinemia were also found in male and female subjects when they are categorized according to BMI (table-5). This findings are consistent with the findings of other study done in abroad [2, 22].
Serum leptin concentration was assessed in relation to Insulin and C-peptide. Our study demonstrated that serum insulin and leptin were positively correlated with each other in both control and diabetic subjects (fig 4). This findings is consistent with the hyperleptinemic/ Hyperinsulinemic or insulin resistant/ leptin resistant hypothesis in obese type2 diabetic subjects [1, 23]. This type of relation was also marked in case of leptin and C-peptide (fig 5).

The kidneys expresses highest leptin receptor and it is one of the important sites for leptin degradation and clearance [9]. So that in the state of renal dysfunction, circulating leptin concentration might be altered. In our study subjects no differences in renal function was found between diabetic and control groups. But when cut off value was used to categorize normoalbuminuria and microalbuminuria among the diabetic subjects, 25 diabetic subjects showed urinary albumin excretion and ACR levels more than the normal range. When the indices of renal function were classified according to BMI and HbA1c, urinary albumin and ACR increases with increasing BMI and HbA1c. Patients having normal BMI showed significantly (p=0.02) lower urinary albumin excretion compared to patients having BMI more than 30. Similar observation was noticed in case of ACR but it is not significant at the level of 5%. Recently many authorities suggest that higher urinary albumin excretion (microalbuminuria) might be an essential component of insulin resistance syndrome, which is commonly associated with obese high BMI group[24].

We also assessed the serum leptin levels among the diabetic subjects with ACR more than 30 mg/gm creatinine. Our study demonstrates no correlation between ACR and circulating leptin concentration (fig 6 and 7). This finding is consistent with the finding of other studies done in abroad [14]". Although at least one study[13] has shown that serum leptin levels has already been increased in earlier stages of nephropathy but our findings contradicts to this result. It's a general agreement by most of the investigators that there are no differences of serum leptin concentration in patients with diabetic nephropathy to that of age, sex and BMI matched control subjects until they reach a very late stage.[10-12].

Conclusion:

From our study it was found that,
1. There is a very little association between serum leptin level and the altered renal function at least in their earlier stages irrespective of glycemic status and BMI. Further study is needed in a large scale to document whether there are changes in serum leptin concentration in early diabetic nephropathy in the stages of Microalbuminuria and Macroalbuminuria.
2. Serum leptin concentration is positively correlated with BMI which was more marked in Diabetic subjects and the female subjects have shown higher values of serum leptin than their male counterpart irrespective of BMI and glycemic status.

3 .Patients with high BMI are associated with increased urinery albumin excretion rate termed as microalbuminuria irrespective of glycemic status which is thought to be an important macrovascular risk factor predictor for cardiovascular and cerebrovascular diseases.

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Conflict of Interest:

There was no conflict of interest with any person, organization, groups, companies, diagnostic laboratories or any other financial institutions in conducting the research works.

Ethical Declaration:

This research work was duly approved by the Institutional Ethical Committee of Sylhet MAG Osmani Medical College, Sylhet and The BIRDEM Academy Dhaka, Bangladesh

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