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RELATIONSHIP BETWEEN PLASMA LEPTIN AND PLASMA INSULIN LEVELS IN TYPE-2 DIABETIC PATIENTS BEFORE AND AFTER TREATMENT WITH GLIBENCLAMIDE AND GLIMEPIRIDE

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Abstract : Type 2 diabetes affects 100 million people throughout the world. Among the various factors implicated in the causation of this disease, the role of leptin, an obesity gene product, is increasingly being investigated. This especially assumes importance in the light of knowledge that obesity confers a minimum of 3–10 fold higher risk of diabetes. This study was planned to investigate the relationship between leptin and insulin levels in type 2 diabetic patients before and after treatment with glibenclamide or glimepiride. 60 type 2 diabetic patients were recruited for the study and were divided into 2 groups-one receiving glimepiride and the other group receiving glibenclamide for duration of 10 weeks. This study demonstrated a highly positive correlation of plasma leptin levels with BMI, plasma insulin and insulin resistance. No gender specific differences were observed in leptin concentrations. The study, however, failed to demonstrate any possible relationship between glycemic control as assessed by blood sugars/ glycosylated hemoglobin (HbA1c) and plasma leptin. The administration of glibenclamide or glimepiride significantly lowered blood glucose levels coupled with a decrease in (HbA1c). Both the drugs increased insulin concentrations. Glibenclamide increased leptin levels but they remained unaltered with glimepiride. Glibenclamide and glimepiride were found to be equally effective in their glucose lowering action. However, the patients receiving glibenclamide experienced higher episode of hypoglycaemic spells than those receiving glimepiride.

Key words : type-2 diabetes glibenclamide glimepiride glycaemic control plasma insulin plasma leptin

INTRODUCTION

Type-2 diabetes affects approximately 100 million people throughout the world and it is predicted that this number will double during the next 10 years (1). In individuals

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with type 2 diabetes, defects in insulin secretion, tissue insensitivity to insulin and abnormalities in adipose tissue metabolism have been well documented (2). Sulphonylureas that act by stimulation of insulin release from the pancreatic P cells form the mainstay of oral anti-diabetic therapy. Glibenclamide, a highly potent agent, is one of the most commonly used members of this class of drug. However, it is known to cause frequent hypoglycemic episodes because of marked fluctuations in insulin levels, which tend to be on the higher side. Glimepiride, on the other hand, is a novel sulphonylurea that achieves steady insulin concentrations, resulting in very low intra-individual variability. Lower insulin levels have been documented in patients treated with glimepiride as compared to other Sulphonylureas (3).

The fact that type 2 diabetes affects certain individuals and not others remains as elusive as ever. Although many biochemical/molecular defects have been described, the genetic basis for this disorder is yet to be delineated. In fact no specific gene has been identified in the etiology of type 2 diabetes. Among the various factors implicated in the causation of this disease, the role of leptin- an obesity gene product, is increasingly being investigated (4). This especially assumes importance in the light of the knowledge that obesity, shown to be a state of hyperleptinemia (5), confers a minimum three to ten fold higher risk of type 2 diabetes (6).

Leptin (*leptos* means thin), a peptide hormone (167 amino acid protein), was discovered at the end of the year 1994 (7). It is produced by adipocytes and acts on the satiety centre on the hypothalamus to suppress appetite, limit food intake and increase energy expenditure (4), whereas, Neuropeptide Y (NPY), a potent stimulator of food intake, is believed to be a mediator of leptin action. Synthesis of NPY is inhibited by leptin (8). A state of hyperleptinemia, possibly due to leptin resistance has been described in majority of obese individuals (5). Obesity is also associated with insulin resistance and hyperinsulinemia (9). Hence obesity, hyperleptinemia, and hyperinsulinemia often coexist and the relationship between insulin and leptin concentrations independent of obesity has been a subject of debate.

Insulin has been found to be a potent regulator of leptin expression in rodents (10, 11) while the data in human are conflicting. There are reports suggesting that in humans, insulin does not stimulate leptin production (12, 13) whereas there is evidence from other clinical and *in vitro* studies that insulin does not have a role in regulation of leptin (11, 14, 15). Leptin, on the other hand, has been shown to improve insulin sensitivity and glucose metabolism in leptin treated rats (16, 17, 18) and a similar response has been reported in human (19). However, relationship between leptin concentrations and insulin resistance has also been cited (20). Therefore it is evident that leptin's influence on insulin action is as debatable as insulin's effect on leptin levels.

In view of the conflicting reports on the correlation of leptin and insulin hormones, this study was planned to investigate the relationship between leptin and insulin levels in type-2 diabetic patients before and after treatment with glibenclamide and glimepiride and to study a possible relationship between glycaemic control and plasma leptin levels.

MATERIAL AND METHODS

Type 2 diabetic patients from the outpatient and endocrine metabolic clinics of Guru Teg Bahadur Hospital, Shahdara, Delhi, India, were recruited for the study.

Inclusion criteria :– Age of patients ≥ 30
Newly diagnosed type 2 diabetic patients (WHO Technical Report series, 1985) (21) who failed to achieve controlled fasting blood sugars (FBS) after four weeks of recommended diabetic diet.

Exclusion criteria :–history of neuropathy, hepatitis, psychiatric disorders or any other illness; pregnancy; patients taking glucocorticoids or other hormones; oral contraceptives; drugs known to interact sulphonylureas and history of allergy to the investigational drugs.

After obtaining written informed consent, a detailed history was taken, a thorough physical examination was carried out and routine hematological/biochemical tests, chest X-ray and ECG were done in order to exclude diseases other than diabetes. Sixty age and gender matched patients of type 2 diabetes were recruited in the study. They were divided in two groups- group A and B. Group A received glibenclamide (minimum 2.5 mg, maximum 15 mg) and group B received glimepiride (4 mg).

The patients once included in the trial were put on minimum dose of the either drug. A particular dose was given for 1 week and then FBS levels were taken. If they were not controlled, the dose of the drug was increased. The dose escalations were done till the attainment of controlled FBS levels or administration of the maximum dose of the drug that was decided for the study (15 mg for glibenclamide and 4 mg for gimepiride). Total duration of drug administration was 10 weeks. All the biochemical investigations and parameters were measured before the administration of the drug and after 10 weeks of treatment.

The following parameters were studied

1. *Weight :*

Patients were weighed using a standard scale that had a precision of 0.1 kg.

1. *Height :*

The heights of the subjects were recorded without footwear, using a vertically mobile scale and expressed to the nearest centimeter.

3. *Body Mass Index (BMI) :*

BMI was calculated from the height and weight as follows;

BMI = weight (kg)/height (m^2).

4. *Waist-Hip Ratio (WHR) :*

The waist and hip circumferences were measured in the erect position, with the abdomen relaxed, the arms at the sides, and the feet together. The measurement was taken at the level of the narrowest part of the torso, as seen from the anterior aspect and was recorded to the nearest 0.1 cm. the hip circumference was measured horizontally at the level of the maximum extension of

the buttocks posteriorly. WHR was then calculated as follows;

WHR = waist circumference (cms)/hip circumference (cms)

5. *Plasma insulin :*

The concentration of insulin in serum samples was estimated using a solid phase Enzyme Amplified Sensitivity Immunoassay (EASIA) MEDGENIX-INS-EASIA kit. In this, insulin was detected in standards and samples using monoclonal antibodies against insulin. An enzyme labeled antibody to insulin was then added followed by substrate. Amount of substrate turnover was then determined colorimetrically which was proportional to the insulin concentration. A sample curve was then plotted and insulin concentrations in samples were determined by interpolation from the standard curve (22, 23).

6. *Plasma leptin :*

Plasma concentrations of leptin were determined using a direct ELISA human leptin kit (Diagnostics Biochem Canad- dbc). The dbc human leptin immunoassay is a 3.5 hour solid phase ELISA designed to measure human leptin in serum or plasma. It contains recombinant human leptin expressed in bacterial cells and antibodies raised against recombinant human leptin (22, 23).

7. *Total glycated haemoglobin :*

Total glycated haemoglobin (HbA1c) was measured with a glycated haemoglobin kit that uses an affinity resin in a disposable column that has an affinity for cis- diols e.g. glucose molecules attached to haemoglobin. An aliquot of whole blood is hemolyzed by mixing with the hemolyzing reagent. The hemolyzed sample is introduced into an affinity resin that binds all glycated hemoglobin. The remaining haemoglobin does not bind and are washed through the column upon addition of a wash buffer. They are then eluted from the column with an elution buffer. The ‘elution’ fraction containing the glycated hemoglobin is collected. The absorption of each fraction is measured at 415 nm. The absorbance is directly proportional to the amount of hemoglobin present in each fraction (24).

Following routine investigations were done

Blood sugars (FBS, PPBS), serum electrolytes, blood urea, serum creatinine, serum uric acid, fasting lipid profile, liver function tests, Hb, TLC, DLC, ESR, ECG, X-ray chest and urine tests- routine (for sugar and albumin) and microscopic examination.

Compliance

The patient’s compliance was determined by asking verbally and by counting the unused tablets on each visit.

Ethics

The study was approved by the institutional ethical committee.

Statistical analysis

The data analyses in this study included Student’s *t* test (paired two tail test), Spearman’s correlation analyses. P value less than 0.05 was considered to be significant. All statistical calculation was done on the SPSS statistical software and GraphPad InStat version 3.00 for Windows 95, GraphPad software, San Diego California USA, www.graphpad.com.

RESULTS

The clinical and metabolic characteristics of the 60 type 2 diabetic subjects at baseline are given in Table I. There were 14 males and 46 females in the study group. Males and females showed no statistically significant differences in any of the baseline characteristics. Mean BMI (24±4.55 kg/m^2) of the recruited subjects were in the normal range. The mean serum insulin concentrations of these sixty type 2 diabetic subjects were 24.43±7.40 µU/ml whereas the serum leptin concentrations ranged from 2.00–6.08 ng/ml; the correlations of serum leptin concentrations with other parameters are listed in Table II. Serum leptin levels correlated positively with BMI, WHR, insulin concentrations and Insulin/Glucose (I/G) ratio. The highly positive correlation of serum leptin with insulin levels as well as with I/G ratio persisted even after adjustments for WHR and BMI (P = 0.0001).

Subjects at baseline did not differ with respect to age, BMI, WHR in terms of the groups to which these were randomized. Mean BMI of the subjects in two groups were within the normal range. The male/female sex ratio in the two groups, though different, was not statistically significant. There was not significant difference in any of the hematological and biochemical parameters in type-2 diabetic subjects of group A and group B (values not shown here) before drug treatment.

Effects of glibenclamide and glimeperide on various clinical and biochemical parameters

There were 30 patients in each group. The clinical and metabolic characteristics were evaluated before the administration of these two drugs. In the group A 5, 8,

TABLE I: Clinical and metabolic parameters of the type 2 diabetic subjects at baseline.

Characteristics	*Mean ± S.D. (n–60)*	*Range (n–60)*
Age (years)	49.5±9.0	34.0–70.0
BMI (kg/m^2)	24.71±4.55	14.00–37.00
WHR	0.93±0.07	0.77–1.12
FBS (mg/dl)	223.33±87.30	117–470
2hPPBS (mg/dl)	303.55±75.00	195–476
GHb (%)	8.72±1.40	6.20–12.50
Fasting Leptin (ng/ml)	3.74±1.02	2.00–6.08
Fasting insulin (µU/ml)	24.43±7.40	11.00–46.75
I/G Ratio	0.13±0.06	0.02–0.29
Lipid profile		
Total cholesterol (mg/dl)	219.28±61.92	90.00–534.00
HDL (mg/dl)	44.85±11.47	19.00–89.00
LDL (mg/dl)	142.67±57.57	48.00–454.00
VLDL (mg/dl)	33.63±23.15	8.00–171.00
TG (mg/dl)	158.68±71.79	41.00–444.00

TABLE II: Correlations of serum leptin levels with other clinical and metabolic characteristics in type 2 diabetic subjects.

Characteristics	*Correlation with leptin concentrations (n–60)*	
	r-value	*P-value*
Age (years)	0.129	0.326
BMI (kg/m^2)	0.448**	0.0001**
WHR	0.301*	0.019*
FBS (mg/dl)	–0.137	0.298
2hPPBS (mg/dl)	–0.124	0.345
GHb (%)	–0.129	0.326
Fasting insulin (µU/ml)	0.676**	0.0001**
I/G Ratio	0.497**	0.0001**
Lipid profile		
Total cholesterol (mg/dl)	0.100	0.449
HDL (mg/dl)	0.020	0.882
LDL (mg/dl)	0.063	0.634
VLDL (mg/dl)	0.069	0.598
TG (mg/dl)	0.1660	0.163

* Correlation is significant at the 0.05 level.
** Correlation is significant at 0.01 level.

TABLE III: Effects of glibenclamide and glimepiride on various clinical and metabolic characteristics.

Characteristics	*Glibenclamide*				*Glimepiride*			
	Mean±SD (n–30)	*P value*	*df value*	*F*	*Mean±SD (n–30)*	*P value*	*df value*	*F*
BMI		0.9976	58	1.271		0.913	58	1.620
Pre treatment	24.627±4.01				24.769±5.097			
Posttreatment	24.630±3.557				24.640±4.005			
WHR		0.6283	58	1.193		0.3769	58	1.223
Pre treatment	0.915±0.076				0.941±0.066			
Posttreatment	0.925±0.083				0.925±0.073			
FBS		<0.0001*	58	1.918		<0.0001*	58	3.644
Pre treatment	236.666±85.374				210.00±88.61			
Posttreatment	153.066±61.645				132.23±46.42			
2hPPBS		<0.0001*	58	1.279		<0.0001*	58	1.772
Pre treatment	314.633±77.650				292.47±71.85			
Posttreatment	214.400±68.660				189.57±53.97			
GHb		0.0120*	58	1.532		0.0166*	58	1.230
Pre treatment	9.190±1.433				8.24±1.22			
Posttreatment	8.110±1.775				7.50±1.10			
Leptin		0.0046*	58	1.987		0.1291	58	1.530
Pre treatment	4.139±0.950				3.337±0.946			
Posttreatment	4.766±0674				3.787±1.170			
Insulin		<0.0001*	58	2.317		0.0160*	58	2.257
Pre treatment	27.734±0.693				21.133±6.401			
Posttreatment	39.028±10.548				36.106±9.617			
I/G ratio		<0.0001*	58	4.203		<0.0001*	58	6.864
Pre treatment	0.134±0.060				0.115±0.050			
Posttreatment	0.291±0.123				0.304±0.131			
Total cholesterol		0.4801	58	1.019		0.3498	58	1.156
Pre treatment	218.40±45.21				220.16±75.86			
Posttreatment	213.93±44.79				198.85±70.57			
HDL		0.0295*	58	2.043		0.0243*	58	2.110
Pre treatment	45.60±13.65				44.20±8.88			
Posttreatment	46.60±9.55				45.30±12.90			
LDL		0.2278	58	1.323		<0.0001*	58	1.271
Pre treatment	142.40±43.20				142.90±57.44			
Posttreatment	134.07±49.69				135.56±16.11			
VLDL		0.1250	58	1.541		0.3089	58	1.206
Pre treatment	37.13±8.69				30.10±16.13			
Posttreatment	22.10±7.00				27.91±14.69			
TG		0.4389	58	1.059		0.1604	58	1.452
Pre treatment	16377±63.40				153.64±80.06			
Posttreatment	159.37±61.60				143.79±66.44			

*Significant P(<0.05) value.

7 and 10 patients received 2.5, 5, 10 and 15 mg of GLB respectively whereas in the group B 8, 7 and 15 patients received 1, 2 and 4 mg of GLI. These drugs were administered to the subjects for duration of 10 weeks. At the end of this follow up period, the clinical and metabolic characteristics were again evaluated to assess and compare the differential effects of these two drugs.

Effects of glibenclamide (GLB):

There was a significant increase in insulin concentrations from 27.7±0.69 to 39.03±10.55 µU/ml (P=0.001), in I/G ratio from 0.1343±0.0602 to 0.2914±0.1236 (P=0.001), in plasma leptin levels from 4.139±0.950 to 4.767±0.674 (P=0.001). The

mean BMI and WHR were increased but the difference was not statistically significant. Both FBS and 2hPPBS levels decreased significantly coupled with decrease in GHb. Glibenclamide significantly increased plasma HDL level (Table III). No significant correlation was observed between leptin levels and the changes in other variables after drug treatment.

Effects of glimepiride (GLI):

There was a significant increase in insulin concentrations from 21.13±6.40 to 36.10±9.6 μU/ml (P=0.0160) and in I/G ratio from 0.115±0.050 to 0.304±0.131 (P=0.0001). There was a significant decrease in both blood sugars (FBS and PPBS). There was neither significant change in serum leptin levels, BMI and WHR nor significant correlation between leptin levels and the other variables after the treatment. However, there was significant change in serum HDL and LDL levels (Table III).

The various symptoms reported by the patients at the basal level as well as during drug treatment were hypoglycemic spells, asthenia, fatigue, headache, abdominal discomfort and pain in extremities. Higher number of patients reporting hypoglycemic episodes was observed during treatment with glibenclamide as compared with glimepiride.

DISCUSSION

The present study in type-2 diabetic subjects shows a positive correlation of plasma leptin levels with BMI. It also provides an evidence of a relationship between plasma leptin and plasma insulin concentrations as a highly positive correlation was demonstrated between two variables. Moreover, the association between leptin and insulin was observed to persist even after corrections for BMI. The present data also showed a strong correlation of leptin levels with markers of insulin resistance i.e. I/G ratio and insulin itself. The gender differences in plasma leptin could not be brought out with good effects in our study. Any significant relationship between leptin concentration and lipid profile as well as with measurements of glycemic control i.e. FBS/2hPPBS/GHb in the diabetic patients could not be demonstrated.

Furthermore, glibenclamide and glimepiride administered to the type-2 diabetic subjects were found to be equally effective in lowering their blood glucose levels. The use of either drug was observed to increase insulin concentrations. Glibenclamide increased leptin levels significantly whereas no such change was observed in glimepiride treated group. A number of workers in the past (6, 14, 25) have demonstrated that leptin circulates at concentrations proportional to BMI and present study has similar findings suggesting that leptin is secreted from adipocytes into circulation and it acts as a lipostat/signaling molecule mainly on the hypothalamus to limit food intake and increase energy expenditure. Higher leptin levels found in obese subjects are probably the results of inadequate leptin signaling for a given leptin concentration, a mechanism akin to leptin resistance.

The results in the study showed positive correlation between fasting leptin and fasting insulin levels and is similar to the previous findings (22, 23). With this, it has been clear that these two variables are associated with one another independent of BMI, a measure

of adiposity. The positive correlation of insulin and leptin with each other (independent of body adiposity) demonstrated here is in consonance with data in past studies (6, 26) and provides more support for the hypothesis that insulin regulates leptin secretion. It has been previously demonstrated that *in vitro* leptin down regulates insulin dependent tyrosine phosphorylation of insulin receptor substrate-1 in Hep G 2 cells (27) and physical concentration of leptin impairs several metabolic actions of insulin in rat adipocytes (28) which suggests that leptin might cause insulin resistance in target tissues. However, another report showed that leptin enhances insulin's ability to inhibit hepatic glucose production thereby improving insulin sensitivity (29). Mohammed Ali et al (20) showed no correlation between leptin levels and insulin sensitivity in type 2 diabetic patients although a positive correlation between leptin and insulin concentrations was demonstrated. The insulin sensitivity in their study was assessed by measuring the metabolic clearance rate of glucose during hyperinsulinemic euglycemic clamp. In this study, we found a strong correlation between plasma leptin and insulin resistance as assessed by measurements of I/G ratio, WHR and insulin concentrations. Hence, based on previous studies and our observation, it could be said that a causal relationship exists between leptin and insulin resistance. Thus, in obese individuals down regulation of hypothalamic receptors leads to an increase in leptin levels which itself could cause insulin resistance or the increased food intake leading to increased adipocyte mass and decreased insulin sensitivity. The former causes further leptin synthesis and release and a further down regulation of hypothalamic receptors leading to the development of vicious cycle with hyperinsulinemia helping drive further adipose tissue accumulation and dyslipidemia. Alternatively, the insulin resistance in type-2 diabetes could be primary, with resultant increase in central adipose tissue mass, enhanced leptin production, loss of appetite control, weight gain and further insulin resistance and hyperleptinemia.

In our study, we could not demonstrate any possible correlation of plasma leptin with parameters of glycemic control i.e. blood sugars and HbA_{1c} and is consistent with past study (30) which showed absence of short term effects of hyperglycemia on plasma leptin levels in man. This result might indicate that short term changes of plasma glucose do not contribute to leptin levels. In contrast, a study in past (24) in seventy type-2 subjects suggested a possibility that hyperglycemia for long periods that was estimated by HbA_{1c}, may have a suppressive action on serum concentrations of leptin. However, the study population of the latter trial included relatively larger number of patients with poorly controlled diabetes. In their study, the values of HbA_{1c} distributed from 4.9–22.1% with 33% of the patients showing HbA_{1c} more than 10.0% whereas in our study all the patients had their HbA_{1c} in the range of 6.2–12.5%. However, in the same study, when data of 59 patients with HbA_{1c} less than 12.0 were re-examined, the correlation between HbA_{1c} and leptin concentrations disappeared. Thus, the discrepancy of the relationship of the two parameters might depend on the distribution of HbA_{1c} values.

Earlier studies (22, 26) have reported a gender difference in leptin concentrations; females having higher leptin levels than males. This has been attributed to a higher

fat content of women of any BMI (6, 25) although gender itself might be associated with leptin independently of body fat mass (31). In this study, however, gender differences in leptin levels could not be brought out with good effects. Female subjects in our study demonstrated higher mean leptin concentrations than the males but the difference was not significant. The discrepancy might be arising from the fact that in our study females far outnumbered males (46 versus 14 respectively). Thus, this is probably not a true reflection of the gender differences in leptin levels. Further studies are thereby needed to explore any possible gender related difference. There was no absence of correlation of leptin with age, total cholesterol, triglyceride, HDL, LDL and VLDL cholesterols, and is similar to earlier findings (20).

As far as various clinical and metabolic variables are concerned, both the drugs glibenclamide and glimepiride significantly decreased blood sugar levels with a concomitant increase in GHb values. Insulin levels also increased post drug therapy. Both the drugs increased plasma leptin and HDL levels, and decreased plasma LDL level. Our study established equal efficacy for both the drugs as has also been previously shown by other workers (32, 33).

The various adverse experiences reported by the type-2 diabetic patients included hypoglycemic episodes, asthenia, fatigue, headache, abdominal discomfort and pain in extremities. The number of patients reporting hypoglycemic episodes was higher in the glibenclamide treated group as compared to the one receiving glimepiride. This is a well recognized adverse effect of glibenclamide probably related to higher dosage, longer duration of action and higher insulin levels resulting in its sustained blood glucose lowering effects (32).

Glibenclamide caused significant increase in leptin concentrations and this was related to the change in insulin levels. This observation is in agreement with the findings of Haffner et al (34) where glibenclamide caused significant increase in leptin concentrations parallel to the changes in insulin levels. No studies have so far been reported regarding the effect of glimepiride on leptin levels. Further studies on similar lines with a larger patient population and over a longer follow up period are probably needed to fully assess the effects of these drugs on plasma levels.

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