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Effects of glibenclamide, metformin or their combination on the correlation between serum leptin levels with glycemic control and insulin levels in type 2 DM

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ABSTRACT: Type 2 diabetes affects millions of 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 study was designed to investigate the relationship between leptin and insulin levels, insulin resistance and glycemic control in newly diagnosed males with type 2 diabetes before and after treatment with either glibenclamide, metformin or their combination. 60 type 2 diabetic patients were recruited for the study and were allocated into 3 groups; one receiving glibenclamide 5mg/day, the second receiving 850mg/day metformin, while the third one treated with combination of them for 90 days. Blood samples withdrawn from each patient at zero time and after 90 days for evaluation of HbA1c and plasma levels of glucose, leptin and insulin. The results demonstrated that although all the treatment approaches significantly improved glycemic control, the use of metformin only affects the correlation between plasma leptin levels with insulin and glycemic control. In conclusion, plasma leptin levels vary in type 2 DM patients beyond the state of adiposity, and can be used as an indicator for the choice of treatment pattern in those patients.
Key words: Type2 DM, leptin, glibenclamide, metformin, glycemic control

INTRODUCTION

Leptin is a hormone produced in adipocytes [1]. It has been suggested that leptin directs metabolic fuels towards utilization and away from storages [2]. The development of Type 2 diabetes in association with obesity, hyperinsulinemia and insulin resistance has been demonstrated. Obesity is associated with a marked increase in circulating leptin concentration [3,4]. However, plasma leptin displays a strong correlation with insulin concentration, insulin resistance, metabolic syndrome, dyslipidemia, even after controlling for measures of body fat mass [5-8]. Leptin, on the other hand, has been shown to improve insulin sensitivity and glucose metabolism in leptin treated rats [9] and a similar response has been reported in human [10]. However, relationship between leptin concentrations and insulin resistance has also been cited [11]. Therefore it is evident that leptin's influence on insulin action is as debatable as insulin's effect on leptin levels. The different severity or condition of type 2 diabetes might result in different degree of insulin concentration and resistance. Many studies have demonstrated that taking oral antidiabetic drugs might change the plasma leptin concentration in type 2 diabetes [12,13]. In view of the conflicting reports on the correlation of leptin with insulin and glycemic control, this study was planned to investigate the relationship between leptin and insulin levels in newly diagnosed type 2 diabetic patients before and after treatment with either glibenclamide, metformin or their combination.

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MATERIALS AND METHODS

This was a single center, open-label, randomized parallel group study conducted at the Specialized Center for Diabetes and Endocrinology/Baghdad, during the period from November 2009 till July 2010. The study was approved by the local research ethics committee and all subjects gave written informed consent to participate in the study. Only male patients with age more than 30 yrs, BMI ranging from (25-29.9) kg/m², glycosylated hemoglobin > 7% and Fasting plasma glucose ≥126mg/dL (7.0 mmol/L) or 2 hr plasma glucose after oral glucose tolerance test ≥200mg/dL (11.1 mmol/L) [14], were included in the study. Patients should not be previously treated with oral hypoglycemic agents before the time of enrollment. Patients with current insulin therapy or received insulin for more than six weeks in last 3 months, who had known hypersensitivity to Biguanides and sulphonylurea, who are on chronic medication known to affect glucose metabolism were excluded from the study. Also the patients with renal disease or renal dysfunction, with congestive heart failure, hepatic insufficiency, alcoholic person and pregnant and lactating women were planned to exclude from the study. A total of 60 newly diagnosed type 2 diabetic patients were enrolled on a treatment program. The patients were given instructions on diabetic diet and asked to monitor their blood glucose level, both fasting and postprandial, glycosylated hemoglobin and lipid profile at the initial visit to the center. The patient's records were maintained for the next three month after their initial visit to hospital. The patients were observed for weight, height and blood pressure measurement. The records of age, sex, family history and other possible associated diseases were also maintained. The records of the weight and height are helpful for the determination of body mass index. The patients were also interviewed for their initial sign and symptoms. As the patients were recruited for the study they were randomized into three groups according to the treatment they received; first group includes 20 male patients treated with 5 mg glibenclamide once daily in the morning for 3 months; second group includes 20 male patients treated with 850 mg metformin single tablet after lunch for 3 months; third group includes 20 male patients treated with a combination of drugs mentioned in previous groups for three months. Another group of 20 healthy male subjects, matched with patients for age, were included and served as control. After 12 hrs fasting, blood samples (10 ml) were obtained from each patient for the evaluation of FPG, HbA1c, leptin and insulin levels at zero time and after three months of treatment using standard methods [15-18]. Body weight and BMI were also evaluated within the same previously mentioned periods. All data are given as mean ± SD. Pre- and post-treatment values were statistically analyzed using paired Student's *t* test, while two ways ANOVA was utilized to compare values between different groups. Pearson's correlation was performed to evaluate the relationship between leptin plasma levels and the values of other parameters as a response to different treatment approaches. Values with $P<0.05$ are considered significantly different.

RESULTS

As shown in table 1, treatment of diabetic males with glibenclamide alone for 90 days increases serum leptin levels significantly (114.5%) compared to pre-treatment value ($P<0.05$). Meanwhile, the use of a combination (glibenclamide and metformin) also increases serum leptin levels (65.4%) but to less extent than that produced by glibenclamide alone. However, the use of metformin alone significantly decreases serum leptin levels (40.6%). Meanwhile, table 1 showed that there is a significant decrease in fasting plasma glucose in all groups of patients treated with either glibenclamide, metformin or their combination for 3 months (49%, 24%, and 42% respectively; $P<0.05$) compared with baseline value. All types of treatment followed produced significant decrease in HbA1c levels ($P<0.05$) compared to pre-treatment values. However, the percent change in HbA1c produced due to the use of a combination (glibenclamide and metformin) was significantly greater (22.9%) than those produced by each one when used alone (18.7% for glibenclamide and 22.3% for metformin), while metformin decreased HbA1c greater than glibenclamide ($P<0.05$). Results presented in table 1 showed that treatment with glibenclamide alone or its combination with metformin significantly increases ($P<0.05$) serum insulin levels, while treatment with metformin alone did not increase insulin levels in the serum compared to pre-treatment values; treatment with glibenclamide produces the highest percent increase in insulin levels (120.8%) which is significantly greater than those produced by metformin alone or the combination of glibenclamide

with metformin (2.6% and 67.1% respectively, $P<0.05$), and the effect of the combination was significantly greater than that produced by metformin alone. Concerning the marker of insulin resistance, glibenclamide and its combination with metformin non-significantly increased the value of insulin resistance compared to pre-treatment levels, though the change due to the use of the combination is less prominent (14.2% *vs.* 1.3%). Meanwhile the use of metformin alone significantly decreases the value of insulin resistance (31.4%) after 90 days treatment compared to pre-treatment values.

Concerning the relationship between long-term glycemic control parameter (%HbA1c) and serum leptin levels, all groups showed very weak non-significant correlation between the two parameters, and only the treatment with metformin alone increases the level of correlation between the two parameters to significant level (r=0.03 *vs.* r=0.49); while glibenclamide, when used alone or in combination with metformin did not show such pattern of changes (Figures 1 and 2). In figures 3 and 4, treatment with metformin demonstrated relatively high correlation between serum leptin levels and IR index value compared to the pre-treatment values; while glibenclamide, when used alone, shifts the correlation between the two parameters toward higher but non-significant value, and when combined with metformin, the value was decreased but not significant.

Table 1. Effects of treatment with glibenclamide, metformin or their combination on glycemic control, insulin level and leptin level in plasma of newly diagnosed males with type 2 DM.

Parameters	Period	Healthy controls	Glibenclamide 5 mg/day	Metformin 850mg/day	Combination GL + MF
Leptin (ng/ml)	*Zero time*	9.4 ± 0.57^{a}	7.6 ± 0.46	8.1 ± 0.70	**7.9 ± 0.74**
	After 3 months		$16.3 \pm 0.93^{*b}$	$4.8 \pm 0.49^{*c}$	**$13.2 \pm 0.70^{*d}$**
FPG (mmol/L)	*Zero time*	4.6 ± 0.13^{a}	10.8 ± 0.53	7.4 ± 0.36	**10.3 ± 0.66**
	After 3 months		$5.5 \pm 0.23^{*b}$	$5.2 \pm 0.16^{*a}$	**$5.9 \pm 0.26^{*b}$**
HbA1c %	*Zero time*	5.1 ± 0.11^{a}	7.4 ± 0.15	7.6 ± 0.15	**7.5 ± 0.18**
	After 3 months		$6.1 \pm 0.16^{*b}$	$5.1 \pm 0.09^{*a}$	**$5.7 \pm 0.11^{*c}$**
Insulin (µIU/ml)	*Zero time*	9.5 ± 0.60^{a}	9.2 ± 0.64	10.7 ± 1.14	**8.8 ± 0.69**
	After 3 months		$20.3 \pm 0.94^{*b}$	10.4 ± 1.14^{a}	**$14.8 \pm 1.04^{*c}$**
IR	*Zero time*	1.9 ± 0.13^{a}	4.3 ± 0.34	3.4 ± 0.37	**3.9 ± 0.37**
	After 3 months		**4.9 ± 0.28^{b}**	**$2.4 \pm 0.25^{*a}$**	**3.9 ± 0.34^{c}**

Values are presented as mean±S.D; number of patients=20 in each group; * significantly different compared to pre-treatment level ($P<0.05$); values with non-identical superscripts (a,b) for the same parameter among different groups are considered significantly different ($P<0.05$).

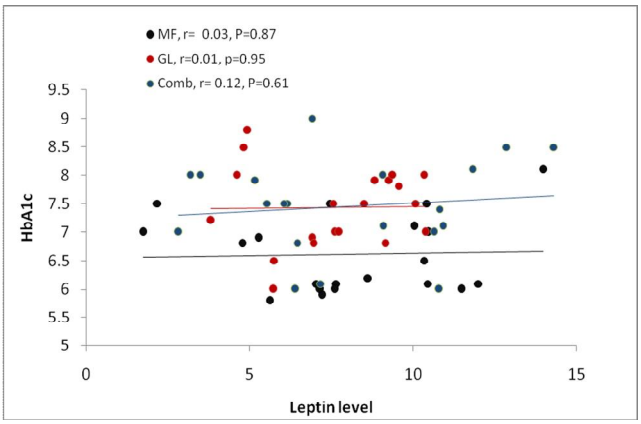

Figure 1. Correlation between serum leptin and HbA1c levels (pre-treatment) with glibneclamide (GL), metformin (MF) or their combination (Comb) in diabetic males.

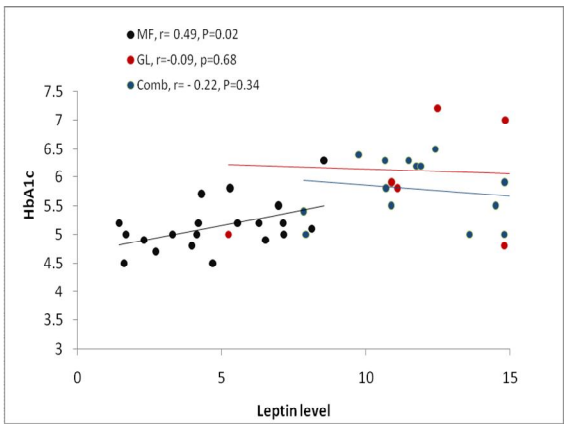

Figure 2. Correlation between serum leptin and HbA1c levels post-treatment with glibneclamide (GL), metformin (MF) or their combination (Comb) for 90 days in diabetic males.

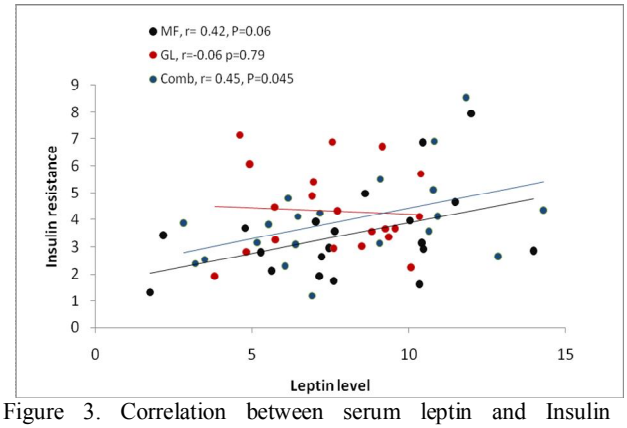

Figure 3. Correlation between serum leptin and Insulin Resistance (IR) (pre-treatment) with glibneclamide (GL), metformin (MF) or their combination (Comb) in diabetic males.

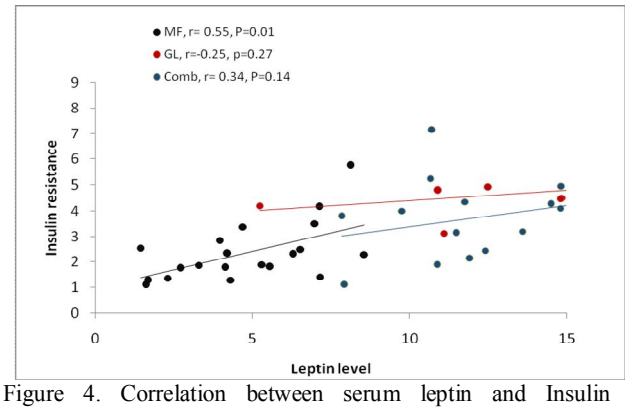

Figure 4. Correlation between serum leptin and Insulin Resistance (IR) (post-treatment) with glibneclamide (GL), metformin (MF) or their combination (Comb) for 90 days in diabetic males.

DISCUSSION

In the present study, although type 2 diabetic subjects are matched for body weight and age, they showed differences in correlation of plasma leptin levels with glycemic control and insulin resistance before initiation of any type of treatment. Many previously reported data has explored the regulators of leptin level in type 2 diabetic subjects [19,20]. The results in the present study showed differences in the pattern of correlation between fasting leptin and insulin resistance marker and is not comparable to previous findings [21,22]. With such finding, 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 in two of the three groups in the present study is in consonance with data in past studies [23,24] and provides more support for the hypothesis that insulin regulates leptin secretion. Many studies have found that leptin levels were influenced by different factors such as adipose fat mass, waist circumflex, BMI, insulin plasma levels or insulin resistance [4,7]. The strong association between insulin and leptin has also been demonstrated [25]. Moreover, it has been reported that taking oral antidiabetic drugs might change the plasma leptin concentration in type 2 diabetes patients [13]. Hence, this study attempted to examine the factors affecting the plasma leptin level in diabetic patients taking the same antidiabetic drugs. The data reported in the present study showed that the pattern of leptin level changes depends on whether the drug used is insulin secretogouge (glibenclamide) or insulin sensitizer (metformin). In our study, we could not demonstrate significant correlation of plasma leptin with parameters of glycemic control measured as HbA1c and this is consistent with previous study [26] which showed absence of short term effects of hyperglycemia on plasma leptin levels in man. However, treatment with metformin alone lead to positive and significant correlation with long-term glycemic control, which seems to be associated with a decrease in insulin resistance. This result might indicate that long term changes of plasma glucose contribute to leptin levels. In contrast, a previous study in seventy type 2 subjects suggested a possibility that hyperglycemia for long periods that was estimated by HbA1c, may have a suppressive action on serum concentrations of leptin [27]. In conclusion, plasma leptin levels vary in type 2 DM patients beyond the state of adiposity, and can be used as an indicator for the choice of treatment pattern in those patients.

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