ORIGINAL ARTICLE

Serum Lipid Peroxidation And Leptin Levels In Male And Female Type 2 Diabetic Patients In Gorgan (South East Of Caspian Sea), Iran

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ABSTRACT

Background: The aim of the present study was to determinate the possible relationship between serum leptin and lipid peroxidation in male and female type 2 diabetic patients in Gorgan, Iran.

Methods: The subjects consisted of fifty type 2 diabetic patients and fifty age and sex-matched control subjects. The concentration of leptin, malondialdehyde, lipid parameters and insulin were measured in all subjects. The results were evaluated by using Independent sample 't' test and Spearman's correlation coefficient test.

Results: Leptin was correlated with BMI (male: r=0.339 and female: r=0.426, p<0.05) and malondialdehyde levels (male: r=0.124 and female: r=0.271, p<0.05) in the type-2 diabetic patients. In the control subjects, only a correlation between leptin and BMI was found (male: r=0.165, female: r= 0.037, p<0.05).

Conclusions: In the correlation analysis using leptin as the dependent variable, BMI was found to be the predictor of leptin in males and females. Increased lipid peroxidation and hyperleptinaemia may play a role in the beginning and development of type 2 diabetes mellitus in this area.

Key Words: Leptin, Lipid peroxidation, Type 2 diabetes mellitus, Gorgan

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Introduction

Diabetes is a major public health problem that is approaching epidemic proportions globally. This metabolic disease is one of the most common endocrine disorders affecting almost 6% of the world's population [1]. The prevalence of type 2 Diabetes mellitus (DM) ranges from 1.2% to 14.6% in Asia, 4.6% to 40% in the Middle East and 1.3% to 14.5 % in Iran [2],[3]. Diabetes mellitus is considered to be one of a rank of free radical diseases. It causes complications, with increased free radical formation [4]. The process of lipid peroxidation is one of the oxidative conversions of polyunsaturated fatty acids to products known as malondialdehyde (MDA). MDA is a highly toxic molecule and its secondary products such as thiobarbutiric acid reactive substance (TBARS), are commonly used to evaluate lipid peroxidation [5]. A major development in energy balance regulation came with the discovery in 1994, of leptin, the protein product of the *ob* gene [6].The functions attributed to leptin are extensive, including the regulation of food intake and energy balance through central hypothalamic pathways, its role as a major signal to the reproductive system in theinhibition of insulin secretion by pancreatic-cells and in the stimulation of glucose transport [7]. Previous studies of leptin in type 2 diabetes have shown no difference in basal levels, apart from expected differences due to BMI [8],[9] or a reduced leptin level, which may be

explained by differences in fat distribution [10]. Importantly however, the relationship between leptin and variables involved in glucose homeostasis and diabetes might show ethnic differences. A recent population study of Peruvian Indians compared with a Caucasian population showed that the Indians had higher insulin and lower leptin levels than the Caucasians [11]. Similarly, Chilean Indians also had higher insulin and lower leptin levels than the Caucasians [12]. Furthermore, Mexican American subjects showed higher levels of leptin as compared to age and sex-matched non-Hispanic whites [13]. These results observed in different ethnic groups reinforce that studies have to be undertaken in different populations. Thus, the aim of the present study was to investigate the possible relationship between serum leptin and the lipid peroxidation of male and female patients diagnosed with type 2 diabetes mellitus in Gorgan,Iran.

Materials And Methods

This study was performed in the Biochemistry and Metabolic Disorder Research Center of Gorgan, Iran, in 2008. We had a study group including 50 patients of type-2 diabetes mellitus who referred to the Department of Diabetes Center in 5th Azar Hospital in Golestan University of Medical Sciences and 50 age and sex matched healthy control subjects. At the point of entry into the study, all diabetic patients underwent clinical and biochemical investigations. The data were collected by trained interviewers. The exclusion criterion was the coexistence of any other serious illness. Type-2 diabetes mellitus was defined as nonketosis diabetes by medical history and it is currently treated with oral agents. None of the patients had micro vascular complications (diabetic nephropathy or retinopathy). Administration of insulin for glycaemic control was considered to be an exclusion criterion. In the controls, diabetes was excluded by the fasting blood glucose test. Ten ml of fasting blood was collected from each subject by veinpuncture. The samples were centrifuged for 10 minutes at 3000rpm. The serum was used for the analyses of malondialdehyde [14], fasting blood sugar [15], lipid profile (total cholesterol [16] including the analysis of triglycerides [17], HDL-cholesterol [18] VLDL-cholesterol and LDL cholesterol [19] in those who had type-2 diabetes mellitus and in controls. Lipids levels were measured by biochemical kits and Lipid peroxidation (the level of lipid peroxidation expressed as malondialdehyde [MDA]) was determined using previously described methods and spectrophotometry techniques (Model JENWAY 6105 UV / VIS) in the Biochemistry and Metabolic Disorder Research Center (Faculty of Medicine). The results were reported as mean± SD. The statistical analysis was done with SPSS version -11.5 software. The results were evaluated by using Independent sample 't' test and Spearman's correlation coefficient test. P values < 0.05 were considered to be statistically significant.

The Measurement Of Serum Leptin

Serum leptin levels were measured using a human leptin ELISA test kit (Biovendor, Research and Diagnostic Products, Czech).

The Measurement Of Serum Insulin

Serum insulin levels were measured using a human insulin ELISA test kit (DiaPlus, Immunoenzymometric assay, USA).

The Measurement Of Serum Malondialdehyde

2.5 ml of trichloroacetic acid was added to 0.5 ml serum and the tube was left to stand for 10 min at room temperature. After centrifugation at 3500 rev. / min for 10 min, the supernatant was decanted and the precipitate was washed once with sulfuric acid. Then, 2.5 ml of sulfuric acid and 3 ml of thiobarbituric acid (TBA) in sodium sulfate were added to this precipitate and the coupling of lipid peroxide with TBA was carried out by heating this mixture in a boiling water bath for 30 min. After cooling in cold water, the resulting

chromogen was extracted with 4 ml of n-butyl alcohol by vigorous shaking. Separation of the organic phase was facilitated by centrifugation at 3000 rev./min for 10 min and its absorbance was determined at awavelength of 530 nm.

Results

The clinical characteristics of the type-2 diabetic patients and control subjects are described in **[Table/Fig 1].** The mean duration of diabetes mellitus in type-2 diabetes mellitus patients was 1.5 years (range 1-3 years). The mean age of male and female patients in the type-2 diabetic and control subjects were 50.38±10.78 (18 males) and 50.46±9.53 (32 females), and 48.40± 10.49 years (20 females) and 48.46± 10.65 years (30 females), respectively. A number of obvious differences were found between the two subjects. The female type 2 diabetes mellitus patients had higher levels of fasting blood sugar, total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, triglycerides, malondialdehyde, leptin and insulin as compared to the male subjects. Notably, the BMI (32.88±5.82 kg/m²), malondialdehyde (2.57±1.38 nmol/ml) and leptin levels (34.99±10.19 ng/ml) were significantly higher in female type-2 diabetes mellitus patients than in diabetic male subjects (25.05±1.85 kg/m², 2.10±0.67 nmol/ml and 11.04±7.76 ng/ml,) (P< 0.05) **[Table/Fig** 1]. The data shown in Table 1 reveals that the BMI (30.60±5.50 kg/m²) and leptin levels (26.34±12.02 ng/ml) was significantly higher in female control subjects than in male subjects (25.97±4.36 kg/m² and 11.26±5.46 ng/ml,) (P< 0.05). There was no significant difference in other parameters in female type-2 diabetes mellitus patients and male subjects. The data of **[Table/Fig 1]**shows that the levels of fasting blood sugar, total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides, malondialdehyde and insulin were significantly higher in male and female (leptin was also higher) type-2 diabetes mellitus patients than in male and female controls (P< 0.05). But this is not the same for HDL-cholesterol, age and BMI in female type-2 diabetes mellitus patients**[Table/Fig 1]**. There were no significant differences in age, BMI, HDL-cholesterol and leptin in male type-2 diabetes mellitus patients and in male control subjects. Leptin correlated positively and significantly with the BMI of diabetic and control males (r=0.339and r=0.165, p<0.05) and females (r=0.426 and r=0.037, p<0.05). Leptin also correlated positively and significantly with malondialdehyde (MDA) in the male and female type-2 diabetes mellitus patients (r= 0.124, r= 0.271, p<0.05) **[Table/Fig** 2].

(Table/Fig 1) Comparison of Fast blood sugar, Lipid profile, Malondialdehyde, Leptin and Insulin between males and females group of type-2 diabetes mellitus patients and control subjects

Parameters	Males (type-2 diabetes mellitus patients) (n=18) Mean±SD	Males (control groups) (n=20) Mean±SD	Females (type-2 diabetes mellitus patients) (n=32) Mean±SD	Females (control groups) (n=30) Mean±SD
Age (years)	50.38±10.78	48.80±10.49	50.46±9.53	48.46±10.65
Body Mass Index (kg/m²)	25.05±1.85[1]	25.97±4.36[2]	32.88±5.82	30.60±5.50
Duration of disease (years)	1.88±1.18	-	1.64±1.16	-
Fast ing Blood Sugar (mg/dl)	164.33±63.56[4]	89.40±6.45	183.64±94.30[3]	88.20±8.27
Total cholesterol (mg/dl)	254.66±81.74[4]	159.85±23.89	271.00±88.86[3]	160.40±27.40
LDL- cholesterol (mg/dl)	169.50±85.86[4]	98.50±25.77	208.70±74.49[3]	101.74±23.11
HDL-cholesterol (mg/dl)	44.44±11.65	46.75±7.48	48.90±11.26	45.06±7.28
VLDLcholesterol (mg/dl)	33.96±15.85[4]	22.14±7.03	34.45±18.45[3]	20.83±8.85
Triglyceride(mg/dl)	169.83±79.28[4]	110.70±35.17	173.40±94.09[3]	104.16±44.28
Malondialdehyde (nmol/ml)	2.10±0.67[1,4]	1.14±0.37	2.57±1.38 [3]	1.07±0.39
Leptin (ng/ml)	11.04±7.76[1]	11.26±5.46[2]	34.99±10.19 [3]	26.34±12.02
Insulin (µIU/ml)	10.40±9.31	4.31±3.09	12.68±9.46[3]	4.24 ±1.84

[1]p<0.05 compared between diabetic males and females, statistically significant.
[2]p<0.05 compared between control males and females, statistically significant.
[3]p<0.05 compared between diabetic females and control females, statistically significant.
[4] p<0.05 compared between diabetic males and control males, statistically significant.

(Table /Fig 2) Correlations of leptin levels with biochemical parameters in type 2 diabetes mellitus patients and control subject s

	Leptin			
	Male		Female	
	Diabetic	Control	Diabetic	Control
Age (years)	-0.071	-0.208	-0.054	0.137
BMI (kg/m²)	**0.339***	**0.165***	**0.426***	**0.037***
Duration (years)	0.165	-	0.394	-
FBS (mg/dl)	0.082	-0.063	-0.193	0.058
TC (mg/dl)	-0.091	0.186	-0.204	-0.305
LDL-C (mg/dl	0.071	0.059	0.066	0.005
HDL-C (mg/dl)	-0.072	-0.119	0.033	-0.385
VLDL-C (mg/dl)	0.323	-0.104	-0.183	-0.233
TG (mg/dl)	0.363	-0.104	-0.017	-0.233
Insulin (µIU/ml)	0.220	-0.242	0.041	0.339
MDA (nmol/ml)	**0.124***	0.219	**0.271***	-0.304

*P<0.05, statistically significant.

Discussion

In the present study, we have observed that the levels of malondialdehyde (MDA), a lipid peroxidation product and a marker

of oxidative stress, is increased significantly in male as well as in female diabetic patients **[Table/Fig** 1]. This apparently shows that diabetic patients are exposed to an increased oxidative stress via lipid peroxidation. Some other researchers have also reported elevated lipid peroxidation products in the blood samples of type 2 diabetic patients [20],[21]. Several studies have shown that lipid peroxidation is increased in diabetes, particularly in type 2 diabetes mellitus [22],[23],[24]. Jain [25] demonstrated that hyperglycaemia stimulates the lipid peroxidation of RBC and Kannan and Jain [26] later showed that it increases oxidative stress in cells in vitro. Contrary to our observations and to that of others, there are several studies which did not find increased oxidative stress in type 2 diabetes mellitus patients [27].

In an animal study, Midaoui and Champlain [28] sufferred the rat from type 2 diabetes mellitus and examined oxidative stress in the model of rat. Notably, they observed that hyperglycaemia alone does not induce oxidative stress unless it was accompanied by insulin resistance; thereby, implying that the involvement of reactive oxygen species is selectively related to insulin resistance [29].

Our results reveal a significant increase in the concentration of MDA in type 2 diabetic patients as compared to the control subjects **[Table/Fig** 1]. This is in agreement with the other published reports [22],[23],[24],[25],[26]. Our results show that BMI, MDA and leptin are statistically increased in female diabetic patients as compared to male subjects **[Table/Fig 1]**.

Many investigators have demonstrated that leptin has a major relationship with BMI [30],[31],[32],[33],[34],[35]. In our study, also, leptin showed a correlation with BMI, both inmales and females with diabetes and in control subjects. Leptin showed a correlation with MDA in both males and females with diabetes. A clear tendency towards being obese and overweight was apparent in female and male diabetic patients (BMI, 30.07±6.08 and 25.05±1.85 kg/m^2) and in control subjects (BMI, 30.60±5.50 and 25.97±4.36 kg/m2). Some of the type 2 diabetes mellitus patients suffered from Hyperlipidaemia. Therefore, our results apparently showed that being obese and overweight gave rise to increased oxidative stress in type 2 diabetes mellitus patients. Being obesity and overweight did not change the oxidative stress in the control subjects. Our study focussed on the association between serum leptin concentration and lipid peroxidation in type 2 diabetics. Recent studies have showed that leptin significantly increases intracellular reactive oxygen species in microvascular endothelial cells, particularly in diabetics [36]. According to our study,increased leptin levels observed in male and female diabetics may be related to increased lipid peroxidation **[Table/Fig** 2].Literature findings on the role of leptin in diabetes is conflicting. Investigators have reported either increased [37], decreased [38],[37],[38],[39] or unchanged [40],[41] leptin levels in diabetics. As Wauters et al. [42] have pointed out, adiposity and gender are the main determinants of leptin levels in normal controls and diabetic patients. Therefore, part of the controversy among previous reports could be related to the difference in the adiposity or the gender of the patients. Many investigators have described leptin alterations only in obese or overweight patients [38], [37],[40). Few workers have studied only men [37] or women [39]. The mechanism by which leptin stimulates oxidative stress conditions is unclear, but it may be related to the fact that leptin stimulates mitochondrial fatty acid oxidation and the increased generation of reactive radicals [43]. Sebnem et al. [44] have observed a significant decrease in the leptin levels in the plasma of streptozotocin- induced diabetic rats. Streptozotocin-induced hypoleptinaemia may be related to a reduced adipose tissue mass and to the reduced assimilation and storage of energy substrates in the fat tissue in insulin deficiency and/or to the direct toxic effect of streptozotocin on the adipose tissue [45]. Panarotto et al. [46] has described lower leptin concentrations in females with diabetes as compared to those in the

control subjects. Our data only appear to be in contrast with this finding; our patients actually had higher leptin levels and higher fasting serum glucose concentrations than those studied by Panarotto's group. Moreover, in correlation analysis, BMI is considered to be a significant predictor of hyperleptinaemia **[Table/Fig** 2]. In the correlation analysis using leptin as the dependent variable, BMI and MDA were found to be significant predictors of leptin. In the present study, it was found that there was a correlation between serum leptin and BMI in males and females in normal and diabetic subjects. Gender specific correlation showed an association between leptin and MDA in diabetic patients. Serum leptin showed a significant relationship to MDA in male and female diabetic patients ($r = 0.124$ and r= 0.271, $p < 0.05$). This trend reflects the increased MDA in males and females. Therefore, serum MDA levels are confounded by leptin or *vice versa,* such that in diabetes. Nakanishi et al. [47] have investigated the association between leptin and oxidative stress and have explained this association exclusively through obesity. Many investigators demonstrated that leptin had a major correlation with BMI [48,[49],[50],[51],[52]. There is still a controversy that leptin concentrations are affected by type 2 diabetes. Surveys of Mexican-American [53] and German [54] subjects showed that leptin did not differ between normal subjects and subjects with type 2 diabetes, with matched BMI in males and females. In another report, it was found that baseline plasma leptin levels in subjects with newly diagnosed or long-standing type 2 diabetes were not significantly different from nondiabetic controls matched for BMI [54]. Other reports comparing plasma leptin levels between controls and weight-matched subjects with type 2 diabetes have led to discrepant conclusions, showing no effect [55],[56] or a decrease in leptin [57],[58]. Our present study shows a similar relationship between leptin and oxidative stress in obese and overweight type 2 diabetes mellitus hyperleptinaemic patients. Finally, our study on individuals who were referred to the Department of Diabetes Center in 5th Azar Hospital in the Golestan University of Medical Sciences on the South East of the Caspian Sea indicated that Type 2 diabetes was associated with higher leptin and MDA levels and BMI. In the correlation analysis, we found a significant relation between leptin levels and BMI in males and females. Increased lipid peroxidation and hyperleptinaemia may play a role in the beginning and in the development of type 2 diabetes mellitus.

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