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Estimation of preptin, myostatin, and some biochemical parameters in diabetic mellitus patients

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ABSTRACT

Type 2 diabetes is an impairment in the way the body controls and utilizes sugar (glucose). This chronic (long-term) illness causes an excess of sugar to circulate in the blood. Over time, issues with the cardiovascular, neurological, and immune systems may result from high blood sugar levels. The purpose of this research was to determine how diabetes mellitus (DM) affected a few biochemical variables as well as how those variables affected one another. This study included 50 patients suffering from diabetic mellitus and another 50 healthy subjects. Insulin, preptin, and myostatin levels were evaluated using the commercial ELISA kit. Glycated hemoglobin, fasting blood sugar (FBS), cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very-low-density lipoprotein (VLDL) were estimated using biochemical tests. Patients suffering from DM showed higher levels of FBS, glycated hemoglobin (HbA1c), insulin, insulin resistance, preptin, cholesterol, triglyceride, HDL, LDL, VLDL, and myostatin than in controls, 156.9880 vs 91.1440, 7.5660 vs 5.1560, 13.9190 vs 6.3408, 8.5928 vs 4.3254, 77.0955 vs 35.8797, 211.5625 vs 120.4149, 215.0857 vs 80.8269, 66.003 vs 26.539, 102.5416 vs 77.7102, 43.0171 vs 16.1654, and 6.303 vs 0.313, respectively. The levels of the studied parameters also showed a significant positive correlation

between each other, except the correlations between HOMO-IR and each of preptin, insulin, triglycerides, LDL, and VLDL.

Keywords: *correlation; Homo-IR; LDL; triglycerides; VLDL*

INTRODUCTION

It is reasonable to think that one of the most ancient human diseases is diabetes mellitus (DM). The metabolic syndrome originally included type 2 diabetes as one of its symptoms in 1988.[1] The characteristics of type 2 diabetes include hyperglycemia, insulin resistance, and a relative insulin deficit, the most common form of DM.[2] Before, type 2 diabetes was thought to be an insulin-independent condition. This condition is caused by the interaction of environmental, genetic, and behavioral risk factors.[3] The primary contributing factors to type 2 diabetes are genetics and lifestyle decisions.[4] The formation of type 2 diabetes is widely acknowledged to be influenced by a number of lifestyle factors, which include binge drinking, smoking, physical inactivity, and sedentary lifestyle.[5] According to reports, obesity is the root cause of around 55% of type 2 DM (T2DM) cases.[6] It is believed that the growth in juvenile obesity between the 1960s and 2000s contributed to the rise in T2DM in children and adolescents.[7] Environmental toxins might be a contributing factor to the recent rise in type 2 diabetes incidence. Bisphenol A, a component of several plastics, has been shown to have a marginally positive correlation with the frequency of type 2 diabetes.[8]

Furthermore, despite the fact that studies have shown a high association between childhood obesity and type 2 diabetes, there is still much to learn about this connection. Although experts are currently looking into the connection between type 2 diabetes and environmental contaminants, it is still evident that there is a significant connection between the growing incidence of juvenile obesity and type 2 diabetes in children and adolescents. Preptin was discovered in rat experiments for the first time in 2001. Along with insulin, the pancreatic beta cells produce this peptide hormone, which has 34 amino acids.[9] Preptin, an endocrine peptide, is thought to activate the insulin-like growth factor receptor 2 (IGF2R), which, together with protein C and phospholipase C, results in calcium-dependent insulin secretion when the level of glucose is high.[10] Similar to insulin, preptin also influences bone metabolism by increasing cellular differentiation and changing the function of osteoblasts and osteoclasts.[11] Preptin is involved in metabolic functions. In T2DM patients, preptin has been the focus of only a very limited number of studies.

METHODS

This study included 50 patients suffering from diabetic mellitus, who attended the Baghdad Teaching Hospital, for therapy or for checking their status. All subjects who were included in this study were notified of the research purpose, and another 50 healthy subjects were involved in the research for comparison of results, as controls.

Blood samples were taken from both controls and patients. The sample collection was done during the fasting status of the subjects using disposable syringes. The drawn blood was divided into two parts, the first part (2 ml) was kept in the EDTA tube (to perform glycated hemoglobin [HBA1c] analysis), while the second part was kept in the gel tube (3 ml) for about 15 min, centrifuged at 1500–2000 ×g for 5 min, and was then transferred into a new plane tube and stored at (−20°C).

Calculation of body mass index (BMI) was done by dividing the square of the height: BMI = weight (kg)/height (m^2).

The fasting blood glucose levels of all patients and controls were checked in accordance with the Braham and Tindoer (1972) theory. According to this theory, glucose oxidase converts glucose to gluconate, which releases hydrogen peroxide. Following this reaction, quinonimine is produced, which is detected spectrophotometrically at 505 nm by reacting hydrogen peroxide with phenol and 4-aminoantipyrine in the presence of peroxidase.

Using the NycoCard Reader II, the amount of HbA1c was measured in all patients and controls.

The total blood cholesterol was measured using the Biolabo laboratory kit; the method of measurement was based on enzymatic hydrolysis. The amount of the produced red dye quinonimide is related to the level of cholesterol; quinonimine absorbance was measured using a spectrophotometer at 500 nm.

Glycerol and fatty acids were digested by enzymes to identify the triglycerides. The amount of red dye quinonimide produced was inversely related to the level of cholesterol. Using a spectrophotometer, the quinonimine absorbance was measured at 500 nm.

Using Friedwald's method, LDL cholesterol may be quantitatively determined from total cholesterol, triglycerides, and the concentration of HDL cholesterol. The formula for calculating LDL is as follows:

LDL = Total Cholesterol − HDL Cholesterol − Triglyceride/5.

VLDL concentration was equal to one-fifth of serum TG.

Insulin levels were estimated by following the instructions provided by the ELISA commercial kit (E0010Hu), the preptin levels were estimated using the kit, E1448Hu, and levels of myostatin in serum were estimated using the commercial kit E0403Hu.

RESULTS

The distribution of the studied samples according to demographic parameters is shown in Table 1. According to age group, the distribution showed nonsignificant difference between the control and patients (chi-square = 1.661, P = 0.894). The results of sample distribution according to the blood groups and gender also showed nonsignificant difference (chi-square = 4.262, P = 0.748 and chi-square = 1.46, P = 0.157, respectively) between studied groups. The patients' samples recorded a significant (P = 0.001) higher BMI (33.3 ± 0.86) compared to that of the control (28.4 ± 0.89).

The results of studied parameters are shown in the table 2. Fasting blood sugar amounts in the patients were considerably (0.001) higher than that of the controls (156.9880 vs 91.1440). Additionally, HbA1c levels in patients were substantially (0.001) higher than in controls (7.5660 vs 5.1560). Insulin levels were somewhat greater in patients compared to healthy subjects (13.9190 vs 6.3408). Insulin resistance levels in patients were much greater than that of controls (8.5928 vs 4.3254). Preptin levels were significantly greater in patients than that of controls (77.0955 vs 35.8797). Patients' cholesterol and triglyceride levels were substantially greater than those of the control group (211.5625 vs 120.4149 and 215.0857 vs 80.8269, respectively). The patients' HDL, LDL, and VLDL values were much greater than those of the control group (66.003 vs 26.539, 102.5416 vs 77.7102, 43.0171 vs 16.1654, respectively). The patients' myostatin levels were also greater than those of controls (6.303 vs 0.313).

Pearson correlation among the studied biochemical parameters was done and the results are summarized in Table 3. The results showed a significant positive correlation among all the lipid profile levels and with insulin level, myostatin, preptin, HbA1c, and FBS, and among each of them. Only the level of insulin resistance failed to show a significant correlation with the other parameters.

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TABLE 1. Distribution of samples according to age, blood group, gender, and BMI difference between patient and control samples.

Parameter		Group		Chi-square	P
		Patients	Control		
Age	<20	2	3	1.661	0.894
	21–30	11	13		
	31–40	6	8		
	41–50	8	9		
	51–60	10	8		
	>61	13	9		
Blood	A^+	7	6	4.262	0.748
	A^-	2	5		
	B^+	4	1		
	B^-	8	7		
	AB^+	12	13		
	AB^-	2	2		
	O^+	15	15		
	O^-	0	1		
Gender	Male	25	31	1.46	0.157
	Female	25	19		
BMI		33.3 ± 0.86	28.4 ± 0.89	-	0.001

BMI, body mass index.

DISCUSSION

Diabetes mellitus is a metabolic illness with many different etiologies, characterized by the presence of persistent hyperglycemia as well as disruptions in the metabolism of proteins, lipids, and carbohydrates, which is brought on by a problem with insulin production, activity, or both.[12]

Protein, lipid, and carbohydrate metabolism are all affected differently by insulin. It promotes the absorption and metabolism of glucose by adipocytes in adipose tissue, while activating glucose uptake by cells and glycogen synthesis in muscles.[13] It also accelerates the utilization of glucose by the liver and its storage as glycogen.[14] This mechanism of action by insulin was supported by the current study which revealed higher insulin levels in the patients. Additionally, the insulin levels were significantly correlated with the elevation of lipid profile tests, preptin levels, and myostatin.

The function of lipoprotein lipase, which needs insulin for tissue production, is to purify plasma lipids. In adipose tissues and the liver, the latter promotes lipogenesis while preventing lipolysis. Additionally, insulin reduces the rate of circulating amino acids by increasing the cellular absorption of amino acids. This is done by stimulating the activation of amino acids and mRNA ribosomal reading, as well as by boosting protein synthesis, which is accomplished by lowering proteolysis.[15] As seen by the findings, this means that insulin deficiency (T1DM) or insulin resistance (T2DM) causes elevated lipoprotein levels.

TABLE 2. Comparison of serum levels of the studied parameter in patients and control.

Group		Mean	SE	P
BS	Patients	156.9880	9.60498	0.001
	Control	91.1440	1.37674	
HbA1c	Patients	7.5660	0.21271	0.001
	Control	5.1560	0.11422	
Insulin	Patients	13.9190	0.61094	0.918
	Control	6.3408	0.50367	
HOMA-IR	Patients	8.5928	1.28206	0.002
	Control	4.3254	0.95238	
Preptin	Patients	77.0955	4.39009	0.001
	Control	35.8797	1.23347	
Cholesterol	Patients	211.5625	3.05717	0.164
	Control	120.4149	4.08512	
Triglyceride	Patients	215.0857	3.55867	0.001
	Control	80.8269	5.77451	
HDL	Patients	66.0038	0.42192	0.001
	Control	26.5392	2.06743	
LDL	Patients	102.5416	3.32350	0.05
	Control	77.7102	4.52219	
VLDL	Patients	43.0171	0.71173	0.001
	Control	16.1654	1.15490	
Myostatin	Patients	6.3033	0.46801	0.001
	Control	0.3130	0.01592	

BS, blood sugar; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

Myostatin levels in diabetic patients have been studied previously, which showed a significant difference in levels between patients and control and also a significant correlation between insulin levels and lipid profile. A previous study showed that compared to normal children, those with T1DM had considerably increased levels of myostatin in blood. The increase in myostatin levels may be attributed to homeostatic mechanism, reduced muscle function, or problems with glucose metabolism.[16] The above results disagreed with the findings of a previous study[17] which revealed a significantly lower myostatin levels (P = 0.001) in DM2 patients compared to healthy controls. Additionally, the levels of myostatin, fasting plasma glucose (P = 0.005), and lipids (P = 0.028) were shown to be negatively correlated, which contradicted with the results of our study. The results of the current study agreed with a previous study that showed these patients had elevated levels of myostatin compared to the controls (2710.60 ± 559.09 vs 2246.37 ± 416.40, $P < 0.001$).[18]

The results also showed a significant elevation of preptin which agreed with a previous study that proved high concentrations of preptin in obese–overweight adults compared with healthy controls. The samples of patients involved in this study revealed them to be overweight (BMI = 33.3 ± 0.86), and the results also correlated with fasting insulin and HOMA-IR.[19] Additionally, our results were consistent with those of Aslan et al. (2011),[17] who discovered a strong positive correlation between preptin concentration and fasting insulin in individuals with gestational diabetes. Additionally, Yang et al.[20] showed that preptin and insulin resistance may be related in individuals who have just been diagnosed with T2DM. Preptin and HOMA-IR were found to be positively correlated in the study findings by Bu et al.,[21] while preptin and insulin were not correlated, indicating that preptin may contribute to the etiology of insulin resistance without influencing insulin production.

CONCLUSION

From the results of this study, we can conclude that patients with DM2 have higher levels of FBS, HbA1c, lipid profile, insulin, preptin, Homo-IR, and myostatin. This increment was also accompanied with a positive correlation among the biochemical parameters as a cascade of biochemical events.

TABLE 3. Correlation of the serum levels among the studied parameters.

Parameter		BS	HBa	Insulin	HOMA-IR	Preptin	Cholesterol	Triglyceride	HDL	LDL	VLDL	Myostatin
BS	R	1	0.783**	0.410**	0.573**	0.318**	0.520**	0.465**	0.494**	0.300**	0.465**	0.558**
	Sig.		0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.002	0.000	0.000
HBa	R		1	0.506**	0.367**	0.396**	0.693**	0.616**	0.609**	0.436**	0.616**	0.645**
	Sig.			0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Insulin	R			1	0.122	0.419**	0.625**	0.570**	0.629**	0.328**	0.570**	0.542**
	Sig.				0.228	0.000	0.000	0.000	0.000	0.001	0.000	0.000
HOMA-IR	R				1	0.051	0.199*	0.182	0.199*	0.105	0.182	0.294**
	Sig.					0.616	0.047	0.070	0.047	0.297	0.070	0.003
Preptin	R					1	0.591**	0.652**	0.584**	0.262**	0.652**	0.444**
	Sig.						0.000	0.000	0.000	0.008	0.000	0.000
Cholesterol	R						1	0.777**	0.782**	0.755**	0.777**	0.693**
	Sig.							0.000	0.000	0.000	0.000	0.000
Triglyceride	R							1	0.779**	0.265**	1.000**	0.682**
	Sig.								0.000	0.008	0.000	0.000
HDL	R								1	0.221*	0.779**	0.701**
	Sig.									0.027	0.000	0.000
LDL	R									1	0.265**	0.335**
	Sig.										0.008	0.001
VLDL	R										1	0.682**
	Sig.											0.000
Myostatin	R											1
	Sig.											

*BS, blood sugar; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein. * significant difference, ** highly significant*

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