**Estimation of Preptin, Myostatin and Some Biochemical Parameters in Diabetic Mellitus Patients**

**Noora Wael Rasheed**

**Department of Medical Laboratory Techniques, Al Rafidain University College, Baghdad, Iraq**

**Corresponding author email:** **Noora.waal@ruc.edu.iq**

**Abstract**

A defect in how the body controls and utilizes sugar is diabetes type 2 (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 affected a few biochemical variables as well as how those variables affected one another. This study included fifty patients suffering from diabetic mellitus and another 50 healthy subjects. Insulin, preptin and myostatin levels have been evaluated by using commercial ELISA kit. Glycated hemoglobin, fasting blood sugar (FBS),  cholesterol,LDL, HDL and VLDL have been estimated by using biochemical tests. levels of fasting blood sugar, HbA1c, insulin, insulin resistance, preptin, cholesterol, triglyceride, HDL, LDL, VLDL, and myostatin showed a higher levels in patient than in control (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), (6.303 vs 0.313), respectively. the levels of the studied parameters also showed a significant positive correlation between each other, except the correlation between HOMO-IR and each of preptin, insulin, triglycerides, LDL, VLDL.

**Keywords; correlation, Homo-IR, LDL, triglycerides and VLDL**

**Introduction**

It's 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. Diabetes type 2 is brought on 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. These include binge drinking, smoking, being inactive physically, and leading a sedentary lifestyle [5]. According to reports, obesity is the root cause of around 55% of type 2 DM patients [6]. It is believed that the growth in juvenile obesity between the 1960s and 2000s contributed to the rise in type 2 DM 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 insulinlike 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 how insulin affects bone metabolism, preptin also influences it by increasing cellular differentiation and changing how osteoblasts and osteoclasts function [11]. Preptin is involved in metabolic functions. In T2DM patients, preptin has only been the focus of a very limited number of studies.

**Methods**

This study included fifty patients suffering from diabetic mellitus who were attending Baghdad Teaching Hospital, asking for therapy or checking their status. All the subjects who included in this study have been notified for the research purpose. Another 50 healthy subjects have involved in the research to perform comparison.

Samples of blood taken from both control and patients. The samples collection were done during the fasting status of the subjects using disposable syringe. The drawn blood divided into two parts, the first one (2ml) kept in the EDTA tube (in order to do the HBA1c) while the second part kept in the gel tube (3ml) for about fifteen minutes then centerfuged at 1500- 2000 Xg for 5 minutes and 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 (m2).

Fasting All patients and controls had their blood glucose levels 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 and 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 glycated hemoglobin (HbA1c) was measured in all patients and controls.

For this reason, total blood cholesterol was measured using a Biolabo laboratory kit; the method of measurement was based on the 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 is 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 LDL = Total Cholesterol - HDL Cholesterol - Triglyceride/5.

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

 Insulin levels have been estimated by following the instructions provided by the commercial kit of ELISA (E0010Hu). And levels of Preptin have been estimated by using the Kit (E1448Hu). And serum levels of Myostatin have been estimated by using the commercial kit (E0403Hu).

**Results**

Distribution of the studied samples according to demographic parameters are shown in table (1). According the age group the distribution showed none-significant difference between the control and patients (chi-square= 1.661, P-Value= 0.894). None-significant difference also shown with the distribution according to the blood groups (chi-square= 4.262, P-Value= 0.748). The results of samples distribution according to gender also showed none-significant difference between studied groups (chi-square= 1.46, P-Value= 0.157). The patients samples recorder a significant (P-value= 0.001) higher BMI (33.3± 0.86) than control (28.4 ± 0.89).

**Table (1); distribution of samples according to Age, blood group, gender. And BMI difference between patient and control samples**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | Group | Chi-square | P-Value |
| 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 |

Fasting blood sugar amounts in the patient were considerably (0.001) upper than those in the control (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 related to healthy subjects (13.9190 vs 6.3408). Insulin resistance levels in patients were much greater than in controls (8.5928 vs 4.3254). Preptin levels were significantly greater in patients than in controls (77.0955 vs 35.8797). Patients' cholesterol levels were substantially greater than those of the control group (211.5625 vs 120.4149). Patients' triglyceride levels were greater than those of the control group (215.0857 vs 80.8269). Patients' HDL values were much greater than those of the control group (66.003 vs 26.539). LDL values were greater in patients compared to controls (102.5416 vs 77.7102). VLDL levels in patients were considerably greater than in controls (43.0171 vs 16.1654). Patients' myostatin levels were greater than those of controls (6.303 vs 0.313).

**Table (2); comparison of serum levels of the studied parameter in patients and control**

|  |  |  |  |
| --- | --- | --- | --- |
| group | Mean | S.E. | P-Value |
| BS | patients | 156.9880 | 9.60498 | 0.001 |
| control | 91.1440 | 1.37674 |
| HbA1c  | patients | 7.5660 | .21271 | 0.001 |
| control | 5.1560 | .11422 |
| INSULIN | patients | 13.9190 | .61094 | 0.918 |
| control | 6.3408 | .50367 |
| HOMA-IR | patients | 8.5928 | 1.28206 | 0.002 |
| control | 4.3254 | .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 | .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 | .71173 | 0.001 |
| control | 16.1654 | 1.15490 |
| Myostatin | patients | 6.3033 | .46801 | 0.001 |
| control | 0.3130 | .01592 |

Pearsons correlation among the studied biochemical parameters have been 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 fasting blood sugar, and among each of them. Only the level of insulin resistance has failed to show a significant correlation with the other parameters.

Table (2); 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 | .783\*\* | .410\*\* | .573\*\* | .318\*\* | .520\*\* | .465\*\* | .494\*\* | .300\*\* | .465\*\* | .558\*\* |
| Sig. |   | .000 | .000 | .000 | .001 | .000 | .000 | .000 | .002 | .000 | .000 |
| HBa | R |   | 1 | .506\*\* | .367\*\* | .396\*\* | .693\*\* | .616\*\* | .609\*\* | .436\*\* | .616\*\* | .645\*\* |
| Sig. |   |   | .000 | .000 | .000 | .000 | .000 | .000 | .000 | .000 | .000 |
| INSULIN | R |   |   | 1 | .122 | .419\*\* | .625\*\* | .570\*\* | .629\*\* | .328\*\* | .570\*\* | .542\*\* |
| Sig. |   |   |   | .228 | .000 | .000 | .000 | .000 | .001 | .000 | .000 |
| HOMA-IR | R |   |   |   | 1 | .051 | .199\* | .182 | .199\* | .105 | .182 | .294\*\* |
| Sig. |   |   |   |   | .616 | .047 | .070 | .047 | .297 | .070 | .003 |
| preptin | R |   |   |   |   | 1 | .591\*\* | .652\*\* | .584\*\* | .262\*\* | .652\*\* | .444\*\* |
| Sig. |   |   |   |   |   | .000 | .000 | .000 | .008 | .000 | .000 |
| cholestrol | R |   |   |   |   |   | 1 | .777\*\* | .782\*\* | .755\*\* | .777\*\* | .693\*\* |
| Sig. |   |   |   |   |   |   | .000 | .000 | .000 | .000 | .000 |
| triglyceride | R |   |   |   |   |   |   | 1 | .779\*\* | .265\*\* | 1.000\*\* | .682\*\* |
| Sig. |   |   |   |   |   |   |   | .000 | .008 | 0.000 | .000 |
| HDL | R |   |   |   |   |   |   |   | 1 | .221\* | .779\*\* | .701\*\* |
| Sig. |   |   |   |   |   |   |   |   | .027 | .000 | .000 |
| LDL | R |   |   |   |   |   |   |   |   | 1 | .265\*\* | .335\*\* |
| Sig. |   |   |   |   |   |   |   |   |   | .008 | .001 |
| VLDL | R |   |   |   |   |   |   |   |   |   | 1 | .682\*\* |
| Sig. |   |   |   |   |   |   |   |   |   |   | .000 |
| Myostatin | R |   |   |   |   |   |   |   |   |   |   | 1 |
| Sig. |   |   |   |   |   |   |   |   |   |   |   |

**Discussion**

Diabetes mellitus is a metabolic illness with many different etiologies that is characterized by the presence of persistent hyperglycemia as well as disruptions in the metabolization of proteins, lipids, and carbohydrates and 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 muscle [14]. It also accelerates the utilization of glucose by the liver and its storage as glycogen [13]. This mechanism of action for insulin support the results this study since the results showed insulin levels was higher in the patients. Additionally, the insulin levels were in a significant correlation 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 tissue 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) cause elevated lipoprotein levels.

Myostatin level in diabetic has been studied previously and showed a significant difference levels in the diabetic, this study also find a significant difference with the between patients and control and also a significant correlation with insilun levels and lipid profile. A previous study showed that In comparison to normal children, those with T1DM had considerably increased blood levels of myostatin. The increased myostatin seen in that study may have been caused by a homeostatic mechanism, reduced muscle function, or problems with glucose metabolism [16].The results of this study disagreed with a previous study [17] that showed a DM2 patients showed significantly lower myostatin levels (p = 0.001) compared to healthy. Additionally, Myostatin and fasting plasma glucose (p = 0.005) and lipid levels (p = 0.028) were shown to be negatively correlated. which contradicted with the results of our study. The results of this study agreed with another previous study that showed these patients had elevated levels of myostatin compared with 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 a high concentrations of preptin in obese–overweight adults compared with healthy controls and the samples of patients of this study were over-weight (BMI=33.3± 0.86). and also agreed with the study regarding the correlation with fasting insulin, and HOMA-IR [19]. Additionally, our results are consistent with those of Aslan and colleagues (2011) [17], who discovered a strong positive correlation between preptin concentration and fasting insulin in individuals with gestational diabetes. Additionally, Yang and colleagues (2009) [20] showed that preptin and insulin resistance may be related in individuals who have just been diagnosed with T2DM. Preptin and HOMA-IR were positively correlated by Bu and colleagues (2012) [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 a higher level of FBS, HbA1c, lipid profile, insulin, preptin, Homo-IR, and myostatin. This increment also accompanied with a positive correlation among the biochemical parameter as a cascade of biochemical events.

**References**

[1] M. Patlak Scientific Advisor, B.C. Hansen, F.R. Naider, D.L. Brautigan, J. Grossman, G. Washington, T.E. Hugli, M.G. Richard Lynch, B.A. Horwitz, FASEB J. 16 (2002) 1853–1853. DOI: 10.1096/FJ.02-0974BKT

[2] A. Olokoba, O. Obateru, L.O.-O. medical journal, undefined 2012, Ncbi.Nlm.Nih.Gov (n.d.).

[3] L. Chen, D.J. Magliano, P.Z. Zimmet, Nat. Rev. Endocrinol. 8 (2011) 228–236. DOI: 10.1038/NRENDO.2011.183

[4] M. Choy, S. Lam, Cardiol. Rev. 15 (2007) 264–271. DOI: 10.1097/CRD.0b013e318123f771

[5] F. Rank, J.O.A. Nn, E.M. Anson, M. Eir, J.S. Tampfer, G. Raham, C. Olditz, A.G.S. Olomon, W. Alter, C.W. Illett, Https://Doi.Org/10.1056/NEJMoa010492 345 (2001) 790–797. DOI: 10.1056/NEJMOA010492

[6] E.W. Gregg, Y.J. Cheng, K.M.V. Narayan, T.J. Thompson, D.F. Williamson, Prev. Med. (Baltim). 45 (2007) 348–352. DOI: 10.1016/j.ypmed.2007.07.020

[7] S.E. Barlow, Pediatrics 120 Suppl 4 (2007). DOI: 10.1542/PEDS.2007-2329C

[8] I.A. Lang, T.S. Galloway, A. Scarlett, W.E. Henley, M. Depledge, R.B. Wallace, D. Melzer, JAMA 300 (2008) 1303–1310. DOI: 10.1001/JAMA.300.11.1303

[9] S. Aydin, Peptides 56 (2014) 94–110. DOI: 10.1016/J.PEPTIDES.2014.03.021

[10] K.C. Cheng, Y.X. Li, A. Asakawa, M. Ushikai, I. Kato, Y. Sato, J.T. Cheng, A. Inui, J. Endocrinol. 215 (2012) 43–49. DOI: 10.1530/JOE-12-0176

[11] G. Chen, L. Shi, L. Cai, W. Lin, H. Huang, J. Liang, L. Li, L. Lin, K. Tang, L. Chen, J. Lu, Y. Bi, W. Wang, G. Ning, J. Wen, Horm. Metab. Res. 49 (2017) 135–141. DOI: 10.1055/s-0042-111325

[12] N. El Houda FERDI, K. Abla, H. Chenchouni, O. El Bouaghi, Iran. J. Public Health 47 (2018) 1119.

[13] M.C. Petersen, G.I. Shulman, Physiol. Rev. 98 (2018) 2133. DOI: 10.1152/PHYSREV.00063.2017

[14] A. Chadt, H. Al-Hasani, Pflugers Arch. 472 (2020) 1273. DOI: 10.1007/S00424-020-02417-X

[15] G.F. Grabner, H. Xie, M. Schweiger, R. Zechner, Nat. Metab. 2021 311 3 (2021) 1445–1465. DOI: 10.1038/s42255-021-00493-6

[16] A. Efthymiadou, I.A. Vasilakis, A. Giannakopoulos, D. Chrysis, Hormones (Athens). 20 (2021) 777–782. DOI: 10.1007/S42000-021-00317-Y

[17] B. García-Fontana, R. Reyes-García, S. Morales-Santana, V. Ávila-Rubio, A. Muñoz-Garach, P. Rozas-Moreno, M. Muñoz-Torres, Endocrine 52 (2015) 54–62. DOI: 10.1007/S12020-015-0758-8

[18] S.N.S. Ahmad, S. Nourollahi, M. Nakhjavani, M. Khojastehfard, M. Mostafazadeh, H. Hajipour, D. Sanajou, Acta Med. Iran. 57 (2019) 160–166. DOI: 10.18502/ACTA.V57I3.1818

[19] M. El-Eshmawy, I.A. Aal, Appl. Physiol. Nutr. Metab. 40 (2015) 218–222. DOI: 10.1139/APNM-2014-0338

[20] G. Yang, L. Li, W. Chen, H. Liu, G. Boden, K. Li, Ann. Med. 41 (2009) 52–56. DOI: 10.1080/07853890802244142

[21] Z. Bu, K. Kuok, J. Meng, R. Wang, B. Xu, H. Zhang, Reprod. Biol. Endocrinol. 10 (2012) 10. DOI: 10.1186/1477-7827-10-10