Comparison Study between Adipsin Levels in Sera of Iraqi Patients with Diabetes and Neuropathy

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Abstract

The current study was designed to compare some of the vital markers in the sera of diabetic and neuropathy patients via estimating Adipsin, Fasting blood Glucose(FBG), Glycated(HbA1c) hemoglobin, Homeostasis Model Assessment Index (Homa IR), Cholesterol, High density lipoprotein (HDL), Triglycerides (T.G), Low-density, and lipoprotein (LDL), Very Low Density Lipoprotein (VLDL), in sera of Iraqi patients with diabetes and neuropathy. A total of ninety subjects were divided into three groups: group I (30 diabetic with neuropathy males) and group II (30 diabetic males without neuropathy), and 30 healthy subjects were employed as control group. The results showed a significant decline in Adipsin levels (p>0.05) in neuropathy, T2DM group with respect to the control group, while, no changes were recorded within both diabetic patients groups. The serum insulin level dropped considerably in neuropathy, T2DM group in a comparison with control group. The Adipsin ROC curve showed a clear cut-off value (4510.813, 3967.08) when calculated in neuropathy, T2DM respectively. The study concluded that Adipsin is a diagnostic factor for T2DM with neuropathy.

Keywords: Adipsin, Homa IR, Insulin, Neuropathy, Type 2 diabetes.

Introduction

Adipokines play a role in several processes, including satiety and appetite, endothelial function, blood pressure, energy expenditure activity, hemostasis, also insulin sensitivity, adipogenesis, energy metabolism in insulin-sensitive tissues, insulin synthesis, and fat distribution in pancreatic -cells are all processes in which adipokines play a part. At first, Adipsin was known as complement factor D which is an adipokine. Adipsin has a role in the synthesis of the (C5–C9) membrane attack complex, as well as the creation of signaling molecules such as anaphylatoxins, complement (3a,C3a, and C5a). Adipsin cuts complement factor B, which catalyzes the synthesis of C3 convertase, resulting in a hydrolysis cascade that results in the creation of C3a, C3b, C5a, and C5b complement fragments. C3a levels have been connected to obesity and metabolic diseases in families, while C3 deficiency has been associated to diabetes risk 1,2.

Adipsin is a serine protease produced by adipocytes in humans. It catalyzes the breakdown of the C3 peptide to create peptide C3a, which acts on -cells to enhance cytosolic free Ca2+ levels and potentiate insulin secretion by augmenting the synthesis of ATP 3. Adipsin has been shown to influence adipocyte development and enhance lipid accumulation, which could explain why it is linked to metabolic diseases. Circulating adipsin was found to be related to obesity, insulin resistance (IR), polycystic ovarian syndrome (PCOS), and the development of coronary artery disease in clinical studies 4,5.

Diabetes mellitus type 2 (T2DM) is a common chronic and systemic metabolic condition that is on the rise. Diabetes mellitus is now the ninth leading cause of death, owing to the numerous chronic complications induced by T2DM 6,8. As a result, early detection and treatment of T2DM chronic consequences is critical. Diabetic peripheral neuropathy (DPN) is one of the microvascular
consequences of type 2 diabetes that manifests in a variety of clinical ways. Peripheral neuropathy, as is well known, is not just a consequence of T2DM but also occurs in CKD, regardless of diabetes. Peripheral neuropathy affects roughly 70% of predialysis patients with CKD. The coexistence of diabetes and CKD is thought to contribute to a faster start of nerve damage in patients. The goal of the study is to find out how much Adipsin, FBG, HbA1c, Homa IR, Cholesterol, T.G, HDL, VLDL, and lipoprotein LDL are present in the sera of Iraqi, diabetics, and neuropathy.

Materials and Methods

Patients
A case-control study in Iraq was conducted to assess the levels of Adipsin and levels in patients with diabetic neuropathy and diabetics without neuropathy. Ninety people between the ages of 45 and 55 were included, between November 2021 and January 2022. Sixty males were recruited from the national diabetes center, Baghdad, Al Mustansiriya University, and compared with thirty healthy (matched by age and gender) controls. The diabetic patients were divided into two groups: group I, which comprised 30 Diabetic neuropathy patients, and group II, which included 30 diabetic neuropathy patients. Controls (n=30) were healthy and free of acute illness. The waist-to-hip ratio (WHR) and BMI were calculated for all groups studied.

Samples:
Ten milliliters of blood were withdrawn via venipuncture, then transferred into a gel tube for serum separation. Serum was obtained by centrifuging the blood samples at 3000 rpm, then the serum was divided into five aliquots, and kept at -20°C until tested.

Sample Analysis
An Adipsin ELISA kit was provided by Abcam - UK to measure serum Adipsin. The serum insulin hormone was measured using an ELISA kit provided by DRG-USA. The estimation run according to the kits’ instructions. Homeostasis Model Assessment (HOMA) IR estimation of insulin resistance using the HOMA index is obtained using the formula [HOMA –IR = (Glucose × Insulin)/405] 12. The glucose level is determined using the method developed by Barham and Trindoer (1972). High performance liquid chromatography was used to detect hemoglobin A1c (HbA1c) (Bio-Rad D-10, Berkeley, USA).

Lipid Profile
Total serum cholesterol was measured using a ready-made laboratory kit. Enzymatically hydrolyzed glycerol and fatty acids were used to calculate triglycerides. Serum High Density Lipoprotein (HDL) levels are measured using a kit. Using Friedwald’s formula, LDL - cholesterol was estimated quantitatively from total cholesterol, triglycerides, and HDL–cholesterol concentration. Only TG concentrations up to (5.32 mmol/L) (400 mg/dl) was calculated using formula according to 15. The concentration of very low density lipoprotein (VLDL) was calculated to be one-fifth of the serum TG.

Statistical Analysis
The data was interpreted as the mean ± standard error and median (25th and 75th percentiles) for normally and non-normally distributed numerical variables respectively. The Shapiro-Wilk test was used to look into the normal distribution of values. An (ANOVA) test was used to see if there was a significant difference between the normally numerical variables. To describe numerical variables that were not normally distributed, the (Mann-Whitney) tests were used. A value of P≤0.05 was used as the significance level. The Spearman's rank coefficient was utilized in a non-parametric evaluation to determine the importance of correlation for the relationship between the two numerical variables. The Adipsin cut-off value was determined using receiver operating characteristic (ROC) curve analysis.

Results and Discussion: The Mean ± SE of Age, BMI and WHR of neuropathy, T2DM patients and, control are listed in Table 1.
Diabetic neuropathy is a common consequence of diabetes mellitus (DM), and it is linked to an increased risk of cardiac arrhythmias and stroke. In multiple large-scale clinical studies, a low lipid profile has been linked to neuropathy development and progression independent of diabetic care, and it has recently been confirmed as a critical independent risk factor for the development of neuropathy. The prevalence of neuropathy in diabetic patients was found to be positively connected to the level of lipid profile testing, and it may also predict the degree of neuropathy when customized to age, medical history, and pharmaceutical history in this study.

Table 1. The serum levels of Age, BMI, and WHR in neuropathy, T2DM patients, and controls.

<table>
<thead>
<tr>
<th></th>
<th>Neuropathy patients Mean ±SE</th>
<th>T2DM patients Mean ±SE</th>
<th>Control Mean ±SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>53.0 ± 6.5</td>
<td>52.0 ± 1.44</td>
<td>50.0 ± 1.06</td>
<td>N.s</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>28.8 ± 0.75</td>
<td>30.5 ± 0.67</td>
<td>23.56 ± 0.212</td>
<td>N.s</td>
</tr>
<tr>
<td>WHR</td>
<td>0.99 ± 0.02</td>
<td>0.96 ± 0.02</td>
<td>0.91 ± 0.006</td>
<td>N.s</td>
</tr>
</tbody>
</table>

Significant difference among three independent means using ANOVA test at 0.05 level

The T.G and, VLDL levels increased significantly in neuropathy patients group (181.0(157.0-210) when compared with control group (153.0 (146.0-159.0) p>0.05 ,while there was no significant difference in Cholesterol when we compared T2DM patients group 171.0 (137.0-208) with control 153.0 (146.0-159.0) .p<0.05, and the result showed a significant difference between patients groups (Neuropathy , T2DM ) when compared among themselves as shown in Table 2. The HDL and, LDL levels showed high significant difference when comparing patients groups (Neuropathy, T2DM) with control.

Table 2. The serum levels of Cholesterol, T.G, HDL, VLDL, and LDL in neuropathy, T2DM patients and control.

<table>
<thead>
<tr>
<th></th>
<th>Neuropathy patients</th>
<th>T2DM patients</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cholesterol(mg/dl)</td>
<td>181.0 a</td>
<td>171.0</td>
<td>153.0</td>
<td>0.01</td>
</tr>
<tr>
<td>T.G (mg/dl)</td>
<td>147.0 a,c</td>
<td>132.0 b</td>
<td>112.0</td>
<td>0.00</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>31.0 a</td>
<td>25.0 b</td>
<td>48.0</td>
<td>0.00</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>29.4 a,c</td>
<td>26.4 b</td>
<td>22.4</td>
<td>0.00</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>124.8 a</td>
<td>132.2 b</td>
<td>83.6</td>
<td>0.00</td>
</tr>
</tbody>
</table>

The median was used to determine whether there was a significant difference between three independent means using the Mann Whitney test at the 0.05 level.

a) Indicates whether there is a significant difference between the control group and the neuropathy group.
b) Determines whether the difference between the control and T2DM groups is statistically significant.
c) Determines whether the neuropathy and T2DM groups have a statistically significant difference.
T.G: Triglycerides, HDL: High density lipoprotein, VLDL: Very Low Density Lipoprotein, LDL: Low-density lipoprotein

Table 3 shows the serum levels of FBG, Insulin, HbA1c, Homa IR, and Adipsin in neuropathy, T2DM patients and control. The FBG and HbA1c, HomaIR levels increased significantly in patients group (Neuropathy, T2DM) when compared with group p<0.05. Serum insulin level significantly decreased when comparing (Neuropathy, T2DM) group with control, and the result showed a significant difference between patients groups (Neuropathy, T2DM) when they were compared among themselves. The result showed high significantly decrease in Adipsin level when we compared (Neuropathy, T2DM) group with control p<0.05, and no significant difference between patients groups (Neuropathy, T2DM) when they were compared among themselves as shown as in Table 3, Fig. 1.

A rise in blood glucose levels is a sign of type 2 diabetes mellitus, which is caused by impaired pancreatic-cell activity, which is necessary for insulin release. Gómez-Banoy et al., discovered that restoring Adipsin in diabetic mice alleviated hyperglycemia while also preserving -cell mass by increasing -cell survival and maintaining -cell transcriptional identity. Furthermore, in humans, higher Adipsin levels are linked to a lower incidence of diabetes. Neuropathy is the most prevalent microvascular complication of diabetes. The most common diabetic consequences are a collection of clinical symptoms induced by damage to the peripheral and autonomic nervous systems. These disorders, which are commonly referred to as different types of neuropathy, are caused by damage to the diffuse and focal nerve systems and affect up to half of all diabetics.

Many adipokines are secreted by adipose tissue, and many of them are involved in energy homeostasis and inflammation. The synthesis of adipokines is dysregulated, which impairs the function of several organs and contributes to the development of metabolic illnesses such as insulin resistance.

**Table 3. The serum levels of FBG, Insulin, HbA1c, Homa IR, and Adipsin in Neuropathy, T2DM patients, and control**

<table>
<thead>
<tr>
<th></th>
<th>Neuropathy patients</th>
<th>T2DM patients</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mg/dl)</td>
<td>178.0 a</td>
<td>158.0 b</td>
<td>98.0</td>
<td>0.00</td>
</tr>
<tr>
<td>Insulin (mg/dl)</td>
<td>1.2 a,c</td>
<td>2.3 b</td>
<td>3.6</td>
<td>0.00</td>
</tr>
<tr>
<td>HbA1c %</td>
<td>9.0 a</td>
<td>7.6 b</td>
<td>5.0</td>
<td>0.00</td>
</tr>
<tr>
<td>Homa IR</td>
<td>0.6 a</td>
<td>0.95 b</td>
<td>0.8</td>
<td>0.53</td>
</tr>
<tr>
<td>Adipsin (pg/ml)</td>
<td>2289.6 a</td>
<td>2070.3 b</td>
<td>8575.2</td>
<td>0.00</td>
</tr>
</tbody>
</table>

The median was used to determine whether there was a significant difference between three independent means using the Mann Whitney test at the 0.05 level.

a) Indicates whether there is a significant difference between the control group and the neuropathy group.
b) Determines whether the difference between the control and T2DM groups is statistically significant.
c) Determines whether the neuropathy and T2DM groups have a statistically significant difference.

FBG: Fasting blood glucose, Glycated hemoglobin, Homa IR: Homeostasis Model Assessment Index, HbA1c: Glycated hemoglobin
Adipsin and other markers in neuropathy and T2DM patients were studied in a correlation research. There was a positive correlation between Adipsin in serum and other parameters including Cholesterol, and LDL (P <0.05) in neuropathy, group, and there was a positive correlation between serum Adipsin and other parameters including BMI, FBG, Homa IR and Insulin in T2DM group as shown in Table 4.

Table 4. Correlation between Adipsin  with BMI, FBG ,Insulin,Homa IR , Cholesterol,and LDL in neuropathy , and T2DM groups .

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Neuropathy group</th>
<th>T2DM group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R value (P value)</td>
<td>R value (P value)</td>
</tr>
<tr>
<td>BMI</td>
<td>0.071 (0.708)</td>
<td>-0.368 (0.046)*</td>
</tr>
<tr>
<td>FBG</td>
<td>0.140 (0.461)</td>
<td>0.363 (0.045)*</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.152 (0.421)</td>
<td>0.361 (0.04)*</td>
</tr>
<tr>
<td>Homa IR</td>
<td>0.033 (0.863)</td>
<td>0.379 (0.039)*</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-0.361 (0.05)*</td>
<td>0.061 (0.750)</td>
</tr>
<tr>
<td>LDL</td>
<td>-0.461 (0.01)*</td>
<td>0.107 (0.573)</td>
</tr>
</tbody>
</table>

* Means significantly significant at P value 0.05
- Mean negatively correlated between adipsin and other markers

ROC curve analysis

The curve analysis was done twice once for neuropathy patients from the healthy subjects and the second for T2DM patients from the healthy subjects as shown below. The capacity of serum Adipsin concentration to distinguish neuropathy sufferers from healthy people was investigated using receiver operating characteristic curve analysis (Table 5; Fig. 2). The receiver operating characteristic curve was significantly lower than the diagnostic test for neuropathy, but it had higher validity (high sensitivity (93.3) and specificity (100.0)).The AUC of the receiver operating characteristic curve for the existence of a neuropathy diagnosis was 0.991 (p ≤ 0.001), which was the optimal level of correct neuropathy prediction.
Table 5. Adipsin's AUC and validity in distinguishing between neuropathy sufferers and healthy controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>AUC</th>
<th>P-Value</th>
<th>cut off value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipsin</td>
<td>0.991</td>
<td>0.001</td>
<td>4510.813</td>
<td>93.3</td>
<td>100</td>
<td>0.9667</td>
<td>100</td>
<td>93.7</td>
</tr>
</tbody>
</table>

The term AUC refers to the area under the curve. The terms negative predictive value (NPV) and positive predictive value (PPV) are used interchangeably.

The ROC curve analysis was used to assess the ability of serum Adipsin concentration to discriminate T2DM patients from healthy people (Table 6; Fig. 3). The ROC curve was significantly lower than the diagnostic test for T2DM, indicating greater validity (high sensitivity (93.3) and specificity (100.0)). The optimal level of correct neuropathy prediction was 0.996 (p ≤ 0.001), as measured by the AUC of the ROC curve for the existence of a neuropathy diagnosis.

Figure 3. ROC curve analysis of serum Adipsin concentration in T2DM patients (n = 30) against healthy subjects (n = 30) (AUC is 0.996), p ≤ 0.001

Table 6. Adipsin's AUC and validity in differentiating between T2DM patients and healthy subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>AUC</th>
<th>P-Value</th>
<th>Optimum cut off value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipsin</td>
<td>0.996</td>
<td>0.001</td>
<td>3967.08</td>
<td>93.3</td>
<td>100</td>
<td>0.933</td>
<td>100</td>
<td>93.7</td>
</tr>
</tbody>
</table>

The term AUC refers to the area under the curve. The terms negative predictive value (NPV) and positive predictive value (PPV) are used interchangeably.

Conclusion

Adipsin is the most specific and sensitive marker in the diagnosis of diabetic patients with neuropathy. Type 2DM can affect the level of Adipsin. High levels of FBG and HbA1c were found correlated in patients with neuropathy. Increased adipsin concentration could result from decreased insulin sensitivity in T2DM with neuropathy. The level of adipsin was positively correlated with IR. This may suggest a potential influence of ADIPSIN level in the diagnosis of T2DM.

Authors' declaration:
- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in Baghdad University.

Authors’ Contribution Statement:
L.O.F. designed, analyzed, proofread, and presented ideas of the research, B.A.A.’s role in this research was collecting samples and analyzing the results, A.S.D.’s role in this research was to do analytics and proof editing.
References


دراسة مقارنة لمستويات الإدبسين في أمصال مرضى السكري والاعتلال العصبي العراقيين

ليلى عثمان فرحان1
بدياء احمد عبد
اشكان شلمان داود1

الخلاصة:
الهدف من الدراسة هو تحديد مستويات الادبسين ، FBG والكولسترول و HDL والإنسولين و HbA1c و الكوليسترولrho و Homa IR في أمصال مرضى السكري والاعتلال العصبي في العراق. شملت الدراسة الحالية تسعين فردًا ، تم تقسيم المرضى إلى مجموعتين (30 من الذكور المصابين باعتلال الأعصاب و السكري) والمجموعة الثانية (30 من الذكور المصابين بالسكري) وتم مقارنة النتائج بمجموعة ثالثة (30 من الرجال الأصحاء). أظهرت النتائج انخفاضًا ملحوظًا في مستويات Adipsin (p> 0.05) عند مقارنة مجموعة (Neuropathy ، T2DM) مع مجموعة التحكم، ولكن لم تحدث تغيرات كبيرة بين مجموعات المرضى (T2DM ، Neuropathy) عند مقارنة بشكل فردي، وانخفاض مستوى الأنسولين في الدم بشكل ملحوظ عند مقارنة مجموعة T2DM مع مجموعة التحكم، وأظهرت النتائج فرقًا كبيرًا بين مجموعات المرضى (T2DM ، Neuropathy) عند مقارنتها بشكل فردي. كانت قيمة Adipsin عند التوالي ز خلصت الدراسة إلى أن Adipsin هو عامل تشخيصي لـ T2DM مع اعتلال الأعصاب.

الكلمات المفتاحية: أدبسين , مقاومة الأنسولين , الأنسولين , الاعتلال العصبي , داء السكري.