Evaluation of Chemerin and Apelin adipokines in Obese and Non-obese Type 2 Diabetes Mellitus Patients

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ABSTRACT
The aim of this study was to investigate whether chemerin and apelin play an important role in the etiology of insulin resistance and type 2 diabetes mellitus. Seventy type 2 diabetes mellitus (T2DM) patients randomly assigned into two subgroups, 35 non obese (BMI <30) diabetic group 1 and 35 obese (BMI >30) diabetic group 2 with another 50 healthy control volunteers, divided into two subgroups, 25 non obese (BMI >30) control group 1 and 25 obese (BMI >30) control group 2. Levels of chemerin, apelin, free fatty acids (FFA), fasting glucose, fasting insulin, HbA1c, homeostasis model assessment of IR (HOMA-IR), homeostasis model assessment of β-cell function (HOMA-%B) were examined. The results showed significant (p<0.05) reduction of chemerin conc. in obese control group 2 as compared to non-obese control group 1. The serum conc. of apelin of non-obese diabetic group 1 was significantly (p<0.05) higher as compared with non-obese control group 1, while it was significantly (p<0.05) lower in obese diabetic group 2 as compared to obese control group 2. There was no significant difference in the serum conc. of FFA between control and diabetic groups. The significant alterations in sera levels of chemerin, apelin of diabetic patients were suggested a possible role of these adipokines in the pathogenesis of the obesity linked insulin resistance and T2DM.

Keywords: Apelin, chemerin, type 2 diabetes mellitus, obesity, insulin resistance.

INTRODUCTION
Adipose tissue secretes a number of bioactive peptides or proteins, collectively named “adipokines”. They play a central role in energy and vascular homeostasis, as well as immunity, and are fundamental to the pathogenesis of the metabolic syndrome1. Adipokines are classified as pro- and anti-inflammatory adipokines according to their effects on inflammatory responses in adipose tissues. Most adipokines show pro-inflammatory activity like chemerin with the noted exceptions of adiponectin, apelin, secreted frizzled-related protein 5 (SFRP5), visceral adipose tissue-derived serine protease inhibitor (Vaspin), and omentin-1. The pro-inflammatory adipokines are increased whereas the anti-inflammatory adipokines are decreased in obese rodents and humans that are associated with insulin resistance2.

Chemerin, described as an adipokine in 20073, was originally known as a chemoattractant for immune cells4. The actions of chemerin, however, remain unclear. Processing of pro-chemerin can lead to active forms with inflammatory or anti-inflammatory properties5. Chemerin function is dependent on cell type. In adipocytes, chemerin potentiates insulin-stimulated glucose uptake concomitant with enhanced insulin signaling6.

In muscle, chemerin induces insulin resistance via IRS-1, Akt signaling and glucose uptake7. Chemerin mediates inflammatory responses, as it is a chemoattractant to induce the infiltration of macrophages, immature dendritic cells, and NK cells in inflammatory disease such as ulcerative colitis and skin lupus8. In 1998, Tatemoto and coworkers purified, from bovine stomach extracts, a peptide binding to the ‘orphan’ APJ receptor, a G protein-coupled receptor (GPCR), now known as the apelin receptor (gene symbol APLNR)9. The identified gene encodes a 77-amino acid polypeptide with a secretory signal sequence. The C-terminal part of this polypeptide that contains the part of the molecule that binds the apelin receptor was called ‘apelin’, for APJ Endogenous Ligand10. In insulin-responsive tissues, apelin receptor is expressed in the adipose tissue, skeletal muscles and heart and at lower levels in the liver11, the main active forms of apelin are apelin-13, -17 and -36 and the pyroglutaminated isoform of apelin-13 (Pyr(1)-apelin-13) characterized by a higher resistance to degradation12. It was recently shown that both short- and long-term apelin treatments improves insulin sensitivity in insulin-resistant obese mice. Indeed, acute Pyr(1)-apelin-13 treatment (200 pmol/kg intravenously) of high-fat diet (HFD) fed C57Bl6/J obese and insulin-resistant mice showed improved glucose tolerance, during euglycemic–hyperinsulinemic clamp.

Thus, apelin is efficient in improving altered glucose metabolism, an effect that was found to be mediated mainly by an increase in glucose uptake in skeletal muscle13. The aim of this study was to investigate whether chemerin and apelin play an important role in the etiology of insulin resistance and type 2 diabetes mellitus and to differentiate between the impact of
obesity and type 2 diabetes on the altered serum chemerin and apelin levels by comparing between obese and non-obese type 2 diabetes patients.

**SUBJECTS AND METHODS**

A case – control study was conducted at diabetic center of Al-Mawana Hospital in Basra from April 2014 till March 2015. All selected Subjects were informed about the aim of study before their written informed consent was obtained. The study was approved by the medical ethical committee of College of Medicine / Al-Nahrain University. This study enrolled 70 type 2 diabetes mellitus (T2DM) patients randomly assigned into two subgroups, 35 non obese (BMI <30) diabetic group 1 and 35 obese (BMI >30) diabetic group 2. Patients were collected according to the inclusion diagnostic criteria of WHO (2011); fasting serum glucose level ≥ 7 mmol/L (≥ 126 mg/dl) and glycosylated hemoglobin (HbA1c) ≥ 6.5%.

All patients had no other disease than T2DM with another 50 healthy control volunteers, divided into two subgroups, 25 non obese (BMI >30) control group 1 and 25 obese (BMI > 30) control group 2. control subjects were collected with Glycemic control inclusion criteria were fasting serum glucose<7.0 mmol/L (<126mg/dl) and HbA1c<6.5%. Subjects were excluded if they have : (1) overt macrovascular complications such as myocardial infarction, angina pectoris, stroke and peripheral vascular diseases. (2) history of hypertension or systolic blood pressure (SBP) >140 mmHg and/or diastolic blood pressure (DBP) >85 mmHg. (3) pregnancy.

5ml blood samples were collected by venepuncture after overnight fasting (10-12 hr) and were divided into two portions, 2 ml in EDTA tube for measurement of HbA1c and 3 ml in plain tube, centrifuged for separation of serum which was divided into two portions, one portion for assay of fasting serum glucose, and lipid profile [Total cholesterol (TC), triglycerides(TG), low density lipoproteins (LDL), high density lipoproteins (HDL), very low density lipoproteins (VLDL)]. and the second portion of serum sample was frozen and stored at(−20°C) for assay of other parameters such as chemerin, apelin, FFA and fasting insulin.

**Statistical Analysis**

All data were expressed as mean ± SD. Student’s t test was used to analyze sample averages. One way analysis of variance (ANOVA) was used to evaluate differences of means between groups. Ratio was compared by the chi square test. Correlations between HOMA-IR, other parameters were analyzed by Pearson’s correlation. P < 0.05 was accepted as statistical difference.

**RESULTS**

The general characteristic of all study groups including age, age range, men/women, address, smoking, BMI, WHR, and physical activity were listed in Table 1. All groups were matched for age, sex and no. of smokers.

Group comparison using student t-test, as shown in Table 2, revealed significant (p<0.05) elevation of serum conc. of chemerin in obese diabetic group 2 as compared to obese control group 2 with significant (p<0.05) reduction of chemerin conc. in obese control group 2 as compared to non obese control group 1.

The serum conc. of apelin-c terminus of non-obese diabetic group 1 was significantly (p<0.05) higher as compared with non obese control group 1, while it was significantly (p<0.05) lower in obese diabetic group 2 as compared to obese control group 2. There was no significant difference in the serum conc. of FFA between control and diabetic groups.

<table>
<thead>
<tr>
<th>Table 1: General Characteristics of Control and Diabetic Groups</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Age Range</td>
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<tr>
<td>Men/women</td>
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<tr>
<td>Address (center/rural)</td>
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<tr>
<td>Smoker/Non smoker</td>
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<tr>
<td>Body mass index (BMI)</td>
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<tr>
<td>Waist to hip ratio(WHR)</td>
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<tr>
<td>Physical activity( min/wk)</td>
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</table>

All values were expressed as mean ± SD; b Significant (p<0.05) as compared with control group 1

b Significant (p<0.05) as compared with control group 2; c Significant (p<0.05) as compared with diabetic group 1
Table 2: Results of serum chemerin, apelin and free fatty acids of control and diabetic groups.

<table>
<thead>
<tr>
<th></th>
<th>Non obese Control Group1 (CG1) n=25</th>
<th>Obese Control Group2 (CG2) n=25</th>
<th>Non obese Diabetic Group1 (DG1) n=35</th>
<th>Obese Diabetic Group2 (DG2) n=35</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemerin (ng/ml)</td>
<td>10.7 ± 3.6</td>
<td>8.1 ± 4.2a</td>
<td>9.4 ± 7.5</td>
<td>11.6 ± 5.6b</td>
<td>0.10685</td>
</tr>
<tr>
<td>Apelin (pg/ml)</td>
<td>94.4 ± 28.1</td>
<td>109.1 ± 30.27</td>
<td>134.8 ±40.42b</td>
<td>36.3 ±17.47abc</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FFA (ng/ml)</td>
<td>253.2 ± 132.9</td>
<td>300.3 ± 218.2</td>
<td>336.7 ± 190.2</td>
<td>287.3 ± 170.4</td>
<td>0.35133</td>
</tr>
</tbody>
</table>

All values were expressed as mean ±SD; a Significant (p<0.05) as compared with control group 1
b Significant (p<0.05) as compared with control group 2; c Significant (p<0.05) as compared with disease group 1

Table 3: Results of Glycemic Control Parameters and HOMA-indexes of Control and Diabetic Groups

<table>
<thead>
<tr>
<th></th>
<th>Non obese Control Group1 (CG1) n=25</th>
<th>Obese Control Group2 (CG2) n=25</th>
<th>Non obese Diabetic Group1 (DG1) n=35</th>
<th>Obese Diabetic Group2 (DG2) n=35</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting serum glucose (mg/dl)</td>
<td>99.1 ± 8.4</td>
<td>99.6 ± 9.3</td>
<td>218.7 ± 72.7ab</td>
<td>234.5 ± 90.1abc</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>-</td>
<td>-</td>
<td>4.8 ± 3.9</td>
<td>2.8 ± 2.2c</td>
<td>0.011</td>
</tr>
<tr>
<td>Family History (positive/negative)</td>
<td>17/8</td>
<td>13/12a</td>
<td>28/7b</td>
<td>27/8b</td>
<td>0.03</td>
</tr>
<tr>
<td>Family History Average scores (mean±SD)</td>
<td>0.7 ± 0.48</td>
<td>0.5 ± 0.51</td>
<td>1.6 ± 1.5ab</td>
<td>1.6 ± 1.36abc</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>15.3 ± 6.37</td>
<td>40.4 ± 25.81a</td>
<td>14.5 ± 12.28b</td>
<td>20.5 ± 15.14abc</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.7 ± 1.5</td>
<td>10.2 ± 7.38a</td>
<td>8.7 ± 8.24a</td>
<td>12 ± 11.07a</td>
<td>0.0021</td>
</tr>
<tr>
<td>HOMA-B%</td>
<td>164.4 ± 94.7</td>
<td>395.1 ± 226a</td>
<td>43.3 ± 34.2ab</td>
<td>52.6 ± 39.15abc</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HbA1c%</td>
<td>5.9 ± 0.35</td>
<td>6 ± 0.36</td>
<td>9.7 ± 2.03ab</td>
<td>9.0 ± 1.97abc</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

All values were expressed as mean ± SD; a Significant (p<0.05) as compared with control group 1
b Significant (p<0.05) as compared with control group 2; c Significant (p<0.05) as compared with diabetic group 1

Table 4: Receiver Operating Characteristic (ROC) as Compared with Disease Duration

<table>
<thead>
<tr>
<th></th>
<th>Cut off Value</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemerin (ng/ml)</td>
<td>&gt;15.1</td>
<td>0.595442</td>
<td>33.33</td>
<td>96.15%</td>
<td>0.2011</td>
</tr>
<tr>
<td>Apelin (pg/ml)</td>
<td>≤29.4</td>
<td>0.699430</td>
<td>74.07</td>
<td>75.00%</td>
<td>0.0043</td>
</tr>
<tr>
<td>FFA (ng/ml)</td>
<td>&gt;308</td>
<td>0.576923</td>
<td>55.56</td>
<td>73.08%</td>
<td>0.2874</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>≤23.2</td>
<td>0.584758</td>
<td>85.19</td>
<td>42.31%</td>
<td>0.2063</td>
</tr>
</tbody>
</table>

The serum insulin concentration (conc.) of diabetic pt.(both DG1 and DG2) was significantly (p<0.05) lower than control group 2 (CG2) but no significant difference as compared with CG1. Also the serum insulin conc. of CG2 was significantly (p<0.05) higher than that of CG1 as shown in Table 3. The pancreatic β-cell function (HOMA-B%) of diabetic pt. groups was significantly (p<0.05) lower than that of control subject groups with no significant difference between diabetic groups (DG1 and DG2) while there was a significant (p<0.05) difference between control subject groups as shown in Table 3. The insulin sensitivity (HOMA-IR) of diabetic pt. was significantly (p<0.05) lower than that of control subjects (CG1) with no significant difference between diabetic groups (DG1 and DG2), and significant (p<0.05) difference between control groups (CG1 and CG2) as shown in Table 3.

The cut off value, area under the curve, sensitivity and specificity of chemerin, apelin, FFA and insulin were listed in Table 4. The cut off value of chemerin was 15.1 ng/ml, whereas that of apelin, FFA and insulin were 29.4mg/dl, 308 ng/ml and 23.2 µU/ml respectively. The sensitivity of chemerin, apelin, FFA and insulin were 33.33%, 74.07%, 55.56% and 85.19% respectively.
DISCUSSION

The data of this study provide evidence that serum chemerin level, a pro-inflammatory adipokine, was elevated in obese diabetic patient as compared to obese control subjects with no significant correlation between serum level of chemerin and body mass index (BMI) and significant reduction of serum chemerin in obese control individuals as compared to non obese control individuals, this indicates that elevated chemerin level was related to diabetes rather than obesity which is consistent with study done by Fatima 13, whom found a higher Chemerin level in obese diabetic patients as compared to controls. Elevated serum Chemerin in obese diabetes mellitus group was a surrogate of impairment in glucose metabolism in obese individual. Also the result of chemerin in this study consistent with work done by YU Shan 14 who found that the baseline level of chemerin in obese T2DM group was significantly higher than normal control group (P<0.001) and then decreased significantly after hypoglycemic agents treatment, therefore; the lower serum chemerin concentrations in non obese diabetic patients as compared to obese diabetic patients may be partly explained by longer duration of hypoglycemic agents treatment and/or lower BMI since chemerin was adipocyte derived peptide.

The non significant correlation between chemerin and BMI was consistent with the study done by Johanna Weigert 15, who found that chemerin was not related to obesity in obese T2DM patients. In contrast, other studies were observed that the level of chemerin correlated positively with BMI 14. Whether elevated chemerin was a cause or a consequence of T2DM still unclear but some studies found insulin resistance-inducing effect of chemerin on murine C2C12 myoblasts through nuclear factor-κB pathway-mediated inflammatory reaction 16. Additionally, Takahashi and coworkers 6 disagreed with this suggestion and postulated that, in adipocytes chemerin has the opposite effect, where it increases insulin stimulated glucose uptake, and so, it stimulates insulin sensitivity. Hence, the increase in the levels of circulating chemerin is a compensatory mechanism in patients with insulin resistance. Thus, chemerin may exert different actions in endocrine and paracrine/autocrine ways. Moreover, Takahashi and coworkers showed that chemerin-deficient mice are glucose intolerant and glucose intolerance was mainly due to increased hepatic glucose production and impaired insulin secretion. They suggested that chemerin and its receptor were expressed in β-cell and chemerin regulates β-cell function and plays an important role in glucose homeostasis in a tissue dependent manner. 17. The results of this study showed a significant reduction of serum apelin concentration, which was a beneficial anti-inflammatory adipokine, of obese diabetic patients as compared to control subjects which were consistent with study done by Kader 26 who observed that apelin level was low in T2DM group as compared to controls, may be due to fact that insulin level was low in diabetic group since insulin stimulate the synthesis and secretions of apelin from adipose tissue as observed by Boucher 29, i.e low apelin level in T2DM was a consequence of pancreatic β-cell dysfunction. This study showed consistent results with those of Erdem and Yu Zhang 20,21 that serum apelin level was lower in obese type 2 diabetic subjects than in healthy control subjects which may be explained by the negative correlation of apelin serum level and BMI as shown in the results which were inconsistent with the positive correlation of circulating apelin with BMI that might be limited to overt obesity while other study did not found any relation of apelin with BMI 19,20,22,23. The results of this study were inconsistent with other studies done by Atif 23 and Cavalle 24 whose found that apelin levels showed higher significant values in obese diabetic groups compared to healthy ones. It may be postulated that a longer duration of diabetes might worsen insulin resistance and secretion, further influencing apelin levels. The obese diabetic patients were in the early stage of their natural history, when the metabolic defects were not fully expressed, as shown by the possible reversal of T2D with lifestyle measures. Therefore the differences in diabetes duration may underlie discrepancies between studies. In contrast to obese T2DM, we found that serum apelin concentration was significantly increased in non obese T2DM as compared to controls which was consistent with the findings of Li and coworkers who observed a significant elevation of apelin level of non obese T2DM as compared to controls 25.

The possible explanation of high apelin level associated with low insulin concentrations in the diabetic patients that apelin may also inhibit the release of insulin, aggravating the disorders of glucose metabolism which was also proved by Beltowski 26 and this may provide evidence that apelin might have a causal role in T2DM story or it may be a compensatory response to further worsening of insulin resistance in chronic diabetic pt. as well as the antidiabetic treatment may be related to this elevation of apelin level specially with chronic use of these drugs as observed by Fan 27 who found that Treatment with metformin and a dipeptidyl peptidase-4 inhibitor elevates apelin levels in patients with type 2 diabetes mellitus.

The non-significant correlation of apelin with HOMA-indexes and glycemic control parameters including fasting glucose, HbA1c and insulin might be due to limited sample size which was consistent with the findings of Atif and inconsistent with YU ZHANG 20,21 who found a negative correlation of apelin with HOMA-IR, fasting glucose and HbA1c suggested a causal role of apelin in insulin resistance and T2DM while Yu Shan 24 observed a positive correlation of apelin with HOMA-IR suggested a compensatory response to insulin resistance.

In this study, it was found that no significant difference of serum FFA observed between non-obese diabetes patients and non obese controls consistent with the findings of Dominique 28, but he found that serum FFA
was significantly elevated only in obese diabetics as compared to non obese controls (inconsistent with the current findings due to comparison with non obese controls in contrast to this study, in which comparison was done between obese diabetics and obese controls), therefore he concluded that serum FFA level was more related to adipose tissue mass than to the presence or absence of the type 2 diabetic state.

This is further supported by the observation that parameters that assess glycemic control were no longer correlated with serum FFA consistent with the findings of this study.

It is clear that insulin resistance, even severe insulin resistance, can exist in obesity without elevation of NEFA concentrations.

It is also clear that elevated NEFA concentrations are not necessarily associated with insulin resistance.

Two commonly seen examples are women versus men and younger versus older subjects.

Women have very significantly raised NEFA concentrations compared with men, yet tend to be more insulin-sensitive and to have better lipid profiles\(^{39}\).

In summary, The significant alterations in sera levels of chemerin and apelin of diabetic patients as compared to controls were suggested a possible role of these adipokines with the presence of obesity, insulin resistance and T2DM and the assay and assessment of sera levels of chemerin and apelin could be beneficial in early detection of T2DM and prevention of its unfavorable consequences especially the cardiovascular complications and atherosclerosis.

It was still unclear whether altered adipokines and inflammatory cytokines were a cause or compensatory mechanism to insulin resistance and T2DM.

REFERENCES


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