Serum chemerin level and its relation to carotid intima media thickness in type 2 diabetic patients

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Abstract

Background: The central pathological mechanism in macrovascular complications of T2DM is the atherosclerotic process. Measuring carotid intima media thickness [CIMT] was found to be suitable to detect the early stages of atherosclerosis. Altered chemerin secretion may be of pathological relevance to various disorders including diabetes mellitus and atherosclerosis.

The aim: to evaluate the relation of serum chemerin levels with early stages of atherosclerosis measured by CIMT in patients with T2DM without established coronary artery diseases (CAD) compared to healthy control subjects.

Patients and methods: The study included 86 subjects divided into two groups: Group I: included 46 T2DM patients without CAD. Group II included 40 healthy control subjects matched for age and sex. All subjects were subjected to the following: full history taking, complete clinical examination, body mass index [BMI] and laboratory assessment included fasting glucose, insulin level, glycated hemoglobin [HbAlc], lipid profile. Serum chemerin levels and homeostasis model assessments (HOMA2-IR). Doppler ultrasound for carotid artery to determine CIMT.

Results: BMI, Fasting plasma glucose, Triglyceride, HbAlc, HOMA2-IR, CIMT and serum chemerin levels were statistically significantly higher in diabetic group than the controls. Serum chemerin level had statistically significant positive correlation with BMI, LDL-c, FBG, HOMA2-IR, HbAlc and CIMT. The CIMT had statistically significant positive correlation with BMI, HbAlc and serum chemerin level.

Conclusions: Serum chemerin level positive correlation with BMI and HOMA2-IR may indicated the relation between chemerin and DM. CIMT and serum chemerin positive correlation to each other and to HbAlc may indicated their relation to atherosclerosis.

Key words
Serum chemerin, carotid intima media thickness, T2DM
Introduction

Diabetes mellitus (DM) is one of the most frequent chronic diseases worldwide, being among the top ten main causes of death in developed countries. It is also becoming epidemic in developing countries. Type 2 diabetes mellitus (T2DM) is the most prevalent form of the disease, representing 90% to 95% of the cases. The risk of macrovascular complications is 2 to 4 fold higher in patients with T2DM than that in persons without diabetes. They are the common causes of morbidity and mortality among people with diabetes. In recent years, non-invasive methods have been developed to measure the intima-media thickness (IMT) of the carotid artery, coronary artery calcification (CAC) and ankle-brachial pressure index (ABPI) as indices for atherosclerosis.

Atherosclerosis though the result from chronic inflammation and injury to the arterial wall in the peripheral or coronary vascular system. Measuring of carotid intima media thickness (CIMT) of the common carotid artery by B-mode ultrasononography was found to be suitable to monitor early stages of atherosclerosis. CIMT has been reported to be an indicator of cardiovascular disease (CVD). On the other hand, increased C-IMT has been observed in patients with T2DM or metabolic syndrome. Therefore, CIMT has been used as a marker of atherosclerosis progression in patients with T2DM.

This allows the detection of minimal lesions of the arterial wall without hemodynamic disturbances.

Although CIMT increase with age, it never exceeds 1.1mm in normal subjects. Moreover, patients with C-IMT>1.1mm were found to have higher prevalence of coronary heart disease (CHD) than patients with C-IMT<1.1mm. Thus, 1.1mm is usually accepted as a cut-off value for the presence of carotid atherosclerosis in T2DM.

The circulating levels of many adipokines are known to change with adiposity and this has been proposed as a contributing factor to the deleterious metabolic changes that often accompany obesity and ultimately lead to the development of T2DM.

Chemerin is one of the newly discovered adipokines, it is also known as reinois acid receptor responder proteins2 (RARRES2).

Early studies established that chemerin has proinflammatory chemoattractant properties elicited through interaction with the G-Protein-coupled receptor chemokine-like receptor 1 (CMKLR1). Chemerin has also been identified as adipokine that regulates adipogenesis and adipocyte metabolism. Chemerin synthesis and secretion increase dramatically with adipocyte differentiation enhancing its function to promote adipogenesis through activation of CMKLR1.

Thus, altered chemerin secretion may be of pathological relevance to various disorders associated with adipose dysfunction, including obesity, dyslipidemia, diabetes mellitus, inflammation, insulin resistance and atherosclerosis.

So, chemerin has a regulatory role in adipogenesis and adipocyte metabolism, and influencing chemerin and chemerin R signaling might lead to novel therapeutic approaches in the treatment of obesity, T2DM and CVD.

Aim of the work to study the relation of circulating chemerin levels with early stages of atherosclerosis, as measured by CIMT, in patients with T2DM without established CAD compared to healthy control subjects.

Subjects:

This study included 86 subjects divided into two groups:

Group I: 46 T2DM patients without established coronary artery disease (CAD).

Group II: 40 healthy control subjects matched for age and sex.

The patients were collected from Cardiology Department, Menoufia University and proved to be free from CAD. Subjects provided an informed consent for study participation. Ethical approval was granted by the ethical committee of Menoufia Faculty of Medicine, Egypt before study beginning.

Methods:

Full history taking and completed clinical examination were done for all patients and controls. Body mass index (BMI) was calculated.

Laboratory assessment: Fasting (10-12 hours overnight) venous blood samples were drawn by sterile vein-puncture. The samples were divided into three fractions: 1) 2 ml of blood were transferred into two EDTA tubes as an anticoagulant; one for estimation of blood HAvC on i-CROMA™ reader (using kits supplied by Boditech Med Inc., United Kingdom) (13) 2) one ml of blood into fluoride tube for FBG (by enzymatic colorimetric test using Spinreact kit, SPAIN) (14) 3) plain tubes left for 30 minute then centrifuged for 10 minutes at 3000 rpm; sera were separated in several aliquots and stored at –20°C until analysis of, lipid profile assessment was performed using Spinreact Kit (Girona, Spain). Low density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald formula. (15) Assay of serum insulin using using DRG ELISA kit, GERMANY and serum chemerin using RayBiotech ELISA kit, Inc, USA.

Homeostasis model assessment 2 (HOMA2) calculator was used to estimate insulin resistance (HOMA-IR).

Ultrasound assessment of Carotid IMT measurement
Intima-media thickness (IMT) of the bilateral common carotid arteries in multiple directions were assessed by a Hitachi 70000 high resolution ultrasound machine equipped with a linear array 12.5 MHz transducer.[17]

Statistical analysis of the data

Data into the computer was done followed by tabulation and analysis using PASW18

Results

The study included 46 patients of T2DM without CAD 26 male, 20 female and 40 healthy control subjects matched for age and sex 22 male, 18 female.

The mean age of diabetic group was 55.25±5.58 and 53.70±4.26 years in the control group. There was no statistically significant difference between the two group regarding age and gender (not shown).

There was a statistically significant difference increase in body mass index, triglyceride, fasting blood glucose, HbAlc levels, HOMA2-IR, CIMT values and serum chemerin levels between the two groups being higher in the diabetic group than in the control group. While, the mean value of HDL-c was statistically significant lower in the diabetic group compared to the control group. table (1).

According to table (2) it was found that chemerin had statistically significant positive correlation with body mass index, LDL, FBG, HOMA2-IR, HbAlc and CIMT. While, CIMT had statistically significant positive correlation with BMI, HbAlc and serum chemerin level.

As shown in table (3) only HOMA2-IR, HbAlc and CIMT have the most independent positive correlation with chemerin.

While, in table only chemerin levels and HbAlc had the most independent positive correlation with the CIMT.

DISCUSSION

Chemerin, a newly discovered adipokine, has been reported to act by its binding to chemerin receptors. Chemerin and its receptors are expressed abundantly in adipose tissue supporting its function in autocrine and paracrine fashion. (18)

Obesity and atherosclerosis are now considered as inflammatory states. Biomarkers that integrate metabolic and inflammatory signals maybe candidates to assess the risk of atherosclerosis and cardiovascular diseases. (19) The accumulation of chemerin in atherosclerotic lesion attract immune cells which add in remodeling of the vessel wall, alteration in insulin sensitivity and glucose uptake in adipocytes and skeletal muscle and the direct inflammatory effect on vascular endothelial cell that could contribute to development of atherosclerosis. (20)

In the present study there was a statistically significant difference between the diabetic patient without CAD and control subjects as regard BMI being higher in the diabetic group. This was in agreement with other researches who reported that obesity as measured by BMI was significantly and independently associated with diabetes. (21,22)

In the present study TG was statistically significant higher in the diabetic group than the control group while HDL-C was statistically significant lower and there was no statistically significant difference between the two groups regarding total cholesterol and LDL-C. this may be due to intake of lipid lowering agents by diabetic patients.

Jeppesen et al reported that patients with diabetic dyslipidemia had a worse prognosis than did those with isolated elevated LDL levels. (23)

The results of Drexel et al, (24) coincide with the results of the present study, as they demonstrated that, low HDL-C and high TG levels rather than high LDL-C level, were more closely linked with the occurrence of CAD and were strong sign of future heart attack or stroke in diabetic population.

Mullugeta et al (25) demonstrated that patients with poor glycemic control have higher serum level of TG than those with good glycemic control. The higher plasma TG is a predictor of CAD and TG enhances the binding of monocytes to endothelial cells. (26)

In the present study the CIMT was statistically significantly higher in the diabetic group than the control group. This is in agreement with other studies that found that CIMT is significantly higher in diabetic patients than non diabetics. (27) In across sectional study assessing the prevalence and predictors of subclinical atherosclerosis (SCA) among asymptomatic individuals in a multiethnic population it was found that 23% of individuals classified to be at low risk have SCA. (28) In other study 38% of asymptomatic young to middle-aged individuals with low risk have abnormal carotid ultrasound findings associated with increased risk for cardiovascular events. (29)

The atherosclerosis risk in communities study has proved that the addition of IMT to the Framingham risk equation led to improved prediction of risk in case of men, (30) and there also other cross sectional studies to suggest that around 20%-60% of patients who are at intermediate risk showed increased IMI values and should be considered as patients at high risk. (31) Yamasoke et al (6) have confirmed that carotid IMT in type 1 and type 2 diabetic patients was larger than that in age-matched non-diabetic individuals.

The present study showed that there was a statistically significant positive correlation between BMI, HbAlc, chemerin and CIMI. In agreement with this, Lakka et al (32) demonstrated that obesity can be associated with progressive increase in
CIMT and other risk factors. De Michele et al (33) also found a significant association between obesity and carotid wall thickness independent on fasting insulin concentration.

The present work suggested that, CIMT is positively correlated with chemerin levels. In accordance with this study Ali et al (19) found a significant positive correlation between serum chemerin levels and each of IL6, TNF, CRP and CIMT.

Yoo et al in disagreement with the results of our study found that (34) serum chemerin level is not correlated to CIMT. They demonstrated that serum chemerin is positively correlated with arterial stiffness represented by Brachial artery pulse wave velocity but not CIMT as CIMT quantitatively measures the arterial morphology consisting of intimal lesions and medial hypertrophy.

The results of the present study showed that HbA1c was independently correlated with CIMT after performing linear regression. This pronounced effect of HbA1c on early carotid atherosclerosis progression corroborates the hypothesis that HbA1c may be causally related to atherosclerosis event at low levels and may explain the positive association between HbA1c and further cardiovascular risk even in non diabetic subjects. (35)

In the present study we found a statistically significant difference between the two groups regarding the serum chemerin level being higher in the diabetic group than the control group. These results are in concordance with Ali et al (19) that serum chemerin levels are elevated in obesity and diabetes and that chemerin exacerbates glucose and that chemerin exacerbates glucose tolerance in these models by decreasing serum insulin levels and glucose uptake by liver tissue. The result of the present study also agreed with that of El-Mesallamy et al (36) who proved that serum chemerin levels were significantly increased in patients with T2DM and in patients with T2DM and ischemic heart disease compared with healthy control subjects.

However the results of the present study disagree with Bozaoglu et al (37) who described that circulating chemerin levels in T2DM were not significantly higher than those in normal control subjects.

The results of the present study showed significant positive correlation between BMI, FBG, HbATC, HoMA-2-IR, CIMT and serum chemerin level. In agreement with Ali et al (19) who found significant positive correlation between serum chemerin levels and each of systolic blood pressure, BMI, HOMA-IR, FBS and post prandial blood glucose.

Sell et al (38) findings were in agreement with our results regarding highly significant increase in FBG and its correlation to chemerin levels in metabolic syndrome group and T2DM which was attributed to the fact that higher chemerin release is associated with insulin resistance by decreasing the rate of auto-phosphorylation and subsequent downstream intracellular signaling cascades of insulin receptor-tyrosine kinase in peripheral tissues.

In the present study we found significant positive correlation between chemerin level and LDL but no correlation between chemerin levels and HDL-C total cholesterol and TG. In disagreement with Ali et al (19) who found significant negative correlation of chemerin level with HDL-C.

The present study showed that HbATC, HOMA-IR and CIMT were independently correlated with serum chemerin levels after performing linear regression.

It can be concluded that serum chemerin level and CIMT are higher in T2DM patients without CAD than in the healthy subjects. Serum chemerin is positively correlated with BMI and HOMA-IR may indicate the relation between chemerin and obesity. CIMT has positive correlation to HbA1c and chemerin levels may indicate their relation to atherosclerosis and may explain the positive association between them and future cardiovascular events.

Disclosures

The authors have no conflict of interest to declare.

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Non

REFERENCES


Table (1): Comparison between the two studied groups as regard all studied parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (n= 46)</th>
<th>Group II (n= 40)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>189.67±24.25</td>
<td>184.70±45.60</td>
<td>0.601</td>
</tr>
<tr>
<td>HDL-c (mg/dl)</td>
<td>37.21±12.72</td>
<td>47.40±9.07</td>
<td>0.035</td>
</tr>
<tr>
<td>LDL-c (mg/dl)</td>
<td>123.93±18.93</td>
<td>117.10±44.97</td>
<td>0.446</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>152.29±58.44</td>
<td>128.73±31.62</td>
<td>0.017</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>147.0±10.95</td>
<td>82.05±8.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.62±0.72</td>
<td>5.09±0.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin (μIU/ml)</td>
<td>13.32±0.72</td>
<td>8.09±0.40</td>
<td>0.31</td>
</tr>
<tr>
<td>HOMA2-IR</td>
<td>3.22±1.72</td>
<td>1.62±1.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Serum chemerin (ng/ml)</td>
<td>381.08±81.15</td>
<td>301.11±62.09</td>
<td>0.005</td>
</tr>
<tr>
<td>Carotid intima media thickness (mm)</td>
<td>0.98±0.19</td>
<td>0.69±0.14</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

HDL-c= high density lipoprotein cholesterol  LDL-c= Low density lipoprotein cholesterol

P≤0.05= statistically significant
### Table (2): Correlation between serum chemerin level and other studied parameters

<table>
<thead>
<tr>
<th></th>
<th>Serum chemerin (ng/ml)</th>
<th>CIMT (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>0.450*</td>
<td>0.003</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>0.074</td>
<td>0.650</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>0.088</td>
<td>0.684</td>
</tr>
<tr>
<td>LDL(mg/dl)</td>
<td>0.838*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG(mg/dl)</td>
<td>0.235</td>
<td>0.318</td>
</tr>
<tr>
<td>Fasting blood glucose(mg/dl)</td>
<td>0.877*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin (μIU/ml)</td>
<td>0.158</td>
<td>0.694</td>
</tr>
<tr>
<td>HOMA2-IR</td>
<td>0.765*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carotid intima media thickness (mm)</td>
<td>0.260*</td>
<td>0.006</td>
</tr>
<tr>
<td>HbAlc(%)</td>
<td>0.864*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

r= Pearson coefficient  
P≤0.05= statistically significant

### Table (3): Multivariate linear regression analysis for serum chemerin levels

<table>
<thead>
<tr>
<th></th>
<th>Serum chemerin (ng/ml)</th>
<th>CIMT (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.049</td>
<td>0.057</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>0.406</td>
<td>0.57</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>0.024</td>
<td>0.067</td>
</tr>
<tr>
<td>LDL(mg/dl)</td>
<td>0.005</td>
<td>0.690</td>
</tr>
<tr>
<td>TG(mg/dl)</td>
<td>0.006</td>
<td>0.048</td>
</tr>
<tr>
<td>FBG</td>
<td>0.619</td>
<td>0.50</td>
</tr>
<tr>
<td>Insulin (μIU/ml)</td>
<td>0.156</td>
<td>0.098</td>
</tr>
<tr>
<td>HOMA2-IR</td>
<td>28.430</td>
<td>12.87</td>
</tr>
<tr>
<td>CIMT (mm)</td>
<td>122.061</td>
<td>43.98</td>
</tr>
<tr>
<td>HbAlc(%)</td>
<td>0.067</td>
<td>0.019</td>
</tr>
</tbody>
</table>

B: Unstandarized Coefficients  
SE: Stander error