Chemerin Novel Biomarker As a Prognostic Factor for Cardiovascular Complications in Type 2 Diabetic Patients

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ABSTRACT
Objective: The present study was aimed to assess chemerin as a prognostic factor for cardiovascular complications in type 2 diabetic patients.

Patients and methods: Forty type 2 diabetic patients without cardiovascular disease, forty type 2 diabetic patients with cardiovascular disease and twenty healthy control counterparts were included in the present study. Chemerin levels were assayed and correlated with clinical pathological parameters. ROC curve analysis was also done for this biochemical marker.

Results: The mean level of chemerin was 57.65 ± 15.69 ng/l in diabetic subjects versus 93.97 ± 26.62 ng/l for the cardio-diabetic subjects (P < 0.0001). The chemerin levels were significantly elevated in the cardio-diabetic patients with increasing-reactive protein (CRP), triglycerides (TG), fasting blood glucose (FBG), glycated hemoglobin (HbA1C), micro-albumin and cholesterol (P < 0.0001, P < 0.0001, P = 0.005, P = 0.04, P = 0.011 and P = 0.0001 respectively). From the ROC curve analysis, it was observed that the area under curve for chemerin was 0.877. This finding indicates the good validity of the above biomarker as a prognostic factor for cardiovascular complication in type 2 diabetic patients.

Conclusion: It could be concluded that chemerin can be used as prognostic biomarker for cardiovascular complications in type 2 diabetic patients.

Keywords: Chemerin, diabetes mellitus, cardiovascular complication, prognosis

INTRODUCTION
Diabetes mellitus is a chronic disease that affects 415 million people worldwide and 5 million people died from diabetes-related complications. Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia, that results from lack of endogenous insulin or resistance to the action of insulin in fat, muscle, and liver in addition to an insufficient pancreatic beta cell response. T2DM is considered as a risk factor for cardiovascular disease (CVD). This is due to a complex group of risk factors associated with T2DM including hypertension, insulin resistance, hyperglycemia, hyperinsulinemia, diabetic dyslipidemia, systemic inflammation and adipose tissue-derived factors. Worth mentioning, the Changes in the mass and metabolism of adipose tissue may be accompanied with visceral obesity and insulin resistance commonly associated with T2DM. Adipocyte is considered as an active endocrine organ and secretes a large number of bioactive mediators (adipokines) that signal to the brain, liver, skeletal muscle, and the immune system, the important metabolic organs in the body. These adipokines include omentin-1, visfatin, and chemerin. Dysregulation of pro-inflammatory and anti-inflammatory adipokines secretion in obesity may serve as a pathogenic link between obesity, insulin resistance and cardiovascular diseases.

Chemerin is considered a proinflammatory cytokine that activates immune cells and contributes to inflammation by activating macrophage adhesion to vascular cell adhesion molecule-1 (VCAM-1) and fibronectin. It is not only a marker of vascular damage but also a prognostic predictor. In addition, chemerin is related to glucose, and lipid metabolism, inflammation, and adipogenesis. All of these lead to the development of cardiovascular complications in diabetic patients, especially atherosclerosis.

PATIENTS AND METHODS
Forty type 2 diabetic patients with cardiovascular disease (cardio-diabetic group) and forty type 2 diabetic patients without evidence of CVD (diabetic group) were included in the current study and collected from clinicians national institute of diabetes and endocrinology. In addition, twenty apparently healthy subjects with no history of type 2 DM, other endocrine dysfunctions, hyperlipidemia, hypertension, or coronary heart diseases were enrolled in the study and served as controls. Patients in the group without vascular disease were T2DM patients who had no history of...
vascular disease and those with normal ECG findings at exercise and normal peripheral artery Doppler ultrasonography findings. Exclusion criteria involved the presence of sustained type 1 DM, acute and chronic infections, malignancy, hepatic or renal disease, diabetic retinopathy and nephropathy, and other endocrine dysfunctions. This study was approved by Ethical Committee of Ethics commission and Scientific Research of the General Authority for hospitals and educational institutes.

Blood and urine samples: venous blood was collected from all participants and each blood sample was divided into two portions. The small portion was collected on EDTA coated tube for determination of HbA1C and the large portion was collected on plain tube for separation of serum. Serum samples were obtained for determination of other parameters. All biochemical variables were measured on the same day of the blood collection. Remaining serum specimens were stored at -20°C until analysis of chemerin. Urine was collected for determination of microalbumin.

Quantitative determination of glucose was carried out colorimetrically using method of Thomas. Quantitative estimation of serum cholesterol was done colorimetrically using method of Richmond. Serum HDL-cholesterol was quantified in serum using method of Okada et al. Triglycerides in serum was measured colorimetrically using method of Jacobs and Van Denmark. Glycated hemoglobin was determined using method described by Trivelli et al. Chemerin was evaluated by solid-phase enzyme-linked immunosorbent assay (ELISA kit) using method of Aronis et al. Serum C-reactive protein (CRP) was measured by ELISA using method of Hedlund. Quantitative estimation of microalbumin in urine was done by immunoturbidimetric assay using method of Mogensen and Schmitz

**STATISTICAL ANALYSIS**

Data were expressed as mean ± SD and analyzed using MedCalc software, version 11. The Student’s t test was used to assess the significance of difference in the levels of chemerin between the patient groups (diabetic and cardio-diabetic) and the control group. The correlation analysis between serum chemerin level and other measured parameters in the different studied groups was performed by correlation coefficient test. The cut-off value was determined for chemerin in the current study according to the best discrimination between diabetic patients and cardio-diabetic patients regarding optimal values of sensitivity and specificity using ROC curves analysis. AUC of the ROC curve was calculated for chemerin. P < 0.05 was accepted as significant.

**RESULTS**

Laboratory assessments of chemerin in the different submitted groups are presented in Table (1). Chemerin levels were significantly higher in diabetic patients than in healthy subjects (P < 0.0001). Likewise, chemerin levels were significantly higher in cardio-diabetic patients than in healthy subjects and diabetic patients (P < 0.0001).

Correlation between serum chemerin level and metabolic parameters in different studied groups were depicted in Table (2). Significant positive correlation between serum chemerin level and cholesterol, TG, CRP, FBG, and HbA1c has been recorded in diabetic patients (P = 0.007, P = 0.001, P < 0.0001, P < 0.0001, and P = 0.031 respectively) and cardio-diabetic patients (P = 0.0001, P < 0.0001, P < 0.0001, P = 0.005, and P = 0.040 respectively), but negative correlation between chemerin level and micro-albumin in diabetic patients (P = 0.026). Moreover, a significant positive correlations has been observed between chemerin level and LDL in diabetic patients (P = 0.045), also, between chemerin and micro-albumin in cardio-diabetic patients (P = 0.011).

The receiving operating characteristic (ROC) curve was designed for chemerin, (Fig. 1). The cut-off values for chemerin, was 75 ng/L. Area under curve (AUC) for chemerin was 0.877. This result indicates the good validity of the above biochemical markerto discriminate diabetic patients than cardio-diabetic patients.

**DISCUSSION**

Diabetes mellitus is a disease that occurs when the body cannot produce sufficient amount of insulin or defect of action of insulin. Patients with type 2 diabetes mellitus have a high risk of cardiovascular morbidity and mortality compared with individuals without diabetes, and are affected by cardiovascular disease. Most of this high risk is related to the prevalence of some risk factors such as dyslipidaemia, obesity and
hypertension in these patients. However the improved cardiovascular disease in patients with type 2 diabetes mellitus cannot be related to the higher prevalence of traditional risk factors only, but, other non-traditional risk factors are important in type 2 diabetic patients. Increased cardiovascular disease in patient with type 2 diabetes mellitus is due to a complex combination of different traditional and non-traditional risk factors that have an important role in the beginning and the evolution of atherosclerosis over its long natural history from endothelial function of clinical events.

The objective of this review is to assess chemerin as prognostic factors for cardiovascular complication in type 2 diabetic patients.

The results obtained in this study showed that chemerin levels were significantly higher in diabetic patients when compared to healthy subjects. Studies of El-Mesallam et al., Hu and Feng, Tarek and Khalid revealed that chemerin level was significantly higher in diabetic subjects when compared to healthy subjects.

Sell et al. explained these findings by the fact that adipose tissue expresses chemerin and chemokine-like receptor-1, and release of chemerin is related to the volume of adipocyte. Furthermore, the release of the high concentration of chemerin is related to insulin resistance at the level of lipogenesis by its reversible binding to the extracellular domain of insulin receptor-tyrosine kinase in peripheral tissues and decreasing the rate of autophosphorylation and subsequent downstream intracellular signaling cascades. Also, Chemerin suppressed phosphorylation of glycogen synthase kinase, an enzyme necessary for synthesis and storage of glycogen, and suppress the uptake of glucose. In addition, chemerin activates extracellular signal-regulated kinase (ERK). Suppression of ERK prevents chemerin-induced insulin resistance, pointing to participate of this pathway in chemerin action. 

Takahashi et al. postulated that chemerin in adipocytes has the opposite effect, where it increases insulin activated glucose uptake, so, it activates insulin sensitivity. Hence, the increase in circulating chemerin level is a compensatory mechanism in patients with insulin resistance. Also, chemerin exert various actions in endocrine, autocrine and paracrine ways. Moreover, Takahashi et al. showed that chemerin-deficient mice are glucose intolerant, and glucose intolerance was essentially due to increased production of hepatic glucose and impaired secretion of insulin. Also, they revealed that chemerin and its receptor were expressed in β-cell, and chemerin regulate function of β-cell and plays an important role in glucose homeostasis in a tissue.

Bozaoglu et al. found that the level of circulating chemerin in diabetic subjects was not significantly higher than in healthy subjects, this because of taking antidiabetic drugs, where Tan et al. showed that metformin (an oral hypoglycemic drug) significantly decreased level of circulating chemerin with a concomitant decrease in insulin resistance in patients with type 2 diabetes.

The serum chemerin level was significantly higher in cardio-diabetic patients when compared to healthy subjects. Study of Ying and Dongying found that serum chemerin levels of coronary artery disease (CAD) patients were significantly higher than that of control subjects, and study of Xiuying et al. found that CAD group showed significantly higher levels of chemerin. In addition, Liang et al. found that the level of chemerin was significantly higher in the acute myocardial infarction (AMI) and unstable angina (UA) groups than in the stable angina (SA) and control groups. This finding explained by Wittamer et al. who showed that chemerin promotes the migration of macrophage and immature dendritic cell. It is well-known that a macrophage-changed to-foam cell switch elicits the initiation and development of atherosclerosis, where, chemerin promote cholesterol uptake and foam cell formation, and increased accumulation of macrophages induce the rupture of plaque and the thrombus formation in advanced atherosclerosis. Therefore, chemerin may be involved in different stages of atherosclerosis through regulating the migration of macrophage. The serum chemerin level was significantly higher in cardio-diabetic patients when compared to diabetic patients. Study of Xiuying et al. found that CAD group showed significantly higher levels of chemerin. Kim et al. revealed that no difference in chemerin levels between asymptomatic type 2 diabetic patients with CAD and without CAD. Our study revealed a significant positive correlation between serum chemerin level and cholesterol, TG, CRP, FBG, and HbA1C in type 2 diabetes mellitus group, and cardio-diabetic patients. Likewise, significant positive correlation between serum chemerin level and

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LDL in diabetic group. Study of Ying and Dongying found a significant positive association between serum chemerin and triglycerides, and high-sensitivity CRP in CAD patients, and Osman et al. found that serum chemerin level was positively correlated with total cholesterol, LDL-C and triglycerides in type 2 diabetes mellitus group.In addition, Qingwei et al. demonstrated that CRP, which is an established marker of inflammation, was positively correlated with chemerin in acute coronary syndrome (ACS) patients. Moreover, Yu-Jin et al. found significant but weak correlations between serum chemerin concentrations and fasting glucose, triglyceride, total cholesterol, LDL-cholesterol and hsCRP in CAD patients.

Our results revealed an negative association between microalbumin and chemerin level in diabetic subjects, but positive association between microalbumin and chemerin level in cardio-diabetic subjects.Wenchao and Ping postulated that no differences were found in the level of serum chemerin between diabetic patients with normo-albuminuria and micro-albuminuria and control subjects. In addition, Christiane and Gunter found that serum chemerin was significantly elevated in type 2 diabetic patients with macro-albuminuria compared with control subjects and diabetic patients with normo-albuminuria and micro-albuminuria.

ROC curve was done to detect the best cut off value of serum chemerin in diabetic and cardio-diabetic patients was 75ng/l with 80% sensitivity and 90% specificity .Studies such as Yao et al. and Lin et al. reported high levels of circulating chemerin in CAD patients, also El-Mesallam Y et al. and Hu and Feng found that level of chemerin was significantly elevated in diabetic patients and in diabetic patients with ischaemic heart disease compared with healthy subjects. Moreover, Kadoglou et al. found that acute myocardial infarction (AMI) group appeared with significantly higher concentrations of chemerin compared with healthy controls . These findings suggesting that chemerin is a biomarker of CAD in patients with type 2 diabetes mellitus.

Measurement of chemerin might provide useful diagnostic and prognostic tools for cardiovascular complication in patients with type 2 diabetes mellitus.

REFERENCES


levels are interrelated in patients with Type 2 diabetes mellitus with or without ischaemic heart disease. Diabet Med.,28 (10):1194-200.
42-Qingwei Ji, Yingzhong Lin, Zhishan Liang, Kunwu Yu, Yuyang Liu, Zhe Fang, Ling Liu, Ying Shi, Quutang Zeng, Chao Chang, Meng Chai and Yujie Zhou (2014): Chemerin is a novel biomarker of acute coronary syndrome but not of stable angina pectoris. Cardiovascular Diabetology, 13:145
Table 1: Laboratory assessments of chemerin in the different studied groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control subject (C)</th>
<th>Diabetic subject (D)</th>
<th>Cardio–diabetic subject (CD)</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemerin (ng/l)</td>
<td>28.80± 6.82</td>
<td>57.65± 5.69</td>
<td>93.97 ± 26.62</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

P1: Diabetic group compared to control group.
P2: Cardio-diabetic group compared to control group.
P3: Cardio-diabetic group compared to diabetic group.

Table 2: Correlation between serum chemerin concentration and metabolic parameters in the different studied groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Serum chemerin level in control groups</th>
<th>Serum chemerin level in diabetic group</th>
<th>Serum chemerin level in cardio-diabetic groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>Cholesterol(mg/dl)</td>
<td>0.258</td>
<td>0.271</td>
<td>0.416</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>0.215</td>
<td>0.362</td>
<td>0.490</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>-0.010</td>
<td>0.966</td>
<td>-0.291</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>0.026</td>
<td>0.912</td>
<td>0.318</td>
</tr>
<tr>
<td>CRP(mg/l)</td>
<td>0.278</td>
<td>0.234</td>
<td>0.761</td>
</tr>
<tr>
<td>FBG(mg/dl)</td>
<td>0.049</td>
<td>0.835</td>
<td>0.802</td>
</tr>
<tr>
<td>HBA1C (%)</td>
<td>0.211</td>
<td>0.370</td>
<td>0.340</td>
</tr>
<tr>
<td>Micro-alb (mg/ml)</td>
<td>-0.275</td>
<td>0.239</td>
<td>-0.349</td>
</tr>
</tbody>
</table>

r: Correlation coefficient, *P<0.05, **P<0.01, not significant(P >0.05)

Fig.1: ROC curves for differentiation between diabetic and cardio-diabetic subjects by chemerin (P=0.0001)