SERUM CHEMERIN AS A PREDICTOR OF CORONARY ARTERY DISEASES IN TYPE 2 DIABETES

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Abstract

Chemerin is an adipokine that has attracted considerable interest due to an increasing body of evidence supporting its roles in adipogenesis, energy metabolism, and inflammation. Our objective was to determine the level of serum chemerin in type 2 diabetic (T2DM) patients and to evaluate its role as a predictor of coronary artery diseases (CAD)in these patients. This case control study was carried out on 30 T2DM patients without CAD, 30 T2DM patients with CAD and 30 apparently healthy, age and sex matched subjects. CAD was diagnosed as presence of one or more coronary arteries with significant stenosis (≥50%) and its severity was evaluated by Gensini score. All studied individuals were subjected to determination of fasting glucose, glycated hemoglobin, lipid profile, high sensitive c reactive protein (hsCRP) and serum chemerin. Our result revealed that serum chemerin was significantly increased in diabetic patients compared to controls and in diabetic patients with CAD compared to those without CAD. It was significantly correlated with hsCRP and Gensini score in CAD patients. Increasing chemerin was an independent predictor of CAD in T2DM. Receiver operating characteristic curve analysis of chemerin revealed an area under curve of 0.949. At cut off value of >375 ng/L, chemerin had 96.7% sensitivity and 86.6% specificity in prediction of CAD in T2DM patients. In conclusion, serum chemerin was increased in type 2 diabetic patients and associated with CAD in these patients. It was correlated with disease severity and can be used in prediction of CAD in type 2 diabetic patients.

INTRODUCTION

Diabetes, because of its vascular complications, is associated with a high risk of morbidity and mortality. The high incidence of cardiovascular complications, including coronary artery, peripheral artery and cerebrovascular diseases has been associated to the hyperglycemia-induced oxidative stress, endothelial dysfunction and lack of endothelial regeneration. This results in abnormal vascular remodeling and impaired neovascularization (American Diabetes Association 2007).

Chemerin is a recently identified adipokine that has attracted considerable interest due to an increasing body of evidence supporting roles for this adipokine in adipogenesis, energy metabolism, and inflammation. In particular, chemerin has been hypothesized as a possible link between obesity and the development of type 2 diabetes mellitus (T2DM) (Kaur et al., 2010). Chemerin is also an identified chemoattractant protein that induces leukocyte migration; in addition, its receptor is expressed by tissue macrophages. These observations suggest that chemerin functions in the development of inflammatory diseases, including atherosclerosis (Ernst et al., 2011).
The aim of the present study was to determine the level of serum chemerin in type 2 diabetic patients and to evaluate its role as a predictor of coronary artery diseases (CAD) in these patients.

**SUBJECTS & METHODS**

**Subjects:**
This case control study was carried out on 90 subjects classified into: 30 type 2 diabetic patients without CAD, 30 type 2 diabetic patients with CAD and 30 apparently healthy, age and sex matched subjects as control group. Type 2 diabetes is defined according to *American Diabetes Association (2016)*. CAD was diagnosed as presence of one or more coronary arteries with significant stenosis (≥50%).

Coronary angiography is performed under local anesthesia using Seldinger technique with right femoral approach (*Seldinger, 1953*). Gensini score was calculated from the number of stenotic coronary artery segments and the degree of lumen stenosis (*Gensini, 1983*).

Patients who had any of the following conditions were excluded from our study: ejection fraction less than 50% calculated by Simpsons method, chronic viral hepatitis, liver cell failure, renal dysfunction, malignancy, myocarditis, pericarditis, active inflammatory disease, acute infections, severe medical illness, and nutritional derangement.

**Laboratory investigations:**
Fasting glucose, lipid profile, HbA1c and hsCRP were analyzed on Cobas6000 analyzer (Roche Diagnostics). Serum chemerin levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Glory Science Co, USA), according to manufacturer’s protocol. The detection limit is 20-1200 ng/L.

**Statistical analysis:**
Sample size was calculated with 95% confidence interval and 80% power (Open Epi). All data were analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA) and MedCalc 13 for windows (MedCalc Software bvba, Ostend, Belgium). Continuous variables were checked for normality by using Shapiro-Wilk test. Continuous variables were expressed as the mean ± standard deviation (SD) or median (range), and the categorical variables were expressed as a number (percentage). One-Way ANOVA was used to compare groups of normally distributed data while Kruskal-Wallis H test was used for non-normally distributed data. Percent of categorical variables were compared using the Chi-square test. Spearman’s rank correlation analysis was done between chemerin and selected study parameters. Univariate regression analysis was done to assess role of chemerin as an independent predictors for CAD in type 2 diabetic patients. Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off values of chemerin and hsCRP with maximum sensitivity and specificity for prediction of CAD. Area Under Curve (AUROC) was also calculated. All tests were two tailed. P < 0.05 was considered statistically significant, p < 0.01 was considered highly statistically significant, and p > 0.05 was considered non-statistically significant.
RESULTS

Table 1: Demographic and clinical characteristics of the studied groups.

| Demographic and Clinical data | Healthy controls (N=30) | Diabetics without CAD (N=30) | Diabetics with CAD (N=30) | p |
|------------------------------|-------------------------|-----------------------------|---------------------------|---|
| Age (years) * | 55.8 ± 6.57 (42 – 66) | 56.1 ± 6.35 (43 – 65) | 57.7 ± 6.40 (45 – 69) | 0.457 |
| Sex | | | | |
| Male | 14(46.7) | 13(43.3) | 20(66.7) | 0.147 |
| Female | 16(53.3) | 17(56.7) | 10(33.3) | |
| Smoking | | | | |
| Non-smoker | 22(73.3) | 20(66.7) | 16(53.3) | 0.257 |
| Smoker | 8(26.7) | 10(33.3) | 14(46.7) | |
| Physical activity | | | | |
| No | 21(70) | 23(76.7) | 27(90) | 0.154 |
| Yes | 9(30) | 7(23.3) | 3(10) | |
| SBP (mmHg) | 120 (110-140) | 120 (110-140) | 140* (120-140) | <0.001 |
| DBP (mmHg) | 80 (70-90) | 80 (70-90) | 90* (80-90) | <0.001 |
| BMI (Kg/m^2) | 27.6 (21.6-39.9) | 27.85 (23.4-35.1) | 27.45 (22.3-39) | 0.994 |

Data are presented as median*, mean± standard deviation# (range) or number(%).
N: Number of subjects.
*Significantly different from other groups.

Table 2: Laboratory findings in the studied groups.

| Laboratory findings | Healthy controls (N=30) | Diabetics without CAD (N=30) | Diabetics with CAD (N=30) | p |
|---------------------|-------------------------|-----------------------------|---------------------------|---|
| Fasting Glucose (mg/dl) | 79.5* (73-94) | 132 (108-343) | 134.5 (106-334) | <0.001 |
| HbA1c (%) | 3.95* (2.3-5) | 4.3 (2.7-6.1) | 4.65 (2.8-6.2) | 0.009 |
| Total cholesterol (mg/dl) | 119 (79-205) | 118 (78-231) | 200.5* (111-306) | <0.001 |
| Triglycerides (mg/dl) | 85.5 (33-183) | 78 (51-199) | 174* (77-443) | <0.001 |
| HDL-C (mg/dl) | 41 (24-61) | 46.5 (28-55) | 34.5* (6-81) | <0.001 |
| LDL-C (mg/dl) | 55.5 (21-130) | 58.5 (20-151) | 125* (53-228) | <0.001 |
| hsCRP (mg/L) | 1.4 (0.2-3.8) | 2.05 (0.8-5.2) | 8.3* (3.5-56.4) | <0.001 |
| Chemerin (ng/L) | 190 (130-240) | 330* (230-690) | 625* (380-985) | <0.001 |

Data are presented as median (range).
N: Number of subjects.
*Significantly different from other groups.
Figure 1: Box-plot for serum chemerin in the studied groups. HS: highly significant.

Table 3: Correlation between chemerin (ng/L) and some studied parameters in type 2 diabetic patients.

| Parameter                  | Diabetics without CAD | Diabetics with CAD |
|----------------------------|-----------------------|--------------------|
|                            | r         | p       | r         | p       |
| Age (years)                | -0.181    | 0.339   | +0.092    | 0.628   |
| SBP (mmHg)                 | +0.094    | 0.623   | -0.010    | 0.957   |
| DBP (mmHg)                 | +0.062    | 0.746   | +0.111    | 0.560   |
| BMI (Kg/m2)                | +0.558    | 0.001*  | +0.183    | 0.332   |
| Fasting glucose (mg/dl)    | +0.620    | <0.001* | +0.125    | 0.509   |
| HbA1c (%)                  | +0.101    | 0.596   | -0.025    | 0.897   |
| Total cholesterol (mg/dl)  | +0.076    | 0.691   | -0.007    | 0.970   |
| Triglycerides (mg/dl)      | +0.153    | 0.421   | -0.083    | 0.662   |
| HDL-C (mg/dl)              | -0.181    | 0.337   | +0.051    | 0.787   |
| LDL-C (mg/dl)              | +0.125    | 0.509   | -0.050    | 0.793   |
| hsCRP (mg/L)               | +0.718    | <0.001* | +0.862    | <0.001* |
| Gensini score              | NA        | +0.687  | <0.001*   |
| Number of affected vessels | NA        | +0.126  | 0.506     |

*Significant.
NA: not applicable
Table 4: hsCRP and chemerin as predictors for CAD in type 2 diabetic patients.

| Parameter          | SN % | SP % | PPV % | NPV % | Accuracy | AUROC (95% CI) |
|--------------------|------|------|-------|-------|----------|----------------|
| hsCRP >3.0 mg/L    | 90%  | 90%  | 90%   | 90%   | 90%      | 0.946 (0.855–0.988) |
| Chemerin >375 ng/L | 96.7%| 86.6%| 88.2% | 96.3% | 91.7%    | 0.949 (0.860–0.989)  |
| Chemerin and hsCRP | 100% | 90%  | 90.9% | 100%  | 95%      | 0.988 (0.858–0.989)  |

SN: Sensitivity.  
SP: Specificity.  
PPV: Positive Predictive Value.  
NPV: Negative Predictive Value.  
AUROC: Area Under Receiver Operating Characteristic curve.  
95% CI: 95% Confidence Interval.

Figure 2: Receiver operating characteristic (ROC) curve of chemerin and hsCRP as predictors for CAD in type 2 diabetic patients.

Table 1 shows demographic and clinical characteristics of the studied groups. In diabetic patients with CAD, the mean± SD (range) of Gensini score was 33.5±32.14 (5-96). Laboratory findings are presented in Table 2. Serum chemerin was significantly increased in diabetic patients compared to controls and in diabetic patients with CAD compared to those without CAD (Figure 1). Serum chemerin was significantly correlated with hsCRP in diabetic groups and with Gensini score in CAD patients (Table 3).

Logistic regression analysis revealed that increasing chemerin level was an independent predictor of CAD in type 2 diabetic patients (β regression Coefficient± standard error is 0.014± 0.004, p <0.001).
ROC curve analysis of hsCRP revealed an AUROC of 0.946. At cut off value of > 3.0 mg/L, 27 out of 30 CAD patients were correctly diagnosed. ROC curve analysis of serum chemerin revealed an AUROC of 0.949. At cut off value of >375 ng/L, 29 out of 30 CAD patients were correctly diagnosed. When using chemerin and hsCRP in combination, all CAD were correctly diagnosed (100% sensitivity) (table 4, figure 2).

DISCUSSION

Early prediction of CAD in diabetic patients is important for early management. Serum biomarkers are important tools for prediction, diagnosis, risk stratification and therapeutic decision-making for patients with cardiovascular disease (Corson et al., 2009). The present study revealed that chemerin level was significantly higher in type 2 diabetic patients compared to control group and in diabetic patients with CAD compared to those without CAD (Ali and Al-hadidi, 2013). Our results confirm previous researches that reported increased serum chemerin in type 2 diabetic patients with atherosclerosis (Elsaid et al., 2013). In contrast, El-Mesallamy et al. (2011) didn’t reveal significant differences in serum chemerin between type 2 diabetic patients with and without CAD.

Higher chemerin release is associated with insulin resistance in skeletal muscles by decreasing the rate of auto phosphorylation and subsequent downstream intracellular signaling cascades of insulin receptor tyrosine kinase activity in peripheral tissues. Chemerin also inhibits glycogen synthase kinase phosphorylation, an enzyme necessary for glycogen synthesis and storage, and thus inhibits glucose uptake. In addition, chemerin activates extracellular signal-regulated kinase (ERK). Inhibition of ERK prevents chemerin induced insulin resistance, pointing to participation of this pathway in chemerin action (Sell et al., 2009).

Logistic regression analysis in this study revealed that increased chemerin is an independent predictor of CAD in type 2 diabetic patients. Similar findings were previously reported in metabolic syndrome patients (Dong et al., 2011 and Askan et al., 2014) and in CAD patients (Xiaotao et al., 2012). We also confirmed the previous results that reported a significant positive correlation between serum chemerin and disease severity evaluated by Gensini score (Yan et al., 2012 and Bozaoglu et al., 2010).

Chemerin activates extracellular signal-regulated kinase (p38 MAPK and ERK1/2 action), stimulates blood vessels migration, invasion, and formation, and leads to angiogenesis (Bozaoglu et al., 2010). Chemerin also promotes migration of inflammatory cells, including monocytes and macrophages, into atherosclerotic plaques by chemotaxis. The activation and penetration of monocytes and macrophages in atherosclerotic plaques are important factors resulting in plaque instability, which induces the rupture of plaque and eventual thrombus formation (Zhao et al., 2015). Moreover, chemerin is expressed in both the foam cells and vascular smooth muscle cells of these atherosclerotic lesions and correlates with overall disease burden. Not only does this support a role for chemerin in the pathogenesis of atherosclerosis, but it lends support to the importance of localized autocrine/paracrine functions of chemerin within the heart (Ernst et al., 2010).

Our study revealed significant positive correlation between chemerin level and hsCRP in type 2 diabetic patients with and without CAD. Similar findings were reported previously in metabolic syndrome (Abdelhaleem et al., 2012 and Lehrke et al., 2009). Endothelial cells express both chemerin and its receptor, CMKLR1, and it is regulated by tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), and other pro-inflammatory cytokines. After being stimulated by inflammatory cytokines, endothelia cells were damaged, triggering macrophages that remain in artery walls by a variety of adhesion and migration activities. Macrophages move lipids into foam cells, promoting the development of atherosclerosis (Kaur et al., 2010).

In the present study, ROC curve analysis of chemerin revealed an AUROC of 0.949 which was comparable with that of hsCRP (0.946). At cut off value of > 375 ng/L, serum chemerin predicted CAD in type 2 diabetic patients with 96.7% sensitivity and 86.6% specificity. When using chemerin and hsCRP in combination, all CAD were correctly diagnosed (100% sensitivity).

In conclusion serum chemerin was increased in type 2 diabetic patients and associated with CAD in these patients. It was correlated with disease severity and can be used in prediction of CAD in type 2 diabetic patient. Further studies on large sample size to confirm these results are recommended.
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