Evaluation of Visfatin in Patients with Obesity, Metabolic Syndrome, Insulin Resistance and Impaired Glucose Tolerance; Case-Control Study

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Introduction: Visfatin is a novel adipokine originally described to be produced predominantly by visceral fat tissue. The aim of the study was aimed to investigate visfatin level in non-diabetic obese patients with or without MetS and prediabetes.

Methods: The cross-sectional study enrolled 169 subjects. Anthropometric measures and metabolic parameters were obtained. Exclusion criteria were pregnancy, smoking, any of the chronic disease. Blood sample was collected overnight fasting and centrifuged, and stored at refrigerator at -70º degree until to be assayed. Visfatin was assayed with ELISA method.

Results: The study enrolled 169 subjects aged 36.9 ± 8.5 years (control group = 52 and obese group: 117; and Male: 28 and Female: 141). Among obese subjects, the frequency of MetS, and PreDM was respectively 35.1% (n = 41) and 70.1% (n = 82). Mean concentration of serum visfatin among subjects with obesity was significantly higher those with normal weight subjects (10.39 ± 2.30 versus 6.9 ± 2.2 ng/mL; p < 0.001). The subjects with IR+ (10.58 ± 2.48 ng/mL ) had significantly higher visfatin concentration than those with IR- (10.58 ± 2.48 ng/mL and 8.47 ± 2.65 ng/mL) (p < 0.001, respectively). Visfatin positively correlated with BMI (r = +0.627 and p < 0.001), WHR (r = +0.203 and p = 0.023), HOMA-IR (r = +0.329 and p < 0.001), and hs-CRP (r = +0.421 and p < 0.001). Mean concentration of serum visfatin was not significantly different between groups of MetS and PreDM.

Conclusion: Visfatin was associated with body mass index and insulin resistance, but not with metabolic syndrome and impaired glucose metabolism.

Key words: Obesity, Metabolic Syndrome, Visfatin, impaired glucose tolerance, insulin resistance.

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University, and Medical Faculty, between January 2015 and May 2015. The study enrolled the obese and non-obese patients admitted to out-patient clinic of Family Medicine. Exclusion criteria were pregnancy, smoking, any of the chronic disease including liver, heart, pulmonary, renal and vascular and endocrine disorders. The patients newly diagnosed were excluded. The participants under treatment of anti-hypertensive, anti-hyperlipidemias and anti-diabetic agents were not enrolled. Informed consent was obtained.

**Anthropometric measurements**

Anthropometric values of all subjects from study and control group were measured according to international protocols: Weight and height were measured with subjects wearing light clothing and without shoes. Height was recorded to the nearest 0.1 cm with GMSC-1 type height tester, stadiometer. Weight was recorded to the nearest 0.1 kg using a balance-beam scale. The waist circumference was measured to the nearest 0.1 cm at the midpoint between bottom of the rib cage and the top of the iliac crest at the end of exhalation using waistline measurer employed with subjects standing without clothing covering the waist area. Hip circumference was measured to nearest 0.1 cm using a tape employed to subjects standing and without clothing except light underwear covering the hip area around the point with the maximum circumference over the buttocks. Body mass index (BMI) was calculated with formulation of weight (kg)/height (m)$^2$ (10)

Blood pressure was measured twice to the nearest 2-3 mm-Hg using a mercury sphygmomanometer, after the subjects were seated at rest at least 5 minutes. The average of two measurements of blood pressure was recorded.

**Biochemical analysis**

All blood and urinary samples were collected in the morning time after 8-12 hours overnight-fasting. After blood was drawn, the tubes were gently shaken and then separated by centrifugation at 3200 rpm for 10-15 minutes. Blood sample for post-prandial glucose level was drawn two hours after meal. Blood sample for complete blood count test was drawn into tube with EDTA. Lipid profile and was assayed with colorimetric method (Cobas 6000 C 501, Roche Diagnostics GmbH, Mannheim, Germany). Insulin and TSH levels were assayed with methods of solid-phase enzyme–labeled chemiluminescent immunometric assay, solid-phase two-site chemiluminescent immunometric assay, solid-phase competitive chemiluminescent enzyme immunoassay, solid-phase chemiluminescent immunometric assay and competitive immunoassay, respectively (IMMULITE 2000, Siemens Healthcare Diagnostics inc. Flanders NJ. 07836 USA). LDL-cholesterol was calculated with Friedewald formulation. Homeostasis model assessment of insulin resistance (HOMA-IR) was also calculated with formulation of fasting serum glucose (mg/dl) x fasting plasma insulin level (µU/ml)/405, excluded if serum glucose level > 200 mg/dl. HOMA-IR was accepted positive, if it is > 2.5 (16) (11). Visfatin and hs-CRP were assayed with ELISA method.

**Determination of Obesity, Metabolic Syndrome and Impaired blood glucose (IFG/IGT):**

Obesity was defined as body mass index value over 29.9 kg/m$^2$, described by WHO (12). MetS was defined, using criteria proposed by NCEP ATP III (13), as three or more of the following variables: (1) fasting blood sugar ≥ 110 mg/dL, (2) triglyceride level ≥ 150 mg/dL, (3) HDL-cholesterol < 50 mg/dL in women, < 40 in men, (4) waist circumference > 88 cm for women and 102 cm for men, and (5) blood pressure ≥ 130/85 mm-Hg. Impaired Fasting Glucose (IFG) and Impaired Glucose Tolerance (IGT) was defined with 75 g oral glucose challenge test as: obese but non-PreDM, obese and PreDM (14).

**Data analysis**

The subjects were grouped as non-obese (BMI < 30 kg/m$^2$) and obese group (BMI ≥ 30 kg/m$^2$) according to BMI class, Prediabetes (PreDM: IFG and/or IGT) and MetS status. Based on OGGT results, the subjects were sub-grouped as PreDM+/PreDM-. The subjects were sub-grouped as MetS+/MetS-. Additionally, the obese patients were sub-grouped IR+/IR- according to HOMAIR value (HOMAIR ≥ 2.5).

All data was entered in the PC software, and analyzed using with SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Firstly of all, obesity, MetS and IFG/IGT were determined according to above and normality test for distribution of variables was tested with Kolmogorov-Smirnov test. Logarithmic transformation was applied for the variables without normal distribution (visfatin, SBP, SBP, TG, HbA1c, insulin, hs-CRP, HOMAIR, ALT, AST, uric acid and WHR). Student-T test was used for comparison of visfatin, metabolic parameters and anthropometric measures between Obesity and Control groups, IR and Control groups. One-way ANOVA (Bonferroni test) was used for comparison of visfatin, metabolic parameters and anthropometric measures between MetS, PreDM and Control groups. For correlation between visfatin and BMI, FBF, PBF, insulin, HbA1c, WHR, ACR, HOMA-IR, hs-CRP, SBP, DBP and age,
Pearson’s correlation analysis was used. Statistically significance was accepted if P value < 0.05.

**Results**

The study enrolled 169 subjects aged 36.9 ± 8.5 years (control group = 52 and obese group: 117; and Male: 28 and Female: 141). Among obese subjects, the frequency of MetS, and PreDM was respectively 35.1% (n = 41) and 70.1% (n = 83). The ratio of subjects with IR+ was 40.2% (n = 68). The number of female was predominant in obese group than non-obese group (88% vs. 73%, p=0.016).

In Table 1, Comparisons of metabolic parameters and anthropometric measures between control and obese groups were given. All variables except HDL-chol were significantly higher among obese subjects compared to normal weight ones. HDL-chol was significantly lower in obese group than control group. Of liver enzymes, AST was not significantly different between groups.

In Figure 1, comparison of serum visfatin concentration between subjects with IR+ and IR-; subjects with health normal weight and obesity was shown. Serum visfatin concentration among subjects with obesity was significantly higher those with normal weight subjects (10.39 ± 2.30 versus 6.9 ± 2.2 ng/mL; p < 0.001). Mean concentration of serum visfatin in subjects with IR+ (10.58 ± 2.48 ng/mL ) was statistically significant and higher than subjects with IR- (8.47 ± 2.65 ng/mL) (p < 0.001).

**Table 1.** Comparison of basic anthropometric measures and metabolic parameters between obese and control groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Obesity (n = 117)</th>
<th>Control (n = 52)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39.3±9.1</td>
<td>32.2± 8.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>37.6 ± 3.2</td>
<td>23.6 ± 3.20</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.95 ± 0.06</td>
<td>0.88 ± 0.11</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SBP (mm-Hg)</td>
<td>129.8 ± 10.5</td>
<td>112.9 ± 7.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DBP (mm-Hg)</td>
<td>84.6 ± 8.7</td>
<td>71.6 ± 8.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
<td>99.8 ± 11.4</td>
<td>92.4 ± 8.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Insulin</td>
<td>9.1 ± 6.6</td>
<td>3.1 ± 1.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>hs-CRP (mg/dL)</td>
<td>6.5 ± 4.9</td>
<td>1.38 ± 1.29</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>4.9 ± 6.4</td>
<td>4.9 ± 1.5</td>
<td>0.025</td>
</tr>
<tr>
<td>LDL-Chol (mg/dL)</td>
<td>112.2 ± 33.7</td>
<td>97.5 ± 33.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HDL-Chol (mg/dL)</td>
<td>44.3 ± 11.8</td>
<td>53.5 ± 13.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>162.5 ± 92.6</td>
<td>104.4 ± 47.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>T-Chol (mg/dL)</td>
<td>188.1 ± 42.2</td>
<td>165.8 ± 42.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.4 ± 2.6</td>
<td>1.6 ± 1.3</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

For statistical analysis, independent unpaired sample T (T-test) was used between obesity and control groups.

**Figure 1.** Comparison of serum visfatin concentration between subjects with healthy normal weight and obesity: subjects with IR+ and IR-.
Figure 2 indicates correlation of serum visfatin concentration with HOMA-IR, BMI, WHR and hs-CRP. It was positively correlated with all. Strong positive correlation was observed with BMI (r = 0.627 and p < 0.001), while weak positive correlation was observed with WHR (r = 0.203 and p = 0.023).

Table 2 represents comparisons of metabolic parameters and anthropometric measures along with mean serum visfatin concentration between MetS status and control group. Accordingly, mean concentration of serum visfatin was not significantly different subjects with Mets+ and Mets- (10.59 ± 2.45 ng/mL and 10.12 ± 2.02 ng/mL).

In table 3, comparisons of mean visfatin concentration, metabolic parameters and anthropometric measures between PreDM status and control group were displayed. Mean serum visfatin concentrations among the subjects with PreDM+ and PreDM- were 10.38 ± 2.11 and 10.34 ± 2.55 ng/mL, they were not significantly different. However, they were significantly higher than those with healthy normal weight (7.04 ± 2.16 ng/mL; p < 0.001).

Discussion

This study provided several ideas regarding relation of visfatin with obesity, metabolic syndrome, impaired glucose metabolism and insulin resistance. Our results indicate that plasma concentration of visfatin increases along with rising insulin resistance and body mass index, but not associated with metabolic syndrome and impaired glucose metabolism.

There have been contradictory findings on the association between visfatin and obesity. Sandrep et al. (16) found that serum visfatin levels are associated with obesity and visceral fat but not with subcutaneous fat on their study enrolling...
sex and age-matched subjects. However, we did not adjust as sex-/age-matched, and evaluate the subcutaneous fat tissue. We evaluated an indicator for visceral fat as anthropometric measure, WHR. Haider et al. (17) also reported that visfatin levels were substantially increased in morbidly obese individuals and its level was reduced after gastric banding surgery. Conversely, Pagano et al. (18) found that plasma levels of visfatin were significantly lower in obese subjects. On the other hand, the negative association we found in obese patients between visfatin and adiposity is not in agreement with data previously reported by Berndt et al. (19). They reported that visfatin plasma concentrations and visceral visfatin mRNA expression correlated with measures of obesity but not with visceral fat mass or waist-to-hip ratio. Correspondingly, Kamiska et al. (20) found elevated levels of visfatin in obese subjects, which did not correlate with the majority of anthropometric parameters, and negative correlation with WHR. We found serum visfatin was correlated with obesity and WHR. However, we did not adjust as sex-/age-matched, and did not evaluate the subcutaneous fat tissue.

Based on previous study reports, it seemed that there was strong positive correlation between visfatin and insulin resistance. However, relationship between them remains controversial. Berndt et al. (19) found that there was no significant correlation between plasma visfatin and fasting plasma insulin concentrations. Additionally, Korner et al. (21) suggested that visfatin was not increased with insulin resistance and not associated with impaired glucose tolerance. We detected that visfatin was correlated with insulin resistance but not with impaired glucose tolerance. Conversely, a recent study conducted by Nourbakhsh et al. (22) revealed that visfatin levels showed positive correlations with fasting glucose and insulin resistance in obese adolescents. Similarly, we found positive correlation between visfatin and insulin resistance in our study.

In our study, serum visfatin concentration was correlated with HOMA-IR, but not associated with MetS. Filippatos et al. (23), on the other hand, revealed that plasma visfatin levels were increased in overweight and obese subjects with MetS compared with those individuals without MetS. In accordance with our finding, Olszanecka-Glinianowicz et al. (24) reported that similar visfatin levels were observed in MetS group, compared to with the non-MetS group. We hypnotized that the increase of visfatin production in obese subjects with IR+ is insufficient to prevent the development of MetS.

### Table 3. Comparison of basic anthropometric measures and metabolic parameters between PreDM+, PreDM- and control groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>PreDM + (n = 83)</th>
<th>PreDM – (n = 34)</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI kg/m²</td>
<td>38.2±7.2</td>
<td>36.2±4.3</td>
<td>23.6±3.2</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>FPG mg/dL</td>
<td>102.3±11.3</td>
<td>94.5±9.3</td>
<td>92.4±8.2</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>SBP (mm-Hg)</td>
<td>130.7±10.8</td>
<td>127.7±9.4</td>
<td>112.9±7.2</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>DBP (mm-Hg)</td>
<td>84.4±8.6</td>
<td>85.2±9.1</td>
<td>71.6±4.6</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>HDL-chol mg/dL</td>
<td>44.6±11.1</td>
<td>43.7±13.5</td>
<td>53.5±13.4</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>TG mg/dL</td>
<td>173.7±97.4</td>
<td>136.7±75.5</td>
<td>104.4±47.5</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>LDL-chol mg/dL</td>
<td>116.1±33.4</td>
<td>103.4±33.2</td>
<td>97.5±33.4</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>Total-chol mg/dL</td>
<td>197.8±37.5</td>
<td>165.9±44.5</td>
<td>165.9±42.6</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>106.1±11</td>
<td>104.2±12.3</td>
<td>84.8±10.7</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>WHR</td>
<td>0.94±0.66</td>
<td>0.94±0.64</td>
<td>0.88±0.12</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.67±2.95</td>
<td>2.49±1.59</td>
<td>1.30±1.58</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>Visfatin (ng/mL)</td>
<td>10.38±2.22</td>
<td>10.34±2.55</td>
<td>7.04±2.16</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>Hc-CRP (mg/dL)</td>
<td>6.92±5.16</td>
<td>5.32±4.27</td>
<td>1.38±1.29</td>
<td>&lt; 0.001**</td>
</tr>
</tbody>
</table>

For statistical analysis, one-way ANOVA (Bonferroni) test was used between PreDM+, PreDM- and control groups * * There was no significant difference between PreDM+ group and PreDM- group. ** There was no significant difference between control and PreDM- groups. *** There was significant difference between three groups.
Based on similar and different results in the studies on visfatin and obesity and obesity related disorders including insulin resistance, metabolic syndrome and impaired glucose metabolism, these variations may be the result of differences in the settings of the studies or ethnic origin of the participants.

The Study Limitation

Our study has some limitations. It is cross-sectional study design, with no longitudinal follow-up. It had small sample size. The subjects in our study were not sex and age-matched. We did not apply the reference method of insulin resistance measurement, i.e. metabolic clamp technique. The study sample is clinical rather than population based.

Conclusion

Visfatin was associated with abdominal obesity and insulin resistance as well as obesity, but not with metabolic syndrome and pre-diabetes mellitus. We suggest that visfatin has effect on development of insulin resistance, but not on metabolic syndrome and impaired glucose metabolism.

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References


