Introduction

Since patients with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) are at high risk for the development of diabetes mellitus (DM) in the future, such patients are currently considered to have subclinical diabetes or pre-diabetes. Early diagnosis and treatment of individuals with subclinical DM are necessary to reduce or delay the likelihood of progression to type 2 DM. Hyperglycemia occurs by the common mechanism in subclinical DM and in type 2 DM. In this mechanism, deficiency of insulin hormone secreted by the pancreatic beta-cells and tissue insulin resistance play an important role. In addition to the parameters that indicate insulin resistance and insulin secretion defects, various markers may also be valuable in identifying individuals who would develop DM.
Recent studies have demonstrated that adipose tissue is not only an energy reservoir but also an active endocrine organ. White adipose tissue stores excess energy as triglyceride in the adipocytes and can rapidly release the stored energy into the circulation when needed. Storage of the energy in the adipocytes and its release are controlled by hormonal signals (e.g., insulin, catecholamines, and glucocorticoids). It has been determined that numerous substances such as leptin, resistin, tumor necrosis factor-α, adiponectin, adipisin, interleukin-6, plasminogen activator inhibitor-1, transforming growth factor-α, angiotensinogen, acylation stimulating protein, insulin-like growth factor 1, prostaglandin I2, and prostaglandin F2α are secreted from the adipocytes[6,8]. These bioactive peptides, which are secreted from white adipose tissue, are generally called as adipokines. Visfatin is a recently discovered novel adipokine, which is synthesized particularly in visceral adipose tissue[5]. Visfatin has insulin-mimetic properties and plasma glucose-lowering effects and it can bind to insulin receptors and activate these receptors[9].

The present study aimed to compare serum visfatin levels among subjects with subclinical diabetes/pre-diabetes (IFG alone and IFG+IGT), subjects with newly diagnosed type 2 DM, and healthy nondiabetic normoglycemic controls and to evaluate the relation of serum visfatin levels with some metabolic parameters and insulin resistance.

Materials and methods

Eighty subjects (53 females and 27 males), who were referred to the Department of Medical Biochemistry for oral glucose tolerance test (OGTT) from Taksim Training and Research Hospital Internal Medicine and Diabetes Polyclinics between January 2009 and April 2009, were included in the present study according to the results of OGTT. The patients were divided into 4 groups according to the fasting and 2nd hour results of OGTT in line with the American Diabetes Association 2003 diagnostic criteria for diabetes as follows:

1) control group including normoglycemic/non-diabetic healthy subjects (n=20),
2) IFG group including subjects with IFG alone (n=20),
3) IFG+IGT group including subjects both with IFG and IGT (n=20),
4) newly diagnosed type 2 DM group including treatment-naive patients with newly diagnosed type 2 DM (n=20).

In addition to fasting and postprandial plasma glucose levels, glycosylated hemoglobin levels, fasting serum insulin and C-peptide levels for the assessment of pancreatic beta-cell functions, metabolic parameters including serum lipid levels (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, and triglyceride), and cortisol levels were measured; the same procedures were performed also for the control group. Furthermore, demographic characteristics (age, gender, waist and hip circumference measurements, and body mass index [BMI]) were recorded for the whole study group. BMI was calculated by dividing body mass in kilograms by height in meters squared (kg/m²).

Insulin resistance was calculated based on the homeostasis model assessment-insulin resistance (HOMA-IR) model, which was defined by Levy et al.[9], according to the following formula: HOMA-IR = Fasting insulin (μU/mL) x fasting glucose (mg/dL) / 405.

Serum visfatin levels were determined by the enzyme-linked immunosorbent assay (ELISA) using an antibody coated 96-well plate human visfatin ELISA kit (BioVision Inc., Milpitas, CA, USA).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL, USA) for Windows was used for the statistical analyses of study data. The Kruskal-Wallis was used to test the normality of data. The Mann-Whitney U test was used to compare non-normally distributed variables between the groups. The results were evaluated at a confidence interval of 95% and the level of significance was set at p<0.05.

Results

The present study included 80 subjects who were evaluated in 4 groups. The demographic characteristics of the study groups are presented in Table 1.

The ages of the IFG, IFG+IGT, and newly diagnosed type 2 DM groups were significantly different from that of the control group (p=0.023,
p=0.002, and p=0.038, respectively). The BMIs of the IFG, IFG+IGT, and newly diagnosed type 2 DM groups were also significantly different from that of the control group (p<0.0001, p<0.0001 and p<0.0001, respectively). In addition, waist circumferences of the IFG, IFG+IGT, and newly diagnosed type 2 DM groups were significantly different from that of the control group (p<0.0001, p=0.004, and p<0.001, respectively). Hip circumferences of the IFG, IFG+IGT and newly diagnosed type 2 DM groups were significantly different from that of the control group (p=0.003, p=0.003, and p=0.003, respectively). No significant differences were determined among the IFG, IFG+IGT and newly diagnosed type 2 DM groups in terms of age, BMI, and waist and hip circumferences.

Table 1: Demographic characteristics of the study groups.
Data are presented as mean±standard deviation, number/number or median (25th-75th percentile), where appropriate.
IFG, impaired fasting glucose; IGT, impaired glucose tolerance; DM, diabetes mellitus; BMI, body mass index.

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Control n=20</th>
<th>IFG n=20</th>
<th>IFG+IGT n=20</th>
<th>Newly diagnosed type 2 DM n=20</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>41.7±7.5</td>
<td>48.9±9.3</td>
<td>51.1±7.2</td>
<td>48.5±7.0</td>
<td>0.002</td>
</tr>
<tr>
<td>Gender (Male/Female)</td>
<td>11-Sep</td>
<td>11-Sep</td>
<td>15-May</td>
<td>15-May</td>
<td>0.319</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>24.0±3.1</td>
<td>30.7±4.9</td>
<td>30.4±4.1</td>
<td>31.7±2.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>86.6±11.3</td>
<td>103.5±16.2</td>
<td>100.1±10.4</td>
<td>101.2±8.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>99.5 (92.5-105.0)</td>
<td>110 (103.5-117.0)</td>
<td>108 (105.0-116.0)</td>
<td>110 (104.0-120)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Table 2: Results of laboratory analyses in the study groups.
Data are presented as mean±standard deviation or median (25th-75th percentile), where appropriate.
IFG, impaired fasting glucose; IGT, impaired glucose tolerance; DM, diabetes mellitus; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; TG, triglycerides; HOMA-IR, homeostasis model assessment-insulin resistance.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Group n=20</th>
<th>IFG Group n=20</th>
<th>IFG+IGT Group n=20</th>
<th>Newly diagnosed type 2 DM Group n=20</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mg/dL)</td>
<td>87.5±7.7</td>
<td>108.8±6.1</td>
<td>111.9±7.9</td>
<td>126.2±14.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>6.8±2.4</td>
<td>13.5±6.5</td>
<td>14.7±5.6</td>
<td>19.5±9.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C-peptide (ng/mL)</td>
<td>2.0±0.5</td>
<td>3.0±1.0</td>
<td>3.1±0.9</td>
<td>3.6±1.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin/C-peptide</td>
<td>3.4±1.0</td>
<td>4.2±0.8</td>
<td>4.5±1.2</td>
<td>5.1±1.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.4±0.3</td>
<td>6.0±0.4</td>
<td>6.0±0.4</td>
<td>6.5±0.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>176.0±18.0</td>
<td>220.0±36.0</td>
<td>221.0±37.7</td>
<td>201.8±38.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>54.0±12.4</td>
<td>47.4±9.4</td>
<td>44.3±13.1</td>
<td>42.9±9.1</td>
<td>0.013</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>103.2±17.4</td>
<td>141.7±31.5</td>
<td>135.5±33.8</td>
<td>125.0±34.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLDL-C (mg/dL)</td>
<td>17 (11.2-23.0)</td>
<td>32</td>
<td>37.5</td>
<td>32.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>82.5 (56.7-112.2)</td>
<td>149.5</td>
<td>186.5</td>
<td>161.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cortisol (µg/dL)</td>
<td>13.8±3.7</td>
<td>14.3±6.2</td>
<td>15.6±4.0</td>
<td>16.7±5.8</td>
<td>0.27</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.4±0.5</td>
<td>3.6±1.7</td>
<td>4.1±1.6</td>
<td>6.0±3.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Visfatin (ng/mL)</td>
<td>0.6±0.6</td>
<td>2.7±1.9</td>
<td>3.2±1.7</td>
<td>3.2±2.2</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
The results of laboratory analyses in the study groups are summarized in Table 2.

**Conclusion**

Subclinical diabetes is defined as IFG, IGT, or state of pre-diabetes. Pre-diabetes is the state of a higher blood glucose level than the normal, which is not high enough to establish the diagnosis of diabetes. In addition to being a risk factor for the development of diabetes and cardiovascular diseases in the future, pre-diabetes is of importance since it is associated with metabolic syndrome. Recent studies have demonstrated that adipose tissue is not only an energy reservoir but also an active endocrine organ.

The reason for the adipose tissue being an active endocrine organ is the fact that it has the function of secreting many cytokines (adipokine/adipocytokine) and hormones. These adipokines have various biological functions including energy balance, glucose homeostasis, lipid metabolism or inflammation. Visfatin, a novel adipokine that has been associated with insulin resistance, has insulin-mimetic property and plasma glucose-lowering effect; moreover, visfatin can bind to insulin receptors and activate these receptors. The mean serum visfatin level, which is the main parameter of the present study, was significantly increased in pre-diabetic (IFG group and IFG+IGT group) and newly diagnosed type 2 DM groups as compared to the healthy controls (Figure 1).

**Figure 1:** Visfatin levels of the study groups. IFG, impaired fasting glucose; IGT, impaired glucose tolerance; DM, diabetes mellitus.

Döğru et al. found the plasma visfatin levels to be higher in newly diagnosed type 2 DM group than in the control and pre-diabetic (IGT alone) groups; however, they determined no difference between the pre-diabetic (IGT) and control groups. They obtained a similar result to that of the present study in terms of high plasma visfatin level found only in the type 2 DM group as compared to the other groups. However, the present study found serum visfatin levels to be different between the pre-diabetic and control groups. This difference between the present study and the study by Döğru et al. may be due to the fact that the pre-diabetic group consisted of patients having IFG alone or IFG and IGT in our study, while the pre-diabetic group consisted of patients having only IGT in their study.

Similar to the present study, in their studies, Takebayashi et al. and Sandeep et al. found the mean plasma visfatin levels to be higher in the type 2 DM group than in the control group; however, different from the present study, these studies had no pre-diabetic groups. Visfatin levels and insulin resistance levels being higher in the pre-diabetic and diabetic patients than in the controls in our study as expected, also considering the above mentioned studies, indicate that IFG and IFG+IGT (pre-diabetic conditions) groups are at risk for the development of diabetes and have increased risk of cardiovascular events.

The presence of a sustained relation between non-diabetic plasma glucose levels and the risk for the development of type 2 DM indicates the importance of early diagnosis and treatment of pre-diabetic cases with insulin resistance particularly for the prevention of macrovascular complications of diabetes. We observed an increase in insulin resistance and accordingly in serum visfatin levels during the progression to diabetes from pre-diabetic state. This increase was more remarkable particularly in the IFG group compared to the non-diabetic healthy controls and less remarkable in the IFG+IGT and type 2 DM groups compared to the IFG group.

In conclusion, visfatin could be considered among therapeutic agents used in the prevention of diabetes and in the prevention or reduction of its critical complications. Since an increase was observed in the visfatin levels in the pre-diabetes and diabetes groups as compared to the normoglycemic healthy controls, we also considered that visfatin could be a valuable marker to predict the possibility of development of metabolic disorders accompanying glucometabolic abnormalities.
References


Author Contributions

Aslan Çelebi and Müjgan Gürler contributed to the design of the study; Müjgan Gürler and Deniz Koç contributed to the conduction of the study; Ali Abbas Özdemir and Ismail Ekizoglu contributed to the data collection; Müjgan Gürler and Şerife Değirmencioğlu contributed to the data analysis; Murat Altay and Müjgan Gürler contributed to the data interpretation; and Müjgan Gürler and Aslan Çelebi contributed to the drafting of the manuscript.

Corresponding author
ASLAN ÇELEBI
GaziosmanpasaTaksim Education and Research Hospital, Karayolları Mahallesi, Osmanbey Caddesi 120 Gaziosmanpaşa Istanbul (Turkey)