Serum Visfatin Level in Gestational Diabetes Mellitus

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ABSTRACT

Background: Visfatin is a newly discovered adipocytokine. There is conflicting data regarding visfatin in gestational diabetes mellitus (GDM).

Aim: The purpose of the present study was to investigate the possible alterations of serum levels of visfatin in patients with GDM.

Subjects and Methods: 50 pregnant women were enrolled in the study, were divided into two groups; group I included 30 pregnant women with GDM and group II included 20 age and body mass index-matched pregnant women with normal glucose tolerance (NGT). Anthropometric and laboratory measurements including serum visfatin levels were assessed, and values were analyzed to compare the differences among the groups.

Results: Regarding fasting visfatin level, GDM group had higher levels in comparison to NGT group (24.73±25.40 ng/ml vs 7.85±2.83 ng/ml in group I and group II respectively, p<0.01), also GDM group had higher 1 hr postprandial visfatin levels than that of NGT group (40.53±25.40 ng/ml vs 16.90±3.02 ng/ml in group I and group II Respectively, p<0.01).There was a significant positive correlation between fasting visfatin and BMI (r=0.50, p<0.01), HbA1C(r=0.655, p<0.01), fasting serum insulin (r=0.386, p<0.05) and HOMA-IR (r=0.554, p<0.01). And, there was a significant positive correlation between 1hour post-prandial visfatin and BMI (r=0.44, p<0.01), HbA1C(r=0.609, p<0.01), fasting serum insulin (r=0.397, p<0.01) and HOMA-IR(r=0.575, p<0.01).

Conclusion: The present study shows that plasma visfatin concentrations are higher in patients with GDM and appear to be associated with glycemic control reflected by HbA1c as well as with insulin resistance.

Keywords: GDM, Visfatin, Insulin Resistance

Research Article

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INTRODUCTION:
Pregnancy presents a unique situation in which transient physiological insulin resistance, approaching levels observed in type 2 diabetes mellitus patients, often forms in order to facilitate nutrient delivery to the developing fetus (1). Important alterations in maternal metabolism and increased insulin resistance coincide with the progressive accumulation of adiposity during the course of normal pregnancy (2). Also, pregnancy is associated with the most dramatic increase in adipose tissue observed during adulthood (3). However, normoglycemia remains due to adequate beta-cell compensation for this higher insulin secretion. When the beta-cell compensation for insulin resistance and hepatic glucose production is inadequate, gestational diabetes mellitus (GDM) ultimately develops (2). Gestational diabetes mellitus is defined as a condition of carbohydrate intolerance with onset or first recognition during pregnancy (4). GDM carries numerous risks for mothers, fetuses, and even offspring. GDM causes vascular and obstetric complications, including diabetic nephropathy, retinopathy, macrosomia, increased operative deliveries, and unexplained fetal demise. Neonatal complications, such as hypoglycemia, hypocalcemia, jaundice, respiratory distress syndrome, and cardiomyopathy, are also more prevalent. Offspring born to women with diabetes have a 1% to 3% risk of cardiovascular and metabolic disorders (5, 6).
Maternal adiposity is an important, modifiable risk factor for the development of GDM (7). Adipocytokines, the bioactive proteins produced by adipose tissue, have recently been implicated in mediating insulin resistance (IR). It has been suggested that hormones secreted by the placenta and cytokines secreted by adipose tissues are related to the development of IR during pregnancy, possibly playing an important role in the pathogenesis of gestational diabetes mellitus (GDM) (3). Visfatin is a newly discovered 52 kDa adipocytokine hormone in humans, and is preferentially produced by visceral adipose tissue (1,8–11). However, it is also found in skeletal muscle, liver, bone marrow, lymphocytes, and placenta (12). Visfatin promotes adipogenesis and exerts insulin-mimetic effects (13). It also upregulates production of proinflammatory cytokines by monocytes (14).
Circulating levels of visfatin are increased in patients with type 1 and 2 DM and obesity (15, 16). However, the association of visfatin with GDM is still unclear (17). In fact, circulating maternal visfatin concentrations of the plasma and serum have been reported to be both higher (1,18–20) and lower (17,21) in GDM patients compared with healthy pregnant women by different studies, contributing to the controversial role of visfatin in GDM.
The aims of this study was to investigate the possible alterations of serum levels of visfatin in patients with GDM, as compared with healthy pregnant women.

SUBJECTS AND METHODS
Study population: This was a cross-sectional study that was conducted at the obstetric clinic, Ain Shams University Hospitals. A total of 50 pregnant women were enrolled in the study, were divided into two groups; group I included 30 pregnant women with GDM and group II included 20 age and body mass index-matched pregnant women with normal glucose tolerance (NGT). All participants were recruited while undergoing regular prenatal follow up visits. GDM was diagnosed during gestational weeks 24–28 according to the American Diabetes Association diagnostic criteria (2011).
Subjects were excluded from the study in the case of
- Multiple pregnancies
- History of diabetes mellitus and/or abnormal glucose readings before pregnancy
- Pregnancy induced hypertension or eclampsia or a history of hypertension before pregnancy
- Smoking
- A history of chronic illnesses
- A history of fetal anomalies
- Patients with GDM who were under treatment with diet or insulin

The study protocol was approved by the ethics committee of our institution and all participants provided a written informed consent before participating in the study.
Measurements of anthropometric indices and blood pressure: A qualified trained staff measured height, weight and blood pressure at the prenatal visit and at the time of blood collection. Body weight was measured to the nearest 0.1 kg using a calibrated manual weighing scale (Seca 709, Les Mureaux, France). Height was measured to the nearest 0.5 cm on a standardized wall-mounted height board. BMI was defined as weight in kilograms divided by height in meters squared (kg/m\(^2\)). For measuring the blood pressure, the subjects remained at rest for at least 15 min then the same staff measured blood pressure on the right arm at the sitting position.

Assays: Venous blood samples for measurement of lipid profile, insulin, visfatin and hemoglobin A1c (HbA1c) concentrations were taken 1 h before oral glucose tolerance test (OGTT) and was carried out after overnight fasting. Plasma samples were stored at -70°C until analysis. All pregnant women underwent screening for GDM between 24 and 28 weeks of gestation as follows: one-step screening was based on 75 gram two-hour oral glucose tolerance test (75 g OGTT) with at least one abnormal result: fasting plasma glucose ≥92 mg/dL (5.1 mmol/L), or one-hour OGTT ≥180 mg/dL (10.0 mmol/L) or two-hour OGTT ≥153 mg/dL (8.5 mmol/L) (22).

Serum visfatin concentration was determined with enzyme-linked immunosorbent assay (EK-003-80; Phoenix Pharmaceuticals, Burlingame, CA, USA). Serum insulin concentration was measured by chemiluminescence assay (Advia Centaur, Siemens Medical Solutions Diagnostics; Tarrytown, USA). Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated using the following formula: Plasma glucose (mg/dL)×fasting plasma insulin (IU/mg/L) in the fasting state divided by 405 (23). Plasma glucose, total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were subsequently measured by using routine laboratory methods. The level of low-density lipoprotein (LDL)-cholesterol was estimated using the formula: total cholesterol - HDL-cholesterol - (Triglyceride/5)

Statistical analysis: Statistical Package for Social Science (SPSS) program version 15 was used for an analysis of data. Data were summarized using mean and standard deviation (mean ±SD) for quantitative and numbers and percentages for categorical variables. Student’s-t test was used to compare the difference between the two groups. Chi-square(χ2)–test was used to compare quantitative data. An ANOVA test was done in comparison between more than two groups. The significance of the test was determined according to the P value to be: not significant (NS) if P > 0.05, significant (Sig) if P < 0.05, highly significant (HS) if P < 0.01.

RESULTS
Our study was conducted on 50 women, selected from obstetric outpatient clinic of Ain Shams University Hospital. They were recruited while undergoing regular prenatal examinations during pregnancy weeks 24–28. They were divided into the following groups: Group I: included 30 women with gestational diabetes (GDM) and Group II: included 20 pregnant women with normal glucose tolerance (NGT).

Our results showed no significant difference between study groups regarding age, weeks of gestation, BMI, systolic and diastolic blood pressure. While, as expected, there was a high significant difference between both groups regarding results of OGTT, with a higher mean among women with GDM compared to healthy pregnant women with NGT (P <0.01). Also, fasting serum insulin was significantly higher in GDM group than in NGT group (9.73±5.45 mU/ml vs 6.00±1.18 mU/ml in group I and group II respectively, p<0.01). And, HOMA IR was significantly higher in GDM group than in NGT group (2.65±1.52 vs 1.21±0.25 in group I and group II respectively, p<0.01). While, there was no significant difference between the study groups regarding lipid profile (Table 1).

As regards fasting visfatin level, GDM group had higher levels in comparison to NGT group (24.73±25.40 ng/ml vs 7.85±2.83 ng/ml in group I and group II respectively, p<0.01), also GDM group had higher 1 hr postprandial visfatin levels than that of NGT group (40.53±25.40 ng/ml vs 16.90±3.02 ng/ml in group I and group II respectively, p<0.01) (Table 1).

There was a significant positive correlation between fasting visfatin and BMI (r=0.50, p<0.01),
HbA1C (r=0.655, p<0.01), fasting serum insulin (r=0.386, p<0.05) and HOMA-IR (r=0.554, p<0.01), total Cholesterol (r=0.212, p<0.01), triglycerides (r=0.106, p<0.01) and LDL-cholesterol (r=0.116, p<0.01), while there was a significant negative correlation with HDL-cholesterol (r=-0.171, p<0.01). And, there was a significant positive correlation between 1 hour post-prandial visfatin and BMI (r=0.44, p<0.01), HbA1C (r=0.609, p<0.01), fasting serum insulin (r=0.397, p<0.01) and HOMA-IR (r=0.575, p<0.01), total Cholesterol (r=0.318, p<0.01), triglycerides (r=0.114, p<0.01) and LDL-cholesterol (r=0.223, p<0.01), while there was a significant negative correlation with HDL-cholesterol (r=-0.108, p<0.01). (Table 2, Figure 1).

Using Receiver Operating Characteristic (ROC) curve, it was shown that fasting visfatin could be used to discriminate diabetic from non-diabetic cases at cut off levels >10, with 73% and 80% sensitivity and specificity respectively (Table 3, Figure 2), and that the 1 hour post-prandial visfatin could be used to discriminate diabetic from non-diabetic cases at cut off levels >22, with 73.3% and 100% sensitivity and specificity respectively (Table 4, Figure 3).

Table (1): Comparison between the study groups regarding demographic data and laboratory investigations.

<table>
<thead>
<tr>
<th>Variants</th>
<th>Group I (GDM) = 30 women</th>
<th>Group II (NGT) = 20 women</th>
<th>Student’s–t test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>P-value</td>
</tr>
<tr>
<td>Age (years)</td>
<td>28.20±5.93</td>
<td>25.95±3.68</td>
<td>0.131</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>26.10±1.54</td>
<td>25.85±1.42</td>
<td>0.565</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.30±5.36</td>
<td>28.80±2.62</td>
<td>0.056</td>
</tr>
<tr>
<td>SBP (mm/Hg)</td>
<td>118.83±11.57</td>
<td>118.50±11.71</td>
<td>0.922</td>
</tr>
<tr>
<td>DBP (mm/Hg)</td>
<td>78.33±8.24</td>
<td>77.50±8.81</td>
<td>0.735</td>
</tr>
<tr>
<td>Fasting Blood Glucose (mg/dl)</td>
<td>112.13±35.78</td>
<td>82.35±7.03</td>
<td>0.001</td>
</tr>
<tr>
<td>30 Min PP Blood Glucose (mg/dl)</td>
<td>150.17±38.92</td>
<td>121.45±13.95</td>
<td>0.001</td>
</tr>
<tr>
<td>1 h PP Blood Glucose (mg/dl)</td>
<td>205.67±53.59</td>
<td>151.75±11.62</td>
<td>0.001</td>
</tr>
<tr>
<td>90 Min PP Blood Glucose (mg/dl)</td>
<td>181.83±54.39</td>
<td>132.00±10.93</td>
<td>0.001</td>
</tr>
<tr>
<td>2 h PP Blood Glucose (mg/dl)</td>
<td>150.50±63.10</td>
<td>117.10±9.87</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting Serum Insulin (mU/ml)</td>
<td>9.73±5.45</td>
<td>6.00±1.18</td>
<td>0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.65±1.52</td>
<td>1.21±0.25</td>
<td>0.001</td>
</tr>
<tr>
<td>HbA1c %</td>
<td>7.19±1.13</td>
<td>5.19±0.25</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting Visfatin (ng/ml)</td>
<td>24.73±19.99</td>
<td>7.85±2.83</td>
<td>0.001</td>
</tr>
<tr>
<td>1 Hour Post-Prandial Visfatin (ng/ml)</td>
<td>40.53±25.40</td>
<td>16.90±3.02</td>
<td>0.001</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>192.63±31.59</td>
<td>191.55±19.97</td>
<td>0.883</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>153.67±37.16</td>
<td>144.75±26.88</td>
<td>0.361</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>64.17±14.80</td>
<td>64.50±13.27</td>
<td>0.936</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>99.00±26.47</td>
<td>98.10±21.20</td>
<td>0.899</td>
</tr>
</tbody>
</table>

BMI, Body mass index; DBP, diastolic blood pressure; GDM, gestational diabetes mellitus; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; LDL-C, low-density lipoprotein cholesterol; NGT, normal glucose tolerance; SBP, systolic blood pressure; P>0.05 is not significant; P<0.05 is significant; P<0.01 is highly significant.
Table (2): Correlations between both fasting visfatin, 1 Hour Post-Prandial Visfatin and other variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fasting Visfatin (ng/ml)</th>
<th>1 Hour Post-Prandial Visfatin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P-value</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.500</td>
<td>0.0001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>0.655</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fastig insulin (mU/ml)</td>
<td>0.386</td>
<td>0.006</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>0.554</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>0.212</td>
<td>0.006</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>0.106</td>
<td>0.009</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>-0.171</td>
<td>0.007</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>0.116</td>
<td>0.009</td>
</tr>
</tbody>
</table>

BMI, Body mass index; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; LDL-C, low-density lipoprotein cholesterol; P>0.05 is not significant; P<0.05 is significant; P<0.01 is highly significant.

Table (3): ROC curve using fasting visfatin to discriminate diabetic from non-diabetic cases.

<table>
<thead>
<tr>
<th>Cutoff value</th>
<th>AUC(CI)*</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>+PV</th>
<th>-PV</th>
<th>P</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;10</td>
<td>0.837(0.705 to 0.926)</td>
<td>73.33</td>
<td>80.00</td>
<td>84.6</td>
<td>66.7</td>
<td>&lt;0.0001</td>
<td>HS</td>
</tr>
</tbody>
</table>

*Area under the curve (confidence interval)

Table (4): ROC curve using 1hour postprandial visfatin to discriminate diabetic from non-diabetic cases.

<table>
<thead>
<tr>
<th>Cutoff value</th>
<th>AUC(CI)*</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>+PV</th>
<th>-PV</th>
<th>P</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;22</td>
<td>0.865(0.739 to 0.945)</td>
<td>73.33</td>
<td>100.00</td>
<td>100.0</td>
<td>71.4</td>
<td>&lt;0.0001</td>
<td>HS</td>
</tr>
</tbody>
</table>

*Area under the curve (confidence interval)
Figure (1): (a) Correlation between fasting visfatin and HbA1c, HOMA-IR. (b) Correlation between 1 hour post-prandial visfatin and HbA1c, HOMA-IR.
DISCUSSION

Epidemiologic studies have revealed that the prevalence of GDM has increased over time (24), along with the increase in the prevalence of obesity (25). Hence, in parallel with the explosion of the obesity and metabolic syndrome in younger adults, incidence of GDM will undoubtedly continue to increase in coming years.

The physiologic role of visfatin in humans has not been fully elucidated; however, it has been proposed that this adipokine has a regulatory role in glucose metabolism and inflammation. Studies regarding maternal circulating visfatin in patients with GDM are scant and inconsistent: both increased (1,18–20) and decreased (17,21) maternal visfatin concentrations were reported. The aims of this study was to

Figure (2): ROC curve using fasting visfatin to discriminate diabetic from non-diabetic cases.

Figure (3): ROC curve using 1 hour postprandial visfatin to discriminate diabetic from non-diabetic cases.
investigate the possible alterations of serum levels of visfatin in patients with GDM, as compared with healthy pregnant women. There was a high significant difference between the two study groups as regard fasting and 1 hr PP visfatin, with a higher mean among the diabetic group.

Our findings are in agreement with those of Krzyzanowska et al. (18) who reported a higher maternal circulating visfatin in 64 patients with GDM than in 30, mostly overweight, normal pregnant women at 28–30 weeks of gestation. Also, Lewandowski et al. (19) reported higher maternal visfatin concentrations in 16 patients with GDM compared to 20 normal pregnant women at 28 weeks of gestation. Subsequently, Ma et al. (26), Coskun et al. (27) and Liang et al. (28) have shown increased serum visfatin levels in patients with GDM.

In contrast, Chan et al. (21) demonstrated that patients with GDM (n=20) in the late second trimester have a lower mean visfatin serum concentration than normal pregnant women (n=20). Haider et al. (17) reported similar results in 10 patients with GDM and 10 aged-matched controls at the same gestational age (24–28 weeks), and Akturk et al. (29) reported that serum visfatin concentrations were significantly lower in women with GDM as compared with healthy pregnant controls. While Telejko et al. (30) reported that visfatin concentrations did not differ in women with GDM and those with NGT between 26 and 33 weeks of gestation, but were significantly lower in GDM than in NGT subjects at term. Also, Görkem et al. (31) found that the visfatin levels of pregnant women with and without GDM were not significantly different.

In the present study there was a significant positive correlation between fasting and 1 hr PP visfatin with HbA1C, fasting serum insulin and HOMA-IR. Also, Lewandowski et al. (19) and Akturk et al. (29) reported a significant positive correlation between visfatin and HOMA-IR. In contrary, Telejko et al. (30), Görkem et al. (31) and Rezvan et al. (32) found no correlation of visfatin with fasting plasma glucose, fasting insulin and HOMA-IR. Berndt et al. (33) demonstrated that visfatin is not correlated with insulin resistance.

The reasons for this discrepancy may be related to differences regarding study design, the number of subjects, gestational age at enrolment, differences in BMI and neonatal birth weights, or even racial differences. Several explanations can account for the association of increased maternal plasma visfatin concentration and GDM. Insulin resistance is accompanied by increased visfatin production and/or secretion. The increase of visfatin in GDM might be a feedback or compensation mechanism that functions in maintenance of the normal metabolic balance. The role of visfatin in insulin sensitivity in GDM patients is supported by previously identified polymorphisms in the visfatin gene that have been clearly associated with insulin resistance in type 2 diabetes mellitus (34). In vivo studies in humans have shown that hyperglycemia increases circulating visfatin concentrations (35). Moreover, circulating visfatin concentrations in patients with type-2 DM are higher than in normal subjects (36-38). Therefore, visfatin could act by a similar mechanism during pregnancy. The current study supports these findings, as serum visfatin levels were shown to be associated with increased blood glucose levels and HOMA-IR.

Visfatin is constitutively expressed in human placental tissue, including amniotic epithelium, mesenchymal cells, the chorionic cytotrophoblast and parietal deciduas (41). The inflammatory cytokine TNF-α (tumor necrosis factor-α) enhances the expression of visfatin in human placenta cells (42). Chronic inflammation, including elevated concentrations of TNF-α, is present in women with GDM (43). Thus the placenta could be a source of increased visfatin in GDM; however, this has to be investigated in experimental studies.

Visfatin might also promote differentiation and maturation of preadipocytes, further promoting glucose transport, lipogenesis and accumulation in visceral fat (33). Cumulatively, these effects all contribute to excessive pregnancy weight gain, consistent with the observed weight gain increases in women with GDM (28).

On the other hand, chronic elevation of visfatin in mice reduces insulin plasma concentrations (12), and it was suggested that visfatin improves insulin sensitivity (39). Visfatin affects the insulin signal transduction pathway by inducing tyrosine phosphorylation of the insulin receptor and IRS1 and
2 (insulin receptor substrate 1 and 2) in the liver. Furthermore, an autocrine/paracrine function on visceral adipose tissue as well as an endocrine role modulating insulin sensitivity in peripheral organs might be modes of action (39).

Also, visfatin has physiological glucose-lowering effects similar to those of insulin and induces the expression of PPAR-γ (peroxisome-proliferator-activated receptor-γ), which might improve insulin resistance (12). So, we can postulate that GDM is a state of temporary insulin resistance and elevated visfatin concentrations in GDM might counteract high glucose levels jointly with increased insulin (18). Furthermore, circulating maternal visfatin has been shown to exert a wide range of autocrine and paracrine effects during pregnancy. So, in GDM, failure to maintain normal glucose levels might result in long-term hyperglycemia, which leads to further stimulation of visfatin secretion as an attempt to regulate glucose and lipid metabolism (40).

CONCLUSION
In conclusion, the present study shows that plasma visfatin concentrations are higher in patients with GDM and appear to be associated with glycemic control reflected by HbA1c as well as with insulin resistance. Further characterization of the mechanism of visfatin expression, regulation, and secretion in placental tissue and adipocytes will be required in order to develop a complete understanding of the relationship between visfatin and GDM. Additionally, future studies are needed with larger populations in gestational diabetic patients to identify whether visfatin, as a biomarker, may be useful for risk evaluation and prediction of GDM. Moreover, future studies should assess the efficacy of exogenous administration of visfatin for the treatment of GDM.

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REFERENCES


34. Zhang YY, Gottardo L, Thompson R, et al. A visfatin promoter polymorphism is associated with
low-grade inflammation and type 2 diabetes. Obesity (Silver Spring) 2006; 14: 2119–2126.

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