Plasma Visfatin Concentrations in Polycystic Ovary Syndrome: Relationships with Indices of Insulin Resistance and Hyperandrogenism

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Abstract

Background: Visfatin, a protein secreted by adipose tissue, is suggested to play a role in pathogenesis of insulin resistance. In polycystic ovary syndrome (PCOS), insulin resistance might be involved in the development of endocrine and metabolic abnormalities.

Objective: The aim of the study was to measure plasma visfatin levels in PCOS women and to assess the relationship between plasma visfatin concentration and indices of insulin resistance and markers of hyperandrogenism in PCOS patients.

Study Design: A total of 50 women were studied. Twenty-five women had PCOS, and the remaining 25 were healthy women with regular menstrual cycles who served as control subjects.

Blood samples were collected between the 3rd and the 5th days of a menstrual cycle in the control group and 3-5 days after a spontaneous menses, or independent of cycle phase in the presence of amenorrhea in the PCOS group for estimation of insulin, glucose, lipid parameters, sex-hormone and visfatin levels.

Results: Plasma visfatin concentrations were significantly higher in the PCOS group (72.94 ± 33.3ng/ml) than in the control group (54.69 ± 31.5ng/ml) (p=0.039). The PCOS group had higher insulin resistance (HOMA-IR) (3.12 ± 0.98) in comparison to the control group (2.27 ± 0.68) (p=0.017). In the PCOS group, plasma visfatin levels were found to be positively correlated with BMI and waist circumference, HOMA-IR as well as with free androgen index, and negatively correlated with LH, total testosterone and sex hormone-binding globulin (SHBG) levels. In the whole study group, plasma visfatin levels was positively correlated with BMI and waist circumference, FSH and SHBG levels as well as with free androgen index, and negatively correlated with LH, total testosterone values.

Conclusion: Visfatin levels are increased in women with PCOS compared to healthy controls. Visfatin is associated with insulin resistance in PCOS patients. Positive correlation was found between visfatin and free androgen index in PCOS patients.

Key Words: Visfatin – PCOS – Insulin resistance – Hyperandrogenism.

Introduction

POLYCYSTIC ovary syndrome (PCOS) is a common heterogeneous disorder in women of reproductive age and the most frequent cause of hyperandrogenism, combined with anovulatory infertility [1]. Its complex pathogenesis involves: (a) hypothalamic-pituitary disturbances in gonadotropin secretion, specifically increased LH levels [2]; (b) disturbed gonadal steroidogenesis, resulting in increased androgen production [3]; and (c) increased resistance to insulin (IR), resulting in compensatory hyperinsulinemia [4], which contributes to PCOS-associated hyperandrogenism by enhancing androgen production [5] and reducing the synthesis of sex hormone-binding globulin (SHBG) [6].

Obesity is present in varying degrees (30-70%) in women with PCOS [7,8], and is usually of the central type [9]. Central obesity, being a prominent feature of the so-called metabolic syndrome, is directly linked to increased peripheral IR [10].

Indeed, unlike all other fat depots, visceral adipose tissue is drained by the portal vein; therefore, portal release of products from visceral fat and their direct effects on the liver could be of particular importance in inducing type 2 diabetes mellitus (T2DM) or in protecting from this disorder [11].

It should be noted that PCOS itself has been shown to confer a risk for IR beyond that caused by obesity alone [12].
Adipose tissue, aside from releasing free fatty acids, which were long thought to be the main culprits in obesity-associated IR, has recently been proven to be a very active protein-secreting organ [13].

The secreted proteins are called adipokines and, most likely, contribute to peripheral insulin sensitivity [14].

Since IR and central obesity are prominent features of PCOS, several studies investigated the role of adipokines in the pathogenesis of the syndrome, and they indicated that resistin and adiponectin, although not primarily involved in PCOS-associated IR, independent of obesity, might interact with steroid synthesis and/or action in polycystic ovaries [15,16,17].

Recently, a novel adipokine called visfatin was described [18]. Visfatin seems to be expressed mainly in visceral adipose tissue and has insulin-like and, therefore, putative anti-diabetogenic properties [11,19,20].

Elevated levels of visfatin have been noted in patients with T2DM or impaired glucose tolerance (IGT) [21].

Exercise has been shown to suppress plasma visfatin concentrations in patients with type 1 diabetes, and increased visfatin levels in morbidly obese individuals are reduced after gastric binding-induced weight loss [22,23].

In contrast, in a recent study, visfatin levels were found to be reduced and not related to IR in obese subjects compared to normal weight controls [24]. Moreover, insulin-sensitizing drugs, namely, thiazolidinediones, have no effect on circulating visfatin and mRNA expression in adipose tissue; both increased and decreased visfatin levels have been reported in women with gestational diabetes [25,26].

PCOS is frequently associated with central obesity, IGT (up to 35%) and T2DM (up to 10%) [27]. In a recent study, both circulating visfatin and adipose tissue visfatin-specific mRNA levels were significantly increased in obese and overweight women with PCOS compared to BMI-matched controls [28].

Panidis D et al., 2008, showed that visfatin levels were positively associated with obesity in healthy women of reproductive age and also their findings favor a possible involvement of increased visfatin levels in PCOS-associated metabolic and hormonal disturbances [29].

In the light of these premises and since visfatin has been suggested as a possible link between intra-abdominal fat accumulation and diabetogenic processes, the present study was designed to measure plasma visfatin levels in PCOS women and also to assess the relationship between plasma visfatin concentration and indices of insulin resistance, and markers of hyperandrogenism in PCOS patients.

Subjects and Methods

Participants: During the period from October 2008 to March 2009, 50 women were enrolled in this observational cross sectional study. The study sample consisted of 25 PCOS cases and 25 control women among who were attending the infertility and internal Medicine clinics at Kasr El-Aini Faculty of Medicine, Cairo University, Cairo, Egypt.

The inclusion criteria of PCOS were based on the Rotterdam criteria (oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries in ultrasonography scan as well as exclusion of other etiologies which mimics the PCOS phenotype) [30]. A patient was considered to have PCOS if she fulfilled two out of three of the above mentioned criteria. A participant was considered having clinical hyperandrogenism or hyperandrogenemia if she presented with hirsutism (≥8 points in Ferriman-Gallwey score) [31] with or without acne (inflammation and obstruction of the pilosebaceous system).

Similarly, a woman was considered to have oligo/amenorrhea and anovulation if she had fewer than six menses during the previous year. Transvaginal ultrasound scan (TVS) was performed for all participants. The morphology of polycystic ovaries was considered if there were 12 or more follicles of 2-9mm in diameter in each ovary and/or enlarged ovary (ovarian volume >10cm³) [32]. The exclusion criteria were all diabetic women (frank history of diabetes mellitus, fasting glucose >125mg/dl or glucose in 120min of OGTT >200mg/dl), women with morbid obesity (BMI >40kg/m²), cases with cardiovascular disease, hypertension, infections or other serious medical problems. All women were non-smokers and they were not taking any anti-inflammatory drugs (within previous 3 months) or drugs known to affect carbohydrate and lipid metabolism.

Methodology: After explanation of the whole procedure, a physical examination and appropriate laboratory tests were performed then TVS was performed on the same day when blood sample was taken for assaying glucose, insulin, visfatin,
lipids and sex-hormone levels. Insulin resistance was evaluated using the homeostasis model assessment (HOMA-IR) index [33]. Studies were performed in regularly cycling women during the early follicular phase (3-5 days) of their menstrual cycle and in the PCOS group, 3-5 days after a spontaneous menses, or independent of cycle phase in the presence of amenorrhea. All analyses were performed after an overnight fast. All subjects gave a written informed consent before entering the study.

**Anthropometry:** BMI was calculated as body weight in kilograms divided by height in meters squared (kg/m$^2$). The waist circumference was measured at the level of iliac crest at the end of normal expiration. The tape should be snug but not indenting the skin [34].

**Biochemical assays:** Whole venous blood samples were taken through a standard venipuncture of the antecubital vein to withdraw about 7ml of fasting (12-14 hours) blood from each subject participating in the study. Samples are divided into 2 aliquots: The 1st aliquot contained about 5ml left to clot and the serum was separated by centrifugation and high-density lipoprotein cholesterol (HDL-C) was precipitated immediately using phosphotungstic acid precipitation [38] and the supernatant was stored at –20°C. Fasting blood glucose was determined immediately using glucose oxidase method and the rest of the serum was stored at –20°C for determination of plasma insulin, total testosterone, estradiol (E$_2$), sex-hormone binding protein (SHBG), FSH and LH. The hormones were determined using immunoanalyzer (Immulate 2000, DPC, Cirrus Inc., Los Angeles, USA) using solid-phase enzyme chemiluminescent immunoassay and the kits were supplied from DPC (96th street, Los Angeles, USA). The 2nd aliquot contained about 2ml of venous blood added into EDTA and centrifuged for 15 minutes at 1000xg within 20 minutes of collection and stored at 20°C for determination of plasma visfatin using ELIZA method [36]. The kit was supplied from BioVendor, LLC (1463 Sand Hill Road, Suite 227, USA). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula [37]: Fasting plasma insulin (µU/ml) x fasting plasma glucose (mg/dl)/405. Free androgen index was calculated as serum testosterone (nmol/l)x100/SHBG (nmol/l) ratio [38].

**Statistical analysis:** Data were statistically described in terms of mean ± standard deviation (±SD), frequencies (number of cases) and relative frequencies (percentages) when appropriate. Comparison of quantitative variables between PCOS and control groups was done using Mann Whitney U test for independent samples. For comparing categorical data, Chi square ($\chi^2$) test was performed. Exact test was used instead when the expected frequency is less than 5. Correlation between serum visfatin levels and various variables was done using Spearman rank correlation equation for non normal variables. A probability value ($p$ value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2003 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

**Results**

Basic clinical, anthropometric and biochemical characteristics of the women studied are summarized in Table (1).

**Table (1): Clinical, anthropometric and biochemical characteristics of the study groups.**

<table>
<thead>
<tr>
<th>Clinical, anthropometric and biochemical characteristics of the study groups.</th>
<th>PCOS (n=25)</th>
<th>Control (n=25)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.42±4.9</td>
<td>26.16±4.6</td>
<td>0.357</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>26.71±3.8</td>
<td>27.3±4.1</td>
<td>0.621</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>89.61±10.3</td>
<td>87.12±8.1</td>
<td>0.593</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>84.44±8.1</td>
<td>83.39±7.9</td>
<td>0.698</td>
</tr>
<tr>
<td>Fasting insulin (µU/ml)</td>
<td>14.41±6.8</td>
<td>12.67±5.9</td>
<td>0.276</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.12±0.9</td>
<td>2.27±0.68</td>
<td>0.017*</td>
</tr>
<tr>
<td>FFA (mmol/l)</td>
<td>0.59±0.26</td>
<td>0.58±0.27</td>
<td>0.492</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>187.30±33.8</td>
<td>183.46±31.9</td>
<td>0.162</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>113.23±23.4</td>
<td>102.92±26.1</td>
<td>0.106</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>51.57±13.0</td>
<td>50.72±12.2</td>
<td>0.732</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>113.92±33.2</td>
<td>108.63±31.7</td>
<td>0.273</td>
</tr>
<tr>
<td>LH (mlU/ml)</td>
<td>11.23±7.2</td>
<td>6.41±2.8</td>
<td>0.016*</td>
</tr>
<tr>
<td>FSH (mlU/ml)</td>
<td>5.76±1.7</td>
<td>6.02±1.8</td>
<td>0.375</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>82.8±73.6</td>
<td>92.4±66.8</td>
<td>0.293</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>2.81±1.1</td>
<td>1.73±0.9</td>
<td>0.009*</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>58.73±41.2</td>
<td>69.21±36.8</td>
<td>0.083</td>
</tr>
<tr>
<td>Free androgen index</td>
<td>6.73±4.1</td>
<td>3.57±2.7</td>
<td>0.007*</td>
</tr>
<tr>
<td>Visfatin (ng/ml)</td>
<td>72.94±33.3</td>
<td>54.69±31.5</td>
<td>0.039*</td>
</tr>
</tbody>
</table>

FFA : Free fatty acids.
HDL : High density lipoprotein.
LDL : Low density lipoprotein.
LH : Luteinizing hormone.
FSH : Follicle stimulating hormone.
SHBG : Sex hormone binding globulin.
* Statistically significant difference.
Studied women were of similar age, with the mean being 25.42 years for the PCOS group and 26.16 years for the control group. There were no major differences between the two groups with respect to BMI, waist circumference, fasting insulin, glucose and lipid parameters levels. However, patients with PCOS had higher concentrations of serum LH, total testosterone and plasma visfatin. Plasma visfatin concentrations were significantly higher in the PCOS group (72.94 ±33.3 ng/ml) than in healthy control group (54.69 ±31.5 ng/ml) (p = 0.039).

Insulin resistance (HOMA-IR) was significantly higher in the PCOS group (3.12 ±0.98) than in healthy control group (2.27 ±0.68) (p=0.017).

Similarly, free androgen index was significantly higher in the PCOS group (6.73 ±4.1) than in healthy control group (3.57 ±2.7) (p=0.007).

Table (2) showed the correlation between plasma visfatin and the different anthropometric and biochemical markers in the whole study population (n=50) and in cases with PCOS (n=25).

Table (2): Univariate correlation between plasma visfatin and the different anthropometric and biochemical markers in the whole study population (n=50) and in cases with PCOS (n=25).

<table>
<thead>
<tr>
<th></th>
<th>All participants</th>
<th>PCOS women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=50)</td>
<td>(n=25)</td>
</tr>
<tr>
<td>Spearman r</td>
<td>p value</td>
<td>Spearman r</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.056</td>
<td>0.350</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.341</td>
<td>0.008*</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.335</td>
<td>0.009*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.359</td>
<td>0.010</td>
</tr>
<tr>
<td>FFA (mmol/l)</td>
<td>0.264</td>
<td>0.064</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>0.165</td>
<td>0.252</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>0.119</td>
<td>0.410</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>–0.349</td>
<td>0.013</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>0.201</td>
<td>0.162</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>0.206</td>
<td>0.151</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>–0.310</td>
<td>0.028*</td>
</tr>
<tr>
<td>LH/FSH ratio</td>
<td>0.290</td>
<td>0.041</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>0.064</td>
<td>0.659</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>0.134</td>
<td>0.354</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>–0.297</td>
<td>0.036*</td>
</tr>
<tr>
<td>Free androgen index</td>
<td>0.359</td>
<td>0.013*</td>
</tr>
</tbody>
</table>

In the whole study group, a statistically significant correlation was observed between plasma visfatin levels and BMI, waist circumference, FSH and SHBG levels as well as with free androgen index, and was found to be negatively correlated with LH, total testosterone values. As for the PCOS group, plasma visfatin levels were found to be positively correlated with BMI and waist circumference, HOMA-IR as well as with as with free androgen index, and negatively correlated with LH, total testosterone and sex hormone-binding globulin (SHBG) levels.

**Discussion**

PCOS is characterized by chronic anovulation, hyperandrogenism, central obesity, and IR, and it is probably the most common endocrine disorder in women of reproductive age [1].

Recently, it has been shown that visceral adipose tissue produces visfatin, which may regulate insulin sensitivity [18]. Higher plasma levels of visfatin in patients with type 2 diabetes mellitus and women with gestational diabetes have been found [25,36].

Visfatin originally was isolated as a secreted factor that synergizes with interleukin-7 and stem cell factors to promote the growth of B cell precursors [18].

Also known as pre B-cell colony-enhancing factor, visfatin has been identified as an adipokine, predominantly expressed in, and secreted from, visceral adipose tissue [18].

It exhibits insulin-like activity and has been shown to activate the insulin receptor in various insulin-sensitive cell types in vitro, stimulate glucose uptake into adipocytes and muscle cells, and suppress glucose release from hepatocytes in vitro [18].

Subsequent observation in human population did not confirm the differences between visfatin expression in visceral and subcutaneous adipose tissue, and the link between plasma visfatin and visceral fat [41].

On the basis of these observations, we studied a group of women with PCOS and a control group, where we measure plasma visfatin levels in both groups and also evaluated the relationships between plasma visfatin levels and indices of insulin resistance and hyperandrogenism which are prominent features of PCOS.

The results we obtained with regard to the basic hormonal profile of women with PCOS, compared
to the control group, are in concordance with well-established evidence on the fundamental characteristics of the syndrome [7,39].

Our data showed that plasma visfatin concentrations were significantly higher in the PCOS group (72.94±33.3ng/ml) than in healthy control group (54.69±31.5ng/ml) (p=0.039). Our results agree with that of Chan et al., 2007, who showed that women with PCOS exhibit higher plasma visfatin levels than control subjects of similar body mass index [40]. Similarly, Panidis et al., 2008, found that plasma visfatin levels were significantly elevated in normal weight women with PCOS compared to BMI-matched controls [29].

Our findings are also in accordance with those by Tan et al., 2006, who reported increased visfatin levels in obese and overweight women with PCOS compared to BMI-matched controls [28].

High visfatin levels in normal weight women with PCOS may suggest an impaired mechanism of visfatin signaling in target tissues in states of IR [36]. Moreover, these findings could reflect a compensatory response to tissue-specific IR and hyperinsulinemia [19], an intrinsic dysregulation in visfatin biosynthesis, or, finally, tissue-specific inflammatory cytokine action [28,36].

In women with PCOS, all of the above may account for the higher visfatin levels in plasma observed in our study and in previously reported studies conducted by Tan et al., 2006, Panidis et al., 2008 [28,29].

Considering that PCOS is associated with a decreased sensitivity to insulin as well as an increased predisposition to IGT and T2DM, our result is in relative agreement with those of previous recent studies in which visfatin levels were found to be significantly increased in patients with T2DM [21,36,41] and positively, correlated with IR [36].

Insulin resistance (HOMA-IR) was significantly higher in the PCOS group (3.12±0.98) than in healthy control group (2.77±0.68) (p=0.017).

In the PCOS group, plasma visfatin levels were found to be positively correlated with HOMA-IR (p=0.046).

Contrary to our results Panidis et al., 2008, demonstrated no correlation between visfatin levels and indices of IR [29].

One explanation for this may be the possibility that, increased visfatin levels may reflect an independent impaired mechanism of visfatin action in peripheral tissues, rather than just a mere compensatory response to IR-related hyperinsulinemia [19,28,36].

In the whole study as well as in the PCOS group, plasma visfatin levels were found to be positively correlated with indices of obesity, namely, BMI and waist circumference. These findings could be attributed to the increased production of visfatin and most adipokines by adipose tissue, although in a single study down-regulation of visfatin in obesity has also been reported [24]. Our results agree with that of Berndt et al., 2005, who demonstrated a significant correlation between plasma visfatin concentrations and BMI [41]. Also Chan et al., 2007, found a significant positive correlation between visfatin levels and BMI in subjects with PCOS but not in normal healthy women [40].

Contrary to our results Chen et al., 2006, demonstrated no correlation between plasma visfatin concentrations and BMI [36].

In the present study, circulating visfatin was found to be negatively correlated with LH, total testosterone, and SHBG levels in the PCOS group.

Our results are in contrast to those of Panidis et al., 2008 [29], Kowalska et al., 2007 [32], who demonstrated that visfatin is positively correlated with LH levels in PCOS patients, but similar to our results Panidis et al., 2008, found that circulating visfatin was negatively correlated with SHBG levels [29].

According to the studies conducted by Nestler et al., 1991 and Ehrmann 2005, they found that the correlation between visfatin and SHBG is most probably indicative of the combined negative effect of obesity and increased androgens on SHBG production, while the positive correlation to LH levels further reflects a putative involvement of this specific adipocytokine in the hypothalamic-pituitary-ovarian axis pathology observed in PCOS [6,7].

Our results are in agreement with of those of Panidis et al., 2008 [29], Kowalska et al., 2007, who demonstrated that visfatin is positively correlated with free androgen index values in PCOS patients.

In conclusion, our data show that women with PCOS exhibit higher plasma visfatin levels than control subjects, and also indicate that visfatin is associated with insulin resistance and free androgen index in PCOS patients.
Further studies are needed to elucidate whether it is possible that, in women with PCOS, increased plasma visfatin levels may not be directly related to PCOS-associated hyperinsulinemia. The actual role of hypervisfatinemia in women with PCOS and its relation to IR, hyperandrogenism need to be clarified by further studies.

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