RESEARCH ARTICLE

Evaluation of insulin resistance and plasma levels for visfatin and resistin in obese and non-obese patients with polycystic ovary syndrome

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ABSTRACT. This study was designed to evaluate insulin resistance and plasma levels of visfatin and resistin in obese and non-obese patients with polycystic ovary syndrome (PCOS).A total of 37 premenopausal PCOS patients with (n = 18, mean (SD) age: 27.5 (5.7 years) or without obesity (n = 19, mean (SD) age: 23.7 (3.1) years) and healthy volunteers (n = 18, mean (SD) age:29.8 (4.1) years) were included in this study. Data on clinical characteristics, glycemic parameters and lipid parameters were recorded for each subject as were plasma visfatin and resistin levels. Mean (SD) HOMA-IR values were significantly higher in obese PCOS patients (3.4 (1.7)) compared with non-obese PCOS patients (2.0 (1.2), p<0.01) and controls (1.6 (0.8), p<0.01). No significant difference was noted between study groups in terms of plasma resistin (ng/mL) or visfatin (ng/mL) levels. There was no correlation between serum plasma visfatin (r = 0.127, p = 0.407) and resistin (r = -0.096, p = 0.544) levels and HOMA-IR. In conclusion, our findings revealed increased likelihood of metabolic and dyslipidemic manifestations in obese compared to non-obese PCOS patients, while no significant difference was noted in visfatin and resistin levels among PCOS patients in terms of co-morbid obesity and in comparison to controls.

Key words: polycystic ovary syndrome, insulin resistance, visfatin, resistin, obesity

Polycystic ovary syndrome (PCOS) is a common, multifaceted endocrinopathy associated with metabolic alterations such as insulin resistance, hyperinsulinemia, dyslipidemia, and obesity, and thereby an increased risk of developing type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) [1-3].

Insulin resistance has been detected in a majority of patients with PCOS beyond that predicted by their body mass index (BMI) [1, 4, 5], while PCOS *per se* has been shown to confer a risk of insulin resistance beyond that caused by obesity alone [5].

The role of insulin resistance and hyperinsulinemia has been increasingly recognized in PCOS pathogenesis [6, 7], while altered production of several novel adipokines, such as leptin, adiponectin, resistin and visfatin, that are predominantly secreted by adipocytes have been suggested to play a role in complex mechanisms linking excess fat to metabolic syndrome via inflammatory processes [8-10].

Resistin is a polypeptide, which contains 94 amino acids. It plays a key role in insulin resistance and glucose homeostasis. There are studies which support a positive correlation between the resistin and insulin resistance in human [8-10]. It has been shown in some studies that people experience increasing levels of resistin due to being overweight and obesity. There are some contradictory publications regarding resistin levels in PCOS. Visfatin is a protein which is expressed in many tissues such as adipocytes, lymphocytes, liver, muscle, bone marrow. Vistafin supresses glucose release from hepatocytes and increases glucose uptake in the muscles. In recent studies, it has been seen that plasma vistafin levels are increased in obesity, type 2 diabetes, and metabolic syndrome. Although there are publications showing an increase in plasma vistafin levels in PCOS, the relationship is not perfectly clear.

Given the potential role of adipokines in forming a link between intra-abdominal fat accumulation and diabetogenic processes in PCOS patients, the present study was designed to evaluate insulin resistance, visfatin and resistin levels in obese and non-obese patients with PCOS.

METHODS

Study population

A total of 37 premenopausal patients with (n = 18, mean (SD) age: 27.5 (5.7 years) or without obesity (n = 19, mean (SD) age: 23.7 (3.1) years), who had been diagnosed with PCOS based on clinical and biochemical

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evaluation in accordance with revised Rotterdam criteria [1] upon admission to our clinic were included in this study. They complained of excessive hair growth and menstrual irregularities. Age-matched, healthy volunteers (n = 18, mean (SD) age: 29.8 (4.1) years) were also. Fourteen out of 37 patients who has been diagnosed with PCOS also had symptoms of oligo-ovulation-anovulation and clinical-biochemical signs of hyperandrogenism. We noted polycystic ovaries, in eight patients with oligo-ovulation-anovulation, and we also saw polycystic ovaries, in 15 patients who had symptoms of oligo-ovulation-anovulation and clinical-biochemical signs of hyperandrogenism. Patients with concomitant diabetes mellitus, hypertension, serum creatinine levels of $\geq$1.5 mg/dL, transaminase levels of $\geq$2.5-fold above the normal value, Cushing syndrome, late-onset congenital adrenal hyperplasia, hypothyroidism, prolactinoma and other types of endocrine disease, a past history of heart failure, malignancy and rheumatological or immunological disease, and patients who had received hormone treatment, including oral contraceptive drugs within the preceding six months were excluded from the study. Fifty two patients who fulfilled the criteria for PCOS were screened. Out of those, 15 patients were excluded from the study. As a result, we began our study with 37 PCOS. To these patients we added 25, age-matched, healthy volunteers who were also screened. Four of these, who did not fulfil the inclusion criteria, and three who rejected to give informed consent were excluded from the study. As a result 18 healthy volunteers were able to be included in the study.

Written informed consent was obtained from each subject following a detailed explanation of the objectives and protocol of the study, which was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the institutional ethics committee. Volunteers, who were age-matched, did not fulfil any of the exclusion criteria, and who gave their written, informed consent, were accepted to join the study, after having been given detailed information about the study.

Study parameters

Data on anthropometrics [weight (kg), height (cm), body mass index (BMI; kg/m^2), waist circumference (cm), hip circumference (cm), waist-to-hip ratio, waist-to-height ratio, body fat ratio), menstrual cycle, smoking status, vital signs [systolic blood pressure (mmHg), diastolic blood pressure (mmHg), pulse (bpm)], glycemic parameters [serum levels for fasting blood glucose (mg/dL), insulin (IU/mL) and C-peptide (ng/mL), homeostasis model assessment insulin resistance index (HOMA-IR) values, 75 g oral glucose tolerance test (OGTT; mg/dL)] and lipid parameters [total cholesterol (mg/dL), HDL-cholesterol (HDL-c; mg/dL), LDL- cholesterol (LDL-c; mg/dL) and triglyceride (mg/dL)] findings were recorded in obese and non-obese PCOS patients, and in control subjects, as were the plasma resistin (ng/mL) and visfatin (ng/mL) levels.

Gynecological parameters

Polycystic ovarian morphology was confirmed in patients by ultrasound. Hirsutism was evaluated using the Ferriman–Gallwey score [11], and menstrual disturbances (none, oligomenorrhea, amenorrhea) were evaluated based on medical history in all subjects.

Anthropometric measurements

The BMI was calculated by dividing weight by the square of the height (kg/m^2). The waist circumference was measured at the narrowest level between the costal margin and iliac crest, and the hip circumference was measured at the widest level over the buttocks while the subjects were standing and breathing normally. The waist-to-hip ratio (WHR) was then calculated as were waist-to-height ratio and body fat ratio.

Insulin resistance

Insulin resistance was calculated using HOMA-IR according to the following formula: fasting plasma glucose (mmol/L) x fasting serum insulin (mU/mL)/22.5 [12].

Assessment of plasma visfatin and resistin levels

All blood samples were drawn after overnight fasting, and centrifuged for 15 minutes at 1000 $\times$ g within 30 minutes of collection. Serum samples obtained were stored in aliquots at -20°C for subsequent visfatin and resistin measurements. RayBio® Visfatin Enzyme Immunoassay (EIA) Kit (RayBiotech, Inc, USA) was used for determination of visfatin in human serum, with an intra-assay CV of <10%, inter assay CV of 15%. This kit is an *in vitro* quantitative assay for detecting visfatin peptide based on the principle of competitive enzyme immunoassay. In this kit, the minimum detectable concentration of visfatin was 0.778 ng/mL, and the detection range 0.1-1,000 ng/mL. Resistin was measured using a commercially available Human Resistin Platinum ELISA kit (eBioscience, Inc. USA) according to the manufacturer's instructions. This kit is an enzyme-linked immunosorbent assay for the quantitative detection of human resistin. The inter-assay variability was 8.1% and the intra-assay variability 5.1%.

Statistical analysis

Statistical analysis was performed using MedCalc Statistical Software (version 12.7.7, MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013). Categorical variables were compared with the χ^2 test, while Student's t test and one-way ANOVA and Tukey *post-hoc* HSD tests were used for the analysis of independent, normally distributed parametric variables, and the Mann-Whitney U test with the Bonferroni correction and Kruskal-Wallis tests were used for the analysis of numerical variables not normally distributed. Data were expressed as "median (min-max)" and percentage (%) where appropriate. $p<0.05$ was considered statistically significant.

RESULTS

Patient characteristics, clinical and glycemic parameters

Data on patient characteristics, anthropometric measurements, and vital signs are summarized in *table 1*.

Table 1
Patient characteristics, anthropometric measurements and vital signs

	PCOS-obese (n = 18)	PCOS-non-obese (n = 19)	Control (n = 18)
Age (year): mean (SD)	27.5 (5.7)*	23.7 (3.1)	29.8 (4.1)**
Anthropometrics: mean (SD)			
Height (cm)	163.2 (5.7)	160.5 (5.3)	161.0 (5.8)
Weight (kg)	89.7 (11.1)**,+	58.0 (6.1)	62.9 (9.2)
Body mass index (kg/m^2)	33.6 (3.0)**,+	22.5 (2.0)	24.2 (2.7)
Waist circumference (cm)	101.1 (8.8)**,+	72.3 (6.1)	76.9 (7.2)
Hip circumference (cm)	115.3 (7.5) **,+	96.3 (6.5)	101.3 (7.1)
Waist-to-hip ratio	0.9 (0.1)**	0.8 (0.1)	0.8 (0.0)
Waist-to-height ratio	0.6 (0.1)**,+	0.5 (0.0)	0.5 (0.0)
Body fat ratio	39.0 (3.3)**,+	23.7 (3.9)	26.9 (7.0)
Menstrual cycle: n (%)			
Regular	6 (33.3)	10 (52.6)	16 (88.9)
Oligomenorrheic	8 (44.4)	8 (42.1)	2 (11.1)
Amenorrheic	4 (22.2)	1 (5.3)	0 (0)
Smoking status: n (%)			
Non-smoker	12 (66.7)	16 (84.2)	12 (66.7)
Active smoker	6 (33.3)	3 (15.8)	6 (33.3)
Vital signs: mean (SD)			
Systolic blood pressure (mmHg)	111.7 (8.6)*	105.8 (6.9)	112.2 (6.5)*
Diastolic blood pressure (mmHg)	69.4 (8.0)	65.8 (6.1)	71.1 (5.8)
Pulse (bpm)	79.1 (3.6)	80.9 (5.7)	80.6 (4.3)

*$p<0.05$ and **$p<0.001$; compared to non-obese polycystic ovary syndrome (PCOS) patients
+$p<0.001$; compared to controls
One-way ANOVA and *post-hoc* Tukey tests

Glycemic and lipid parameters

Mean (SD) insulin levels were significantly higher in obese PCOS patients (16.1 (5.3) IU/mL) compared with non-obese PCOS patients (9.8 (3.9) IU/mL) and controls (7.9 (3.6) IU/mL) ($p<0.001$ for each). Mean (SD) HOMA-IR values were significantly higher in obese PCOS patients (3.4 (1.7)) compared with non-obese PCOS patients (2.0 (1.2), $p<0.01$) and controls (1.6 (0.8), $p<0.01$) (*table 2*). In obese PCOS patients, mean (SD) HDL-c levels were significantly lower than controls (43.7 (7.9) mg/dL *versus* 51.7 (8.6) mg/dL, $p<0.01$), while triglyceride levels were significantly higher compared with non-obese PCOS patients (133 (61.8) mg/dL *versus* 80.6 (35.9) mg/dL, $p<0.01$) (*table 2*).

Plasma visfatin and resistin levels

No significant differences were noted between obese PCOS patients, non-obese PCOS patients, and controls in terms of plasma visfatin (ng/mL) and resistin (ng/mL) levels (*table 3*). No correlation of serum plasma visfatin ($r = 0.127$, $p = 0.407$) and resistin ($r = -0.096$, $p = 0.544$) levels with HOMA-IR was noted.

DISCUSSION

Our findings revealed significantly higher levels for plasma insulin and HOMA-IR values in obese PCOS patients when compared to non-obese PCOS patients and controls, which seem in agreement with the statement that excess body weight in women with PCOS may accelerate progression toward diabetes by exacerbating both insulin resistance and compensatory enhanced insulin response [13-15]. Moreover, insulin has been suggested to contribute to the promotion and/or the maintenance of PCOS, particularly in obese women [16], while obesity has been shown to act in concert with the intrinsic and apparently distinctive defects in insulin action in PCOS, and thus enhance the risk of diabetes linked with hyperinsulinemia, insulin resistance and beta-cell dysfunction [16, 17].

Higher serum levels of triglycerides and SBP, and lower values for HDL-c in our obese PCOS patients seem in line with the indication of higher rates for dyslipidemia and metabolic syndrome in obese young women with PCOS than in weight-matched controls [18-20], and the indicated role of co-morbid obesity in augmenting this link [21].

Accordingly, albeit the actual mechanisms involved have not yet been clarified [21], based on significantly higher insulin secretion, insulin resistance and dyslipidemia in obese PCOS patients, our findings support the notion that adiposity plays a crucial role, not only in the development, but also in the maintenance of manifestations of the clinical, biochemical, and metabolic features of PCOS [16, 22].

Consistent with several recently published studies [23-25], our findings revealed no difference in plasma visfatin levels between patients with PCOS and control groups, and no correlation between HOMA-IR and visfatin levels, which supports the idea that circulating visfatin levels may not be a useful clinical biomarker for metabolic traits [26].

However, it should be noted that although data from past studies on the role of these adipokines in insulin sensitivity and obesity yielded inconsistent results, a significant relationship between the plasma concentration of visfatin and the expression of visfatin in visceral adipose tissue and insulin resistance in women with PCOS has been reported

Table 2
Glycemic and lipid parameters in study groups

		PCOS-obese (n = 18)	PCOS-non-obese (n = 19)	Control (n = 18)
Glycemic parameters: mean (SD)				
Fasting blood glucose (mg/dL)		87.1 (14.1)	86.9 (8.4)	81.7 (7.8)
Insulin (IU/mL)		16.1 (5.3)$^{**,++}$	9.8 (3.9)	7.9 (3.6)
HOMA-IR		3.4 (1.7)$^{**,+}$	2.0 (1.2)	1.6 (0.8)
Oral glucose tolerance test (mg/dL)	0-hour	88.6 (23.4)	81.7 (22.4)	
	2-hour	104.0 (28.0)	95.5 (16.6)	
C-peptide (ng/mL)		2.6 (1.0)	2.0 (0.6)	2.0 (0.7)
Lipid parameter: mean (SD)				
Total cholesterol (mg/dL)		180.7 (33.1)	182.4 (34.1)	173.3 (48.1)
HDL-cholesterol (mg/dL)		43.7 (7.9)$^{+}$	50.0 (9.4)	51.7 (8.6)
LDL-cholesterol (mg/dL)		110.3 (28.8)	117.7 (32.2)	105.8 (21.0)
Triglyceride (mg/dL)		133 (61.8)*	80.6 (35.9)	106.4 (50.6)

HDL: high density lipoprotein; HOMA-IR: homeostatic method of assessment-insulin resistance; LDL: low density lipoprotein; PCOS: polycystic ovary syndrome
$^{*}p<0.01$ and $^{**}p<0.001$; compared to non-obese PCOS patients
$^{+}p<0.01$ and $^{++}p<0.001$; compared to controls
One-way ANOVA with *post-hoc* Tukey test for glycemic parameters, Mann-Whitney U test with Bonferroni correction for lipid parameters)

Table 3
Plasma resistin and visfatin levels in study groups

	PCOS-obese (n = 18)		PCOS-non-obese (n = 19)		Control (n = 18)	
	Mean (SD)	**Median (min-max)**	**Mean (SD)**	**Median (min-max)**	**Mean (SD)**	**Median (min-max)**
Resistin (μg/L)[1]	5.8 (4.7)	4.0 (0.1-18.2)	6.6 (3.3)	5.9 (1.8-13.5)	6.3 (4.7)	5.3 (1.5-19.9)
Visfatin (μg/mL)[2]	67.1 (18.8)	64.2 (40.3-112.5)	76.7 (32.4)	73.2 (2.9-142.8)	62.7 (18.4)	72.2 (27.9-84.4)

[1]p = 0.442 and [2]p = 0.433; Kruskal-Wallis test

in several studies [27-31]. In a study, investigating the association of insulin resistance and visfatin levels of patients with PCOS, visfatin levels of the control group and patients with PCOS were seen to be similar, but no association was determined regarding insulin resistance and visfatin levels (23). In a study comparing the visfatin levels of 57 PCOS patients with the control group, no statistically significant disparity was determined; also no relationship was determined between visfatin levels and obesity, and other metabolic markers (24). In a study reporting results different from our own, it was shown that visfatin levels were statistically significantly higher in normal, glucose-tolerant, lean 27 PCOS patients compared to an age- and BMI-matched, healthy volunteer control group (27). Also in the same study, we saw a positive correlation between visfatin levels and insulin resistance.

While all these studies indicate that visfatin was associated with insulin resistance, and thereby may participate in the pathophysiology of PCOS [8], it seems noteworthy that despite evidence of increased expression of visfatin mRNA in both omental fat and peripheral blood mononuclear cells (PBMCs) in women with PCOS compared with controls, only the expression of visfatin in omental adipose tissue, but not that in PBMCs, has been related to the insulin resistance index in women with PCOS [8].

Our findings revealed similar levels for plasma resistin in PCOS patients and controls, and consistent with plasma resistin levels reported in the general population, and in obese and non-obese PCOS patients in the literature [32, 33]. Based on lack of a significant difference between our study groups in terms of plasma resistin levels, and the lack of a correlation between plasma resistin levels and HOMA-IR, our findings are in accordance with the lack of difference between BMI-matched PCOS patients and control groups in terms of serum resistin levels, reported in several studies [34-37], despite the differences in insulin resistance and glucose-to-insulin ratio between PCOS and control groups [34, 36], and between insulin-resistant and non-insulin-resistant PCOS groups [37]. In the study by Pandis and colleagues, it was established that the resistin levels were higher in patients who had been diagnosed with PCOS and with a BMI$\geq$25 kg/m^2, than in patients who had also been diagnosed with PCOS, but with a BMI<25 kg/m^2 (34). When comparing patients who had been diagnosed with PCOS and a BMI$\geq$25 kg/m^2 with healthy volunteers with a BMI<25 kg/m^2, we also saw higher resistin levels. A comparison of patients who had been diagnosed with PCOS and a BMI<25 kg/m^2, and healthy volunteers with a BMI<25 kg/m^2, we saw no statistically significant difference. In this study, as well as our own, it was concluded such that there is no association between resistin and PCOS-related insulin resistance.

However, data regarding the levels of resistin in PCOS patients remain controversial as some studies have indeed revealed a positive correlation between resistin and insulin resistance [9, 38], and significantly higher serum resistin levels have been reported in obese than in non-obese PCOS patients and controls [33].

Notably, while resistin mRNA levels have been reported to be two-fold higher in adipocytes from PCOS patients than

in those from normal controls, it has been suggested that adipocyte-produced resistin does not play a key role in the body, since adipocytes are not a major source of circulating resistin in humans [36].

In this regard, given the lack of metabolic and dyslipidemic manifestations in our lean PCOS patients, along with the lack of change in visfatin and resistin levels regardless of co-morbid obesity, our findings emphasize the role of obesity, but not dysregulated adipokine levels, in the heterogeneity of clinical manifestations seen in PCOS patients.

In conclusion, our findings revealed an increased likelihood of metabolic and dyslipidemic manifestations in obese compared to non-obese PCOS patients, while no significant alteration was noted in visfatin and resistin levels among PCOS patients, with respect to co-morbid obesity, as well as in comparison to controls. Further investigations would be helpful to elucidate the role of obesity in the etiology of PCOS, as well as the regulatory role of adipokines that may act as a link in the interplay between obesity and PCOS.

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