Evaluation of the relationship between serums Visfatin and Resistin levels with BMI in PCOS young women

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a common endocrine / metabolic disorder in women of reproductive age .Abdominal adiposity and obesity are frequently present in PCOS. It now appears that, obesity is associated with a low-grade inflammation of white adipose tissue.
Adipokines play a significant role in the pathogenesis of a low-grade inflammation associated with obesity. Among variety of adipokines, resistin and visfatin are proposed as important pro inflammatory mediators and they have recently been suggested to be associated with obesity related diseases.
The aim of this study is to evaluate the correlation of visfatin and resistin serum levels and the ratio of these two adipokines with BMI in PCOS women under age of 35 years old. Twenty eight young women with clinically confirmed PCOS disease (14 lean and 14 obese), and 12 young, healthy and lean women with stable weight and BMI<25 were enrolled. Blood was obtained from the included persons, and visfatin and resistin were assessed by ELISA method. We did not observe any significant differences in serum visfatin and resistin concentrations and also in the Visfatin/Resistin ratio between PCOS and control group. Also we did not found a significant correlation between visfatin and resistin with BMI. This study demonstrated that serum resistin and visfatin levels do not seem to be directly involved in the pathology of PCOS.

Keywords: Polycystic ovary syndrome; Visfatin, Resistin; BMI; Obesity.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in premenopausal women with 6–7% prevalence's worldwide [1–3]. PCOS should be considered a life span disorder. Aside from the cutaneous and reproductive manifestations that appear peripubertally, this disorder is frequently associated with obesity [4], insulin resistance and type 2 diabetes [5,6], low-grade chronic inflammation [7], oxidative stress [8], and increased in cardiovascular risk [9, 10].
Currently PCOS is considered a complex multigenic disorder, in which multiple genetic, epigenetic, and environmental factors play a significant role in the development of the hyperandrogenic, reproductive, and metabolic phenotype of the syndrome [7]. Substantial evidence indicates that obesity plays a pivotal role in the pathogenesis of PCOS. In obesity mouse models, severe macrophage invasion was observed in the vascular⁄stromal compartment of adipose tissue, suggesting that excess adiposity is associated with chronic inflammation [11, 12]. In patients with PCOS, dysfunction of adipose tissue has been observed with the over-production of some pro-inflammatory adipokines. Resistin and visfatin are cytokines that secreted by visceral adipose tissue, and recently suggested that these cytokines are associated with obesity related diseases [13, 14]. The expression of human resistin is predominantly localized in macrophages and stroma cells in adipose tissue

rather than adipocytes [15, 16]. As a secreted circulating protein, resistin can exert its functions in both endocrine and paracrine manners [16].

In human studies, resistin circulating levels or gene expression have varied from increased to unchanged in obesity, PCOS or type 2 diabetes mellitus [17-24]. Visfatin, an adipokine isolated by Fukuhara et al. [25], corresponds to a protein identified previously as pre-B cell colony-enhancing factor (PBEF), a 52 kDa cytokine expressed and secreted by lymphocytes [26]. Visfatin appears to be an important mediator of inflammation [27]. There are also conflicting data on visfatin circulating levels in obese humans. Some studies showed the increased levels of visfatin [28, 29], but there are studies that didn't confirm this [30]. The status of the circulating levels and gene expression of these cytokines in PCOS is also controversial [31, 32].

The aim of this study was to evaluate the serum levels of visfatin and resistin in PCOS patients under the age of 35 years old and highlighting the probable correlation of them with BMI.

MATERIALS AND METHODS

Subjects

A group of 28 women with PCOS (14 normal weight and 14 obese) based on NIH diagnostic criteria were evaluated. The clinical criteria included oligomenorrhoea or amenorrhoea dating from menarche. The biochemical criteria were increased LH concentration, LH/FSH (luteinizing hormone/follicle stimulating hormone) ≥2~3, and elevated T (testosterone) levels. Clinical hyperandrogenism was quantified by the modified Ferriman–Gallwey score and hirsutism was defined when the score was ≥8.

Normal weight was defined as body mass index from 18.5 to 24.9 kg/m^2 and obesity as BMI ≥ 25.0 kg/m^2. The women with PCOS were under 35 years old (mean±SD of age =28.21±3.61).

Twelve healthy women with age<35 (mean±SD of age = 26.91±3.31) years and BMI<25 (mean= 22.67±1.52) were enrolled as the control group. Since this study was aimed at exploring further the contribution of obesity, only lean subjects were included in control group and the data analysis. Subjects who were obese (BMI>25) were excluded.

The controls had a regular menstrual cycle and no chronic or acute disease. In our study, participants with Cushing's syndrome, thyroid dysfunction, androgen-secreting tumor, and enzyme deficiency (21-hydroxylase in particular) were excluded. The patients were not undergoing pharmacological treatment. Other exclusion criteria included smoking and alcohol abuse. In all subjects anthropometric measurements (body mass and height) were determined, and body mass index (BMI) was calculated according to the standard formula. The study was conducted after obtaining informed consent from each participant and approved by the Ethical Committee of Shahid Beheshti University of Medical Sciences. All of the women were studied between days 3 and 6 of the menstrual cycle. Blood samples were taken from the participants between 7:00 AM and 9:00 AM in the morning while sitting, using vacutainer tubes after a 12-hour overnight fast. Samples were centrifuged within 30 to 45 minutes of collection and stored at -80°C.

Laboratory procedures

Visfatin (CSB-E08940h) and resistin (CSB-E06884h) were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) method and using materials provided by Cusabio Inc.

The lower detectable concentrations were 0.156 (ng/ml) for visfatin and 0.078 (ng/ml) for resistin. The respective inter- and intra-assay coefficients of variation were <10% and <8% for both visfatin and resistin according to the manufacturer claims.

Statistical analysis

Statistical analyses were performed using 16.0 PC package (SPSS Inc., Chicago, IL). The Kolmogorov-Smirnov test was applied to both groups to test the normal distribution for each variable. Only resistin had non-normal distribution. Anthropometric data and hormonal values were presented as mean±SD. The Kruskal-Wallis Test was used for subgroups comparisons of mean serum resisitin levels and the Visfatin / Resistin ratio. Mean values of serum visfatin were compared with the analysis of variance (ANOVA) test. Univariate correlation coefficients were calculated according to Spearman rank-order equation for relationship between resistin and Visfatin / Resistin ratio with BMI, and Pearson's correlation coefficient for relationship between visfatin and BMI. We considered $p<0.05$ as statistically significant.

RESULTS

The clinical characteristics of the studied groups are shown in Table 1.

Patients with PCOS and controls were in similar age. There was no difference in BMI between normal weight PCOS subgroup and controls. As expected, BMI were significantly higher in the obese PCOS subgroup than in the normal weight PCOS subgroup and the controls.

The hormonal characteristics of the studied groups are shown in Table 2.

Mean serum of resistin was higher in women with PCOS in both subgroups than the controls, whereas no statistically significant difference existed between them. But there was a significant difference in mean resistin levels between all PCOS and the controls (p=0.46).

We did not observe any significant differences in mean serum visfatin concentrations and the Visfatin / Resistin ratio between PCOS subgroups and in comparison to the control group.

We did not found any Correlations between serum resistin and visfatin levels with BMI. And also no correlation between visfatin/resistin ratio and BMI has been observed (Table 3).

Clinical characteristics	**All PCOS**	**Lean PCOS**	**Obese PCOS**	**Controls**
N	28	14	14	12
Age (years)	28.21±3.61	26.92±3.64	29.50±3.20	26.91±3.31
BMI (kg/m^2)	25.56±3.58	22.72±1.40	28.39±2.71*	22.67±1.52

Table 1. Clinical characteristics of the studied groups

* p < 0.001 obese PCOS vs. controls.

Table 2. Hormonal characteristics of the studied groups

Hormonal characteristics	**All PCOS**	**Lean PCOS**	**Obese PCOS**	**Controls**
N	28	14	14	12
MEAN Resistin (ng/ml)	2.08±2.80*	1.98±3.14	2.19±2.53	0.74±0.54
MEAN Visfatin (ng/ml)	3.24±0.99	3.29±0.89	3.19±1.12	3.28±0.82
Ratio (V/R)^	4.77±6.97	3.95±2.92	5.59±9.54	9.04±10.20

* p < 0.05 all PCOS vs. control.

^ Ratio (V/R); visfatin / resistin of each individuals

Table 3. Correlation between BMI and remaining parameter

Correlation		**Resistin**	**Visfatin**	**Ratio (V/R)**
BMI	**Lean PCOS**	0.14	0.91	0.21
	Obese PCOS	0.14	.076	0.18
	Controls	0.77	0.24	0.44

DISSCUSSION

The results of our study showed no significant difference in resistin serum levels between groups, which is supported by several other studies [23, 33-36].

Seow et al. was reported that resistin mRNA levels were twofold higher in adipocytes from PCOS than in those from normal controls [21]. These results may indicate that resistin may have a local paracrine action in adipose tissue of PCOS patients. As in humans, resistin is predominantly expressed in macrophages and adipocyte precursor cells in visceral fat [37, 38]. Although we did not observe any correlation between serum resistin levels and BMI, Xita et al. were showed that the resistin gene polymorphism is associated with BMI in women with PCOS. These findings suggesting that resistin might be related to adiposity in a different regulatory mechanism in PCOS (39).

In the present research we did not find a difference in serum visfatin levels between patients with PCOS in both subgroups and the control group.

And also several recently published studies also did not find a difference in plasma or serum visfatin levels between patients with PCOS and the control group [40-42].

Zwirska-Korczala et al also did not detect a difference in visfatin levels between lean PCOS subjects and lean controls [43].

Lajunen et al. found no association between circulating full-length visfatin levels and PCOS, obesity or metabolic markers, and suggested that visfatin may act as a pro-inflammatory cytokine [41].

As many studies also demonstrated that visfatin displayed pro-inflammatory properties and modulated immune functions [27, 44]. Our results showed no correlation between serum visfatin and BMI, which is also reported in Lajunen's et al. study [41]. But the others demonstrated higher serum visfatin levels in lean PCOS subjects than the lean controls and the correlation of this hormone with BMI [45-48]. The Seow's et al study indicated that PCOS is associated with increased visfatin mRNA concentrations in PBMCs and in omental adipose tissue. However, only visfatin mRNA concentration in omental adipose tissue is closely correlated with BMI [49].thus these result may indicate that serum visfatin may not reflect the event of its role in omental adipose tissue.

CONCLUSION

This study demonstrated that serum resistin and visfatin levels do not seem to be directly involved in the pathology of PCOS. It is possible that these hormones might act with their pro-inflammatory characteristics as local determining factor in adipose tissue that is not reflected in patient's serum.

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