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Relationship between hyperandrogenism, obesity, inflammation and polycystic ovary syndrome

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Abstract

This prospective study aimed to determine the status of circulating levels of C-reactive protein (CRP), tumor necrosis factor α (TNF-α), IL-27, IL-35, IL-37, α-1 acid glycoprotein in patients with polycystic ovary syndrome (PCOS) compared with controls and to evaluate their relation with hyperandrogenism and obesity. Forty-eight patients with PCOS (29 obese, 19 lean) and 40 healthy controls (20 obese, 20 lean) were enrolled. CRP, TNF-α, IL-27, IL-35, IL-37, α-1 acid glycoprotein, sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEA-S) levels were measured. Levels of total testosterone, A4, DHEA-S were significantly higher in patients with PCOS than in controls both in the obese and lean groups, while levels of SHBG were significantly lower in all patients with PCOS than in all ($p < 0.05$). Free androgen index (FAI) values were significantly higher in all patients with PCOS than in all controls (all $p < 0.05$). Levels of CRP, TNF-α, α-1 acid glycoprotein were significantly increased in all patients with PCOS compared with all controls (all $p < 0.001$). FAI had a positive correlation with CRP, TNF-α, α-1 acid glycoprotein, a negative correlation with IL-27, IL-25, IL-37 (all $p < 0.01$). Body mass index had a negative correlation with IL-27, IL-35, IL-37, a positive correlation with α-1 acid glycoprotein, FAI ($p < 0.05$). The findings confirm the proinflammatory state of PCOS. Moreover, obesity along with PCOS significantly elevates the inflammatory status and hyperandrogenism.

Keywords

Hyperandrogenism, inflammation, obesity, polycystic ovary syndrome

History

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Introduction

Polycystic ovary syndrome (PCOS) is a very common endocrinopathy that affects 4–10% of women of reproductive age [1]. Its characteristics are polycystic ovaries, oligo- or anovulation and hyperandrogenism. With respect to hyperandrogenism, there is confounding data in the earlier literature [2–5]; some authors documented increased levels of testosterone in obese patients with PCOS [2,4,5], whereas others have found increased levels of androstenedione in lean patients with PCOS [3,5]. The reason for increased levels of dehydroepiandrosterone sulfate (DHEA-S) found in patients with PCOS has yet to be understood.

Obesity is commonly observed in women with PCOS [6]. For a definite diagnosis of PCOS, two out of these three characteristics have to be identified in the patient [7].

Levels of several inflammatory mediators (i.e. cytokines and glycoproteins) can be increased in patients with PCOS, which suggests that it is a proinflammatory state. On the other hand, there is an ongoing debate as to which mediators may be increased in patients with PCOS. For instance, previous studies have documented increased levels of C-reactive protein (CRP) in patients with PCOS in support of this argument [1,8,9]. Imbalances between proinflammatory such as tumor necrosis factor α (TNF-α) and anti-inflammatory cytokines are argued to have an impact on PCOS [10]. IL-27, IL-35, IL-37 are a new triad of anti-inflammatory cytokines that have recently been identified and not been evaluated in patients with PCOS [11]. In a recent study that evaluated metabolic profiles of patients with PCOS, N-acetyl glycoproteins and mainly acute phase α-1 acid glycoprotein provided the most consistently increased signal intensity in the spectroscopy spectra [12,13].

The relationship between inflammation, hyperandrogenism and PCOS is still poorly understood. Hence, we embarked on a study to determine the status of circulating levels of inflammatory mediators, namely CRP, TNF-α, IL-27, IL-35, IL-37 and α-1 acid glycoprotein in patients with PCOS compared with healthy controls, and to evaluate their relation with hyperandrogenism and obesity, taking into consideration that PCOS is a complex disease with several factors involved in its pathogenesis. We hypothesized that circulating levels of these markers would be different between patients with PCOS and healthy controls, and between obese and lean patients with PCOS.

Materials and methods

Patients and blood samples

Forty-eight patients with PCOS (29 obese and 19 lean) and 40 healthy controls (20 obese and 20 lean) who attended the obstetrics and infertility clinics of Istanbul University School of Medicine (Istanbul, Turkey) were enrolled into this prospective study.

The diagnosis of PCOS made based on clinical and chemical findings, and imaging tests in accordance with the 2003 Rotterdam classification [7]. Specifically, patients were

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diagnosed as having PCOS if they satisfied, at least, two of the following three criteria: (1) oligomenorrhea/oligo-ovulation, (2) biochemical or clinical hyperandrogenism and (3) the existence of polycystic ovaries. In all patients with PCOS, polycystic ovaries were morphologically identified by the existence of 12 or more follicles (2–9 in size) in each ovary using pelvic ultrasound [14]. Patients with PCOS and insulin resistance (IR), which was evaluated with the homeostasis model assessment of IR [15], were excluded from the study. We excluded patients with PCOS and IR to avoid bias between obese and lean PCOS sub-groups as lean patients with PCOS tend to have no IR [16].

Healthy controls were ovulatory as confirmed by the existence of regular menstrual cycles and having a progesterone level of >5 ng/ml in the luteal phase. Pelvic ultrasound was used to confirm the absence of PCOS morphology in all controls.

Obesity was defined as having a body mass index (BMI, kg/m^2) of 30–40, while normal weight was defined as a BMI of 18–25 [17]. The free androgen index (FAI) was calculated as the percentage ratio of total testosterone (TT) to sex hormone-binding globulin (SHBG) values. Biochemical hyperandrogenism was defined as an FAI value of >4.5.

None of the participants had taken medications that could alter the immune function or carbohydrate metabolism over the last 3 months, neither had they taken regular exercise over the last 6 months.

All blood samples were collected in the morning (08:00–10:00 AM) and during early follicular phase (d 3–5) in women with regular menstrual cycles or any other day in women with amenorrhea after they gave informed consent. Approval from the ethics committee of Istanbul University School of Medicine, Istanbul Faculty of Medicine was obtained for this study.

Plasma measurements

Immediately after collection, blood samples were centrifuged at 3000 rpm for 10 min at 4 °C to aliquot the supernatants, which were then stored at −80 °C until required. When the supernatants were thawed for analysis, they were centrifuged at 10 000 rpm for 5 min at 4 °C to remove any precipitate.

CRP, TNF-α, IL-27, IL-35, IL-37 and α-1 acid glycoprotein levels were measured using commercially available enzyme-linked immunosorbent assay kits (MyBioSource, Inc., San Diego, CA). Measurements were performed using a Wallac 1420 VICTOR3 Multilabel microplate reader (PerkinElmer, Life Sciences, Milan, Italy). All the inter- and intra-assay coefficients of variation were <5% and <10%, respectively. TT, androstenedione (A4), SHBG and DHEA-S levels were measured using electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN).

Statistical analysis

Continuous variables were presented as the mean and standard deviation (SD). All variables were normally distributed based on their histogram. Comparisons between the two groups were performed using the independent *t*-test. Group variables were analyzed using one-way analysis of variance. Pair-wise correlation of the hormonal and inflammatory mediators was performed using Pearson's correlation coefficient. Binary logistic regression analysis was utilized to study the association between the inflammatory and hyperandrogenism markers separately and PCOS outcome. The effect of the above-mentioned mediators on PCOS was expressed through an odds ratio and 95% confidence interval. Then, an ROC curve analysis was carried out on the predicted probabilities. Cutoffs were evaluated at 97.96% specificity. Analyses were performed using version 20.0 of the Statistical Package for the Social Sciences software (SPSS, Inc., Chicago, IL). A p value of <0.05 was considered significant.

Results

All participants were Caucasian and from the metropolitan area of Istanbul. The demographic characteristics of the participants are presented in Table 1. There were no statistical differences between the BMI of patients with PCOS who were obese and controls with obesity and lean patients with PCOS and lean controls (all $p > 0.05$). Age was similar in the four groups of patients with PCOS and controls ($p > 0.05$). The FSH levels of patients with PCOS who were obese were lower than those of controls with obesity ($p < 0.05$). FSH levels were similar between

Table 1. Demographic and hormonal characteristics of the obese and lean PCOS and control groups.

	Obese groups (BMI = 30–40 kg/m^2)		Lean groups (BMI = 15–25 kg/m^2)		Overall ANOVA test (F)
	PCOS patients n=29	Controls n=19	PCOS patients n =20	Controls n=20	p
Demographics					
Age, years	27.62 ± 3.26	28.89 ± 3.46	28.55 ± 4.06	28.70 ± 4.22	NS
BMI, kg/m^2	33.28 ± 3.05	31.78 ± 2.56	22.91 ± 1.06	21.98 ± 1.79	0.001
FAI	6.00 ± 3.59[a]	1.88 ± 1.24[a]	5.10 ± 2.44 [b]	1.50 ± 0.89[b]	0.001
Hormonal panel					
FSH, mIU/ml	5.67 ± 1.16[a,c]	6.85 ± 1.11[a]	6.42 ± 1.07[c]	6.72 ± 1.44	0.004
LH, mIU/ml	13.75 ± 2.82[a]	6.28 ± 1.77[a]	14.36 ± 4.36[b]	6.55 ± 1.62[b]	0.001
TT, ng/ml	0.39 ± 0.19[a]	0.23 ± 0.10[a]	0.38 ± 0.15[b]	0.27 ± 0.13[b]	0.001
A4, ng/ml	2.82 ± 0.65[a]	0.78 ± 0.34[a]	2.65 ± 0.52[b]	0.89 ± 0.44[b]	0.001
SHBG, nmol/l	25.55 ± 7.89[a]	48.10 ± 12.10[a]	27.91 ± 7.09[b]	65.62 ± 12.68[b]	0.001
DHEA-S, ng/ml	287.41 ± 86.71[a]	142.61 ± 27.17[a]	289.37 ± 72.88[b]	155.01 ± 29.64[b]	0.001
Inflammatory mediators					
CRP, mg/l	1.55 ± 0.25[a]	0.61 ± 0.18[a]	1.56 ± 0.19[b]	0.62 ± 0.12[b]	0.001
TNF-α, ng/ml	148.49 ± 27.32[a]	66.68 ± 18.72[a]	160.65 ± 18.44[b]	67.65 ± 9.17[b]	0.001
IL-27, ng/ml	35.22 ± 9.35[a,c]	228.67 ± 41.01[a]	89.06 ± 6.67[b,c]	182.11 ± 14.29[b]	0.001
IL-35, ng/ml	2.79 ± 1.02[a,c]	6.52 ± 0.99[a]	4.91 ± 0.66[b,c]	7.40 ± 1.12[b]	0.001
IL-37, ng/ml	21.16 ± 5.19[a,c]	73.29 ± 10.09[a]	48.95 ± 7.63[b,c]	83.32 ± 10.91[b]	0.001
α-1 acid glycoprotein, mg/l	226.21 ± 42.30[a,c]	71.91 ± 12.24[a]	126.90 ± 16.59[b,c]	72.95 ± 12.75[b]	0.001

BMI, body mass index; FAI, free androgen index; NS: non-significant. Mean $p > 0.05$.
Values are expressed as mean ± SD.
[a,b,c,d]Similar superscripts indicate a statistically significant difference ($p < 0.05$).

lean patients with PCOS and lean controls ($p > 0.05$). LH levels of patients with PCOS were higher than controls in both the obese and lean groups ($p < 0.05$). Levels of TT, A4 and DHEA-S were also significantly higher in patients with PCOS than in controls both in the obese and lean groups, but levels of SHBG were significantly lower in patients with PCOS than in controls in the obese and lean groups (all $p < 0.05$). FAI values were significantly higher in patients with PCOS than in controls in the obese and lean groups (all $p < 0.05$).

Levels of inflammatory mediators (CRP, TNF-α, α-1 acid glycoprotein) were significantly increased in patients with PCOS and obesity compared with controls with obesity and in lean patients with PCOS compared with lean controls (all $p < 0.001$) (Table 1). Levels of cytokines with anti-inflammatory properties (IL-27, IL-35 and IL-37) were significantly decreased in patients with PCOS and obesity compared with controls with obesity, and in lean patients with PCOS compared with lean controls (all $p < 0.001$). When obese and lean patients with PCOS were compared, levels of IL-27, IL-35, IL-37 were significantly lower, and levels of α-1 acid glycoprotein were significantly higher in patients with PCOS and obesity compared with lean patients with PCOS (all $p < 0.001$).

FAI had a strong positive correlation with CRP, TNF-α, α-1 acid glycoprotein and a negative correlation with IL-27, IL-25, IL-37 ($r = 0.63$, 0.48, −0.61, −0.60, 0.49, respectively; all $p < 0.01$). BMI had a strong negative correlation with IL-27, IL-35, IL-37, a strong positive correlation with α-1 acid glycoprotein ($r = -0.26$, −0.47, −0.49, 0.49, respectively; all $p < 0.01$), and a positive correlation with FAI ($r = 0.22$; $p < 0.05$).

Binary logistic regression analyses were carried out for each mediator hypothesized to be associated with PCOS and revealed IL-35, IL-37, TT, SHBG and DHEA-S were significant single predictors of PCOS ($p < 0.001$) (Table 2).

ROC plots were drawn to compare the predictive performance of IL-35, IL-37, TT, SHBG and DHEA-S, and to determine cutoff levels that best discriminated between women with PCOS. The ROC curves, ROC area under the curve (AUC) values and cutoff values (Table 3) showed that the predictive performance of performance of IL-35, IL-37, SHBG and DHEA-S were excellent (AUC 0.971, 0.991, 0.968, 0.994, respectively; all $p < 0.001$).

Table 2. Probability ratios of parameters for prediction of PCOS in patients.

	Odds ratio	95% Confidence interval	*p*
CRP	0.001	0.001–0.002	0.98
TNF-α	0.006	0.001–0.662	0.98
IL-27	1.936	0.001–0.575	0.993
IL-35	9.693	3.207–29.298	0.001
IL-37	1.339	1.120–1.600	0.001
α-1 acid glycoprotein	0.044	0.001–0.834	0.98
TT	0.002	0.001–0.065	0.001
A4	0.001	0.001–0.120	0.986
SHBG	1.320	1.153–1.511	0.001
DHEA-S	0.848	0.759–0.947	0.003

Discussion

The data in the present study confirm that PCOS is a proinflammatory state. Furthermore, both obesity and PCOS caused increased plasma levels of inflammatory mediators. Hyperandrogenism is evident in both obese and lean patients with PCOS, but obesity seems to exacerbate the problem.

PCOS is considered to be a metabolic disorder as well as a reproductive disorder owing to its association with obesity, hyperandrogenism and cardiovascular diseases. Reproductive disorder characteristics of PCOS were present in our study as an increased gonadotropic dysfunction, identified by increased LH levels in both obese and lean patients with PCOS, a finding that has also been found in previous studies [18,19].

Several studies in the literature have evaluated changes in the inflammatory state in PCOS and documented an increase in CRP levels regardless of the BMI in patients with PCOS [1,3,8,9,20]. In the present study, levels of CRP were increased both in obese and lean patients with PCOS compared with the obese and lean controls. Levels of CRP were similar between obese and lean patients with PCOS. These findings support the argument that levels of CRP increase in PCOS with or without the presence of obesity. We documented similar findings for TNF-α, which is a proinflammatory cytokine and one of the principal mediators of inflammation. Other studies in the literature have also presented increased levels of TNF-α in PCOS [21,22].

IL-27, IL-35 and IL-37 are recently discovered anti-inflammatory cytokines [11,23]. Levels of this triad of cytokines were significantly decreased in obese and lean PCOS patients compared with obese and lean controls. These findings seem to be in line with the argument that PCOS is a proinflammatory state because there appears to be an imbalance between pro and anti-inflammatory mediators in patients with PCOS.

Glycoproteins are associated with several conditions such as inflammation and diabetes [24]. In a relatively recent study on obese mice, acute phase α-1 acid glycoprotein was induced in adipose tissue to suppress the inflammation, which was a result of metabolic signals and increased levels of TNF-α [25]. In a study by Sun et al., acute phase α-1 acid glycoprotein provided the most consistent increased signal intensity in the spectroscopy spectra of the plasma samples of patients with PCOS [13]. In the present study, increased levels of acute phase α-1 acid glycoprotein were observed both in obese and lean patients with PCOS compared with obese and lean controls, again underlining the proinflammatory state of PCOS.

Obesity certainly has a role in inflammation because adipose tissue is the main location of inflammatory cytokine production [26]. However, the increase in proinflammatory cytokines and

Table 3. Predictive accuracy of IL-35, IL-37, TT, SHBG and DHEA-S.

	AUC	Optimal cutoff point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IL-35	0.971	5.90 ng/ml	97.96	87.18	90.57	97.14
IL-37	0.991	60.15 ng/ml	97.96	94.87	96.00	97.37
TT	0.730	0.31 ng/ml	67.35	69.23	73.33	62.97
SHBG	0.968	39.22 nmol/l	97.96	92.31	94.12	97.30
DHEA-S	0.994	200.85 ng/ml	97.96	100.00	100.00	97.50

AUC, area under the ROC curve; PPV, positive predictive value; NPV, negative predictive value.

decrease in anti-inflammatory cytokines cannot solely be attributed to obesity, because there seems to be a strong association between inflammation and PCOS. The results of the present study indicated that levels of proinflammatory cytokines in patients with PCOS and obesity were significantly more increased than in obese controls, but levels of anti-inflammatory cytokines were significantly more decreased than in obese controls. Hence, inflammation seems to be affected both by obesity and PCOS.

In the present study, androgen levels were increased both in obese and lean patients with PCOS compared with obese and lean controls and had hyperandrogenism as their FAI values were above 4.5. We found that *T* levels were increased in obese and lean patients with PCOS compared with controls, whereas T levels were similar between obese and lean patients with PCOS. Some studies in the literature found increased T levels in patients with PCOS and obesity compared with lean patients with PCOS [4,5], and other studies found similar levels of T to those in our study [19,27,28]. Our findings on A4 levels were similar to T as increased in obese and lean patients with PCOS compared with controls, whereas the levels were similar between obese and lean patients with PCOS. There are conflicting studies in the literature that both support [4,19] and contradict [5,29] these observations. The differences in T and A4 levels with some studies may have been due to different PCOS inclusion criteria, the inclusion of different phenotypes, differences in BMI cutoff points, and various race and ethnic characteristics. Levels of DHEA-S were increased in obese and lean patients with PCOS compared with obese and lean controls in the present study, which was in line with earlier findings [30].

It has been previously stated that patients with PCOS face a vicious cycle in which increased androgen levels lead to abdominal adiposity and in return, increased androgen levels stimulate androgen secretion [31], both of which contribute to hyperandrogenism [32]. Furthermore, as a result of the vicious cycle mentioned above, the synthesis of SHBG is inhibited [33]. Supporting this argument, SHBG levels in the present study were lower in patients with PCOS.

It is not clear if increased androgen levels in PCOS cause the proinflammatory state or if inflammation triggers the androgens, which subsequently results in hyperandrogenism [34]. Some researchers have argued that increased levels of androgens cause a proinflammatory state in PCOS because hyperandrogenism leads to increased mononuclear cell sensitivity to ingested glucose [22].Obesity is a state of chronic inflammation. Weight gain in women who have a genetic predisposition to PCOS may result in the amplification and unmasking of this disorder. In addition, our study indicated strong correlations between BMI and FAI and inflammatory mediators, which mean that both inflammation and hyperandrogenism are worsened in patients with PCOS who are obese. Accordingly, it has been previously documented that features of hyperandrogenism were significantly improved in patients PCOS even with modest weight loss [35].

The major limitation of this study was the inclusion of a single population, namely Caucasian Turkish women. The variability of PCOS across populations has been previously stated. For instance, ethnic variability has made a difference in androgen levels of women with PCOS in some studies [36,37]. Hence, it may be useful to include women with different ethnic backgrounds in future studies.

In conclusion, the findings of the present study further confirm the proinflammatory state of PCOS. Moreover, obesity along with PCOS significantly elevates the inflammatory status and hyperandrogenism.

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Declaration of interest

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