Original Article

Changes of biochemical parameters in normal weight and overweight/obese women with polycystic ovary Syndrome

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ABSTRACT

Objectives: Polycystic ovary syndrome (PCOS) is an endocrine disease in reproductive-age women, which interferes with fertility, menstruation, and body composition. Some biochemical parameters change in women with PCOS, a feature which can be a consequence of the disease itself or of the accompanying obesity. This study investigated the glucose and lipid profiles, sex hormones, and hsCRP in normal weight and overweight/obese Iranian women with and without PCOS.

Methods: 314 women with PCOS and 138 healthy and fertile women were recruited for the study. The patients and controls were divided according to body mass index (BMI) into two groups as follows: BMI<25 Kg/m^2 and BMI≥ 25 Kg/m^2. Blood samples were collected from all participants to assess fasting blood glucose, fasting insulin, lipid profile, FSH, LH, free testosterone, and hs-CRP levels. The homeostasis model of insulin resistance (HOMA-IR) was calculated for assessing insulin sensitivity.

Results: BMI, fasting insulin, HOMA-IR, triglyceride, FT, LH, and hs-CRP levels were significantly higher in the PCOS group, while lower levels of FSH were observed in comparison to non-PCOS women. Among overweight PCOS women, hs-CRP levels were significantly elevated compared to normal-weight PCOS women. However, there was no significant difference in circulating hs-CRP levels between normal-weight and overweight participants in the control group.

Conclusion: The study results suggest that both insulin resistance and an increase in hs-CRP may be relevant to PCOS. Moreover, the findings indicated that chronic inflammation in PCOS is not solely dependent on the pathogenesis of the disease but may be exacerbated by increased body weight.

Keywords: Polycystic Ovary Syndrome, Hs-CRP, Insulin Resistance, Obesity

Abbreviations: BMI: Body mass index; CVD: cardiovascular disease; FBG: fasting blood glucose; FSH: follicle-stimulating hormone; FT: free testosterone; HOMA-IR: homeostasis model of insulin resistance; HDL-C: high-density lipoprotein cholesterol; hs-CRP: high sensitive-CRP; IR: insulin resistance; LH: luteinizing hormone; LDL-C: low-density lipoprotein cholesterol; MetS; metabolic syndrome; NAFLD: non-alcoholic fatty liver disease ; PCOS: Polycystic ovary syndrome; PCOM: polycystic ovarian morphologic; TG: triglycerides; T2DM: type II diabetes mellitus

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Introduction

Polycystic ovary syndrome (PCOS) is a common polygenic, multifactorial, inflammatory, endocrine disorder, affecting 4–21% of women of reproductive age (1, 2). Recent studies have highlighted the involvement of genetics, epigenetic changes, environmental factors, oxidative stress, chronic low-grade inflammation, mitochondrial dysfunction, and metabolic disorder in the pathogenesis of PCOS (3-6). The pathogenesis of PCOS shares common features with metabolic syndrome (MetS) including dysfunctional adipose tissue, obesity, insulin resistance (IR), and an increased risk of developing type II diabetes mellitus (T2DM), cardiovascular disease, dyslipidemia, and non-alcoholic fatty liver disease (NAFLD) (7-9). PCOS is a complex condition characterized by the presence of at least two of three Rotterdam criteria, which include hyperandrogenism (manifested as hirsutism or hyperandrogenaemia), oligo-ovulation or anovulation, and polycystic ovarian morphologic (PCOM) features (10, 11). Elevated levels of insulin and androgens disrupt follicular growth, leading to the irregular menstrual cycle and the accumulation of immature follicles (12). In particular, obese PCOS women are more susceptible to infertility. Obesity contributes to infertility in PCOS patients through various mechanisms such as the exacerbation of hyperandrogenism, alteration of luteinizing hormone (LH) secretion, and increased insulin resistance (13, 14). High body mass index (BMI) and IR significantly elevate the risk of developing PCOS in women (15). Insulin resistance and compensatory hyperinsulinaemia (HI) are prevalent in 65–95% of women with PCOS, primarily among those who are overweight or obese. Moreover, IR has been observed in half of PCOS women with normal weight, indicating that IR can occur independently of obesity (16, 17). Obesity and insulin resistance, commonly observed in individuals with PCOS, trigger a chronic inflammatory process (18-20). High sensitive-CRP (hs-CRP) is an acute phase protein secreted from the liver, which is stimulated by interleukin-6 and originating from the adipose tissue (21). It has been reported that serum hs-CRP levels increase in inflammatory conditions such as obesity, hypertension, dyslipidemia, cancer progression, type 2 diabetes mellitus, and atherosclerosis (22, 23). A few studies have investigated CRP levels in women with PCOS (21), but the results have not been consistent. While some studies report elevated CRP levels in women with PCOS (24, 25), it is suggested that serum CRP levels are more closely associated with obesity rather than with the presence of PCOS per se. Thus, further research is needed to determine whether serum CRP levels are indeed increased in women with PCOS, and to identify the variables that predict serum CRP levels (26). In this context, the study aimed to investigate the circulating concentrations of glucose, lipid profile, sex hormones, and hs-CRP in both normal weight and overweight/obese infertile women with PCOS, compared with healthy controls among Iranian women.

Materials and methods

Study participants

The study was approved by the Ethics Committee of the University of Tehran, Tehran, Iran. All women signed written informed consent before participation in the investigation. Subjects were selected from the Obstetrics and Gynecology Department of Ibn Sina Infertility Center, Tehran, Iran. Venous blood was collected from each subject at the early follicular phase of their menstrual cycle. The subjects previously participated in the studies, and some of the data have been used in recent publications. So, the present study is a secondary analysis of PCOS and non-PCOS data bank (27-29). A total of 452 women (314 PCOS and 138 healthy fertile controls) were included in this case-control study. The inclusion criterion included PCOS diagnosis based on the 2003 Rotterdam Criteria (10). Women who smoked, were pregnant or lactating, consumed alcohol, or with a history of diabetes mellitus (DM), cardiovascular diseases (CVD), endocrine disorders including hyperprolactinemia, thyroid diseases, premature ovarian failure, congenital adrenal hyperplasia, Cushing's syndrome, and adrenal tumors (28) were excluded. The control group consisted of women who were fertile, had regular menstrual cycles, and exhibited no clinical or biochemical hyperandrogenism (28). Moreover, participants who have been taking the following medications for the past three months were not included: anti-hypertensive, weight loss agents, anti-inflammatory, lipid-lowering, insulin-sensitizing, antioxidant supplements, and oral contraceptives (28).

Anthropometrics and biochemical measurements

Anthropometric data were recorded for each subject. BMI was computed as weight (kg) divided by height squared (m^2). After a 10 h overnight fast, at the follicular phase of their menstrual cycle, five milliliters of venous blood was collected from each participant as previously described (27). The serum samples were immediately centrifuged (1000× g for 15 min), aliquoted, and stored at -80℃.

Biochemical analyses included fasting blood glucose (FBG), lipid profile (triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)), and high sensitivity C-reactive protein (hs-CRP) measured using reliable enzymatic techniques (27-29). The serum levels of fasting insulin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and free testosterone (FT) were measured using ELISA kits as described previously (27-29). The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated using the following formula: ([FBG (mg/dl)]×[fasting

serum insulin (μU/ml)]/405) (30).

Statistical analysis

All statistical analyses were carried out using the SPSS 16 software (SPSS, Chicago, IL, USA). First, the Shapiro–Wilk test was used to test the normal distribution of the variables. Before further analysis, skewed variables were logarithmically transformed to approximate normality. Normal variables were presented as mean with standard deviation (SD) and compared between non-PCOS and PCOS groups using Student's T-test. The non-PCOS and PCOS groups were also stratified according to BMI as normal weight (BMI < 25 Kg/m^2) and overweight/obese (BMI ≥ 25Kg/m^2), and compared using one-way ANOVA, supplemented with Bonferroni post hoc test. A p-value of less than 0.05 was considered statistically significant.

Results

The clinical features of the study population in both non-PCOS and PCOS groups are presented in Table 1, some data have been reported in former studies (27-29). There were statistically significant differences in terms of age and BMI between groups (P < 0.01). Regarding the glucose metabolism parameters, circulating levels of fasting insulin and HOMA-IR were significantly higher in the PCOS group when compared to the non-PCOS group (P < 0.001). Moreover, the lipid profile showed significantly elevated levels of TG in the PCOS group (P < 0.05) than the non-PCOS group. There was no significant difference in levels of LDL-C, HDL-C, and TC between groups. Moreover, the PCOS group exhibited higher levels of FT (p < 0.001), LH (P < 0.05), and lower levels of FSH (P < 0.001). Furthermore, the level of the circulating concentrations of hs-CRP (mg/L) was significantly higher in the PCOS group (3.96±1.2) when compared to the non-PCOS group (2.5±0.93) (P < 0.001) (Fig.1).

Subsequently, the non-PCOS and PCOS groups were stratified according to BMI: normal-weight (BMI < 25 Kg/m2) and overweight/obese (BMI ≥25 Kg/m^2) for further analysis (Table 1). Results indicated that hs-CRP levels were significantly higher in normal-weight and overweight/obese PCOS women (3.51 ± 1.11 and 4.29 ± 1.17 mg/L, respectively) when compared to normal-weight and overweight/obese controls (2.40 ± .92 mg/L and 2.60 ±.94, respectively) (P < 0.001). Furthermore, hs-CRP levels were significantly elevated in overweight/obese PCOS women when compared to normal-weight PCOS women (P < 0.001). However, there was no significant difference in circulating levels of hs-CRP between normal-weight and overweight/obese participants in the control group (Fig. 1).

Table 2 provides an overview of the clinical and laboratory parameters among women categorized as normal weight and overweight/obese, with or without PCOS. A statistically significant difference was observed between the overweight/obese non-PCOS group and PCOS subgroups in terms of age (P < 0.001). Fasting insulin level and HOMA-IR values were significantly higher in overweight/obese PCOS groups when compared to the non-PCOS subgroups (P < 0.001). HOMA-IR showed a higher value in normal weight PCOS compared to normal weight non-PCOS patients (P < 0.001). Furthermore, normal weight PCOS subjects had significantly elevated fasting insulin level

Table 1: Clinical features of the study population

Variables	Non-PCOS (n = 138)	PCOS (n = 314)	p-value
Age (years)	31.86±5.12	29.9±4.56	<0.001
BMI (Kg/m^2)	25.42±3.96	26.7±4.49	0.004
FBG (mg/dL)	90.3±9.66	89.67±9.51	0.517
Fasting Insulin (μU/mL)	3.26±1.9	5.62±3.83	<0.001
HOMA-IR	0.71±0.47	1.1±0.76	<0.001
TG (mg/dL)	117.41±39.8	127.26±57.21	0.035
TC (mg/dL)	164.92±39.74	172.15±35.61	0.055
LDL-C (mg/dL)	99.2±30.86	98.28±29.15	0.760
HDL-C (mg/dL)	46.66±7.22	45.04±11.35	0.068
Free Testosterone (pg/mL) $^{\Delta}$	1.53±0.33	3.24±1.13	<0.001
LH (IU/L) $^{\$}$	6.68±2.62	7.56±4.83	0.025
FSH (IU/L) $^{\$}$	8.44±2.35	6.56±3.54	<0.001

Data are given as mean ± standard deviation. Independent t test for comparison between PCOS and Non-PCOS groups.
$^{\Delta}$ Parameter was compared between 86 non-PCOS and 172 PCOS women.
$^{\$}$ Parameters were compared between 86 non-PCOS and 320 PCOS women.

PCOS, Poly Cystic Ovary Syndrome; BMI, Body Mass Index; FBG, Fasting Blood Glucose; HOMA-IR, Homeostatic Model Assessment for Insulin; TG, Triglyceride; TC, Total Cholesterol; LDL-C, Low Density Lipoprotein Cholesterol; HDL-C, High Density Lipoprotein Cholesterol; LH: luteinizing hormone; FSH: follicle-stimulating hormone

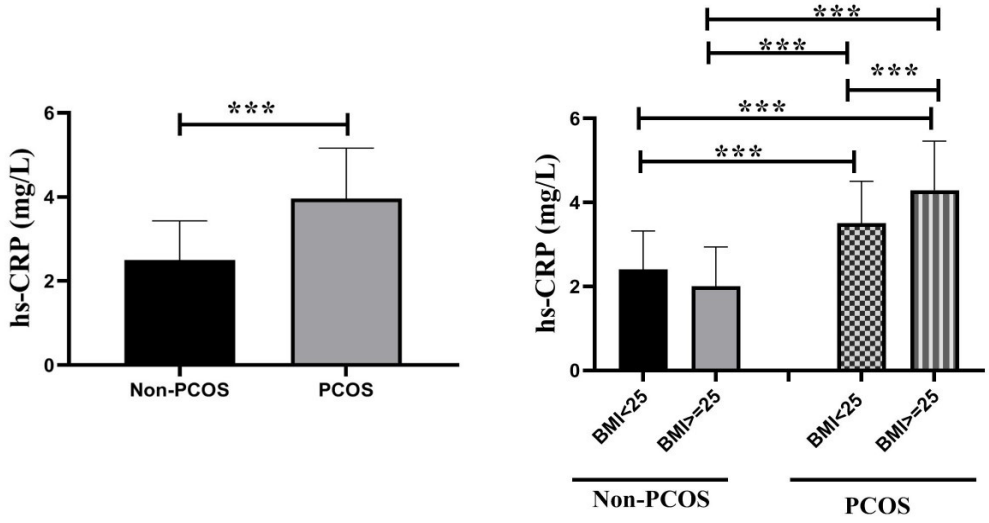

Figure 1: Serum levels of hs-CRP in PCOS (n=172) and non-PCOS (n=86) women with normal weight and overweight/obese. Comparison was made using student t test and one-way ANOVA supplemented with Bonferroni test.
PCOS: Polycystic ovary syndrome; BMI: Body Mass Index; hsCRP, high sensitive reactive protein.

Table 2: Clinical and laboratory parameters of normal weight and overweight/obese women with or without PCOS

Parameters	Non-PCOS		PCOS		p-value
	BMI <25	BMI ≥25	BMI <25	BMI ≥25	
Age (years)	31.14±5.3	32.53^{ab}±4.89	29.75^{b}±4.40	30.2^{a}±4.61	<0.001
BMI (Kg/m^2)	22.46^{ac}±1.92	28.19^{ab}±3.32	22.51^{bd}±2.73	29.18^{cd}±3.34	<0.001
FBG (mg/dL)	88.18±7.78	92.07±10.51	88.99±8.47	90.22±10.16	0.070
Fasting Insulin (µU/mL)	2.98^{cd}±1.85	3.49^{ab}±1.92	4.98^{bd}±3.76	5.99^{ac}±3.82	<0.001
HOMA-IR	0.59^{bc}±0.37	0.82^{a}±0.52	0.99^{c}±0.71	1.18^{ab}±0.80	<0.001
TG (mg/dL)	115.26±37.95	119.55±42.20	118.18±54.31	132.61±58.51	0.031
TC (mg/dL)	162.60±36.85	168.08±42.85	168.61±36.08	174.44±35.45	0.127
LDL-C (mg/dL)	99.30±31.65	99.73±30.86	96.79±29.62	99.84±29.03	0.844
HDL-C (mg/dL)	48.06±6.52	45.81±7.51	46.33^{a}±10.6	43.80^{a}±9.30	0.005
Free Testosterone (pg/mL)	1.56^{cd}±0.36	1.51^{ab}±0.30	3.24^{bd}±1.11	3.24^{ac}±1.16	<0.001
LH (IU/L)	6.49±2.73	6.862.53	7.83±5.06	7.42±4.63	0.318
FSH (IU/L)	8.47^{cd}±2.40	8.41^{ab}±2.34	6.61^{bd}±3.38	6.59^{ac}±3.68	<0.001

Parametric data are analyzed with One-way ANOVA test and given as mean ± standard deviation.
P < 0.05 is statistically significant.
Significant differences among pairwise groups with Bonferroni's approach indicated by similar uppercase letters (such as a, b and c).
PCOS: Polycystic ovary syndrome; BMI: Body mass index; FBG: Fasting blood glucose; TG: Triglyceride; TC: Total cholesterol; LDL-C: Low density-lipoprotein cholesterol; HDL-C: High density-lipoprotein cholesterol; LH: Luteinizing hormone; FSH: Follicle-stimulating hormone.

when compared to normal weight and overweight/obese non-PCOS groups. Overweight/obese PCOS women exhibited significantly lower level of serum HDL-C in the PCOS group (P < 0.01). Free testosterone was significantly elevated in normal weight and overweight/obese cases with PCOS in comparison to normal weight and overweight/obese cases without PCOS (P < 0.001). Normal weight and overweight/obese patients with PCOS had lower FSH hormone levels than normal weight and overweight/obese cases without PCOS (P < 0.001).

Discussion

Nowadays, the genetic origins of PCOS are acknowledged, with numerous genes involved in the pathogenesis of hyperandrogenemia, dyslipidemia, insulin resistance, inflammation, disturbed folliculogenesis, and metabolic syndrome (31). Currently, there are four commonly recognized phenotypes of PCOS (32), with IR present in all phenotypes (33). IR in women with PCOS is influenced by circulating androgen levels (34). Androgens also regulate lipid metabolism and adipocyte differentiation, and induce the accumulation of abdominal adipose tissue. Animal models have shown that prenatal and postnatal exposure to androgens can lead to enlargement of adipocytes, accumulation of visceral fat, and decreased insulin sensitivity in women (35). Increased androgen production in adipose tissue and subsequent lipid accumulation can lead to systemic

IR in patients with PCOS (36).

Increased adipose tissue and its dysfunction may exacerbate physiological factors and cytokine levels, thereby promoting low-level inflammation, interfering with insulin signaling, causing adipose tissue to release free fatty acids, increasing ectopic fat deposition, and aggravating IR on one's own and other organizations (33, 37). In the present study, obese and non-obese groups of PCOS had a significant increase in HOMA-IR and fasting insulin levels in comparison to BMI-matched controls. In addition, normal weight and overweight/obese PCOS women had significantly higher fasting insulin levels when compared to normal and overweight/obese non-PCOS group, however, the elevated amount of insulin in overweight/obese PCOS compared to normal weight PCOS did not reach a significant level. These results were in agreement with former studies which stated that the majority of women with PCOS have insulin resistance which is independent of body weight changes (38, 39). Former investigations documented abnormalities of insulin metabolism in both lean and overweight patients with PCOS, and that therapeutic strategies targeting IR in PCOS improve clinical features and might reduce long-term sequelae including diabetes and cardiovascular diseases (40, 41). In line with a study done by Bjekić-Macut et al. (31), in our research, PCOS women had significantly higher BMI in comparison to non-PCOS women. Weight loss has shown clear benefits on PCOS outcomes, including reproductive function, glucoregulatory status, androgen status, and lipid profiles (42, 43).

PCOS is a proinflammatory disease and several studies on PCOS show increased levels of circulatory inflammatory markers (44-46). Chronic inflammation caused by hyperinsulinemia and excessive fat accumulation in the visceral compartment is linked to PCOS disorders. In our study, women with PCOS, whether normal weight or overweight, had higher hs-CRP than BMI-matched control groups. Meta-analysis studies revealed that hs-CRP levels are nearly 100% higher in PCOS patients than in controls (47, 48). The underlying mechanism of elevated serum hs-CRP in PCOS has not been disclosed yet, and it remains unclear whether it is associated with PCOS itself or the accompanying obesity and accumulation of visceral fat. A meta-analysis highlighted elevated WBC and hs-CRP levels independent of obesity in PCOS; however, it was reported that the meta-analysis had a limitation of inadequate BMI matching regarding the publications included (49). In addition, a few more investigations did not find a relationship between obesity and serum hs-CRP levels in PCOS (50, 51).

In addition, it was shown that hs-CRP levels were significantly higher in overweight/obese PCOS women when compared to normal-weight PCOS women, indicating that chronic inflammation in PCOS could be accentuated by increased body weight, which agreed with previous investigations (21, 52). Ün and colleagues reported that hs-CRP values were significantly increased in women with obesity when patients with PCOS were considered as obese/non-obese (53). In contrast, some studies found that the increase in the inflammatory marker hs-CRP in PCOS patients was solely caused by obesity, i.e., that PCOS status per se had no impression on the severity of these patients (54, 55). In view of the significantly higher mean hs-CRP values in women with PCOS compared to controls, it seems that routine evaluation of hs-CRP levels in all patients with PCOS may be useful for a successful PCOS treatment plan. The other inflammatory markers such as specific cytokines can be considered hold promise as markers of inflammation but are constrained by their instability, insufficient performance, lack of commercial assays applicable to the routine laboratory setting, and lack of standardization. Therefore, hs-CRP was measured in this study (56).

However, the present investigation possesses several limitations. First, the sample size could be larger. Second, the results might only relate to the Iranian people and cannot be generalized to other populations. Third, utilizing other adiposity parameters such as WHR, WC, or the use of body composition analyses might have been more informative than the mere use of BMI. Finally, the use of the euglycemic/hyperglycemic clamp is the gold standard for the evaluation of IR; however, HOMA-IR remains commonly approved.

Conclusion

The current study results reveal that both IR and inflammation may be linked to PCOS. Furthermore, the findings indicate that chronic inflammation in PCOS is not solely dependent on PCOS but may be exacerbated by elevated body weight. In view of the significantly higher mean hs-CRP values in women with PCOS compared to controls, it seems that routine assessment of hs-CRP values in all cases with PCOS may be warranted and requires more analysis.

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Conflict of Interest

The authors declared that they have no conflict of interest.

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