Original Article

Homocysteine and C-reactive Protein Levels in Women with Polycystic Ovary Syndrome

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Abstract

Objectives: Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in infertile women characterized by both reproductive and metabolic dysfunctions of different degrees. Furthermore, it has been associated with increased cardiovascular disease (CVD) risk and related long-term health sequela. The aim of this study is to evaluate serum homocysteine (Hcy) and C-reactive protein (CRP) levels in women with PCOS and to evaluate their relationship with clinical and laboratory parameters in women with PCOS.

Materials and Methods: The prospective single-center study included 45 women with PCOS (study group) and 41 control subjects. Demographic variables and Hcy, CRP, fasting blood glucose, insulin, follicle-stimulating hormone, luteinizing hormone, estradiol, total and free testosterone, dehydroepiandrosterone sulfate, thyroid-stimulating hormone levels, and lipid profiles of the subjects were recorded. homeostatic model assessment for insulin resistance (HOMA-IR) indexes were calculated.

Results: Fasting plasma glucose, insulin, HOMA-IR, free and total testosterone levels, and clinical hirsutism were significantly higher in the study group. There was no statistically significant difference in lipid profile between groups. Hcy and CRP levels were higher in the study group, which was not statistically significantly different ($P > 0.05$).

Conclusion: Some of the parameters that are correlated with CVD risk were found to be higher in women with PCOS, although the difference for Hcy and CRP did not reach statistical significance. However, the current study reveals that the CVD risk associated with PCOS deserves more comprehensive prospective studies with long-term outcomes.

Keywords: C-reactive protein, homocysteine, polycystic ovary syndrome

Introduction

The prevalence of polycystic ovary syndrome (PCOS) is 5%–15% in reproductive age women according to Rotterdam criteria that include chronic menstrual dysfunction, clinical or biochemical hyperandrogenism, and polycystic ovaries confirmed by ultrasonography (≥10 follicles and ≥10 ml ovarian volume).[1] It is one of the most common etiological factors for female anovulatory infertility but also accompanied by metabolic disorders such as obesity, insulin resistance, gestational diabetes mellitus, Type II diabetes mellitus, and long-term adverse health outcomes.[2]

There is an increased risk of cardiovascular disease (CVD) in women with PCOS. Not only myocardial infarction is approximately seven times higher in this population,[3] but also extensive coronary artery disease and carotid atherosclerosis are more common in women with PCOS.[4,5] CVD risk factors such as dyslipidemia, diabetes mellitus, and obesity have also been more common in women with POCS. However, it is not known whether increased cardiovascular events in women with PCOS are due to accompanying

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obesity, higher body mass index (BMI), or other metabolic disorders independently.

Homocysteine (Hcy) is a nonessential amino acid, which is not present in the daily diet but synthesized in our body from methionine, another amino acid produced by metabolization of proteins. Hcy, once produced from methionine, can be removed in two ways which are reconversion to methionine by remethylation or to cysteine by transsulfuration.

Hyperhomocysteinemia is associated with an increased risk of aging-related diseases such as atherosclerotic and thromboembolic events besides CVDs.[6-8] Hcy is hypothesized to increase CVD risk, by both its effects on vascular wall structures and blood coagulation system.[7,9,10] Therefore, hyperhomocysteinemia has been accepted as one of the CVD risk factors. Independent of the Framingham risk scoring system, which is frequently used to estimate CVD risk, it is proposed that, for each increment in Hcy concentrations of 5 μmol, the risk of cardiovascular events increases by about 20%.[11] In addition, hyperhomocysteinemia reduces the synthesis of nitric oxide in endothelial cells. Nitric oxide plays a role in almost all stages of female reproductive processes, such as ovulation, early embryo development, implantation, pregnancy, and birth contractions.[12] From this point of view, higher Hcy levels might be correlated with adverse fertility outcomes.[13]

C-reactive protein (CRP) is a well-known marker for infection but also used to follow up low-grade chronic inflammation which provides the pathophysiological basis for atherosclerosis and is a good predictor of vascular events. CRP causes atherogenesis directly via adhesion molecule expression and complement activation.[14] Therefore, CRP has been accepted as one of the criteria for cardiovascular risk assessment by the Centers for Disease Control and Prevention and American Heart Association[15,16] and also different cutoff values for determination and classification as mild, moderate, and high risks have been defined.[17] Increased CRP levels are observed in women with PCOS reflecting the chronic inflammatory aspect of the disease. This increased inflammatory activity can theoretically affect folliculogenesis, and ovulation as the anovulation is the main defect of patients with PCOS, resulting in infertility.[18]

Although, women with PCOS are mostly presented with anovulatory infertility, PCOS is also associated with long-term adverse outcomes such as metabolic syndrome and CVD. Many markers indicating increased metabolic activity including Hcy and CRP levels have been shown to increase in women with PCOS necessitating further research to overcome the long-term adverse health outcomes in these women. From this point of view, in our study, we aimed to evaluate levels of two different markers acting through different pathways – Hcy: associated with endothelial inflammation and CRP: associated with chronic low-grade inflammation – and to evaluate their relationship with clinical and laboratory parameters in women with PCOS.

Materials and Methods

The study was performed in the Obstetrics and Gynecology Outpatient Clinic of Ankara University Hospital. It was a prospective, single-center study. Our study population comprised two groups of women (45 with PCOS and 41 age- and BMI-matched controls). The Ethical Committee of the University Hospital approved the study (113-2990), and informed consent was taken from all participants. Among 100 patients, data of 86 who accepted to participate in the study were included. The study group ($n = 45$) consisted of women who were diagnosed with PCOS according to the Rotterdam criteria.[19] Furthermore, subgroup analysis of the two groups regarding BMI, homeostatic model assessment for insulin resistance (HOMA-IR), and the presence of clinical hirsutism was held. Exclusion criteria were the presence of hyperprolactinemia, thyroid dysfunction, adrenal dysfunction, diabetes mellitus, hypertension, pregnancy, and smoking. None of the participants were on hormonal medications during the study period.

Venous blood samples were collected (2nd or 3rd day) during the menstrual cycle after overnight fasting for at least 12 h. In cases with oligo-anovulation, blood samples were obtained after progesterone withdrawal bleeding. Levels of fasting blood glucose, insulin, and hormone profile (follicle-stimulating hormone, luteinizing hormone, estradiol [E2], total and free testosterone [total-T and free-T, respectively], dehydroepiandrosterone sulfate, and thyroid-stimulating hormone) were evaluated. Fasting insulin and fasting plasma glucose (FPG) levels were used for the calculation of HOMA-IR (insulin × glycemia in μmol/L/22.5).[20] Insulin resistance was accepted as HOMA-IR ≥2.1.[21] Lipid profiles (total cholesterol, low-density lipoprotein, high-density lipoprotein [HDL], and very-low-density lipoprotein) of two groups were recorded. CRP levels were evaluated by competitive ELISA technique (Calbiochem-Novabiochem, California, USA), and Hcy levels were measured by fluorescence polarization immunoassay (IMX System, Abbott Diagnostics, USA). Clinical hirsutism was evaluated by the modified Ferriman–Gallwey scoring system, and a total score of 8 or over was defined as clinical hirsutism.[22] Biochemical hirsutism was defined as total testosterone level >80 ng/dL or DHEAS level >350 ng/Dl.[22]

Statistical analysis

Statistical analysis was performed using SPSS for Windows version 22.0 (SPSS Inc., Chicago, IL, USA). Data were shown as mean ± standard deviation or median (minimum–maximum)

and numerical or percentile where applicable. Distribution of numerical variables was evaluated by Kolmogorov–Smirnov test, and similarity between-group variances were evaluated by Levene's test. The difference between numerical variables of two groups was analyzed by *t*-test as a parametric test and Mann–Whitney *U*-test otherwise. The categorical variables were compared by Chi-square test. Correlation between numeric variables was reported by either Spearman or Pearson correlation coefficient. P <0.05 was considered statistically significant.

Results

The clinical characteristics and hormonal and biochemical parameters are shown in Table 1. There was no significant difference between groups in age and BMI ($P > 0.05$). FPG, insulin levels, and HOMA-IR index values were significantly higher in the study group compared to the control group (87.3 ± 9.1 vs. 81.4±0.7 mg/dL, $P = 0.004$; 11.9 ± 5 vs. 3.7 ± 1.5, $P < 0.001$; and 2.6 ± 1.2 vs. 0.8 ± 0.3, $P < 0.001$, respectively). Free and total testosterone levels (2.5 ± 0.1 vs. 1.3 ± 0.4 pg/mL, $P < 0.001$, and 0.62 ± 0.25 vs. 0.49 ± 0.13 ng/mL, $P = 0.009$, respectively) and clinical hirsutism ($n = 13$ vs. $n = 0$, $P < 0.001$, respectively) were statistically significantly higher in the study group.

Table 1: Clinical and laboratory findings of polycystic ovary syndrome and control group patients

	PCOS (n=45)	Control (n=41)	P
Age (years)	23.9±3.6	24±2.9	0.884
BMI (kg/m^2)	23.2±3.1	22.4±3.1	0.293
Clinical hirsutism, *n* (%)	13 (37.1)	-	<0.001*
FPG (mg/dL)	87.3±9.1	81.4±6.7	0.004*
Fasting insulin (µU/mL)	11.9±5	3.7±1.5	<0.001*
HOMA-IR	2.6±1.2	0.8±0.3	<0.001*
FSH (mIU/L)	5±1.4	7.1±1.3	<0.001*
LH (mIU/L)	7.5±3.5	6.2±1.9	0.061
TSH (µIU/mL)	2.2±1	1.8±0.6	0.073
Total testosterone (ng/mL)	0.62±0.25	0.49±0.13	0.009*
Free testosterone (pg/mL)	2.5±1.1	1.3±0.4	<0.001*
17OH-progesterone (ng/mL)	1.2 (0.4-3.3)	0.9 (0.4-1.9)	0.003*
DHEAS (µg/dL)	259±99.8	230.1±55.9	0.147
Total cholesterol (mg/dL)	167.5±31.1	157.5±25.2	0.157
Triglyceride (mg/dL)	73.5±20.9	77.2±28.8	0.554
HDL (mg/dL)	58.1±13.1	54.2±8	0.160
LDL (mg/dL)	96.9±22.9	88.5±15.8	0.083
VLDL (mg/dL)	15 (8-36)	14 (8.2-34)	0.410
E2 (pmol/L)	43 (23-374)	41 (27-64)	0.292
Homocysteine (µmol/L)	8.7 (4.8-18)	7.7 (5.5-18.6)	0.181
CRP (mg/L)	1.9 (0.3-21.9)	0.9 (0.5-5.4)	0.164

*P<0.05. FPG: Fasting plasma glucose, CRP: C-reactive protein, HOMA-IR: Homeostatic model assessment for insulin resistance, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, TSH: Thyroid-stimulating hormone, DHEAS: Dehydroepiandrosterone sulfate, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very-low-density lipoprotein, BMI: Body mass index, E2: Estradiol, PCOS: Polycystic ovary syndrome

There was no statistically significant difference between lipid profiles (total cholesterol, low-density lipoprotein, HDL, and very-low-density lipoprotein) between the two groups ($P > 0.05$). There was no statistically significant difference in Hcy and CRP levels between the study and control groups, although Hcy and CRP levels were higher in the study group (8.7 [4.8–18] vs. 7.7 [5.5–18.6] µmol/L, $P = 0.181$, and 1.9 [0.3–21.9] vs. 0.9 [0.5–5.4] mg/L, $P = 0.164$, respectively).

According to the correlation analysis, there was no statistically significant correlation between biochemical parameters and Hcy or CRP values ($P > 0.05$). In subgroup analysis, the study group was divided into two subgroups according to BMI (BMI <25 vs. BMI ≥25), insulin resistance (HOMA-IR <2.1 vs. HOMA-IR ≥2.1), and clinical hirsutism (present vs. not present). There was no statistically significant difference in Hcy and CRP levels between subgroups ($P > 0.05$).

Discussion

The main findings of the study were that many biochemical factors thought to cause CVD were higher in patients with PCOS. Hcy and CRP have been accepted as biochemical markers for possible future CVD. Most of the previous literature found that Hcy levels were higher in women with PCOS than in controls. This study also revealed higher Hcy levels in women with PCOS, but the difference was not statistically significant between study and control subjects. Similarly, CRP levels were higher in the study group, but the difference did not reach a statistical significance. Besides, there was no statistically significant correlation between two markers of concern and other biochemical/clinical variables.

PCOS, which is a frequent disease of reproductive-age women, is shown to have a correlation with long-term adverse health outcomes and cardiovascular disorders. There are available data suggesting the increased incidence of CVD in women with PCOS,[11] but the causality has not been elucidated in detail yet. There is a plenty of data in the literature concerning higher Hcy levels in women with PCOS. A meta-analysis concerning CVD markers in PCOS population, including 24 case–control and cross-sectional studies, reported that Hcy levels were statistically significantly higher in subjects with PCOS, although long-term outcomes of this descriptive conclusion were not known.[11] Another meta-analysis concerning levels of oxidative stress markers including Hcy in women with PCOS reported that the presence of PCOS was accompanied by a 23% increment in Hcy levels.[23]

Previous studies reported various interfering factors with Hcy levels such as, folate, Vitamin B6, and B12 levels, smoking status, medications such as antilipidemic drugs and genetic enzymatic deficiencies.[24-27] Interestingly, some of the studies[28-30] have not evaluated these intervening factors as a limitation which might be a possible explanation for conflicting results in the literature about the subject. These factors need to be taken into consideration while evaluating levels of Hcy before reaching clear conclusions. Furthermore, Hcy levels were shown to be higher in subjects with metabolic syndrome[31] which is a quite frequent clinical situation in women with PCOS. However, most of the literature did not adjust their findings for the presence of metabolic syndrome while evaluating Hcy levels. In addition, women with PCOS are classified in four different phenotypes with a different severity of metabolic and cardiovascular outcomes, and some clinical conditions such as metabolic syndrome were more prevalent among PCOS phenotypes with hyperandrogenism.[32] A relatively low number of subjects with clinical and biochemical hirsutism in the current study population might be another possible explanation for the higher but statistically unsignificant difference between the groups. Phenotyping women with PCOS in future studies might serve for more precise conclusions.

Besides CVD risk, high Hcy levels have been associated with adverse fertility outcomes such as lower oocyte and embryo quality, lower fertilization and implantation rates, and adverse fetal outcomes.[33] But also, it has been demonstrated that Hcy levels are significantly correlated with obesity and insulin resistance which have a role in causality of anovulatory infertility in women with PCOS.[34] In this study, there was no statistically significant correlation between Hcy levels and metabolic variables which might be attributed to the relatively normal BMI and young age of the included subjects.

As previously mentioned, CRP has been used to follow up low-grade inflammation which is correlated with CVD risk. A meta-analysis of 48 studies (case–control and cross-sectional) concerning CRP levels in women with PCOS reported that CRP levels were significantly higher in the study group.[11] Contradictory to these findings, although the mean CRP level in women with PCOS was higher than the control group in the current study, there was no statistically significant difference. A point that should be considered is that mean CRP levels of the study population (1.9 [0.3–21.9] mg/L) were classified as intermediate risk (1–3 mg/L)[17] and mean CR*P* values of the control group (0.9 [0.5–5.4] mg/L) would be classified as low risk (<1 mg/L)[17] if these values were taken into consideration for CVD risk assessment defined according to the Centers for Disease Control and Prevention and American Heart Association.[18] CRP levels were affected by the menstrual cycle[16,35] and most of the studies preferred to obtain blood sampling for CRP measurement with other biochemical parameters which were at the early days of the menstrual cycle. We also obtained blood samples in the 2nd and 3rd day of the menstrual cycle, but for both groups, excluding the possible intervening effect of the active menstruation on the CRP values.

Insulin resistance is associated with atherosclerotic processes.[36] Insulin resistance and obesity are seen in women with PCOS, but insulin resistance may be seen equally in both obese and nonobese women with PCOS.[37,38] In our study, insulin resistance levels were higher in women with PCOS than controls. Obesity is another contributing factor for CVD. It is accepted as one of the hallmarks of PCOS which becomes the risk factor for CVD in future.[39] Hcy is increased in both obese and nonobese women with PCOS in accordance with the findings of the current study.

Hyperandrogenism might a possible risk for CVDs. Free testosterone might be a major contributing factor to CVD risk.[40] In addition, abnormal lipid metabolism might be an important factor for CVDs. There were different results in the literature regarding dyslipidemia in women with PCOS. Some studies reported similar lipid parameters in women and PCOS and controls[39] while others reported higher triglyceride and lower HDL levels.[41,42] In our study, although lipid parameters were higher in PCOS, the difference did not reach statistical significance, which might be attributed to the normal mean BMI and young age of the subjects.

The limitations of this study might be evaluated under a few headings. First of all, it was a single-center study. The study consisted of a limited number of patients. Second, it was designed as a descriptive study rather than an interventional study searching for effects of those interventions. Finally, the cross-sectional design of the study does not let to conclude about long-term alterations in the variables of CVD risk.

Conclusion

Many factors important for the development of CVD, including Hcy and CRP, are increased in women with PCOS. Heterogeneous clinical presentation and multiple aspects of PCOS are possibly the main limitations to reach final clear conclusions. Therefore, more comprehensive studies on homogeneous study groups with long-term follow-up are needed.

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Conflict of interest

There are no conflicts of interest.

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