Original Study

The Predictive Role of Serum Cystatin C Levels in Polycystic Ovary Syndrome in Adolescents

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A B S T R A C T

Study Objective: To evaluate the correlation between serum cystatin levels and clinical parameters in adolescents with polycystic ovary syndrome (PCOS).

Design, Setting, Participants, Interventions, and Main Outcome Measures: This prospective case-control study included 89 adolescents with PCOS. Demographic characteristics and hormonal and biochemical parameters were compared between study (89 patients with PCOS) and control (84 subjects without PCOS) groups. Risk factors recorded were age, body mass index (BMI), waist to hip ratio (WHR), Ferriman–Gallwey score, triglyceride, total cholesterol, high-density lipoprotein, low-density lipoprotein (LDL), high-sensitivity C-reactive protein, cystatin C, follicle stimulating hormone, luteinizing hormone, estradiol, dehydroepiandrosterone sulfate, homeostatic model assessment insulin resistance index, free testosterone, and progesterone levels.

Results: BMI, WHR, Ferriman–Gallwey score, and triglyceride, LDL, total cholesterol, estradiol, dehydroepiandrosterone sulfate, free testosterone, luteinizing hormone, high-sensitivity C-reactive protein, and cystatin C levels, and homeostatic model assessment insulin resistance index scores were significantly higher, and high-density lipoprotein levels were lower in the PCOS patients compared with healthy subjects ($P < .05$). We also found positive correlations between the cystatin C levels and BMI, WHR, estradiol, high-sensitivity C-reactive protein, and LDL levels in the study group.

Conclusion: The serum cystatin C level is a promising marker for diagnosing adolescent patients with PCOS and suggests an inflammatory etiology for these patients. Further studies with more participants should examine this potential association with inflammation.

Key Words: Cystatin C levels, Etiology, Inflammation, Polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy causing anovulatory infertility, and it affects approximately 5% of women of reproductive age.[1] The clinical spectrum of PCOS includes hyperandrogenism, menstrual irregularities, and infertility.[2] Rotterdam consensus criteria (2 of the 3 following criteria: oligo or anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic ovaries) are used to diagnose PCOS.[3] PCOS might be a proinflammatory state related to inflammation at the molecular level and insulin resistance.[4,5]

The extracellular cysteine protease inhibitor, cystatin C, belongs to the cystatin superfamily and consists of 122 amino acids. It is a basic, low molecular weight, cationic protein, known for its involvement in intracellular protein catabolism. Recent studies have shown that cystatin C is not only a more sensitive indicator of renal function but also a strong independent predictor of cardiovascular disease, diabetes, and all-cause mortality.[6,7] Cystatin is related to low-intensity inflammation and tumor metastasis.[8]

In this prospective case-control study, we evaluated the association between the clinical parameters of PCOS and serum cystatin levels in adolescents, to examine a possible inflammatory pathogenesis.

Materials and Methods

Ethics Statement

This study was approved by the Ethics Committee of Ankara Zekai Tahir Burak Women's Health Research and Education Hospital. Informed consent was obtained from each participant before enrolling in the study.

Study Population and Design

In this prospective case-control study we enrolled 89 adolescents seen between January 1, 2014 and December 31, 2015 at the outpatient adolescence clinic of Ankara Zekai Tahir Burak Women's Health Research and Education Hospital, a tertiary referral hospital, and a group of 84 healthy control participants.

The World Health Organization defines adolescence as the ages of 10 to 19 years.[9] The control group had normal menstrual cycles, with no evidence of hyperandrogenism and normal ovarian morphology on pelvic ultrasonography examination and a Ferriman–Gallwey score (FGS) of less

The authors indicate no conflicts of interest.

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than 8. The diagnosis of PCOS was on the basis of the Rotterdam criteria.[3] Patients were excluded from the study if they were pregnant, had diabetes, thyroid disease, adrenal, hepatic, or cardiovascular disorders, abnormal renal function (a glomerular filtration rate of <60 mL/min), or other causes of hyperandrogenism, such as hyperprolactinemia, Cushing syndrome, or congenital adrenal hyperplasia. No subjects were treated with corticosteroids, statins, aspirin, oral contraceptives, or any other medication for at least 3 months before study participation.

Individual blood samples were collected from the participants in the morning, after fasting overnight for at least 12 hours. Serum cystatin C and high-sensitivity C-reactive protein (hs-CRP) levels were measured using a sensitive immunonephelometric method (Dade Behring, Marburg, Germany).[10] The assay detection limits were 0.175 mg/L for hs-CRP and 50 ng/mL for cystatin C.

A fasting early morning endocrine profile (including pituitary hormones, ovarian, and adrenal steroids), and serum lipids, glucose, and insulin levels were measured in the follicular phase of a spontaneous or progesterone-induced menstrual cycle. Progesterone levels were measured on the 21st day of the cycle.

Plasma glucose was determined using the glucose oxidase/peroxidase method (Gordion Diagnostic, Ankara, Turkey). Serum levels of follicle-stimulating hormone, luteinizing hormone (LH), estradiol, progesterone, prolactin, dehydroepiandrosterone sulfate (DHEA-S), free testosterone, insulin, cortisol, and thyroid stimulating hormone were measured with specific electrochemiluminescence immunoassays (Elecsys 2010 COBAS; Roche Diagnostics, Mannheim, Germany). The total cholesterol, high-density lipoprotein (HDL), and triglyceride levels were determined with enzymatic colorimetric assays using spectrophotometry (BioSystems, Barcelona, Spain). Low-density lipoprotein (LDL) was calculated using the Friedewald formula. Insulin resistance was calculated using the homeostatic model assessment insulin resistance index (HOMA-IR) according to the formula HOMA-IR = fasting plasma glucose (mmol/L) × fasting serum insulin (mU/mL)/22.5.[11]

Body mass index (BMI; calculated as weight [kg]/height [m^2]) and waist to hip ratio (WHR) were measured in each subject. Hirsutism was evaluated using the FGS. The BMI, WHR, and hirsutism scores were assessed by a single investigator for all subjects (A.T.).

Transvaginal ultrasonography was performed by the same investigator using a 7.5-MHz transvaginal probe and a LOGIQ ultrasound system. Antral follicles were measured in 3 dimensions, and those with a mean diameter of 2 to 9 mm were counted.

Statistical Analyses

Statistical analyses were performed using SPSS 15.0 for Windows (SPSS, Chicago, IL). The mean and SD were calculated for continuous variables. The normality of the variables was analyzed using the Kolmogorov–Smirnov test. An independent samples *t* test was used to evaluate associations between continuous variables. All variables were included in the backward stepwise procedure. The Pearson correlation coefficient was used to test associations between clinical parameters. Receiver operator characteristic (ROC) curve analysis was used to establish the cutoff value for the cystatin levels. Two-sided *P* values were considered statistically significant at *P* less than .05.

Results

In Table 1 the demographic, clinical, and laboratory findings of the subjects are summarized. Patients with PCOS had significantly higher BMI, WHR, and FGS compared with the control participants ($P < .05$). The triglyceride and LDL cholesterol levels were higher and the HDL cholesterol levels were lower in the PCOS patients ($P < .05$). The HOMA-IR index scores and DHEA-S, hs-CRP, free testosterone, and cystatin C levels were significantly higher in the study group ($P < .05$).

Table 1
Comparison of Demographic, Analytical, Clinical, and Laboratory Characteristics between Subjects with and without PCOS

Variable	PCOS (n = 89)	Healthy Volunteers (n = 84)	Test Values
Age, years	18.31 ± 0.21	18.81 ± 0.21	$t = 1.15$; $P = .251$
Body mass index	24.6 ± 0.4	21.3 ± 0.3	$t = 5.26$; $P < .001$
WHR	0.80 ± 0.7	0.77 ± 0.06	$t = 3.34$; $P < .001$
FG score	11.31 ± 0.5	5.11 ± 0.3	$t = 7.61$; $P < .001$
Triglyceride, mg/dL	112.1 ± 6.1	87.3 ± 3.7	$t = 1.81$; $P < .001$
Cholesterol, mg/dL	159.4 ± 3.6	147.3 ± 3.4	$t = 0.41$; $P < .001$
HDL, mg/dL	62.31 ± 1.2	66.2 ± 0.9	$t = 3.09$; $P < .001$
LDL, mg/dL	74.4 ± 2.7	66.3 ± 2.7	$t = 0.89$; $P < .001$
Hs-CRP, mg/L	4.3 ± 0.47	2.8 ± 0.2	$t = 0.86$; $P < .001$
HOMA-IR	2.7 ± 0,1	1.4 ± 0.1	$t = 5.15$; $P < .001$
Cystatin C, ng/mL	0.68 ± 0.01	0.61 ± 0.01	$t = 3.59$; $P < .001$
FSH, mIU/mL	6.62 ± 0.19	6.62 ± 0.26	$t = 0.01$; $P = .98$
LH, mIU/mL	9.24 ± 0.55	6.88 ± 0.59	$t = 2.90$; $P = .004$
Estradiol, mIU/mL	39.61 ± 2.11	26.70 ± 1.61	$t = 4.71$; $P < .001$
DHEA-S, μg/dL	366.02 ± 20.64	272.98 ± 14.62	$t = 3.45$; $P < .001$
f-T, pg/mL	1.83 ± 0.10	1.54 ± 0.08	$t = 2.11$; $P = .036$
Progesterone, ng/mL	1.06 ± 0.35	0.70 ± 0.56	$t = 0.97$; $P = .335$

DHEA-S, dehydroepiandrosterone sulfate; FG, Ferriman–Gallwey; FSH, follicle-stimulating hormone; f-T, free testosterone; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment insulin resistance index; Hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein cholesterol; LH, luteinizing hormone; PCOS, polycystic ovary syndrome; WHR, waist to hip ratio
Data are presented as mean ± SD except where otherwise noted. $P < .05$ is accepted to be statistically significant.

Table 2
Correlation between CC and Variables

Variable	BMI		Estradiol		CRP		WHR		LDL	
	CC	*P*	CC	*P*	CC	*P*	CC	*P*	CC	*P*
Cystatin C	0.168	.027	0.308	<.001	0.224	.021	0.346	.046	0.311	<.001

BMI, body mass index; WHR, waist to hip ratio
$P < .05$ is accepted to be statistically significant.

Correlation analysis identified positive correlations between the cystatin C levels and BMI, WHR, estradiol, LDL, and hs-CRP in the study group (Table 2).

According to the ROC curve analysis (Fig. 1), the cystatin C level was a discriminative parameter in PCOS patients. The area under the curve, cutoff value, sensitivity, and specificity are listed in Table 3. The cutoff value for cystatin was 0.54 ng/L, with a sensitivity of 92.1% and a specificity of 76.7%.

Discussion

In this study we evaluated the possible association between inflammation and the pathogenesis of PCOS. To our knowledge, this is the first study to evaluate the association between adolescent PCOS and the proinflammatory marker cystatin C. The clinical parameters evaluated were age, BMI, WHR, FGS, triglyceride, total cholesterol, HDL, LDL, hs-CRP, cystatin C, follicle-stimulating hormone, LH, estradiol, HOMA-IR, DHEA-S, free testosterone, and progesterone levels.

The BMI, WHR and FGS, triglyceride, LDL, total cholesterol, estradiol, DHEA-S, free testosterone, LH, hs-CRP, cystatin C, and HOMA-IR were significantly higher and the HDL cholesterol levels were lower compared with healthy subjects ($P < .05$). We also found positive correlations between the cystatin C levels and BMI, WHR, estradiol, hs-CRP, and LDL levels in the study group. According to the ROC curve analysis, the cystatin C level was a discriminative parameter in PCOS patients.

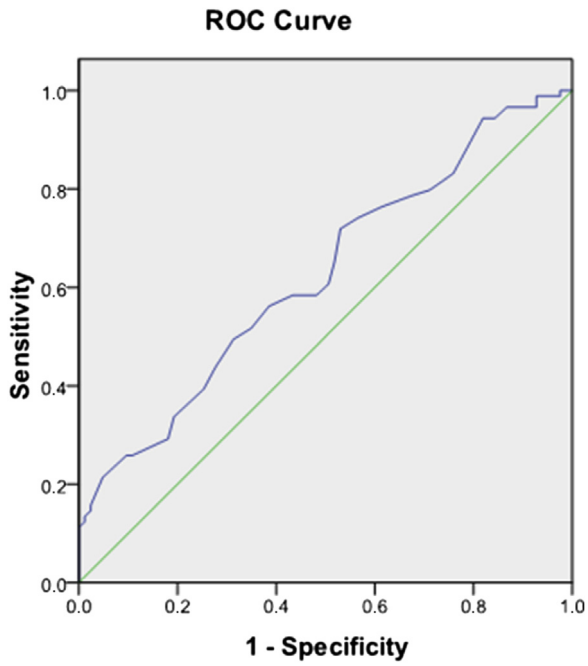

Fig. 1. Receiver operator characteristic (ROC) curve for the cystatin C variable (area under the curve, 0.625; $P < .0001$; 95% confidence interval, 0.542-0.708) revealing its diagnostic potential for adolescent polycystic ovary syndrome.

The etiology of PCOS, which is one of the most common endocrine disorders in women of reproductive age, is associated with chronic anovulation, insulin resistance, and androgen excess.[12,13] PCOS has also been linked to insulin resistance and cardiovascular disease; both of these complications are related to inflammation, which is thought to be the key feature in endothelial dysfunction and atherosclerosis.[14] This low-intensity inflammation sets the background for the development of dyslipidemias and atherosclerosis.[15]

Roe et al[16] found higher cholesterol levels among PCOS patients, but reported no correlation between androgen levels and increased cholesterol levels. Further, they found no correlations between inflammation markers and increased androgen levels. Similarly, we did not find any correlations between inflammation markers, such as hs-CRP and cystatin C levels, and increased androgen levels and FGS in adolescents with PCOS.

In a study of the association between insulin resistance and lipid profile and metabolic syndrome in patients with PCOS, Mamaghani et al[17] found a significant correlation between higher LDL cholesterol levels and increased androgen levels. We also found a significant correlation between higher LDL levels and cystatin C levels. This result supports the role of inflammation in the etiology and pathogenesis of PCOS.

Cystatin C is a cysteine protease inhibitor produced by all nucleated cells. Recent studies show that in inflammation, cystatin C decreases endogenous cysteine protease and neutrophil migration activity. Cystatin is an early determinant of cardiovascular disease.[18,19] Gozashti et al[20] found that cystatin had a prognostic value in patients with PCOS and suggested that it might be a useful clinical marker providing complementary information to establish risk determinants. The positive correlation between hs-CRP and cystatin C levels found in our study supports the suggestion that the cystatin C level is a good predictor of inflammation in patients with PCOS.

Table 3
The AUC, Cutoff Values, and Sensitivity and Specificity for Cystatin Levels in Patients with Adolescent PCOS

	AUC	SE	95% CI	Cutoff Value	Sensitivity-Specificity, %
Cystatin C	0.625	0.042	0.542-0.708	0.54	92.1-76.7

AUC, area under the curve; CI, confidence interval; PCOS, polycystic ovary syndrome; SE, standard error

In a previous study, cystatin C was associated with PCOS status, but not with systolic and diastolic blood pressure or any of the lipid profile variables or demographic characteristics. This suggests that the information provided by cystatin is not just a marker of glomerular filtration. Burrows et al[21] showed that adolescent PCOS patients without cardiovascular risk factors have subclinical early coronary atherosclerosis. On the basis of our results, we postulate that cystatin C is one of the early predictors of adverse clinical outcomes in adolescent PCOS, along with increased BMI, WHR, HOMA-IR, triglyceride, and cholesterol levels.

In conclusion, the cystatin C level appears to be a promising marker of complications such as insulin resistance and cardiovascular disease, both related to inflammation. The limitations of our study are a small number of participants and a nonrandomized study design. Further studies with more participants and a randomized design should provide more information on the role of cystatin C in patients with PCOS.

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