Observational Study

Medicine

OPEN

Serum calprotectin levels as markers of inflammation, insulin resistance and hyperandrogenism in women with polycystic ovary syndrome

Necati Hancerliogulları, MD[a,*], Aytekin Tokmak, MD[a], Gurhan Guney, MD[b], Meryem Kuru Pekcan, MD[a], Esin Merve Koc, MD[a], Tuba Candar, MD[c], Yaprak Engin Ustün, MD[a]

Abstract

Polycystic ovary syndrome (PCOS) is a common, complex endocrine, and metabolic disorder. Inflammation has been thought to play an important role in PCOS pathogenesis in recent years, and various inflammatory markers have been investigated; however, no definite conclusion has been reached. As a multifunctional regulatory protein in different inflammatory processes, calprotectin may play a role in the etiology of PCOS. Therefore, based on this hypothesis, we aimed to determine serum calprotectin concentrations in women with PCOS and to compare them with healthy controls. This cross-sectional study was conducted at a tertiary referral center during the study period. Forty-three women (n = 43) with PCOS and 47 women (n = 47) in the control group were enrolled in this cross-sectional study. Serum calprotectin concentrations were measured using enzyme-linked immunosorbent assay and compared with markers of glucose and lipid metabolism. Clinical characteristics and hormonal parameters were evaluated in both groups. Levels of serum calprotectin were measured as 347 ± 28.8 and 188 ± 15.3 ng/mL in the PCOS and healthy control groups, respectively ($P = .009$). The mean homeostatic model assessment for insulin resistance[1] index and total testosterone levels were significantly higher in the PCOS group than in the control group (both $P < .001$). Spearman's correlation test demonstrated linear correlations between calprotectin and C-reactive protein, waist circumference, insulin resistance index, and total testosterone levels in the PCOS group (all $P < .05$). Serum calprotectin levels were higher in women with PCOS. This biomarker may be an indirect sign of insulin resistance, hyperandrogenism, or chronic inflammation in women with PCOS.

Abbreviations: BMI = body mass index, CRP = C-reactive protein, DHEA-S = Dehydroepiandrosterone Sulfate, HC = hip circumference, HOMA IR = Homeostatic Model Assessment for Insulin Resistance, LH = luteinizing hormone, PCOS = polycystic ovary syndrome, TT = total testosterone, WC = Waist Circumference.

Keywords: Calprotectin, chronic inflammation, hyperandrogenism, insulin resistance, polycystic ovary syndrome

1. Introduction

Polycystic ovary syndrome (PCOS) is a common, complex endocrine and metabolic disorder affecting 12% to 18% of reproductive age women, and due to the Rotterdam ESHRE/ASRM criteria, is characterized by anovulation, hyperandrogenism, and polycystic ovarian morphology.[2] Insulin resistance, dyslipidemia, abdominal obesity, and atherosclerosis are more common in women with PCOS than in healthy women. Long-term metabolic effects are thought to be related to hyperinsulinemia, hyperandrogenism, and low-grade chronic inflammation in women with PCOS.[3]

Calprotectin, an antimicrobial protein in the cytoplasm of neutrophil granulocytes, is a multifunctional regulator protein in different inflammatory processes that is released from intracellular cytoplasmic granules when endothelial adhesion of monocytes or neutrophil activation occurs.[4] PCOS is closely related to chronic inflammation in the adipose tissue and considering the activation of monocytes and neutrophils during inflammation, calprotectin secreted from these cells may also be associated with PCOS.[5,6]

Recent studies have shown that this inflammatory environment may be associated with adverse metabolic events in PCOS, such as insulin resistance and cardiovascular disorders, as this inflammation simultaneously impairs ovarian steroidogenesis and folliculogenesis. Based on this relationship, we hypothesized that the serum levels of the inflammatory marker calprotectin may play a role in the chronic inflammatory milieu in PCOS and may be linked to insulin resistance and hyperandrogenemia, which are common endocrinological problems associated with PCOS.[7]

The authors have no funding and conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

[a] *Department of Obstetrics and Gynecology, Bilkent City Hospital, University of Health Sciences, Ankara, Turkey,* [b] *Department of Reproductive Endocrinology and Infertility, Balikesir University Medical Faculty, Balikesir, Turkey,* [c] *Department of Clinical Biochemistry, School of Medicine, Ufuk University, Ankara, Turkey.*

** Correspondence: Necati Hancerliogullari, Department of Obstetrics and Gynecology, Bilkent City Hospital, University of Health Sciences, Universiteler Mahallesi 1604.cadde no:9, Cankaya Ankara 06800, Turkey (e-mail: necatihancerliogullari@gmail.com).*

Copyright © 2022 the Author(s). Published by Wolters Kluwer Health, Inc.

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Hancerliogulları N, Tokmak A, Guney G, Pekcan MK, Koc EM, Candar T, Ustün YE. Serum calprotectin levels as markers of inflammation, insulin resistance and hyperandrogenism in women with polycystic ovary syndrome. Medicine 2022;101:51(XXX).

Received: 22 October 2022 / Received in final form: 24 November 2022 / Accepted: 29 November 2022

http://dx.doi.org/10.1097/MD.0000000000032326

2. Methods

2.1. Study design

In our sample size analysis using the GPower software (Universitat Kiel, Kiel, Germany), we found that at least 42 patients in each group (α: 0.05, effect size: 0.80) were required to achieve a power of 95% (1 – β: 0.95). We performed this cross-sectional study by selecting and comparing 43 PCOS patients with 47 healthy age- and body mass index (BMI)-matched controls who visited our outpatient gynecology clinic between June 2017 and September 2017. Our study complied with the requirements of the Declaration of Helsinki, and the Ethics Committee of Dr Zekai Tahir Burak Training and Research Hospital approved this study (number 37/2018). All patients provided written informed consent before participating in the study and we selected our study participants randomly in order to avoid bias. According to the European Society of Human Reproductive and Embryology and the American Society of Reproductive Medicine (Rotterdam ESHRE/ASRM—Sponsored PCOS Consensus Working Group) decision in 2004, PCOS was defined as the presence of two of the following 3 criteria: polycystic appearance of the ovaries on ultrasound imaging, presence of 12 or more follicles with a diameter of 2 to 9 mm and ovarian volume >10 cm^3, biochemical and clinical manifestations of anovulation, oligo-ovulation, and hyperandrogenism. Clinical hyperandrogenism was defined as a Ferriman-Gallwey score above 8, whereas biochemical hyperandrogenism was defined as a total testosterone (TT) and/or dehydroepiandrosterone sulfate (DHEA-S) level above the 95th percentile. Oligomenorrhea was defined as a menstrual cycle duration of more than 45 days, and amenorrhea was defined as the absence of menstruation for more than 3 sequential months.

2.2. Participants

Adolescents under the age of 19 and patients over the age of 40 were not included in the study. The same investigator evaluated all subjects using the same ultrasound device. In addition, thin patients with a BMI below 18, obese patients with a BMI over 30, those with acute and/or chronic diseases, those with endocrinological and autoimmune diseases, patients with any hematological disorders, and patients with liver and/or kidney failure were excluded from the study.

2.3. Laboratory Studies

Each subject provided blood at 9 AM after an overnight fast on the 2nd or 3rd day of the menstrual cycle and was centrifuged at 4000/rpm for 10 minutes as soon as the blood samples were collected. While there was fasting glucose, insulin and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) values, lipid profile (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides), calprotectin, and C-reactive protein (CRP) in the biochemical evaluation, the main basal hormones such as follicle stimulating hormone, luteinizing hormone (LH), estradiol, TT, DHEA-S, 17-hydroxyprogesterone, thyroid stimulating hormone, and prolactin were in the hormonal evaluation.

Complete blood count parameters were included in the hematological evaluation. The glucose hexokinase method was used to measure serum glucose concentration (Roche Hitachi Cobas 6000; Roche Diagnostics GmbH, Mannheim,Germany). We measured basal hormone levels, such as follicle stimulating hormone, LH, estradiol, TT, DHEA-S, thyroid stimulating hormone, and prolactin, using an electrochemiluminescence immunoassay (Roche Cobas e 601; Roche Diagnostics GmbH). The colorimetric method (Beckman Coulter Fullerton, CA) was used to measure all lipid profile parameters (total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides).

C-reactive protein was measured by nephelometry using a BN II System (Erlangen,Germany). To measure insulin resistance, HOMA-IR was calculated using the following formula: fasting glucose (mmol/l) × fasting insulin × 0.055/22.5.[8] A HOMA-IR index greater than 2.5 was accepted as insulin resistance.[9] A Coulter LH-780 hematology blood analyzer (Beckman Coulter Inc., Brea, CA) was used to measure complete blood count parameters. A human CALP (Calprotectin) enzyme-linked immunosorbent assay kit (catalog number: E-EL-H2357; Elabscience, Houston,TX) was used for calprotectin measurement using a Chemwell 2900 Chemistry Analyzer (Awareness Technology, Inc., Palm City, FL). Intra-assay and inter-assay variation of coefficient values were 3.90 and 4.74 ng/mL, respectively.

2.4. Statistical analysis

We used SPSS version 25.0, (Chicago, IL) for statistical analysis of the data. We used the Shapiro–Wilk test to assess whether the data were normally distributed. We evaluated the homogeneity of the variance using Levene's test. Statistical evaluation was expressed as the standard deviation from the mean or number (%). To compare the groups, we used the *t* test for independent variables with normal distribution and the Mann-Whitney *U* test for those without normal distribution. The chi-square test was used to compare categorical variables between groups. Spearman's correlation analysis was used to examine correlations between variables. We used a 2-tailed perception rate for statistical hypothesis evaluations.

3. Results

This study included 43 PCOS patients and 47 non-PCOS control patients. The mean age of the patients with PCOS

Table 1

Clinical parameters in PCOS patients and controls.

Variables	PCOS (n = 43)	Control (n = 47)	P
Age (yr)**	28.5 ± 5.6	29.3 ± 5.4	.145
BMI (kg/m^2)*	25.1 ± 3.6	24.8 ± 3.2	.234
WC (cm)*	84.6 ± 7.7	80.3 ± 8.3	.299
HC (cm)*	100.3 ± 6.2	100.2 ± 5.8	.125
WHR*	0.83 ± 0.07	0.82 ± 0.07	.753
FGS**	12.2 ± 6.1	5.3 ± 3.8	<.001

Data are shown as mean ± SD. Groups were compared using Student's *t* test and **Mann-Whitney *U* test, as appropriate. Statistical significance was defined as $P < .05$.

BMI = body mass index, FGS = Ferriman-Gallwey score, HC = hip circumference, PCOS = polycystic ovary syndrome, WC = waist circumference, WHR = waist-to-hip ratio.

* Student's t-test.

** Mann-Whitney U-test.

was 28.5 ± 5.6 years, and the mean age of the control patients was 29.3 ± 5.4 years and with no significant difference between the groups (P = .145). There were no significant differences in the mean values of waist-to-hip ratio, hip circumference (HC),[10] BMI, and waist circumference (WC)[11] between the PCOS and control groups (P > .05). Table 1 shows the demographic and anthropometric characteristics of all patients. The basal hormone levels and biochemical markers are shown in Table 2. Serum levels of calprotectin were measured as 347 ± 28.8 in the PCOS group and 188 ± 15.3 ng/mL in the control group, respectively (P = .009). The mean values of TT and HOMA-IR index in the PCOS group were significantly higher than those in the control group (P < .001). There were 22 patients with insulin resistance and 21 without insulin resistance in the PCOS group. The mean calprotectin concentrations were 432 ± 31.2 and 255 ± 22.4 ng/mL in PCOS patients with and without insulin resistance, respectively (P = .017). While the mean serum calprotectin levels of 8 patients with insulin resistance (HOMA-IR index > 2.5) in the control group were 207 ± 12.3, the mean serum calprotectin levels of 38 patients in the control group without insulin resistance (HOMA-IR index < 2.5) were in the range of 193 ± 16.9 and there was no statistically significant difference in serum calprotectin levels between subjects with and without insulin resistance in the control group (P = .821).

Spearman's correlation analysis showed that calprotectin levels were positively correlated with CRP, WC, HOMA-IR, and TT values in the PCOS group (all P < .05). However, we found that age, estradiol level, and neutrophil-to-lymphocyte ratio did not correlate well with serum calprotectin levels, as shown in Table 3. The receiver operating characteristic curve analysis demonstrated a modest discriminative value for PCOS. The area under the receiver operating characteristic curve of serum calprotectin for predicting PCOS was 0.660 with a 95% confidence interval of 0.547 to 0.773 (P = .009), as shown in Figure 1.

4. Discussion

In this study, we aimed to evaluate whether serum calprotectin concentration was elevated in patients with PCOS and whether there was a correlation between serum calprotectin levels and insulin resistance indices and hyperandrogenism. According to our findings, serum calprotectin levels were higher in patients compared to the healthy controls. HOMA-IR and TT levels were significantly and positively correlated with serum calprotectin concentrations.

Many studies have shown that there is a significant relationship between PCOS and inflammation, and that PCOS cases with high CRP, which is one of the indicators of inflammation, have a high risk of developing type 2 DM and cardiovascular disease, regardless of whether they were obese or not.[12,13] In another study by Ganie et al,[14] it was stated that there was no elevation of CRP levels in PCOS and that CRP might have increased if there was an abnormality in BMI and other anthropometric measurements. In our study, there was no significant difference in either BMI or CRP levels between the groups. Another study stated that the inflammatory and anti-inflammatory balance was impaired in PCOS, and many cytokines that provided this balance might have played a role in PCOS pathogenesis.[15] Calprotectin, which may have a role as an inflammatory marker in PCOS had a positive correlation with CRP in our study and this finding made us think that there might be a subclinical inflammatory process that has not yet been reflected in CRP levels in our PCOS cases.

There are 2 studies in the current literature on the relationship between PCOS and calprotectin levels. In the first study, Chen et al[16] found that serum calprotectin levels were significantly increased in PCOS patients and positively correlated with the HOMA-IR index, similar to our study. However, its relationship with hyperandrogenism was not indicated in this study, unlike that in our study. Hyperandrogenemia itself causes a low-grade chronic inflammatory state, and at the same time this inflammatory state stimulates androgen production in the ovaries as shown in both in vivo and in vitro conditions.[17,18] In vitro

Table 2

Comparison of the biochemical and endocrinological features of the groups.

Variables	PCOS (n = 43)	Control (n = 47)	*P*
FBG (mg/dL)*	89.0 ± 5.8	86.0 ± 6.6	.025
FBI (mU/L)**	13.8 ± 6.7	5.1 ± 3.2	<.001
HOMA-IR**	3.1 ± 2.3	1.7 ± 0.5	<.001
HDL-C*	61.5 ± 7.6	64.6 ± 8.1	.251
TG**	105.2 ± 54.9	102.2 ± 53.1	.860
LDL-C**	76.2 ± 31.8	62.7 ± 23.4	.061
TC*	158.3 ± 37.1	146.1 ± 29.4	.048
LH (U/L)**	9.8 ± 6.9	7.3 ± 6.0	.036
FSH (U/L)**	6.5 ± 1.8	7.1 ± 1.8	.096
E2 (pg/mL)**	39.4 ± 10.0	38.5 ± 16.3	.806
TSH (U/L)**	2.3 ± 1.0	2.2 ± 1.0	.888
PRL (ng/mL)**	16.5 ± 9.1	15.3 ± 7.2	.062
DHEAS (mg/dL)**	370.9 ± 181.1	327.1 ± 149.3	.282
Total-T**	0.6 ± 0.2	0.4 ± 0.2	<.001
17OH-P (ng/mL)**	1.4 ± 1.2	1.3 ± 0.7	.507
CRP (mg/L)**	3.8 ± 2.5	2.9 ± 2.3	.786
WBC (10^3/mL)*	8.7 ± 2.5	8.5 ± 2.9	.903
Neutrophil (%)*	61.0 ± 9.9	61.6 ± 11.6	.787
Lymphocyte (%)**	28.5 ± 8.8	29.3 ± 9.8	.928
NLR**	2.6 ± 1.6	2.5 ± 1.3	.750
Calprotectin (ng/mL)**	347 ± 28.8	188.8 ± 15.3	.009

Data are shown as mean ± SD. Groups were compared using Student's *t* test and **Mann-Whitney *U* test, as appropriate. Statistical significance was defined as P < .05.

17OH-P = 17-hydroxyprogesterone, CRP = serum C-reactive protein, DHEAS = dehydroepiandrosterone sulfate, E2 = estrogen, FBG = fasting blood glucose, FBI = fasting blood insulin, FSH = follicle-stimulating hormone, HDL = high-density lipoprotein, HOMA-IR = homeostatic model assessment of insulin resistance, LDL = low-density lipoprotein, LH = luteinizing hormone, NLR = neutrophil-lymphocyte ratio, PCOS = polycystic ovary syndrome, PRL = prolactin, TC = total cholesterol, TG = triglyceride, total-T = free testosterone, TSH = thyroid-stimulating hormone, WBC = white blood cell.

* Student's t-test.

** Mann-Whitney U-test.

Table 3

Correlation of calprotectin and other variables in PCOS group.

Variable		
Age	*r*	0.044
	P	.697
BMI	*r*	0.273
	P	.192
WC	*r*	0.212
	P	.045
HC	*r*	0.206
	P	.041
WHR	*r*	0.072
	P	.570
Neutrophil	*r*	0.048
	P	.708
Estradiol	*r*	0.075
	P	.515
DHEA-S	*r*	0.139
	P	.262
Glukose	*r*	0.054
	P	.616
CRP	*r*	0.422
	P	.151
NLR	*r*	0.060
	P	.638
TT	*r*	0.245
	P	.021
HOMA-IR	*r*	0.295
	P	.005

Statistical significance was defined as $P < .05$.
BMI = body mass index, CRP = serum C-reactive protein, DHEA-S = dehydroepiandrosterone sulfate, HC = hip circumference, HOMA-IR = Homeostatic Model Assessment for Insulin Resistance, NLR = neutrophil-lymphocyte ratio, PCOS = polycystic ovary syndrome, *r* = correlation coefficient, TT = total testosterone, WC = waist circumference, WHR = waist-to-hip ratio.

studies have shown that proinflammatory stimuli also stimulate steroidogenesis in ovarian cells.[19] Ovarian steroidogenic enzymes are regulated by proinflammatory stimuli and inhibited by anti-inflammatory agents (resveratrol and statins).[20] These findings suggest that inflammation directly stimulates androgens in PCOS patients. In our study, a significant relationship was observed between testosterone and calprotectin levels. This supports the assertion that high androgen levels can increase inflammation or vice versa.

In a second study on the relationship between calprotectin and PCOS, Li et al[21] found that exosomes carrying S100 calcium-binding protein A9 (Calprotectin) were able to activate the nuclear factor kappa B pathway, increase inflammation, and disrupt steroidogenesis, which is probably involved in the occurrence of PCOS. In addition, they stated that calprotectin is not only secreted into granulosa cells and follicle fluid but can also be secreted into distal regions. In our own study, we found high blood levels of calprotectin in PCOS patients, but if we had analyzed the follicle fluid, we could have found it to be high as well.

We also found a positive correlation between calprotectin and WC and HC, but not between calprotectin and BMI. Although calprotectin levels have been shown to be correlated with BMI in a number of studies, a number of studies have shown that BMI might not always correlate well with visceral adipose tissue mass.[22–24] The calprotectin levels in our study had a positive correlation with WC and HC, suggesting that adipose tissue might be responsible for the increased calprotectin levels in PCOS patients. Likewise, Catalán et al[25] stated that calprotectin, which is secreted from visceral adipose tissue, might be responsible for the sequestration of macrophages to the adipose tissue and the subsequent inflammation due to its chemotactic property. Some studies have revealed that inflammation in the adipose tissue of non-obese PCOS patients causes insulin resistance by disrupting the insulin-signaling mechanism. In related studies, hyperglycemia resulting from insulin resistance was the main substrate used by mononuclear cells, and reactive oxygen molecules released after glucose consumption induced the release of proinflammatory mediators.[17,26] Similar to the studies mentioned above, the PCOS patients in our study were not obese, had high HOMA-IR indices, and showed a strong correlation between calprotectin and HOMA-IR levels.

The major drawback of our study was its cross-sectional design. The patients also belonged to only 1 ethnic group. Blood samples were collected in the early follicular phase. Repeated sampling of serum calprotectin levels was not performed during the menstrual cycle, and we were unable to check its reproducibility. Additionally, the assay method may have affected the final results. Further prospective studies that longitudinally observe patients in larger groups should be performed to validate the clinical usability of calprotectin levels in PCOS. In conclusion, serum calprotectin levels were significantly higher in normal-weight patients with PCOS than in controls; insulin resistance and hyperandrogenism were also more prevalent in normal-weight patients with PCOS. Although other nonspecific inflammatory markers were similar between the groups, calprotectin levels were higher in PCOS patients and were significantly correlated with adipose tissue, insulin resistance, and hyperandrogenemia. Calprotectin may be an indicator of an inflammatory state in PCOS patients and may be used as an adjunct diagnostic marker for hormonal and metabolic disturbances.

Calprotectin levels can be studied in a large group of patients with PCOS, and if a strong relationship with inflammation is detected, the use of anti-inflammatory agents may also be demonstrated. This situation should be considered when treating PCOS patients.

Acknowledgements

We are grateful to all the study participants.

Author contributions

NH, AT, and GG contribute to the conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, and writing the original manuscript. MKP and EMK contribute to the data curation, methodology and software. TC contributes to formal analysis and investigation. YEU contributes to the supervision and writing and editing the manuscript.

Conceptualization: Necati Hancerliogullari, Aytekin Tokmak.
Data curation: Necati Hancerliogullari, Aytekin Tokmak, Esin Merve Koc.
Formal analysis: Necati Hancerliogullari, Aytekin Tokmak, Tuba Çandar.
Funding acquisition: Necati Hancerliogullari, Aytekin Tokmak.
Investigation: Necati Hancerliogullari, Aytekin Tokmak, Meryem Kuru Pekcan, Tuba Çandar.
Methodology: Necati Hancerliogullari, Aytekin Tokmak, Meryem Kuru Pekcan, Esin Merve Koc.
Project administration: Necati Hancerliogullari, Aytekin Tokmak, Meryem Kuru Pekcan.
Resources: Necati Hancerliogullari, Aytekin Tokmak, Meryem Kuru Pekcan, Esin Merve Koc.
Software: Necati Hancerliogullari, Aytekin Tokmak, Meryem Kuru Pekcan, Esin Merve Koc.
Supervision: Necati Hancerliogullari, Aytekin Tokmak, Gurhan Guney, Yaprak Engin Ustün.
Validation: Necati Hancerliogullari.
Visualization: Necati Hancerliogullari.
Writing – original draft: Necati Hancerliogullari, Gurhan Guney.
Writing – review & editing: Gurhan Guney, Yaprak Engin Ustün.

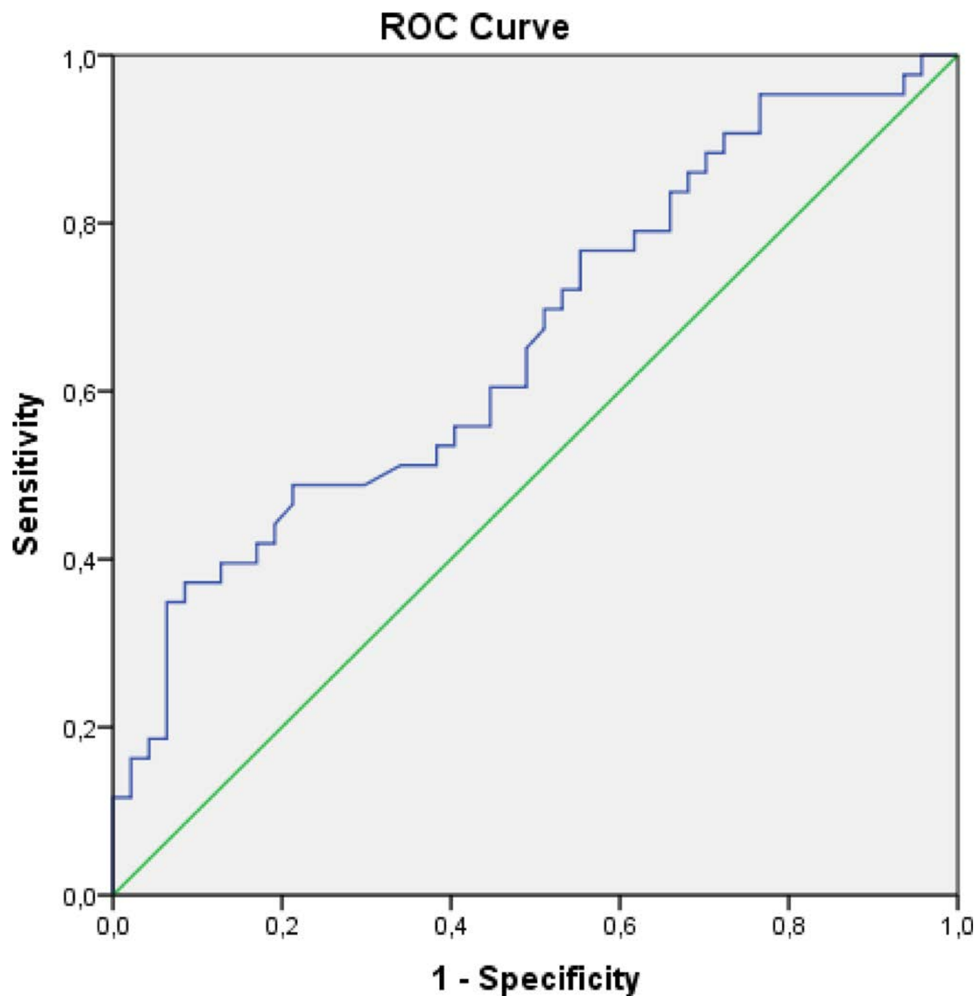

Figure 1. ROC curve analysis of serum calprotectin concentrations for discriminating PCOS cases. PCOS = polycystic ovary syndrome, ROC = receiver operating characteristic.

References

[1] Fernando S, Wallace EM, Rombauts L, et al. The effect of melatonin on ultrasound markers of follicular development: a double-blind placebo-controlled randomised trial. Aust N Z J Obstet Gynaecol. 2020;60:141–8.

[2] Teede HJ, Misso ML, Costello MF, et al.; International PCOS Network. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Fertil Steril. 2018;110:364–79.

[3] Anagnostis P, Tarlatzis BC, Kauffman RP. Polycystic ovarian syndrome (PCOS): long-term metabolic consequences. Metabolism. 2018;86:33–43.

[4] Shabani F, Farasat A, Mahdavi M, et al. Calprotectin (S100A8/S100A9): a key protein between inflammation and cancer. Inflamm Res. 2018;67:801–12.

[5] Guney G, Taskin MI, Baykan O, et al. Endotrophin as a novel marker in PCOS and its relation with other adipokines and metabolic parameters: a pilot study. Ther Adv Endocrinol Metab. 2021;12:20420188211049607.

[6] Rudnicka E, Suchta K, Grymowicz M, et al. Chronic low grade inflammation in pathogenesis of PCOS. Int J Mol Sci. 2021;22:3789.

[7] Moghetti P, Tosi F. Insulin resistance and PCOS: chicken or egg? J Endocrinol Invest. 2021;44:233–44.

[8] Lisowska-Myjak B, Skarżyńska E, Płazińska M, et al. Relationships between meconium concentrations of acute phase proteins. Clin Exp Pharmacol Physiol. 2018;45:1218–20.

[9] Tang Q, Li X, Song P, et al. Optimal cut-off values for the homeostasis model assessment of insulin resistance (HOMA-IR) and pre-diabetes screening: developments in research and prospects for the future. Drug Discov Ther. 2015;9:380–5.

[10] Reichart B, Meiser B, Vigano M, et al. European Multicenter Tacrolimus (FK506) Heart Pilot Study: one-year results – European Tacrolimus Multicenter Heart Study Group. J Heart Lung Transplant. 1998;17:775–81.

[11] Bernardi L, Valenti C, Wdowczyck-Szulc J, et al. Influence of type of surgery on the occurrence of parasympathetic reinnervation after cardiac transplantation. Circulation. 1998;97:1368–74.

[12] Rostamtabar M, Esmaeilzadeh S, Tourani M, et al. Pathophysiological roles of chronic low-grade inflammation mediators in polycystic ovary syndrome. J Cell Physiol. 2021;236:824–38.

[13] Khichar A, Gupta S, Mishra S, et al. Assessment of inflammatory markers in women with PCOS and their correlation with insulin resistance. Clin Lab. 2021;67.

[14] Ganie MA, Hassan S, Nisar S, et al. High-sensitivity C-reactive protein (hs-CRP) levels and its relationship with components of polycystic ovary syndrome in Indian adolescent women with polycystic ovary syndrome (PCOS). Gynecol Endocrinol. 2014;30:781–4.

[15] Abraham Gnanadass S, Divakar Prabhu Y, Valsala Gopalakrishnan A. Association of metabolic and inflammatory markers with polycystic ovarian syndrome (PCOS): an update. Arch Gynecol Obstet. 2021;303:631–43.

[16] Chen S, Jiang M, Ding T, et al. Calprotectin is a potential prognostic marker for polycystic ovary syndrome. Ann Clin Biochem. 2017;54:253–7.

[17] González F. Inflammation in polycystic ovary syndrome: underpinning of insulin resistance and ovarian dysfunction. Steroids. 2012;77:300–5.
[18] Aboeldalyl S, James C, Seyam E, et al. The role of chronic inflammation in polycystic ovarian syndrome-A systematic review and meta-analysis. Int J Mol Sci. 2021;22:2734.
[19] Zafari Zangeneh F, Naghizadeh MM, Masoumi M. Polycystic ovary syndrome and circulating inflammatory markers. Int J Reprod Biomed. 2017;15:375–82.
[20] Ortega I, Villanueva JA, Wong DH, et al. Resveratrol potentiates effects of simvastatin on inhibition of rat ovarian theca-interstitial cells steroidogenesis. J Ovarian Res. 2014;7:21.
[21] Li H, Huang X, Chang X, et al. S100-A9 protein in exosomes derived from follicular fluid promotes inflammation via activation of NF-κB pathway in polycystic ovary syndrome. J Cell Mol Med. 2020;24:114–25.
[22] Al-Ghadban S, Bunnell BA. Adipose tissue-derived stem cells: immunomodulatory effects and therapeutic potential. Physiology (Bethesda). 2020;35:125–33.
[23] Nijhuis J, Rensen SS, Slaats Y, et al. Neutrophil activation in morbid obesity, chronic activation of acute inflammation. Obesity. 2009;17:2014–8.
[24] Xia MF, Chen Y, Lin HD, et al. A indicator of visceral adipose dysfunction to evaluate metabolic health in adult Chinese. Sci Rep. 2016;6:38214.
[25] Catalán V, Gómez-Ambrosi J, Rodríguez A, et al. Increased levels of calprotectin in obesity are related to macrophage content: impact on inflammation and effect of weight loss. Mol Med. 2011;17:1157–67.
[26] Maktabi M, Jamilian M, Asemi Z. Magnesium-Zinc-Calcium-Vitamin D Co-supplementation improves hormonal profiles, biomarkers of inflammation and oxidative stress in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Biol Trace Elem Res. 2018;182:21–8.