



OPEN Evaluation of serum calprotectin levels in patients with polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age. PCOS is associated with many chronic complications such as endometrial thickening, endometrial cancer, breast cancer, infertility, dyslipidemia, and long-term cardiovascular disease. Therefore, many parameters and markers should be used in the evaluation of patients diagnosed with PCOS. Calprotectin has also been shown to be an important marker of destruction in many diseases that progress with inflammatory processes. The objective of our study was to examine the diagnostic efficacy of calprotectin in the context of polycystic ovary syndrome. In this study, 39 patients diagnosed with polycystic ovary syndrome and 41 healthy controls were analyzed. Serum calprotectin levels were compared between patients with different phenotypes of PCOS and a control group. Our study demonstrates significant differences in hormonal and inflammatory parameters between PCOS patients and healthy controls. PCOS patients exhibited significantly lower FSH levels and higher LH levels, resulting in a marked increase in the LH/FSH ratio. Additionally, DHEA-S and total testosterone levels were significantly elevated in the PCOS group, while SHBG levels were notably lower. The free androgen index (FAI) and serum calprotectin levels were also significantly higher in the PCOS group, highlighting the potential role of calprotectin in the pathophysiology of the disorder. Our study demonstrated that serum calprotectin levels are significantly higher in patients with PCOS compared to healthy controls. This finding underscores the potential role of calprotectin in the pathophysiology of PCOS and suggests that this marker could provide valuable insights into the inflammatory processes associated with the condition. However, further large-scale and long-term studies are necessary to better understand the impact of calprotectin on different PCOS phenotypes and its potential application in clinical practice.

Keywords Calprotectin, Polycystic ovary syndrome, Phenotype

Polycystic ovary syndrome (PCOS) is a significant cause of irregular menstrual cycles and hyperandrogenism (HA) in women. It was initially described by Stein and Leventhal in 1935¹. PCOS affects 1 in every 10 women worldwide². The Endocrine Society Clinical Practice Guidelines define PCOS (using the Rotterdam criteria) as having at least two of the following criteria: ovulatory dysfunction, or polycystic ovaries. Although not part of the official diagnostic criteria for hyperandrogenism (HA), insulin resistance and hyperinsulinemia are typical clinical findings and significant etiological factors for the hormonal disturbances seen in PCOS³. The Amsterdam ESHRE/ASRM-sponsored 3rd PCOS Consensus Workshop Group identified different phenotypes characterised by HA and chronic anovulation that involve ovarian dysfunction and polycystic morphology⁴. The consensus recommended that screening for insulin and glucose tolerance should be performed by OGTT (75 g, 0- and 2-hour values) in the presence of phenotypes characterised by HA, anovulation, obesity, and a family history of diabetes.

Calprotectin is a neutrophil cytosolic, calcium-binding protein belonging to the S100 protein family. It exhibits antimicrobial, immunoregulatory, and antiproliferative properties⁵. As a biomarker of neutrophil migration, elevated levels are observed in several inflammatory processes⁶. S100 proteins in the cytoplasm have been demonstrated to regulate a number of cellular processes, including cell proliferation and differentiation, apoptosis, signal transmission and cell motility⁷. Despite being a commonly observed endocrinopathy, PCOS requires further investigation due to discrepancies in the diagnostic criteria and the identification of patients at risk, as well as the lack of a sensitive and specific marker that is universally accepted. The frequent occurrence

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of pathologies such as hyperandrogenemia, insulin resistance and dyslipidaemia following inflammatory processes in PCOS suggests a possible role for calprotectin in PCOS and accompanying pathologies. Significant associations were observed for diseases of inflammatory nature such as inflammatory bowel disease, rheumatoid arthritis and familial Mediterranean fever.

The objective of this study was to assess the efficacy of measuring serum calprotectin levels, a protein involved in inflammatory processes, in identifying the risk of developing insulin resistance and diagnosing PCOS.

Materials and methods

Ethical approval was obtained from the Pamukkale University Clinical Research Ethics Committee (No: 60116787-020/24503). The study was conducted in accordance with the Declaration of Helsinki and relevant regulations, and informed consent was obtained from all participants or their legal guardians.

The study group consisted of women aged between 14 and 35 years who visited the Obstetrics and Gynecology outpatient clinic at Pamukkale University Hospital and met the Rotterdam criteria for PCOS (chronic oligo/anovulation, clinical/biochemical HA, and the presence of 12 or more subcapsular follicles in ovaries as determined by transvaginal ultrasonography). Patients with the following conditions were excluded: diabetes mellitus, Cushing's syndrome, androgen secreting tumors, endocrinopathy including late-onset 21-hydroxylase deficiency, infectious diseases, hypertension, thyroid dysfunction, hyperprolactinemia, chronic liver disease, use of medication that interferes with insulin secretion and function, sex hormones, and lipid profile. In addition, 11 patients with PCOS who were initially evaluated but demonstrated serum estradiol levels above 30 on Day 2 were excluded from the study. Only patients with estradiol levels below 30 were included.

The same exclusion criteria employed for the PCOS study group were applied to the control group as well. Healthy women of reproductive age (18–39) with regular menstrual cycles (periods lasting 2 to 7 days occurring every 25 to 34 days) were enrolled in the control group. Venous blood samples were obtained from all participants on Days 3–5 of spontaneous or progesterone-induced cycles. Fasting serum glucose (FSG), insulin, thyroid-stimulating hormone (TSH), prolactin, triglycerides (TG), low-density lipoprotein (LDL), high-density lipoproteins (HDL), dehydroepiandrosterone sulfate (DHEAS) The levels of dehydroepiandrosterone sulfate (DHEAS), total testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, anti-mullerian hormone (AMH), and calprotectin were studied in the specimens acquired. Insulin resistance was quantified using the homeostasis model assessment (HOMA-IR) score, calculated as the fasting insulin concentration (mIU/L) multiplied by the glucose concentration (mmol/L) divided by 405. Patients with HOMA-IR scores > 2.4 were considered to have insulin resistance.

The free androgen index (FAI) was calculated using the formula total testosterone (nmol/L)/SHBG (nmol/L) x 100. Scores between 0 and 7 were considered within the normal range, while scores of ≥ 8 were considered indicative of hyperandrogenemia.

Serum calprotectin levels were quantified using a calprotectin enzyme-linked immunosorbent assay (ELISA) kit (CALPRO lot no: SEK504Hu). The kit's detection range was 31.2–2000 pg/mL. The minimum detectable dose for the kit is typically below 13.3 pg/mL. The microplate provided with the kit contains antibodies that have been pre-bound to S100A8. Subsequently, standards or samples were added to the appropriate microplate wells with biotin-conjugated antibodies specific to S100A9. Subsequently, horseradish peroxidase (HRP) conjugated avidin is added to each microplate well and incubated. Following the addition of the TMB substrate solution, wells containing only calprotectin, biotin-conjugated antibodies, and enzyme-conjugated avidin will undergo a color change. Subsequently, the enzyme-substrate reaction is terminated through the addition of a sulfuric acid solution, and the color change is then measured spectrophotometrically at a wavelength of 450 nm \pm 10 nm. The concentration of calprotectin in the specimens is determined by comparing the optical density (OD) to a standard curve.

On the day that serum samples were acquired, measurements were taken of the waist circumference, hip circumference, height, and weight of the patients. The waist-hip ratio and body mass index (BMI) (kg/m^2) were calculated. The statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS), version 20.0. The data were presented as mean \pm standard error (SE). Given that numerous parameters exhibited a normal Gaussian distribution, a parametric method, specifically the T-test, was employed for the assessment of the data. The correlation between the parameters was calculated using the Pearson correlation coefficient. A p-value of less than 0.05 was deemed statistically significant for all analyses.

Results

A total of 39 patients diagnosed with polycystic ovary syndrome (PCOS) and 41 healthy controls were enrolled in the study. Table 1 presents the basic demographic and anthropometric features of the patient and control groups. There were no significant differences between the two groups in terms of age, weight, waist circumference, hip circumference, or waist-hip ratio. The results were not statistically significant.

The patients were evaluated based on the findings of the clinical examination, which included the assessment of hair loss and acanthosis nigricans. Additionally, both the physical examination and laboratory tests were utilized to assess hirsutism. Patients presenting with acne and hair loss complaints and exhibiting a score of 8 or above on the Ferriman-Gallwey scale were considered to have clinical HA. Patients with FAI and/or DHEA-S levels above the reference range, as determined by the reference values of the laboratory test performed, were considered to have laboratory HA. Subsequently, 26 of the 39 patients diagnosed with PCOS demonstrated acne, 8 demonstrated hair loss, and 2 demonstrated acanthosis nigricans. The control group exhibited three (7.3%) participants with acne and no cases of hair loss or acanthosis nigricans. The mFGS scores were elevated in 24 patients from the PCOS group, while six participants from the control group exhibited high scores. Ultrasound scans revealed the presence of polycystic ovaries in 34 patients from the PCOS group and in 5 participants from

Parameters	PCOS patient group (n = 39) $\bar{X} \pm SE$	Control group (n = 41) $\bar{X} \pm SE$	p values
Age (year)	21.10 ± 0.47	22.44 ± 0.51	0.058
Weight (kg)	64.73 ± 2.45	64.83 ± 2.31	0.976
BMI (kg/m ²)	29.14 ± 5.75	24.31 ± 0.77	0.384
Waist (cm)	73.76 ± 2.47	74.73 ± 1.92	0.754
Hip (cm)	100.65 ± 1.90	101.71 ± 1.32	0.642
Waist/Hip ratio	0.73 ± 0.011	0.73 ± 0.011	0.806
FSH (mIU/mL)	6.01 ± 0.27	8.22 ± 0.57	0.001
LH (mIU/mL)	11.48 ± 1.20	7.39 ± 0.35	0.001
LH/FSH	1.99 ± 0.21	1.01 ± 0.06	<0.001
DHEA-S (ng/mL)	396.01 ± 25.74	227.53 ± 15.95	<0.001
T. testosterone (µg/L)	0.41 ± 0.04	0.26 ± 0.02	0.002
SHBG (nmol/L)	31.43 ± 3.05	48.75 ± 2.85	<0.001
FAI	6.70 ± 1.62	2.27 ± 0.29	0.007
FSG (mg/dL)	90.11 ± 1.21	88.56 ± 0.70	0.261
HOMA-IR	4.18 ± 0.50	3.21 ± 0.29	0.087
HDL (mg/dL)	57.92 ± 3.54	50.00 ± 2.35	0.070
LDL (mg/dL)	83.28 ± 5.79	72.40 ± 6.57	0.227
Triglycerides (mg/dL)	90.29 ± 10.19	84.62 ± 7.23	0.648
Calprotectin (pg/mL)	322.45 ± 36.54	205.19 ± 16.23	0.004

Table 1. Demographics, anthropometric measurements and biochemical parameters of the patient and control groups.

Condition	Patient group N (%)	Control group N (%)	p value
Acne	26 (66.7%)	3 (7.3%)	<0.001
Hair loss	8 (20.5%)	0 (0%)	0.002
A.N.	2 (5.1%)	0 (0%)	0.234

Table 2. Status of acne, hair loss and acanthosis nigricans among patient and control groups.

Parameters	PCOS patients with insulin resistance (n = 22) $\bar{X} \pm SE$	PCOS patients without insulin resistance (n = 17) $\bar{X} \pm SE$	P
Calprotectin (pg/mL)	357.20 ± 40.02	277.49 ± 65.79	0.285

Table 3. Association of insulin resistance and serum calprotectin results in PCOS patient group.

the control group. In total, 30 (76.9%) of the 39 patients in the PCOS group were classified as having clinical HA, while 26 (66.7%) were considered to have laboratory HA (Table 2).

The levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), dehydroepiandrosterone sulfate (DHEA-S), and total testosterone were found to be significantly higher in the polycystic ovary syndrome (PCOS) group compared to the control group. Conversely, the levels of FSH were significantly lower in the PCOS group than in the control group. In addition, sex hormone-binding globulin (SHBG) levels were found to be lower in the PCOS group, while the free androgen index was statistically higher in the PCOS group. Although higher fasting serum glucose, HOMA-IR, and triglyceride levels were observed in the PCOS group, the difference between the patient and control groups did not reach statistical significance. Both groups were tested for serum calprotectin levels. The results demonstrated no significant difference between the patient and control groups (Table 1).

Additionally, serum calprotectin levels were evaluated in relation to the HOMA-IR index in order to assess insulin resistance in PCOS groups. A HOMA-IR score of 2.4 or higher was considered indicative of insulin resistance. Although serum calprotectin levels were found to be higher in patients with higher HOMA-IR scores, the difference did not reach statistical significance (Table 3).

A statistically significant positive correlation was observed between serum calprotectin levels and serum AMH levels for the PCOS patient group (Fig. 1) ($p = 0.019$).

The objective of this study was to compare calprotectin and AMH among PCOS patients, with the aim of calculating the sensitivity and specificity of each marker upon detection of a positive correlation between the two. A cut-off value of 204.54 pg/mL for serum calprotectin in PCOS yielded a sensitivity of 66.70% and a

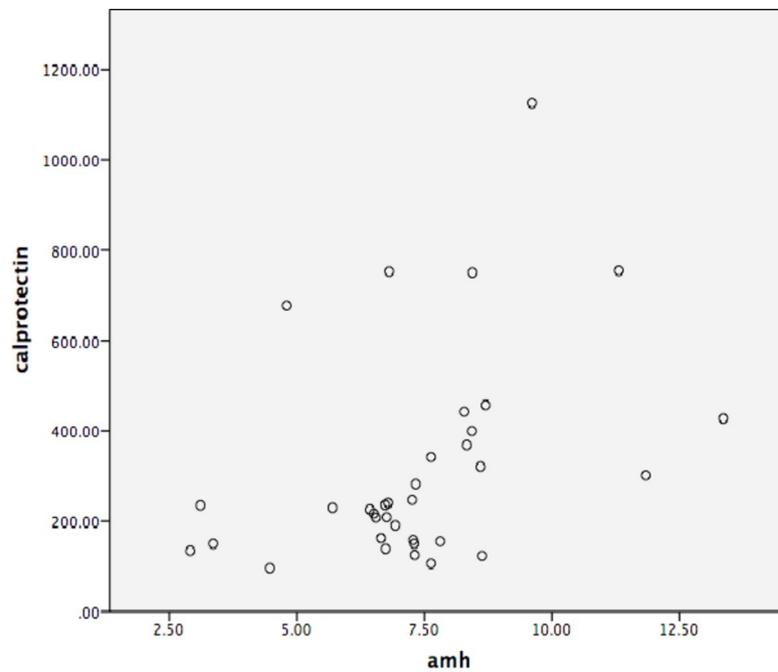


Fig. 1. Correlation analysis of serum AMH levels and serum calprotectin results.

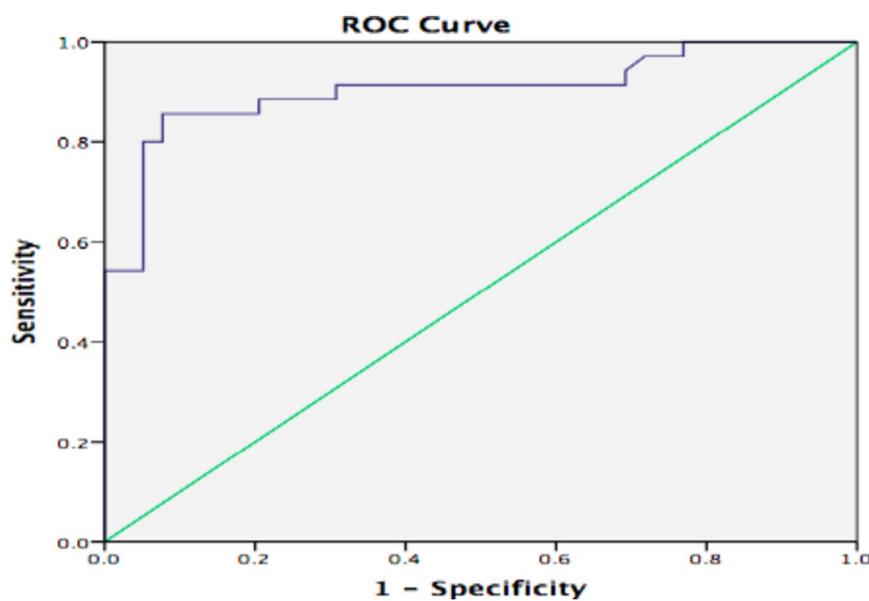


Fig. 2. Serum AMH level ROC curve analysis in PCOS patient group.

specificity of 54.70%. The cut-off value of 4.34 ng/mL for AHM in PCOS yielded a sensitivity of 91.40% and a specificity of 69.20% (Fig. 2).

The investigation of the association between serum calprotectin and BMI, waist-hip ratio, LH/FSH ratio, and SHBG among the PCOS group revealed no significant correlation. The p-values for these correlations were 0.277, 0.670, 0.981, and 0.364, respectively.

Correlation analysis of serum calprotectin with the HOMA-IR index among patients with PCOS yielded no statistically significant results, despite patients with higher HOMA-IR scores demonstrating a higher frequency of calprotectin detection.

The serum calprotectin levels of patients with and without polycystic ovary morphology were compared in ultrasound scans. Although patients with polycystic ovaries demonstrated higher calprotectin levels, the observed difference was not statistically significant ($p=0.051$).

Discussion

Polycystic ovary syndrome (PCOS) is a condition characterized by the presence of hyperandrogenism (HA), oligo-anovulation, clinical and/or biochemical findings of and polycystic ovaries on ultrasound⁸. In our study, the PCOS patient group exhibited increased levels of oligomenorrhea-amenorrhea, hirsutism, the free androgen index, serum total testosterone, and dehydroepiandrosterone sulfate (DHEA-S) compared to the control group.

Mahde et al. reported biochemical HA in 43.5% and clinical HA in 60.9% of the cases⁹. Our findings revealed a rate of 76.9% for clinical HA and 66.7% for laboratory HA among our PCOS patient group. These results indicate that the majority of clinical HA cases are accompanied by laboratory HA, thereby supporting the clinical diagnosis.

Sachdeva et al. report more common insulin resistance among obese PCOS group¹⁰. In this study, correlation analysis of BMI as a marker of obesity and HOMA-IR as a marker of insulin resistance yielded statistical significance.

Elevated levels of calprotectin have been observed in patients with inflammatory pathologies. To ascertain the impact of insulin resistance on serum calprotectin levels, a study was conducted to examine the relationship between HOMA-IR, a marker of insulin resistance, and serum calprotectin values in a cohort of patients with polycystic ovary syndrome. Despite higher levels of serum calprotectin among high HOMA-IR patients, statistical significance was not achieved. Upon analyzing the correlation between HOMA-IR and serum calprotectin, it was found that although the calprotectin was higher in high HOMA-IR patients, the *p* value of 0.101 yielded no significant correlation.

Calprotectin is a significant marker of the inflammatory response, particularly as a protein released following neutrophil activation. Chronic low-grade inflammation is known to contribute to the pathophysiology of PCOS. Several studies indicate that patients with PCOS may exhibit elevated levels of proinflammatory cytokines and inflammatory markers, which are linked to insulin resistance, hyperandrogenemia, and metabolic disorders. It is believed that calprotectin may be influenced by these mechanisms, with insulin resistance stimulating its production through increased neutrophil activation, thereby intensifying the inflammatory response. Additionally, visceral adipose tissue is considered a key inflammatory source in PCOS, and the proinflammatory mediators released from this tissue may further contribute to the elevated levels of calprotectin. Some studies have demonstrated that elevated calprotectin levels may serve as a marker of catabolism in a multitude of inflammatory diseases¹¹. In their investigation into the potential association between PCOS and calprotectin, Shouzen Chen and colleagues observed that calprotectin levels were significantly higher in PCOS patients compared to controls¹². Our findings also revealed significantly elevated serum calprotectin levels in the PCOS patient cohort. While these results highlight calprotectin as a potential marker in PCOS, further research is required to evaluate different phenotypes separately in conditions with multiple phenotypes, such as PCOS. The detection of patients with low or high serum calprotectin levels in the PCOS group may take on a new meaning with further investigation of subgroups. Therefore, calprotectin can be used more efficiently.

Laven et al.¹³ observed a significant positive correlation between serum AMH levels and LH, testosterone, androstenedione, free androgen index, and number of follicles. Our study revealed a significant positive correlation between serum calprotectin levels and serum AMH levels, as demonstrated by correlation analysis. The correlation of calprotectin levels with AMH levels, an established marker that has been shown to provide significant clinical information in PCOS, is a valuable finding. This condition necessitated a comparison between the sensitivity and specificity of these two markers in PCOS. In their study evaluating AMH in PCOS, Le et al.¹⁴ found 78.50% sensitivity and 75.83% specificity. In our study, the AMH cut-off value for PCOS was determined to be 4.34 ng/mL, resulting in a sensitivity of 90.40% and a specificity of 69.20%. Similarly, a serum calprotectin cut-off value of 204.54 pg/mL yielded a sensitivity of 66.70% and a specificity of 54.70%. These results indicate that neither marker is sufficient for PCOS diagnosis on its own, and that both can be used in conjunction with clinical and laboratory evidence for diagnosis. In contrast, Chen et al.¹² report a sensitivity of 75.6% and specificity of 85.2% in identifying PCOS for serum calprotectin. While they found a higher sensitivity, this is still below the levels for AMH. Therefore, it is not possible to conclude with certainty that AMH outperforms calprotectin in the diagnosis of PCOS. The extensive prior use of AMH in studies and clinical settings allows for the collection of larger data sets. A meta-analysis involving future calprotectin studies may provide an objective evaluation.

Although the study provides strong data with its prospective design, it has limitations that may arise from a single centre and small sample size. Due to the multivariate clinical nature of PCOS, a large subgroup analysis can be performed in the evaluation of the parameters and the results can be more meaningfully examined for diagnostic performance. In addition, as there were not enough studies evaluating calprotectin performance in PCOS during the study period, the analysis compared the results with other previously investigated parameters.

Conclusion

In this study, serum calprotectin levels were found to be significantly higher in the PCOS group compared to the control group. Correlation analysis of the PCOS group revealed a significant positive correlation between serum calprotectin and serum AMH levels. A possible explanation for the conflicting results between the studies could be the number of cases and different exclusion criteria. Additionally, the heterogeneous structure of the PCOS group and different PCOS phenotypes may have contributed to these results. Consequently, further research is required to evaluate the PCOS phenotypes and patient subgroups in greater depth in order to elucidate the role of calprotectin in PCOS pathophysiology.

Data availability

Data is provided within the manuscript or supplementary information files.

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Author contributions

The conceptualisation and design of this study were conducted with the input of all authors. The material preparation, data collection and analysis processes were conducted by Rifat Şener, Süleyman Erkan Alataş and Ömer Tolga Güler. The initial draft of the article was drafted by Rifat Şener and Süleyman Erkan Alataş, and all authors provided feedback on previous versions. Ömer Tolga Güler made substantial contributions to the analysis and interpretation of the data. All authors read and approved the final version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

Ethical approval was gained from the Pamukkale University Clinical Research Ethics Board (No:60116787-020/24503).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Additional information

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