

Diagnostic and endocrine-metabolic significance of serum kallistatin and vasorin in women with polycystic ovary syndrome from Southern Iraq

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Abstract

Objective: Polycystic ovary syndrome (PCOS) is a common endocrine disorder with heterogeneous metabolic and reproductive features. Novel circulating biomarkers may improve phenotypic characterization. This study aimed to compare serum kallistatin and vasorin levels between women with PCOS and healthy controls and to examine their associations with body mass index (BMI), insulin resistance (IR), and reproductive hormones.

Methods: This case-control study enrolled 120 women aged 18–45 years from Southern Iraq: 60 diagnosed with PCOS by Rotterdam criteria and 60 healthy controls. Serum kallistatin and vasorin were measured by enzyme-linked immunosorbent assay. Metabolic and hormonal parameters were assessed using automated biochemical and electrochemiluminescence immunoassays. Statistical analyses included independent t-tests, Mann–Whitney U tests, Spearman correlations, and receiver operating characteristic (ROC) curve analysis.

Results: Women with PCOS had significantly higher serum kallistatin (3.37 ± 0.85 vs. 2.13 ± 0.96 ng/mL, $p < 0.001$) and lower serum vasorin (62.72 ± 9.86 vs. 94.92 ± 10.31 pg/mL, $p < 0.001$) than controls. Kallistatin was significantly higher in those with elevated BMI (3.55 ± 0.82 vs. 2.77 ± 0.67 ng/mL, $p = 0.003$) and with high IR (3.62 ± 0.85 vs. 2.72 ± 0.40 ng/mL, $p < 0.001$). Vasorin showed no significant association with BMI or IR but was lower in women with high testosterone (60.27 ± 9.82 vs. 63.53 ± 9.84 pg/mL, $p = 0.006$). ROC analysis yielded areas under the curve of 0.829 for kallistatin and 0.988 for vasorin.

Conclusions: Serum kallistatin and vasorin are altered in PCOS and show distinct association patterns: kallistatin with metabolic features (obesity, insulin resistance) and vasorin with androgen status. These findings support further investigation of both molecules as adjunctive biomarkers, pending validation in independent cohorts.

Keywords: Polycystic ovary syndrome, Kallistatin, Vasorin, Insulin resistance, Testosterone

Plain English Summary

Polycystic ovary syndrome (PCOS) is a common hormone disorder that affects women of childbearing age. It often causes irregular periods, excess hair growth, and difficulties with fertility, and it is linked to weight gain and diabetes risk. Researchers are looking for new substances in the blood that could help understand or identify PCOS. In this study, we measured two such substances, kallistatin and vasorin, in the blood of 60 women with PCOS and 60 healthy women from Southern Iraq. We found that women with PCOS had higher levels of kallistatin and lower levels of vasorin than healthy women. Kallistatin was particularly high in women who were overweight or had signs of insulin resistance (a condition that can lead to diabetes). Vasorin was lower in women with high levels of the male hormone testosterone. Our results suggest that kallistatin might be linked to the metabolic problems of PCOS, while vasorin

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might be more related to hormone imbalances. These are early findings, and more research is needed before these tests can be used in routine care.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders affecting women of reproductive age, with a global prevalence estimated between 10% and 13% depending on the diagnostic criteria applied (1, 2). The syndrome is characterised by a combination of clinical and biochemical features, including hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology on ultrasound (3). Beyond reproductive manifestations, PCOS is increasingly recognised as a multisystem condition associated with metabolic disturbances such as insulin resistance, dyslipidaemia, obesity, and an elevated long-term risk of type 2 diabetes and cardiovascular disease (4, 5).

The pathophysiology of PCOS is complex and not fully understood, but insulin resistance is considered a key driver. Hyperinsulinaemia amplifies ovarian androgen production and reduces hepatic sex hormone-binding globulin (SHBG) synthesis, thereby increasing free androgen levels (6, 7). Obesity, particularly central adiposity, exacerbates insulin resistance and worsens the hormonal and metabolic profile of affected women (8). Given this heterogeneity, there is growing interest in identifying circulating biomarkers that could help distinguish between metabolic and reproductive phenotypes of PCOS and potentially guide personalised management (9).

Kallistatin, a serine protease inhibitor, has anti-inflammatory, anti-oxidative, and vasoprotective properties (10). Recent studies have suggested that kallistatin may be altered in PCOS, but findings have been inconsistent. Yurtkal and Canday reported lower serum kallistatin in women with PCOS compared with controls (11), while other work has pointed to a possible relationship with metabolic parameters. Vasin is a transmembrane glycoprotein involved in cellular signalling and tissue remodelling; it has been implicated in ovarian function, and a single exploratory study found significantly reduced serum vasin levels in PCOS with good diagnostic discrimination (12, 13).

To date, no study has simultaneously examined kallistatin and vasin in the same PCOS population from the Middle East, where the prevalence of obesity and metabolic syndrome is high. Therefore, this case-control study was designed to evaluate serum kallistatin and vasin levels in women with PCOS from Southern Iraq and to explore their associations with body mass index (BMI), insulin resistance,

lipid profile, testosterone, and selected reproductive hormones.

Materials and Methods

Study design and setting

This was a case-control study conducted at Basra Women and Children Teaching Hospital, Basra, Iraq, between September 2025 and January 2026. The study protocol was approved by the research ethics committee of Southern Technical University and the Basra Health Department (approval number 2119, dated 2 September 2025), and the study adhered to the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants before enrolment.

Participants

A total of 120 women aged 18–45 years were enrolled: 60 women with a confirmed diagnosis of PCOS and 60 apparently healthy women as controls. All participants were recruited from the gynaecology outpatient clinic and through community advertisements.

The diagnosis of PCOS was established according to the Rotterdam criteria, requiring the presence of at least two of the following three features after exclusion of other androgen-excess or ovulatory disorders: (i) oligo-anovulation (menstrual cycles >35 days or <8 cycles per year), (ii) clinical and/or biochemical hyperandrogenism (modified Ferriman-Gallwey score ≥ 8 or elevated total or free testosterone), and (iii) polycystic ovarian morphology on transabdominal or transvaginal ultrasound (≥ 12 follicles of 2–9 mm and/or ovarian volume >10 mL). All diagnoses were confirmed by a consultant gynaecologist.

Control participants were healthy women without any clinical or biochemical evidence of PCOS, regular menstrual cycles (21–35 days), no signs of hyperandrogenism, and normal ovarian morphology on ultrasound when available. Women with any of the following conditions were excluded from both groups: cancer, liver disease, renal impairment, thyroid disorders, diabetes mellitus, Cushing syndrome, pregnancy, current use of hormonal medications (including oral contraceptives, anti-androgens, or insulin sensitisers), or other ovarian or uterine pathologies (e.g., endometriosis, ovarian tumours). Routine laboratory investigations, including renal function tests, liver function tests, and thyroid-stimulating hormone, were used to support the exclusion criteria where appropriate.

Sample size consideration

No formal power calculation was performed a priori; the sample size ($n = 60$ per group) was based on feasibility and similar published studies on biomarkers in PCOS. Post-hoc, with $\alpha = 0.05$ and 80% power, the achieved sample size was adequate to detect a moderate effect size (Cohen's $d \approx 0.5$) in the primary comparison of kallistatin between groups.

Data collection and clinical measurements

Demographic and clinical data were collected through direct interviews and physical examinations. Height and weight were measured in light clothing without shoes, and body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). A BMI ≥ 25 kg/m^2 was defined as high BMI (overweight/obese) for subgroup analysis, consistent with regional definitions.

Laboratory measurements

Venous blood samples were collected after an overnight fast of at least 8 hours. Samples were centrifuged at 3000 rpm for 10 minutes, and serum was separated into two aliquots. The first aliquot was used immediately for routine biochemical and hormonal analyses, while the second was stored at $-20^\circ C$ for subsequent measurement of kallistatin and vasorin. All samples were subjected to only one freeze-thaw cycle before analysis.

Biochemical and hormonal assays

Fasting glucose, insulin, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured using a fully automated chemistry analyser (Cobas Integra 400, Roche Diagnostics). Hormonal parameters – luteinising hormone (LH), follicle-stimulating hormone (FSH), prolactin, total testosterone, and dehydroepiandrosterone (DHEA) – were measured by electrochemiluminescence immunoassay on a Cobas e411 platform (Roche Diagnostics). Insulin resistance was estimated using the homeostatic model assessment (HOMA-IR) with the formula: fasting glucose (mg/dL) $\times$ fasting insulin ($\mu IU/mL$) / 405.

Kallistatin and vasorin assays

Serum kallistatin and vasorin concentrations were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Kallistatin was assayed using a kit (Cat. No. E3392Hu, Sunlong Biotech, China) with a sensitivity of 0.022 ng/mL and an intra-assay coefficient of variation (CV) of $<8\%$ and an inter-assay CV of $<10\%$. Vasorin was measured

using a kit (Cat. No. E7987Hu, Sunlong Biotech, China) with a sensitivity of 5.52 pg/mL and intra-assay CV $<8\%$ and inter-assay CV $<12\%$. All samples were run in duplicate, and the mean values were used for analysis.

Statistical analysis

Data were analysed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Normality of continuous variables was assessed by the Shapiro–Wilk test and visual inspection of histograms. Normally distributed variables were expressed as mean $\pm$ standard deviation (SD), and non-normally distributed variables as median with interquartile range.

Between-group comparisons for normally distributed variables were performed using Student's t-test (or Welch's t-test when variances were unequal). The Mann–Whitney U test was used for non-normally distributed variables. For subgroup analyses (according to IR status, BMI status, and testosterone status), Welch's t-test was applied given the unequal sample sizes and variances. Correlations between biomarkers and hormonal parameters were assessed using Spearman's rank correlation coefficient (r). The diagnostic performance of kallistatin and vasorin to distinguish PCOS from controls was evaluated by receiver operating characteristic (ROC) curve analysis, with calculation of the area under the curve (AUC), optimal cutoff values (maximising Youden's index), sensitivity, specificity, and overall accuracy. Given the exploratory nature of the subgroup analyses, no adjustment for multiple comparisons was applied, and these results should be interpreted as hypothesis-generating. A two-tailed $p < 0.05$ was considered statistically significant.

Results

Baseline characteristics of the study population

The clinical, metabolic, hormonal, and biomarker characteristics of the 60 women with PCOS and 60 healthy controls are summarised in Table 1. The two groups were similar in age (24.47 ± 5.67 vs. 25.82 ± 5.03 years, $p = 0.170$). Women with PCOS had significantly higher BMI (31.13 ± 8.29 vs. 23.76 ± 2.80 kg/m^2 , $p < 0.001$) and higher HOMA-IR values (3.11 ± 1.93 vs. 1.87 ± 0.76 , $p < 0.001$) compared with controls.

Regarding the lipid profile, LDL-C was significantly higher in the PCOS group (105.21 ± 31.33 vs. 91.17 ± 22.68 mg/dL , $p = 0.031$), while HDL-C was significantly lower (46.70 ± 9.98 vs. 54.86 ± 10.63 mg/dL , $p < 0.001$). Total cholesterol and triglyceride levels did not differ significantly between groups. Hormonal analysis showed significantly higher LH (14.99 ± 12.34 vs. 6.98 ± 5.63 $\mu IU/mL$, $p < 0.001$) and testosterone (42.04 ± 24.40 vs.

21.94 ± 10.31 ng/dL; note unit correction, $p < 0.001$) in the PCOS group, and lower FSH (5.45 ± 2.09 vs. 6.63 ± 2.77 μ IU/mL, $p = 0.009$). Prolactin and DHEA did not differ significantly.

Women with PCOS had significantly lower serum vasorin (62.72 ± 9.86 vs. 94.92 ± 10.31 pg/mL, $p < 0.001$) and significantly higher serum kallistatin (3.37 ± 0.85 vs. 2.13 ± 0.96 ng/mL, $p < 0.001$) compared with controls.

Biomarker comparisons

Table 1: Baseline clinical, metabolic, hormonal, and biomarker characteristics of the study groups

Variable	PCOS group (n = 60)	Control group (n = 60)	p value
Age (years)	24.47 ± 5.67	25.82 ± 5.03	0.17
BMI (kg/m ²)	31.13 ± 8.29	23.76 ± 2.80	<0.001
HOMA-IR	3.11 ± 1.93	1.87 ± 0.76	<0.001
Total cholesterol (mg/dL)	151.61 ± 38.80	151.60 ± 26.35	0.935
Triglycerides (mg/dL)	105.97 ± 56.11	85.81 ± 32.89	0.095
LDL cholesterol (mg/dL)	105.21 ± 31.33	91.17 ± 22.68	0.031
HDL cholesterol (mg/dL)	46.70 ± 9.98	54.86 ± 10.63	<0.001
LH (μ IU/mL)	14.99 ± 12.34	6.98 ± 5.63	<0.001
FSH (μ IU/mL)	5.45 ± 2.09	6.63 ± 2.77	0.009
Prolactin (ng/mL)	17.48 ± 5.81	17.62 ± 9.80	0.283
Testosterone (ng/dL)	42.04 ± 24.40	21.94 ± 10.31	<0.001
DHEA (μ g/dL)	238.10 ± 139.43	219.38 ± 67.19	0.642
Vasorin (pg/mL)	62.72 ± 9.86	94.92 ± 10.31	<0.001
Kallistatin (ng/mL)	3.37 ± 0.85	2.13 ± 0.96	<0.001

Data are mean ± standard deviation. PCOS: polycystic ovary syndrome; BMI: body mass index; HOMA-IR: homeostatic model assessment of insulin resistance; LDL: low-density lipoprotein; HDL: high-density lipoprotein; LH: luteinising hormone; FSH: follicle-stimulating hormone; DHEA: dehydroepiandrosterone

Biomarker associations with insulin resistance

Table 2 shows the comparison of biomarker levels according to HOMA-IR status among women with PCOS, using a cutoff of HOMA-IR >2.5 to define high insulin resistance. Serum vasorin levels did not differ significantly between

women with normal IR (n = 17) and those with high IR (n = 43): 63.71 ± 9.42 vs. 62.33 ± 10.11 pg/mL, $p = 0.501$. In contrast, serum kallistatin was significantly higher in the high-IR group (3.62 ± 0.85 vs. 2.72 ± 0.40 ng/mL, $p < 0.001$).

Table 2: Biomarker levels according to insulin resistance status in women with PCOS

Biomarker	Normal HOMA-IR (n = 17)	High HOMA-IR (n = 43)	p value
Vasorin (pg/mL)	63.71 ± 9.42	62.33 ± 10.11	0.501
Kallistatin (ng/mL)	2.72 ± 0.40	3.62 ± 0.85	<0.001

Data are mean ± standard deviation. High HOMA-IR defined as >2.5. PCOS: polycystic ovary syndrome

Biomarker associations with body mass index

The relationship between biomarkers and BMI status among PCOS women is presented in Table 3. Using a BMI cutoff of 25 kg/m², 14 women had a normal BMI, and 46 had a high BMI. Vasorin levels did not differ significantly

between the two groups (66.21 ± 9.48 vs. 61.65 ± 9.82 pg/mL, $p = 0.119$). However, kallistatin levels were significantly higher in women with high BMI (3.55 ± 0.82 vs. 2.77 ± 0.67 ng/mL, $p = 0.003$).

Table 3: Biomarker levels according to body mass index status in women with PCOS

Biomarker	Normal BMI (n = 14)	High BMI (n = 46)	p value
Vasorin (pg/mL)	66.21 ± 9.48	61.65 ± 9.82	0.119
Kallistatin (ng/mL)	2.77 ± 0.67	3.55 ± 0.82	0.003

Data are mean ± standard deviation. High BMI is defined as ≥25 kg/m². PCOS: polycystic ovary syndrome

Biomarker associations with testosterone status

When women with PCOS were stratified by testosterone levels (normal ≤45 ng/dL, high >45 ng/dL), both biomarkers showed significant

differences (Table 4). Women with high testosterone (n = 15) had lower vasorin levels than those with normal testosterone (n = 45): 60.27 ± 9.82 vs. 63.53 ± 9.84 pg/mL, $p = 0.006$.

Interestingly, kallistatin was also significantly lower in the high-testosterone group (3.24 ± 0.71 vs. 3.41 ± 0.90 ng/mL, $p = 0.014$).

Table 4: Biomarker levels according to testosterone status in women with PCOS

Biomarker	Normal testosterone (n = 45)	High testosterone (n = 15)	p value
Vasorin (pg/mL)	63.53 ± 9.84	60.27 ± 9.82	0.006
Kallistatin (ng/mL)	3.41 ± 0.90	3.24 ± 0.71	0.014

Data are mean $\pm$ standard deviation. High testosterone is defined as >45 ng/dL. PCOS: polycystic ovary syndrome

Correlations between biomarkers and reproductive hormones

Spearman correlation analysis among women with PCOS (Table 5) revealed weak but statistically significant negative correlations between vasorin and both LH ($r = -0.279$, $p = 0.031$) and FSH ($r = -0.274$, $p = 0.034$). No significant correlations were observed between

vasorin and kallistatin, prolactin, testosterone, or DHEA. Kallistatin showed no significant correlations with any of the measured reproductive hormones. As expected, testosterone was strongly positively correlated with DHEA ($r = 0.682$, $p < 0.001$), and LH was moderately correlated with FSH ($r = 0.499$, $p < 0.001$).

Table 5: Spearman correlations between biomarkers and reproductive hormones in women with PCOS

Variable	Kallistatin (ng/mL)	Prolactin (ng/mL)	Testosterone (ng/dL)	DHEA (μ g/dL)	LH (μ IU/mL)	FSH (μ IU/mL)
Vasorin (pg/mL)	$r = -0.114$, $p = 0.387$	$r = 0.192$, $p = 0.142$	$r = -0.008$, $p = 0.951$	$r = 0.025$, $p = 0.852$	$r = -0.279$, $p = 0.031$	$r = -0.274$, $p = 0.034$
Kallistatin (ng/mL)	—	$r = 0.047$, $p = 0.720$	$r = -0.036$, $p = 0.782$	$r = -0.222$, $p = 0.088$	$r = 0.175$, $p = 0.181$	$r = 0.186$, $p = 0.156$
Prolactin (ng/mL)	—	—	$r = 0.173$, $p = 0.185$	$r = 0.093$, $p = 0.481$	$r = -0.203$, $p = 0.119$	$r = -0.149$, $p = 0.257$
Testosterone (ng/dL)	—	—	—	$r = 0.682$, $p < 0.001$	$r = 0.188$, $p = 0.151$	$r = 0.255$, $p = 0.049$
DHEA (μ g/dL)	—	—	—	—	$r = 0.080$, $p = 0.545$	$r = 0.172$, $p = 0.188$
LH (μ IU/mL)	—	—	—	—	—	$r = 0.499$, $p < 0.001$

PCOS: polycystic ovary syndrome; DHEA: dehydroepiandrosterone; LH: luteinising hormone; FSH: follicle-stimulating hormone

Diagnostic performance of kallistatin and vasorin
ROC curve analysis was performed to evaluate the ability of each biomarker to distinguish women with PCOS from healthy controls. For serum kallistatin (Figure 1A), the area under the curve (AUC) was 0.829 (95% confidence interval [CI]: 0.754–0.904). At an optimal cutoff value of 2.55 ng/mL, kallistatin yielded a sensitivity of 85.0%, a specificity of 68.3%, and an overall accuracy of 76.7%.

For serum vasorin (Figure 1B), the AUC was 0.988 (95% CI: 0.973–1.000). At a cutoff of 73.0 pg/mL, vasorin achieved a sensitivity of 100%, a specificity of 90.0%, and an overall accuracy of 95.0%. These AUC estimates, particularly for vasorin, should be considered optimistic because they were derived from the same dataset used to select the cutoffs; internal validation (e.g., bootstrap or cross-validation) would be required to obtain less biased estimates.

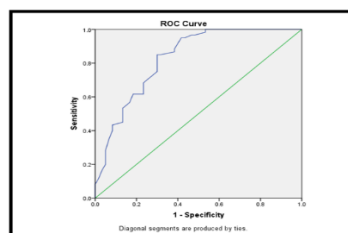


Figure 1A: Receiver operating characteristic (ROC) curve of serum kallistatin for distinguishing women with polycystic ovary syndrome from healthy controls

The area under the curve (AUC) was 0.829 (95% confidence interval: 0.754–0.904). At an optimal cutoff of 2.55 ng/mL, sensitivity was 85.0%, specificity was 68.3%, and overall accuracy was 76.7%

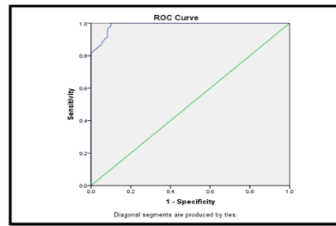


Figure 1B: Receiver operating characteristic (ROC) curve of serum vasorin for distinguishing women with polycystic ovary syndrome from healthy controls

The area under the curve (AUC) was 0.988 (95% confidence interval: 0.973–1.000). At an optimal cutoff of 73.0 pg/mL, sensitivity was 100%, specificity was 90.0%, and overall accuracy was 95.0%

Discussion

This case-control study from Southern Iraq provides new evidence that serum kallistatin and vasorin are significantly altered in women with PCOS compared with healthy controls, and that they show distinct patterns of association with metabolic and reproductive parameters. Specifically, kallistatin was elevated in PCOS and was higher in women with obesity and insulin resistance, whereas vasorin was reduced in PCOS and showed stronger associations with testosterone status. These findings suggest that the two biomarkers may reflect different pathophysiological domains within the heterogeneous syndrome of PCOS.

Comparison with previous literature

Our observation of elevated serum kallistatin in PCOS contrasts with the study by Yurtkal and Canday, who reported lower kallistatin levels in Turkish women with PCOS compared with age- and BMI-matched controls (11). There are several possible explanations for this discrepancy. First, our control group was not matched for BMI; women with PCOS had substantially higher BMI (31.13 vs. 23.76 kg/m²). Given that we found kallistatin to be significantly associated with BMI, the between-group difference might be partly driven by adiposity rather than PCOS per se. Second, ethnic and dietary differences between Iraqi and Turkish populations may influence baseline kallistatin expression. Third, the degree of insulin resistance and inflammatory burden likely differs across cohorts. Kallistatin has known anti-inflammatory and anti-oxidative functions, and its circulating levels may rise as a compensatory response to metabolic stress (10). Thus, in a cohort with high obesity and insulin resistance (as in the present study), elevated kallistatin could reflect an adaptive mechanism, whereas in a leaner PCOS phenotype, different regulatory pathways might dominate.

In contrast, our vasorin findings are remarkably consistent with the only other published study on this biomarker in PCOS. Keyif and colleagues reported significantly lower serum vasorin in women with PCOS (AUC = 0.918) and no significant association with BMI or insulin resistance (12). The present study replicates these findings and further extends them by showing a significant relationship between vasorin and testosterone status. The excellent discriminatory ability of vasorin in both studies (AUC = 0.988 here vs. 0.918 in the Turkish cohort) is striking and suggests that vasorin might be a robust indicator of PCOS-related pathobiology, possibly through pathways involving androgen signalling or ovarian remodelling (13, 14).

Pathophysiological interpretation

Insulin resistance is widely accepted as a central mechanism linking obesity, hyperandrogenism, and ovulatory dysfunction in PCOS (6, 7). Our finding that kallistatin is elevated in women with high HOMA-IR and high BMI supports the hypothesis that kallistatin is more closely tied to the metabolic dimension of PCOS. A recent comprehensive review highlighted that visceral adiposity aggravates insulin resistance and hyperandrogenism, and that weight reduction improves endocrine and metabolic outcomes (8). Within this framework, kallistatin might serve as a circulating marker of metabolic stress or a compensatory anti-inflammatory response. However, we caution that the association does not imply causation, and unmeasured confounders (e.g., dietary intake, physical activity, inflammatory cytokines) could explain part of the observed relationship.

The lower vasorin levels in PCOS and their association with high testosterone, but not with BMI or IR, suggest a different biological role. Vasorin is a transmembrane protein that modulates transforming growth factor-beta

(TGF- β) signalling and is expressed in ovarian tissue (13, 14). Reduced circulating vasorin might reflect altered ovarian function or androgen-mediated downregulation. The weak negative correlations of vasorin with LH and FSH further hint at a possible link with gonadotropin dynamics. However, the magnitude of these correlations ($r \approx -0.28$) is modest and should not be overinterpreted.

Importantly, our subgroup analysis by testosterone status showed that both vasorin and kallistatin were lower in the high-testosterone group. This was unexpected because kallistatin was higher in the overall PCOS group and higher in those with obesity/IR. The most plausible explanation is that the high-testosterone subgroup ($n = 15$) had different metabolic characteristics – possibly lower BMI or different IR status – but we did not perform stratified analyses by both testosterone and BMI simultaneously due to small sample sizes. Alternatively, the direction of association between kallistatin and testosterone may be non-linear, or the finding may be a chance occurrence due to multiple testing.

Diagnostic performance and clinical implications

The high AUC values, especially for vasorin, raise the possibility that these biomarkers could eventually serve as adjunctive tools in PCOS diagnosis. However, we emphasise three important caveats. First, the ROC analysis was performed in the same dataset that was used to define the optimal cutoffs, leading to substantial optimism bias. Without independent validation or internal resampling (e.g., bootstrap or cross-validation), the reported sensitivity and specificity are likely overestimates. Second, the control group was not BMI-matched, and the large difference in adiposity between groups may have contributed to the discriminatory power of kallistatin. Third, PCOS diagnosis remains a clinical construct based on established criteria; no single biomarker has yet proven sufficient for diagnosis (9, 15). Therefore, we regard these findings as preliminary and hypothesis-generating, not as evidence for clinical implementation.

Strengths and limitations

Strengths include the simultaneous measurement of two novel biomarkers in a well-characterised PCOS cohort from an understudied Middle Eastern population, the use of standardised diagnostic criteria, and the comprehensive assessment of metabolic and hormonal parameters.

Limitations must be acknowledged. First, this was a single-centre study with a relatively modest sample size, particularly for subgroup analyses

(e.g., only 14 women with normal BMI, 15 with high testosterone). The small subgroup sizes render these comparisons unstable and vulnerable to type I and type II errors; accordingly, they should be considered exploratory. Second, the lack of BMI matching between cases and controls means that some of the between-group differences in biomarkers, particularly kallistatin, may be attributable to adiposity rather than PCOS itself. Multivariable adjustment would have been desirable, but the sample size limited such analyses. Third, the case-control design precludes causal inference. Fourth, we did not measure SHBG or calculate free testosterone, which might have provided more precise estimates of androgen bioactivity. Fifth, the HOMA-IR calculation assumes fasting samples, which we obtained, but we did not repeat the measurement to account for day-to-day variability. Sixth, we did not assess inflammatory markers (e.g., C-reactive protein, interleukin-6) that could help elucidate the mechanisms linking kallistatin and vasorin to PCOS phenotypes. Seventh, the extremely high AUC for vasorin (0.988) should be interpreted with caution because it may reflect overfitting; validation in an independent cohort is essential. Future directions: Large, multicentre, prospective studies with BMI-matched controls and standardised phenotyping are needed to validate the diagnostic utility of kallistatin and vasorin. Longitudinal studies could examine whether these biomarkers change with weight loss or insulin-sensitising treatments. Mechanistic studies using ovarian tissue or cell culture models would help clarify whether vasorin and kallistatin play active roles in PCOS pathogenesis or are merely downstream markers.

Conclusion

In this case-control study of women from Southern Iraq, PCOS was associated with elevated serum kallistatin and reduced serum vasorin. Kallistatin showed significant associations with obesity and insulin resistance, suggesting a closer link to the metabolic phenotype of PCOS, whereas vasorin was associated with testosterone status and exhibited excellent preliminary discriminatory ability. Neither biomarker is ready for routine clinical use, but both warrant further investigation in well-designed validation studies. Our findings contribute to the growing literature on novel circulating markers in PCOS and highlight the importance of considering metabolic and reproductive phenotypes separately.

List of Abbreviations

AUC: Area under the curve

BMI: Body mass index
DHEA: Dehydroepiandrosterone
ELISA: Enzyme-linked immunosorbent assay
FSH: Follicle-stimulating hormone
HDL: High-density lipoprotein cholesterol
HOMA IR: Homeostatic model assessment of insulin resistance
IR: Insulin resistance
LDL: Low-density lipoprotein cholesterol
LH: Luteinising hormone
PCOS: Polycystic ovary syndrome
ROC: Receiver operating characteristic
SD: Standard deviation
SHBG: Sex hormone binding globulin
TGFβ: Transforming growth factor beta

Declarations

Ethics Approval and Consent to Participate

The study protocol was approved by the research ethics committee of Southern Technical University and the Basra Health Department (approval number 2119, dated 2 September 2025). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before enrolment.

Consent for Publication

All authors have given their consent for the publication of this work under the Creative Commons Attribution Non-Commercial 4.0 license.

Data Availability

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

Competing Interests

The authors declare that they have no competing interests.

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Author Contributions

ATAD worked on the conceptualisation, methodology, formal analysis, investigation, data curation, writing the original draft and visualisation.

MMA also worked on the conceptualisation, methodology, validation, investigation, resources, supervision, project administration, writing the review and editing.

MWF worked on the methodology, validation, investigation, resources, funding acquisition, writing the review and editing.

All authors have read and agreed to the published version of the manuscript.

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