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ORIGINAL PAPER

Evaluation of hormonal and adipokine biomarkers in the diagnosis of polycystic ovary syndrome – a case-control study

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ABSTRACT

Introduction and aim. Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder that significantly affects women of reproductive ages. This study evaluated the diagnostic potential of hormonal biomarkers such as anti-Müllerian hormone (AMH), total and free testosterone, ratio of luteinizing hormone (LH/FSH) ratio, and adipokines, including visfatin and kisspeptin, in distinguishing PCOS patients from healthy controls.

Material and methods. In this case-control study, 50 women diagnosed with PCOS were compared with 50 controls of the same age. Demographic and clinical data were collected through structured interviews and physical examinations. Physical activity levels was assessed using the International Physical Activity Questionnaire short form as well as anthropometric measurements were performed using a calibrated digital scale Seca 803. Blood samples were analyzed for AMH, total and free testosterone, LH/FSH ratio, visfatin, and kisspeptin levels using enzyme-linked immunosorbent assay.

Results. PCOS patients exhibited significantly higher levels of AMH (8.1±2.3 ng/mL vs. 4.07±1.1 ng/mL, p<0.001), Free testosterone (4.55±0.95 pg/mL vs. 2.47±0.46 pg/mL, p<0.001), visfatin (86.6±11.02 ng/mL vs. 49.53±10.25 ng/mL, p<0.001), and kisspeptin (9.88±1.96 ng/mL vs. 4.84±1.07 ng/mL, p<0.001) compared to controls. Logistic regression showed that elevated levels of AMH (odds ratio [OR]=2.95, p=0.0056), visfatin (OR=1.7, p=0.0043) and kisspeptin (OR=18.3, p=0.0015) were strongly associated with PCOS.

Conclusion. These findings confirmed the significant role of AMH, testosterone, visfatin, and kisspeptin in the diagnosis of PCOS. Integration of adipokine markers, particularly visfatin and kisspeptin, with traditional hormonal markers enhances diagnostic accuracy.

Keywords. anti-Müllerian hormone, diagnosis, kisspeptin, LH/FSH ratio, PCOS, testosterone, visfatin

Introduction

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders affecting women of reproductive age, with an estimated prevalence ranging from 6% to 20%, depending on the diagnostic criteria used.[1] PCOS is a multifaceted condition with various clinical presentations, commonly involving hyperandrogenism, ovulatory irregularities, and polycystic ovarian

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morphology.[2] These symptoms not only affect reproductive health, but are also associated with metabolic disturbances such as insulin resistance, obesity and an increased risk of developing type 2 diabetes and cardiovascular disease.[3]

The pathophysiology of PCOS involves systemic changes, including metabolic and hormonal dysfunction, as changes in the well as central nervous system (CNS), such as dysregulation of the hypothalamic-pituitary-gonadal (HPG) axis and abnormal secretion of gonadotropin-releasing hormone (GnRH) secretion.[4] Evidence suggests that the CNS plays a role in PCOS pathophysiology through the dysregulation of GnRH secretion, which disrupts the pulsatile release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). This dysregulation contributes to hyperandrogenism and anovulation observed in PCOS patients.[5] Additionally, chronic anovulation results in irregular menstrual cycles and infertility, while metabolic complications contribute to the development of insulin resistance and obesity in many PCOS patients.[6]

PCOS is typically diagnosed using one of three main criteria: the National Institutes of Health (NIH) 1990 criteria, the Rotterdam 2003 criteria, or the Androgen Excess and PCOS Society (AE-PCOS) 2006 criteria.[7] Rotterdam criteria are the most widely used and require the presence of two of the following three characteristics: hyperandrogenism, oligo or anovulation, and polycystic ovaries on ultrasound.[8]

Despite established diagnostic criteria, the identification of reliable biomarkers for PCOS remains a key challenge. Hormonal markers, including LH, FSH and androgens (total and fee testosterone), have long been used to assess reproductive and endocrine dysfunction in PCOS patients.[9] More recently, anti-Müllerian hormone (AMH) has emerged as a promising biomarker due to its correlation with the number of ovarian follicles, reflecting ovarian reserve and hyperandrogenism in PCOS.[10,11] Elevated AMH levels are commonly observed in PCOS patients, making it a valuable diagnostic tool, although it may not be universally applicable in all phenotypes.[12]

In addition to these hormonal markers, emerging research has focused on the role of adipokines, signaling proteins secreted by adipose tissue, and metabolic dysfunction observed in PCOS. Adipokines such as visfatin and kisspeptin have been implicated in insulin resistance, inflammation, and the regulation of reproductive hormones.[13,14] Visfatin, in particular, is associated with insulin resistance and has been shown to be elevated in PCOS patients, suggesting a link between metabolic disturbances and reproductive dysfunction.[15] Kisspeptin, on the other hand, plays a critical role in the regulation of GnRH secretion, and its dysregulation in PCOS contributes to the abnormal hormonal environment characteristic of the syndrome.[13]

Given the complexities of PCOS, a multimarker approach integrating both hormonal and adipokine profiles may improve diagnostic accuracy and provide greater insight into the pathophysiology of the syndrome. This study aimed to evaluate the diagnostic utility of key hormonal markers (AMH, testosterone, LH/FSH ratio) and adipokines (visfatin, kisspeptin) in differentiating PCOS patients from healthy controls. We hypothesized that elevated levels of AMH, visfatin, and kisspeptin, along with an increased LH/FSH ratio and hyperandrogenism, could serve as reliable markers for the diagnosis of PCOS. By incorporating adipokine profiles into the diagnostic framework, we sought to enhance the early detection of PCOS and provide a more comprehensive understanding of its metabolic and reproductive components.

Material and methods

Study design and participants

A case-control study was conducted between January 2023 and December 2024 at [Al-Sadr Teaching Hospital in Najaf, Iraq], following approval of the Institutional Review Board (Approval Number: 34328). Written informed consent was obtained from all participants prior to inclusion, in accordance with the principles of the Declaration of Helsinki.[16] The sample size (n) was determined using the following formula to compare the two proportions in case-control studies:[17]

$$n = \left\{ \left(Z_{\left\{\frac{\alpha}{2}\right\}} + Z_{\{\beta\}} \right)^2 \cdot \left[p_{1(1-p_1)} + p_{2(1-p_2)} \right] \right\} / \{ (p_1 - p_2)^2 \}$$

- $Z_{\left\{\frac{\alpha}{2}\right\}} = 1.96$ for a 95% confidence level,
- $Z_{\{\beta\}} = 0.84$ for 80% power,
- $p_1 = 0.70$ (proportion of a specific marker in the PCOS group based on previous studies)
- $p_2 = 0.30$ (proportion in controls).

A total of 100 women aged 18 to 40 years were recruited and divided into two groups: 50 women diagnosed with PCOS according to the revised Rotterdam criteria and 50 age-matched healthy controls of the same age without PCOS.[18] The diagnostic criteria for PCOS include at least two of the following: (1) oligo or anovulation, (2) clinical and/or biochemical signs of hyperandrogenism and (3) polycystic ovarian morphology on ultrasound, excluding other endocrine disorders such as congenital adrenal hyperplasia, Cushing syndrome, thyroid dysfunction, or androgen-secreting tumors. Control participants were recruited from the general population and had regular menstrual cycles (21–35 days), no clinical or biochemical signs of hyperandrogenism, and normal ovarian morphology on transvaginal ultrasound.

Exclusion criteria for all participants included pregnancy, lactation, use of hormonal medications or insulin sensitizing agents within the last three months, smoking, alcohol abuse, and chronic systemic diseases such as diabetes mellitus, hypertension, or cardiovascular disease.

Data collection

Demographic and clinical data were collected through structured interviews and physical examinations performed by trained clinicians. The information gathered included age, body mass index (BMI) and physical activity levels assessed using the International Physical Activity Questionnaire (IPAQ) short form.[19]

Anthropometric measurements were performed with the participants wearing light clothing and no shoes. Weight was measured to the nearest 0.1 kg using a calibrated digital scale (Seca 803; Seca GmbH & Co. KG, Hamburg, Germany), and height was measured to the nearest 0.1 cm using a wall mounted stadiometer (Seca 217). BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2).

Clinical evaluations included detailed menstrual history (age at menarche, cycle duration, and regularity), reproductive history (eg pregnancies and miscarriages), and evaluation of signs of hyperandrogenism, such as hirsutism. Hirsutism was evaluated using the modified Ferriman-Gallwey scoring system, with a score ≥8 indicating hirsutism.[20]

Hormonal and metabolic assessments

Venous blood samples were collected from all participants between 8:00 a.m. and 9:00 a.m. after an overnight fast of at least 8 h. For women with regular menstrual cycles, samples were collected during the early follicular phase of the menstrual cycle (days 2–5). For women with oligomenorrhea or amenorrhea, samples were collected on a random day and progesterone levels were measured to confirm the absence of ovulation. Blood samples were centrifuged at 3,000 rpm for 10 min at 4°C, and the serum was separated and stored at − 80°C until analysis.

Serum levels of key hormonal markers, including AMH, total testosterone, LH, and FSH, were measured. These were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits: AMH: human AMH ELISA kit (catalog number: E-EL-H0317, Elabscience, Houston, TX, USA), total testosterone: testosterone ELISA kit (catalog number: E-EL-0072, Elabscience, Houston, TX, USA). Free testosterone levels were quantified using a commercially available free testosterone ELISA kit (Catalog Number: ab178663, Abcam, Cambridge, UK). LH: LH ELISA Kit (catalog number: ab178658, Abcam, Cambridge, UK). FSH: FSH ELISA kit (catalog number: E-EL-H1143, Elabscience, Houston, TX, USA). The assays were performed according to the manufacturer's instructions. The luteinizing hormone / follicle stimulating hormone ratio was calculated.

Adipokine and neuropeptide measurements

Serum levels of visfatin and kisspeptin were measured to assess their roles in the pathophysiology of PCOS. Quantification was performed using specific ELISA kits. Visfatin: human visfatin ELISA kit (catalog number: ab267658, Abcam, Cambridge, UK) and kisspeptin (catalog number: MBS165884, MyBioSource, Inc., San Diego, CA, USA).

All assays were performed according to the manufacturer's instructions. The intra- and inter-assay coefficients of variation were less than 10% and<15%, respectively, for all assays.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS, IBM, Armonk, NY, USA) program was used to detect the effects of different groups (patients and controls) on the study parameters. A t-test was used to compare the means. The Chi-square test was used to compare the percentages (0.05 and 0.01 probability). Estimation of the correlation coefficient and multiple linear regression between variables. Sensitivity and specificity of parameters in the patient and control groups. Cutoff values for biomarkers were determined using receiver operating characteristic (ROC) curve analysis to maximize sensitivity and specificity. The Youden index is used to identify the optimal threshold for each parameter.[21]

Results

Demographic characteristics of study groups

These findings highlight notable differences in metabolic, reproductive, and cardiovascular health between women with PCOS and healthy controls. Although no age differences were observed ($p=0.22$), the PCOS group had a significantly higher body mass index ($p<0.001$) and longer menstrual cycles ($p<0.001$). Analysis of pregnancies in the study population (Table 1) revealed significant differences between PCOS patients and controls ($p<0.001$). In the control group, 36% had one pregnancy, 30% had two pregnancies, and 34% had no previous pregnancies. On the contrary, 38% of PCOS patients had one pregnancy, 28% had two pregnancies, and 34% had three or more pregnancies. None of the PCOS patients was nulliparous. Elevated hirsutism scores ($p<0.001$) indicated more severe androgenic symptoms in the PCOS group. Furthermore, both Systolic and Diastolic blood pressures were significantly higher ($p<0.001$), suggesting an increased cardiovascular risk. These results highlight notable metabolic, re-

productive, and cardiovascular differences between the groups.

Table 1. Demographic characteristics of control subjects and patients with PCOS[a]

Characteristic	Control n=50	PCOS n=50	p
Age (years)			
Mean±SD	27.88±6.12	29.4±6.6	0.22 I NS
Range	18–39	18–39	
BMI (kg/m^2)			
Mean±SD	24.4±2.1	31.98±3.21	<0.001 I***
Range	20.27–29.9	25.46–39.9	
Cycle length (days)			
Mean±SD	30.86±2.06	43.4±4.02	<0.001 I***
Range	28–34	35–50	
Pregnancies			
Non pregnancies, n (%)	0 (0%)	17 (34%)	<0.001 F***
Pregnancies (1), n (%)	19 (38%)	18 (36%)	
Pregnancies (2), n (%)	14 (28 %)	15 (30%)	
Pregnancies (3), n (%)	17 (34 %)	0 (0%)	
Hirsutism (FG score)			
Mean±SD	3.06±0.9	8.2±1.94	<0.001 I***
Range	1.31–5.03	4.13–14.2	
Systolic (mmHg)			
Mean±SD	119±9.98	128.5±10.25	<0.001 I***
Range	97.5–143	106.8–157.8	
Diastolic (mmHg)			
Mean±SD	74.98±5.1	85.64±4.59	<0.001 I***
Range	65.4–86.1	75.4– 96.2	

[a] Pregnancies were classified as follows: non-pregnancies – no previous pregnancy, pregnancies (1) one previous pregnancy, pregnancies (2) two previous pregnancies, and pregnancies (3) three or more previous pregnancies; n number of cases, SD – standard deviation, BMI body mass index, Fisher's exact test; I independent samples t-test; NS not significant (p≥0.05)

Comparison of mean hormonal values among control group and patients with PCOS

Table 2 shows significant hormonal imbalances in PCOS patients compared to controls, with elevated free testosterone (p<0.001) and AMH levels (p<0.001). The LH/FSH ratio was significantly higher in PCOS patients (3.44±0.47) than in controls (1.54±0.42, p<0.001). The mean serum concentrations of LH and FSH in PCOS patients were 10.32±2.4 mIU/mL and 3.00±0.95 mIU/mL, respectively, while in the control group, LH was 5.60±1.5 mIU/mL and FSH was 3.64±0.8 mIU/mL. The FSH/LH ratio was higher in PCOS patients than in controls; however, this finding is consistent with historical observations and has limited diagnostic significance compared to AMH and adipokines. PCOS patients also exhibited lower total testosterone levels (p<0.001). These findings underscore key hormonal disruptions in PCOS, as shown in Figure 1.

Table 2. Comparison of hormonal markers between control and PCOS patients[a]

Characteristic	Control (n=50)	PCOS (n=50)	p
LH (mIU/mL)			
Mean±SD	5.60±1.5	10.32±2.4	<0.001 I***
Range	3.5–8.2	7.0–15.6	
FSH (mIU/mL)			
Mean±SD	3.64±0.8	3.00±0.95	<0.001 I***
Range	2.4–5.2	1.8–5.0	
LH/FSH Ratio			
Mean±SD	1.54±0.42	3.44±0.47	<0.001 I***
Range	0.58–2.5	2.4–4.63	
Total testosterone (ng/dL)			
Mean±SD	45.3±9.2	81.4±16.5	<0.001 I***
Range	22.6–63.08	26.48–118.6	
Free testosterone (pg/mL)			
Mean±SD	2.47±0.46	4.55±0.95	<0.001 I***
Range	1.36–3.188	1.922–7.26	
AMH (ng/mL)			
Mean±SD	4.07±1.1	8.1±2.3	<0.001 I***
Range	1.59–6.68	2.57–11.2	

[a] anti-Müllerian hormone, LH – luteinizing hormone, FSH follicle-stimulating hormone, statistical significance is indicated by ***p<0.001, I – independent sample t-test

Analysis of adipokine variations between the control and PCOS groups

Table 3 reveals significantly elevated levels of adipokines in PCOS patients compared to controls, with visfatin (86.6±11.02 vs. 49.53±10.25 ng/mL) and kisspeptin (9.88±1.96 vs. 4.84±1.07 ng/mL) both showing higher mean values in the PCOS group (p<0.001). These findings highlight notable differences in adipokine regulation in PCOS, as shown in Figure 2.

Table 3. Comparative analysis of adipokine levels in control and PCOS patients[a]

Characteristic	Control (n=50)	PCOS (n=50)	p
Visfatin (ng/mL)			
Mean±SD	49.53±10.25	86.6±11.02	<0.001 I***
Range	31.64–68.9	62.5–115.3	
Kisspeptin (ng/mL)			
Mean±SD	4.84±1.07	9.88±1.96	<0.001 I***
Range	2.58–7.33	6.3–15.13	

[a] Statistical significance is indicated by ***p<0.001, I independent sample t-test

Analysis of adipokine and hormonal correlations in PCOS patients

Table 4 shows the significant positive correlations between adipokines (Visfatin and Kisspeptin) and hormonal markers in PCOS patients. For example, visfatin was strongly correlated with kisspeptin (r=0.720, p<0.001), indicating its potential interdependence in the pathophysiology of PCOS. Similarly, both adipokines

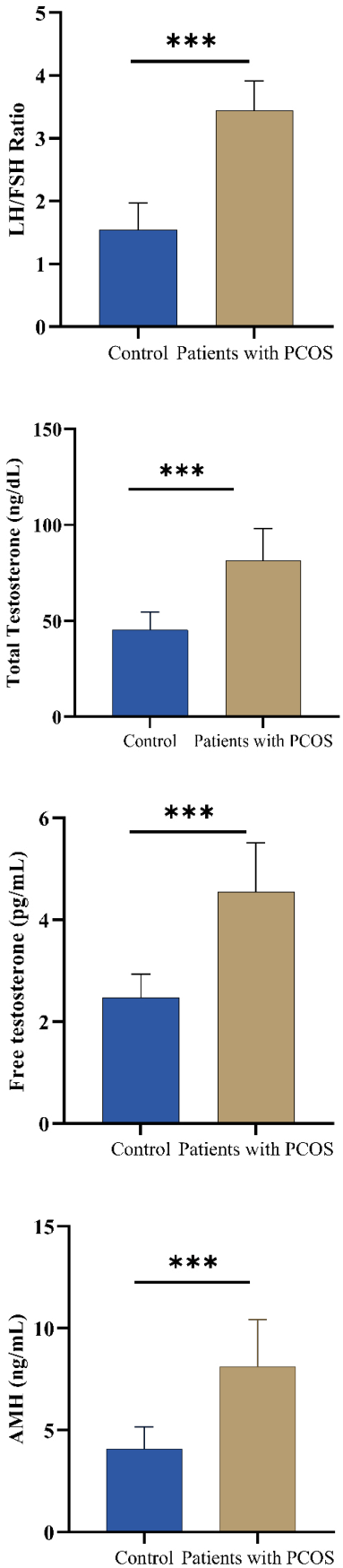

Fig. 1. Bar chart showing the mean comparison among control group and patients with PCOS

showed strong correlations with AMH and testosterone levels, suggesting their roles in hyperandrogenism and ovarian dysfunction.

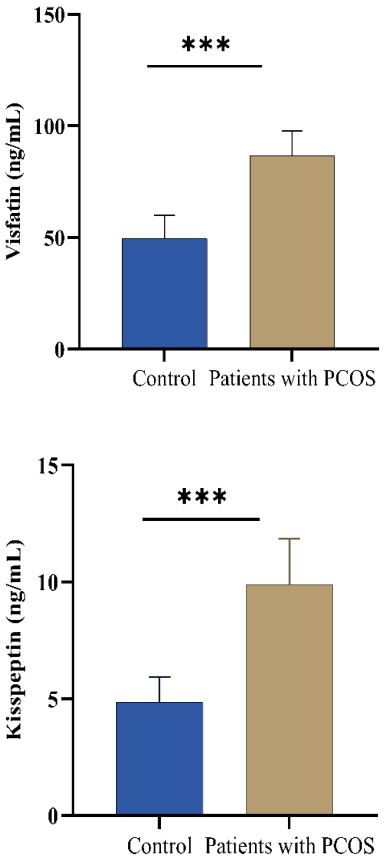

Fig. 2. Bar chart showing the mean comparison among control group and patients with PCOS

Table 4. Correlations of adipokines and hormone levels in patients with PCOS.

	Correlation coefficient (r) and p				
	Visfatin	Kisspeptin	Free testosterone	Total testosterone	AMH
Visfatin (ng/mL)	1	r= 0.720 p<0.001	r=0.699 p<0.001	r=0.711 p<0.001	r=0.695 p<0.001
Kisspeptin (ng/mL)	r= 0.720 p<0.001	1	r= 0.692 p<0.001	r= 0.667 p<0.001	r= 0.647 p<0.001
Free testosterone (pg/mL)	r= 0.699 p<0.001	r= 0.692 p<0.001	1	r= 0.668 p<0.001	r= 0.551 p<0.001
Total testosterone (ng/dL)	r=.711 p<0.001	r= 0.667 p<0.001	r= 0.668 p<0.001	1	r= 0.637 p<0.001
AMH (ng/mL)	r= 0.695 p<0.001	r= 0.647 p<0.001	r= 0.551 p<0.001	r= 0.637 p<0.001	1

Diagnostic efficacy of hormonal and adipokine markers in PCOS

Table 5 highlights various hormonal and adipokine markers for PCOS, all showing significant predictive power with p values of 0.001. For AMH, a cut-off value of >5.74 ng/mL achieved 86% sensitivity and 94% specificity, reflecting its high diagnostic utility for PCOS. Similarly, visfatin and kisspeptin demonstrated excel-

lent predictive power, with cut-off values of >68.91 ng/mL and >6.96 ng/mL, respectively, achieving sensitivities and specificities exceeding 95% (Fig. 3).

Table 5. Analysis of the ROC curve for hormones and adipokines in the diagnosis of PCOS a

Variables	Cut-off value	Sens**%	Spec%	Ppv**	Npv	Accuracy	AUC%	p (AUC= 0.05)
LH/FSH ratio	>2.38	100	96	98	100	98	100	0.001**
Total testosterone (ng/dL)	>63.07	92	100	100	92.7	92	96	0.001**
Free testosterone (pg/ml)	> 3.09	96	98	98	96	94	97.6	0.001**
AMH (ng/ml)	>5.74	86	94	93.5	87	80	91	0.001**
Visfatin (ng/ml)	>68.91	98	100	100	98	98	99.7	0.001**
Kisspeptin (ng/ml)	>6.96	96	98	100	96	94	99.6	0.001**

[a] Sens sensitivity, Spec – specificity, PPV positive predictive value, NPV negative predictive value, accuracy [(Sensitivity + Specificity) - 1], AUC area under the curve

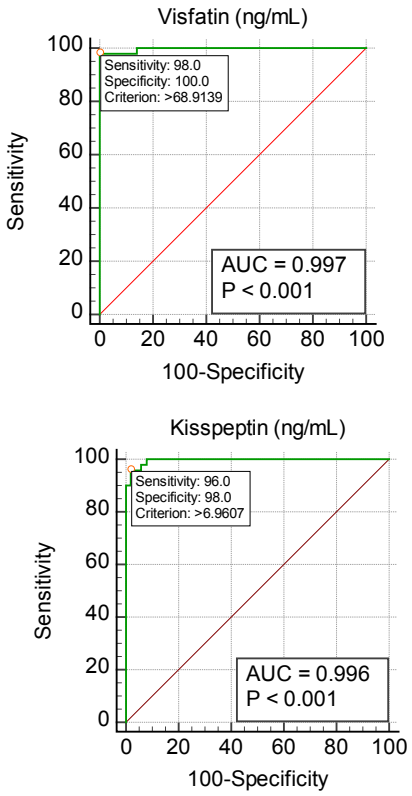

Fig. 3. The ROC curve for adipokines

Statistical Analysis of adipokines and insulin resistance in PCOS

Table 6 presents the logistic regression analysis showing a strong model fit (R^2=0.87) to explain the relationship between adipokines and PCOS. Visfatin and kisspeptin were significantly associated with an increased risk of PCOS, with odds ratios of 1.7 and 18.3, respectively, both of which were statistically significant. These findings suggest that high levels of adipokines strongly predict the probability of PCOS.

Table 6. Logistic regression analysis of adipokines in PCOS patients (model 1, R^2=0.87)[a]

Variables	B (coef)	Wald	Odds ratio	95% CI for odds ratio	p
Visfatin	0.53	1.8	1.7	1.18 to 2.45	0.0043**
Kisspeptin	2.3	9.1	18.3	3.59 to 180.81	0.0015**

[a] B (coef) regression coefficient, CI – confidence interval

Evaluation of hormonal profiles in predicting PCOS

Table 7 presents the results of the logistic regression analysis identifying the significant hormonal predictors of PCOS. AMH was strongly associated with PCOS, with an odds ratio (OR) of 2.95 (95% CI: 1.33–5.28, p=0.0056). This underscores the utility of AMH as a reliable biomarker for the diagnosis as it reflects ovarian reserve and correlates with polycystic ovarian morphology. The total testosterone level also had a significant effect (odds ratio=1.19, p=0.0002). On the contrary, the historically significant LH / FSH ratio was not a statistically significant predictor in this model (p=0.3168). This finding aligns with the current literature, suggesting that the clinical utility of the LH/FSH ratio has decreased due to its variability across PCOS phenotypes and the influence of confounders, such as obesity and age.

Table 7. Hormonal predictors of PCOS a logistic regression model (model 2, R^2=0.9)[a]

Variables	B (coef)	Wald	Odds ratio	95% CI for odds ratio	p
AMH	0.97	6.7	2.95	1.33 to 5.28	0.0056**
Total testosterone	0.17	14	1.19	1.088 to 1.309	0.0002***
LH/FSH ratio	0.180	0.067	0.455	0.0972 to 2.1274	0.3168

[a] B (coef) regression coefficient, CI – confidence interval

Discussion

This study provides a comprehensive evaluation of the hormonal and adipokine profiles in women with PCOS, advancing our understanding of their diagnostic utility and clinical relevance. Our findings revealed that AMH, testosterone, visfatin, and kisspeptin, when used in combination, significantly improved the accuracy of PCOS diagnosis. These results are consistent with a growing body of evidence, although some discrepancies with previous studies highlight the complex nature of PCOS and its diverse phenotypes.

Our study confirmed that AMH, testosterone (both total and free), visfatin, and kisspeptin levels were significantly elevated in patients with PCOS compared to healthy controls. These findings are consistent with well-established theories regarding the characteristics of PCOS endocrine and metabolic dysfunction. Additionally, logistic regression analysis demonstrated that these markers, particularly AMH and adipokines, were strong

predictors of PCOS with excellent diagnostic precision, as shown by ROC curve analyzes.

AMH has emerged as a crucial biomarker for PCOS due to its role in reflecting ovarian reserve and follicular activity.[22] In our study, AMH levels were significantly higher in PCOS patients (8.1±2.3 ng/mL) than in controls (4.07±1.1 ng/mL), which is consistent with previous studies that have also reported elevated levels of AMH in PCOS patients.[23,24] Begum et al. found that AMH is a surrogate marker for antral follicle count, with levels typically 2 to 3-fold higher in women with PCOS than in women without PCOS. Our findings support this and reinforce the idea that AMH is strongly correlated with polycystic ovarian morphology, making it a valuable diagnostic tool.

However, AMH remains a cornerstone in the diagnosis of PCOS due to its strong correlation with ovarian reserve and antral follicle count. Our findings, which showed a significant association between elevated AMH levels and PCOS (OR, 2.95; p=0.0056), further strengthen its diagnostic value. Similarly, total testosterone levels were significantly associated with PCOS (OR, 1.19; p=0.0002), reflecting the role of hyperandrogenism in pathophysiology. Although our study found that AMH is a reliable predictor of PCOS, other studies have raised concerns about its variability across different phenotypes of PCOS. Bahadur et al. and Alsolaiman et al. noted that AMH diagnostic performance of AMH may be lower in women with milder forms of PCOS or those without overt polycystic ovarian appearance.[25,26] Therefore, while AMH was a useful marker in our cohort, it may not be universally applicable to all PCOS subgroups.

Elevated androgen levels are a hallmark of PCOS, and our study confirmed this, with both total testosterone (81.4±16.5 ng/dL) and free testosterone (4.55±0.95 pg/mL) being significantly higher in PCOS patients. These findings are consistent with the extensive literature on hyperandrogenism in PCOS.[27,28] Hyperandrogenism contributes to clinical manifestations such as hirsutism, acne, and alopecia and is one of the key diagnostic criteria for PCOS.

In our logistic regression model, the total testosterone level had a modest but significant association with PCOS (OR=1.19, p=0.0002). This is consistent with studies by Grassi et al. and Ye et al., highlighting the role of hyperandrogenism as a critical driver of this syndrome.[29,30] However, the diagnostic utility of testosterone, particularly in milder cases of PCOS, has been questioned. Some studies, such as those of Pace and Azziz, reported that androgen levels could not be elevated in all PCOS patients, particularly those with less severe phenotypes or without clinical signs of hyperandrogenism.[31]

Although the FSH/LH ratio has historically been considered a marker for PCOS, its diagnostic utility is now considered limited compared to newer biomarkers such as AMH, visfatin, and kisspeptin. In our study, the LH/FSH ratio was significantly higher in the PCOS group (3.44±0.47) than in controls (1.54±0.42), consistent with the results of previous studies.[5,32] However, in our logistic regression analysis, the LH / FSH ratio did not emerge as a statistically significant predictor of PCOS (p=0.3168), suggesting that while it may serve as a useful clinical marker, it may not be as robust as other markers such as AMH or testosterone.

This result aligns with recent literature questioning the reliability of the LH / FSH ratio in all PCOS phenotypes, particularly in obese patients or those with insulin resistance.[33] These findings suggest that the LH/FSH Ratio may be more variable than previously thought and its diagnostic utility may be limited when used alone.

Chemokines play a crucial role in PCOS pathophysiology by mediating inflammatory responses and metabolic dysfunctions. Elevated chemokine levels in PCOS patients have been associated with increased insulin resistance and systemic inflammation, which contribute to the breakdown of reproductive and metabolic homeostasis. These findings suggest that chemokines act as intermediaries in the crosstalk between adipose tissues and the reproductive system.

Visfatin and kisspeptin have emerged as promising biomarkers in PCOS, particularly in light of their roles in metabolic and reproductive dysfunction. Visfatin, an adipokine secreted by visceral adipose tissue, is strongly associated with insulin resistance and systemic inflammation, which are the two hallmark features of PCOS. In this study, visfatin levels were nearly double in PCOS patients compared to controls, underscoring the potential role of visfatin in linking metabolic disturbances to reproductive dysfunction. This finding is supported by previous studies that reported elevated visfatin levels in women with PCOS, linking it to insulin resistance and inflammation.[15,34] Koleva-Tyutyundzhieva et al. found that visfatin levels were significantly higher in patients with insulin-resistant PCOS, which may explain the metabolic disturbances commonly observed in this syndrome.

Kisspeptin is a neuropeptide that plays a pivotal role in the regulation of the HPG axis by stimulating the release of GnRH. Dysregulation of kisspeptin signaling has been implicated in abnormal gonadotropin secretion characteristics of PCOS. The elevated levels of kisspeptin observed in this study may reflect compensatory mechanisms to counteract disrupted HPG axis signaling in PCOS. These findings highlight the dual role of kisspeptin as a metabolic and reproductive biomarker in PCOS.[35] In our study, kisspeptin emerged as a strong predictor of PCOS (OR=18.3, p=0.0015), highlighting its potential as a biomarker of reproductive dysfunction in PCOS. Studies by Yeung et al. and Gao et al. demonstrated that elevated kisspeptin levels are associated with

abnormal gonadotropin secretion in PCOS, further supporting our findings.[36,37]

Recent studies have highlighted the role of novel biomarkers such as callistatin in PCOS. Callistatin, a member of the kallikrein-related peptide family, has been shown to play a regulatory role in inflammation and metabolic dysfunction, which are key features of PCOS. Evidence suggests that callistatin exerts anti-inflammatory effects by modulating cytokine release and mitigating oxidative stress, making it a potential biomarker for identifying and managing PCOS.[38]

While our study focused on hormonal and adipokine markers, such as AMH, visfatin, and kisspeptin, the integration of emerging biomarkers, such as callistatin, could provide a more comprehensive diagnostic framework for PCOS. The inclusion of callistatin in future studies may help elucidate its interplay with established markers and its potential utility in predicting PCOS phenotypes and associated metabolic risks.

The combined use of hormonal and adipokine markers provided excellent diagnostic accuracy in our study, with ROC analysis showing AUC values of 0.95 or higher for the LH/FSH ratio, visfatin, and kisspeptin. These findings highlight the value of integrating adipokine markers into traditional diagnostic criteria for PCOS. By combining markers that reflect both reproductive and metabolic dysfunction, this multimarker approach could provide a more comprehensive diagnostic tool, particularly for identifying women with atypical or mild PCOS.

Our findings align with those of Liu et al. and Ruan et al., who suggested that novel biomarkers such as visfatin and kisspeptin could improve the early detection of PCOS, especially in women with metabolic abnormalities.[39,40] The high sensitivity and specificity of these markers in our study suggest that they could be incorporated into clinical practice to improve both diagnostic accuracy and potential for early intervention.

Study limitations and strengths

One of the main strengths of our study is the comprehensive analysis of hormonal and adipokine markers, which provides a more holistic view of endocrine and metabolic disturbances in PCOS. The use of logistic regression and ROC curve analyzes strengthened the statistical validity of our findings, and the relatively large sample size and inclusion of well-matched controls increased the reliability and generalizability of our results. However, this study has some limitations. This case-control design limits our ability to establish causal relationships between elevated markers and the development of PCOS. Longitudinal studies are necessary to assess whether changes in these biomarkers can predict the onset of PCOS over time. It is important to note that, while testosterone levels are elevated in many patients with PCOS, this may not be universally observed in all phenotypes. This variability underscores the need for a multimarker approach to PCOS diagnosis. Furthermore, the study population may not represent all PCOS phenotypes, particularly those with normal weight or those without insulin resistance. More research is required to evaluate the utility of these markers in diverse populations.

Future directions

Future studies should explore the longitudinal dynamics of these biomarkers in women at risk of developing PCOS. Furthermore, the role of adipokines, such as visfatin and kisspeptin, in the metabolic aspects of PCOS warrants further investigation, particularly with respect to their potential as therapeutic targets. Expanding the panel to include additional adipokines and inflammatory markers may also provide a more nuanced understanding of the metabolic and reproductive interactions in PCOS.

Conclusion

These findings confirmed the significant role of AMH, testosterone, visfatin, and kisspeptin in the diagnosis of PCOS. Integrating adipokine markers, particularly visfatin and kisspeptin, with traditional hormonal markers improves diagnostic accuracy and provides a more comprehensive understanding of the pathophysiology of PCOS. These results suggest that a multimarker approach could be beneficial in clinical practice for early detection and management of PCOS. In addition to the hormonal and adipokine biomarkers evaluated in this study, recent advances have identified callistatin as a new marker with potential diagnostic and therapeutic implications in PCOS[38]. Future research should explore the integration of callistatin with other emerging biomarkers to develop a more holistic approach to diagnose and managing PCOS.

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Declarations

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Author contributions

Conceptualization, R.D.A.A., K.H.H., W.R.A-H. and B.R.Y.; Methodology, R.D.A.A., K.H.H., W.R.A-H. and B.R.Y.; Investigation, R.D.A.A., K.H.H., W.R.A-H. and

B.R.Y.; Data Curation, R.D.A.A., K.H.H., W.R.A-H. and B.R.Y.; Writing – Original Draft Preparation, R.D.A.A., K.H.H., W.R.A-H. and B.R.Y.; Writing – Review & Editing, R.D.A.A., K.H.H., W.R.A-H. and B.R.Y.; Supervision, R.D.A.A., K.H.H., W.R.A-H. and B.R.Y.

Conflicts of interest

The author declare that they have no competing interests.

Data availability

The data sets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

Ethical clearance was granted by the Institutional Review Board of the Medical Laboratory Techniques, College of Health and Medical Techniques at Al-Furat Al-Awsat Technical University, Al-Kufa, Iraq (Approval Number: 31003).

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