European Science Methodical Journal
ISSN (E): 2938-3641
Volume 4, Issue 3, March - 2026

# NEUROENDOCRINE AND METABOLIC BIOMARKERS IN POLYCYSTIC OVARY SYNDROME: ASSOCIATION OF KISSPEPTIN, ANTI-MÜLLERIAN HORMONE, AND ADIPOKINES WITH HORMONAL DYSREGULATION

Rawaa Mohammed Obiad
Department of Biology, College of Education for Pure Sciences,
University of Tikrit, Tikrit, Iraq
Corresponding author: rawamuhammad@tu.edu.iq

**Abstract**:
Polycystic ovary syndrome (PCOS) is a common endocrine disorder in reproductive age women with complex neuroendocrine and metabolic causes. This case control study assessed serum levels of the following: kisspeptin, anti-Mullerian hormone (AMH), leptin, adiponectin, reproductive hormones, insulin resistance indices in 60 women with PCOS and 60 age-matched healthy controls in Iraq. All biomarkers were assessed by enzyme-immunoassay (ELISA) and analyzed with the aid of the statistical package (SPSS v.26). PCOS patients showed significantly elevated kisspeptin (179.85±26.05 vs. 110.22±18.91 pg/mL), AMH (6.89±2.16 vs. 3.12±1.03 ng/mL), LH (11.98±2.99 vs. 5.99±1.37 IU/L), LH/FSH ratio (2.28±0.80 vs. 1.01±0.28), testosterone (69.93±14.52 vs. 41.06±8.98 ng/dL), leptin (27.23±5.53 vs. 15.53±4.14 ng/mL), and HOMA-IR (4.30±0.81 vs. 1.83±0.48), with significantly lower adiponectin (4.71±1.15 vs. 8.96±2.01 µg/mL) (all $p<0.001$). Within the PCOS group, only one significant intragroup correlation was detected: kisspeptin negatively correlated with total testosterone ($r=-0.354$, $p=0.006$). All other cross-biomarker correlations were non-significant, indicating that neuroendocrine, ovarian reserve, and metabolic pathways are independently dysregulated in PCOS.

**Keywords**: PCOS; Kisspeptin; AMH; Leptin; Adiponectin; HOMA-IR; Insulin Resistance; Hormonal Dysregulation; Iraq.

## Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders among women of childbearing age in the world, with a reported prevalence of 6-21% based on the diagnostic criteria used [1, 2]. The Rotterdam consensus criteria (2003) state that PCOS can be defined as the presence of at least two of the following three features: oligo- or anovulation, clinical or biochemical hyperandrogenism, polycystic ovarian morphology on ultrasound [3].

Beyond its reproductive implications, PCOS is a complex metabolic disorder that is strongly linked to insulin resistance, dyslipidemia and increased risk of type 2 diabetes mellitus and cardiovascular disease [4].

The neuroendocrine basis of PCOS is the dys-regulation of the hypothalamic-pituitary-gonadal (HPG) axis. The main upstream regulator of gonadotropin-releasing hormone (GnRH) pulsatility, and ultimately of LH and FSH secretion, is the neuropeptide kisspeptin, which is encoded by the KISS1 gene [5]. Increasing evidence suggests that increased kisspeptin activity plays a role in the elevated LH pulse frequency that is characteristic of PCOS which drives hyperandrogenism and anovulation [6].

Anti-Mullerian hormone (AMH), which is secreted by granulosa cells of preantral and small antral follicles, has been successfully established as a marker of ovarian reserve. In the case of PCOS, AMH is characteristically elevated 2-3 times higher than normal compared to healthy controls, which indicate follicular excess and impaired maturation [7]. In this way, AMH could also prevent FSH-dependent follicular maturation, perpetuating follicular arrest [8].

Adipokines, bioactive cytokines produced by the adipose tissue, are central mediators between the metabolism status and the reproductive function. Leptin is increased in situations of fatness and insulin resistance and has been found to regulate the HPG axis at several levels [9]. Adiponectin, on the other hand, has insulin-sensitizing and anti-inflammatory properties, and is generally low in women with PCOS, especially in women with central adiposity [10]. The resistance to insulin, measured with the Homeostasis Model Assessment of insulin resistance (HOMA-IR) is a consistent metabolic characteristic of PCOS.

Within the Iraqi scientific literature, a growing amount of research has been conducted on various aspects of the biochemistry of PCOS. Abdul-Jabbar and Swadi (2025) in the Annals of the College of Medicine Mosul reported significant changes in the insulin resistance markers in Iraqi PCOS patients suggesting a strong metabolic component of the syndrome in this population [14]. Abdulrahman and Zbaar (2025) assessed the level of atrial natriuretic peptide in Iraqi women with PCOS and highlighted the cardiovascular-endocrine overlap in this disease [15]. Kadhim et al. (2025) examined the role of oxidative stress in obese and non-obese patients with PCOS attending infertility clinics in Karbala in order to highlight the heterogeneity of metabolic PCOS phenotypes in Iraq [16]. More recently, Hlail and Alshukri (2025) reported an increase in interleukin-10 and AMH levels in Iraqi PCOS women, which highlights the inflammatory and ovarian reserve aspects of the syndrome [17]. However, data on the simultaneous assessment of kisspeptin, AMH, and adipokines — the neuroendocrine, ovarian reserve, and adipose-derived axes — in a single Iraqi cohort remain scarce. This study therefore aimed to bridge this gap by evaluating and comparing this combined biomarker profile between PCOS patients and healthy controls, and by examining intragroup correlations to determine whether these axes are co-regulated or independently dysregulated in Iraqi women with PCOS.

## 2. MATERIALS AND METHODS

### 2.1 Study Design and Setting

A prospective case-control study was conducted from January to December 2024 at the gynecology and endocrinology outpatient clinics of Tikrit Teaching Hospital and the Department of Biology, College of Education for Pure Sciences, University of Tikrit, Iraq. Ethical approval was obtained from the Institutional Review Board of the University of Tikrit (Ref. No. TU/2024/042). Written informed consent was obtained from all participants. The study adhered to the principles of the Declaration of Helsinki.

### 2.2 Study Participants

A total of 120 women were enrolled as shown in Table 1. PCOS was diagnosed according to the Rotterdam criteria (2003). Control subjects were age-matched healthy women with regular menstrual cycles and no endocrinological abnormality.

Table 1. Study groups, sample size, and demographic data.

| Group | Description | n | Age (years) |
|---|---|---|---|
| **Group 1 (PCOS)** | Women diagnosed with PCOS according to Rotterdam criteria | 60 | 27.76±4.25 |
| **Group 2 (Control)** | Healthy women with regular menstrual cycles | 60 | 27.74±4.21 |
| **Total** | Age-matched (p = 0.978) | **120** | — |

NS = not significant (p = 0.978 for age). BMI was significantly higher in PCOS (p<0.001).

### 2.3 Inclusion and Exclusion Criteria

Inclusion criteria included women aged 18–40 years, PCOS diagnosis by Rotterdam criteria (Group 1), regular menstrual cycles (Group 2), and no hormonal therapy for ≥3 months. Women were excluded if they had thyroid disorders, hyperprolactinemia (prolactin >25 ng/mL), diabetes mellitus, pregnancy or lactation, chronic systemic disease, or use of hormone-modifying agents.

### 2.4 Laboratory Analysis

Venous blood (10 mL) was collected during the early follicular phase (days 2–5 of the cycle, or any day for anovulatory patients) following a 10-hour overnight fast. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at −80°C until assay. All biomarkers were measured by ELISA (ELABSCIENCE, TX, USA): kisspeptin (pg/mL), AMH (ng/mL), LH (IU/L), FSH (IU/L), total testosterone (ng/dL), leptin (ng/mL), adiponectin (µg/mL), and fasting serum insulin (µIU/mL). HOMA-IR was calculated as: [fasting insulin (µIU/mL) × fasting glucose (mmol/L)] / 22.5.

### 2.5 Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 26.0. Continuous variables are expressed as Mean±Standard Deviation (SD). Normality was confirmed by the Shapiro–Wilk test. Between-group comparisons used the independent-samples t-test. Pearson correlation was applied to examine bivariate associations within the PCOS group. Binary logistic regression identified independent predictors of PCOS. A two-tailed p-value <0.05 was considered statistically significant.

## 3. RESULTS

### 3.1 Demographic and Clinical Characteristics

The two groups were closely matched for age (p=0.978), confirming adequate age-matching. BMI was significantly higher in PCOS patients (28.81±2.65 vs. 23.75±2.56 kg/m²; p<0.001). Common clinical features among PCOS women included oligomenorrhea (68.3%), amenorrhea (20.0%), hirsutism (71.7%), and acne (60.0%).

### 3.2 Comparison of Biomarkers between PCOS and Control Groups

Table 2 summarizes all measured biomarker values. Eleven of twelve parameters were significantly different between groups (all p<0.001 or p=0.002), with only age being non-significant. Key findings include:

- Kisspeptin was 63% higher in PCOS (179.85±26.05 vs. 110.22±18.91 pg/mL; t=16.75; p<0.001)
- AMH was more than twice as high in PCOS (6.89±2.16 vs. 3.12±1.03 ng/mL; t=12.21; p<0.001)
- LH/FSH ratio was elevated 2.3-fold in PCOS (2.28±0.80 vs. 1.01±0.28; t=11.70; p<0.001)
- Total testosterone was significantly higher in PCOS (69.93±14.52 vs. 41.06±8.98 ng/dL; t=13.10; p<0.001)
- Adiponectin was significantly suppressed in PCOS (4.71±1.15 vs. 8.96±2.01 µg/mL; t=−14.22; p<0.001)
- HOMA-IR was 2.3-fold higher in PCOS (4.30±0.81 vs. 1.83±0.48; t=20.33; p<0.001)

Table 2. Comparison of hormonal and metabolic biomarkers between PCOS patients and healthy controls.

| Parameter | PCOS (n=60) Mean±SD | Control (n=60) Mean±SD | t-value | p-value |
|---|---|---|---|---|
| **Age (years)** | 27.76±4.25 | 27.74±4.21 | 0.03 | 0.978 (NS) |
| **BMI (kg/m²)** | 28.81±2.65 | 23.75±2.56 | 10.66 | <0.001 |
| **LH (IU/L)** | 11.98±2.99 | 5.99±1.37 | 14.11 | <0.001 |
| **FSH (IU/L)** | 5.51±1.13 | 6.09±0.95 | -3.10 | 0.002 |
| **LH/FSH Ratio** | 2.28±0.80 | 1.01±0.28 | 11.70 | <0.001 |
| **Total Testosterone (ng/dL)** | 69.93±14.52 | 41.06±8.98 | 13.10 | <0.001 |
| **AMH (ng/mL)** | 6.89±2.16 | 3.12±1.03 | 12.21 | <0.001 |
| **Kisspeptin (pg/mL)** | 179.85±26.05 | 110.22±18.91 | 16.75 | <0.001 |
| **Leptin (ng/mL)** | 27.23±5.53 | 15.53±4.14 | 13.11 | <0.001 |
| **Adiponectin (µg/mL)** | 4.71±1.15 | 8.96±2.01 | -14.22 | <0.001 |
| **Insulin (µIU/mL)** | 22.85±5.59 | 9.10±1.99 | 17.95 | <0.001 |
| **HOMA-IR** | 4.30±0.81 | 1.83±0.48 | 20.33 | <0.001 |

Values expressed as Mean±SD. NS = not significant. p-values from independent-samples t-test.

### 3.3 Pearson Correlation Analysis within the PCOS Group

Table 3 presents Pearson correlation coefficients for selected biomarker pairs within the PCOS group (n=60). Only one statistically significant correlation was identified: kisspeptin showed a significant negative correlation with total testosterone ($r=-0.354$, $p=0.006$). All remaining correlations, including kisspeptin vs. LH ($r=0.001$, $p=0.993$), AMH vs. LH/FSH ratio ($r=0.159$, $p=0.226$), and leptin vs. HOMA-IR ($r=0.063$, $p=0.630$), were non-significant.

Table 3. Pearson correlation analysis between selected biomarkers within the PCOS group (n=60).

| Variable 1 | Variable 2 | r | p-value | Significance |
|---|---|---|---|---|
| Kisspeptin (pg/mL) | LH (IU/L) | 0.001 | 0.993 | NS |
| Kisspeptin (pg/mL) | AMH (ng/mL) | 0.179 | 0.172 | NS |
| Kisspeptin (pg/mL) | Total Testosterone (ng/dL) | -0.354 | 0.006 | * |
| Kisspeptin (pg/mL) | Adiponectin (µg/mL) | 0.059 | 0.657 | NS |
| AMH (ng/mL) | LH/FSH Ratio | 0.159 | 0.226 | NS |
| Leptin (ng/mL) | HOMA-IR | 0.063 | 0.630 | NS |
| Adiponectin (µg/mL) | HOMA-IR | -0.020 | 0.876 | NS |
| Leptin (ng/mL) | Adiponectin (µg/mL) | -0.099 | 0.451 | NS |

* Statistically significant ($p<0.05$). NS = not significant.

## 4. DISCUSSION

This study provides a comprehensive real-patient multi-biomarker profile from women with PCOS in Iraq, demonstrating consistent and statistically significant between-group differences across neuroendocrine, ovarian reserve, and metabolic parameters. These findings are broadly concordant with the published literature. The most clinically informative pattern, however, emerged from intragroup correlation analysis: despite each biomarker being individually dysregulated, cross-biomarker correlations within the PCOS group were largely absent, suggesting compartmentalized rather than unified dysregulation.

The 63% elevation of kisspeptin in PCOS is consistent with reports by Calik et al. [6] and Prabhakar et al. [11], who attributed kisspeptin neuronal hyperactivity to impaired estradiol negative feedback at the hypothalamic level. This dysregulation is postulated to drive elevated GnRH and LH pulse frequency. However, the absence of a significant kisspeptin–LH correlation within the PCOS group (r=0.001, p=0.993) suggests that single-point peripheral kisspeptin measurements do not linearly track LH secretion, possibly because pulsatile GnRH-LH dynamics are not captured by static serum sampling [6].

AMH was more than twice as elevated in PCOS patients (6.89 vs. 3.12 ng/mL), consistent with its established role as a marker of follicular excess and impaired maturation in PCOS [7, 8]. These values are in agreement with the Iraqi data reported by Hlail and Alshukri (2025) which documented a significantly elevated AMH levels in Iraqi women with PCOS compared to controls [17], reinforcing the applicability of AMH as a reliable ovarian reserve marker in the Iraqi PCOS population. The lack of a significant AMH-LH/FSH correlation (r=0.159, p=0.226) in the PCOS group is in contrast with some published data and may represent phenotypic heterogeneity in the cohort, or statistical power in n=60.

Insulin resistance as judged from significantly higher HOMA-IR (4.30+-0.81 vs. 1.83+-0.48) and fasting insulin (22.85+-5.59 vs. 9.10+-1.99 uIU/mL) was one of the most consistent metabolic features observed in our cohort. This pattern is very similar to that reported for Iraqi data where Abdul-Jabbar and Swadi (2025) confirmed that insulin resistance indices were significant in PCOS patients in Mosul and suggested that insulin-sensitizing therapies might be especially useful in the Iraqi PCOS population [14]. The higher BMI in the patients with PCOS in our study (28.81 vs. 23.75 kg/m2) is a likely one, as visceral adiposity promotes the hepatic insulin resistance and the secondary hyperinsulinemia which in turn activates the theca cell androgen synthesis. This insulin-androgen amplification loop is well proven and seems to be operative in our cohort of patients as well [4].

The adipokine profile in PCOS, where leptin is increased and adiponectin is suppressed, is similar to that of the metabolic disturbance involving insulin resistance and higher adiposity. These results were in line with a study conducted by Kadhim et al. (2025) which evaluated patients with PCOS in Karbala and showed both obese and non-obese patients with PCOS had oxidative stress and metabolic disorder with GluCur excess in obese phenotype [16]. Within the PCOS group of the present study, however, the results of the correlations between leptin and HOMA-IR (r=0.063, p=0.630) and adiponectin and HOMA-IR (r=-0.020, p=0.876) were

non-significant and can imply that leptin elevation may be primarily reflecting adiposity rather than insulin resistance severity in this cohort of patients. Similar cardiovascular-metabolic overlap was reported by Abdulrahman and Zbaar 2025 among Iraqi PCOS women, which adds further evidence for the multi-system nature of this disorder [15].

The most novel finding was that there was a significant negative correlation with the kisspeptin and total testosterone in the PCOS group (r= -0.354, p=0.006). This inverse association might at first seem to be a paradox, since the kisspeptin should trigger LH-induced androgen production. One plausible reason is that there may be a compensatory down-regulation of kisspeptin activity in women with more severe androgenic PCOS as a result of altered sex steroid feedback dynamics or distinct sub-phenotypes of PCOS with high kisspeptin/moderate androgenics and low kisspeptin/high androgenics [1]. This finding is consistent with the concept of heterogeneity of PCOS and needs to be replicated in larger stratified cohorts.

Taken together, the pattern of individually dysregulated but largely uncorrelated biomarkers within PCOS suggests the seven axes which are disrupted in PCOS, rather than a single mastered upstream pathology. This observation is especially important in the Iraqi setting where recent studies have shown phenotypic diversity in the presentation of PCOS in different Iraqi provinces and patient populations [14, 15, 16, 17]. These converging lines of Iraqi evidence, together with the present data, suggest that the standardized multi-biomarker assessment may be critical for accurate PCOS phenotyping in clinical practice in Iraq.

Study limitations include the single-center design, a sample size that may be underpowered for some correlation analyses, the use of single-point serum measurements for pulsatile hormones, and the absence of SHBG, free androgen index, and inflammatory markers such as IL-6 or CRP, which could further characterize the immune-metabolic axis of PCOS.

## 5. CONCLUSION

PCOS is characterized by simultaneous neuroendocrine, ovarian reserve, and metabolic dysregulation, with largely independent biomarker pathways. In this Iraqi cohort of 120 women, all measured parameters — kisspeptin, AMH, LH, LH/FSH ratio, testosterone, leptin, insulin, and HOMA-IR — were significantly elevated in PCOS, while adiponectin was significantly suppressed. Within the PCOS group, these biomarkers were largely uncorrelated, with the sole exception of a significant inverse kisspeptin–testosterone association (r=−0.354, p=0.006). These findings reinforce the need for a multi-target diagnostic and therapeutic approach in the clinical management of PCOS, particularly within the Iraqi healthcare context where metabolic comorbidities are prevalent.

**Funding:** This research received no external funding.

**Ethical Approval:** Approved by the IRB of the University of Tikrit (Ref. No. TU/2024/042). Informed consent obtained from all participants.

## REFERENCES

[1] Lizneva D, Suturina L, Walker W, et al. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. Fertil Steril. 2016;106(1):6–15.
[2] Azziz R, Carmina E, Chen Z, et al. Polycystic ovary syndrome. Nat Rev Dis Primers. 2022;8(1):1–18.
[3] Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81(1):19–25.
[4] Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev. 2012;33(6):981–1030.
[5] Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M. Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol Rev. 2012;92(3):1235–1316.
[6] Calik MW, Calik J, Bogumil R. Kisspeptin and its role in polycystic ovary syndrome. Adv Clin Exp Med. 2021;30(4):463–472.
[7] Dewailly D, Andersen CY, Balen A, et al. The physiology and clinical utility of anti-Müllerian hormone in women. Hum Reprod Update. 2014;20(3):370–385.
[8] Pigny P, Jonard S, Robert Y, Dewailly D. Serum anti-Mullerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. J Clin Endocrinol Metab. 2003;91(3):941–945.
[9] Quinton ND, Smith RF, Clayton PE, et al. Leptin binding activity changes with age: the link between leptin and puberty. J Clin Endocrinol Metab. 1999;84(7):2336–2341.
[10] Toulis KA, Goulis DG, Farmakiotis D, et al. Adiponectin levels in women with polycystic ovary syndrome: a systematic review and a meta-analysis. Hum Reprod Update. 2009;15(3):297–307.
[11] Prabhakar VK, Lahari T, Jayashree K. Serum kisspeptin levels in women with polycystic ovary syndrome: a case control study. Int J Reprod Contracept Obstet Gynecol. 2023;12(2):456–461.
[12] Sahmay S, Aydogan Mathyk B, Sofiyeva N, et al. Serum AMH levels and insulin resistance in PCOS. Eur J Obstet Gynecol Reprod Biol. 2021;253:167–171.
[13] de Roux N, Genin E, Carel JC, et al. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci USA. 2003;100(19):10972–10976.
[14] Abdul-Jabbar IA, Swadi AAJ. Evaluation of insulin resistance markers in polycystic ovary syndrome patients receiving metformin, finasteride or both. Ann Coll Med Mosul. 2025;47(2):200–210.
[15] Abdulrahman MA, Zbaar SA. Assessment of atrial natriuretic peptide serum level in patients with polycystic ovary syndrome in Iraqi population. Thi-Qar Med J. 2025;29(2):1–9.
[16] Kadhim YI, Jasim AA, Abbas MR, Muhammad FJ, Mohameed SH. The role of oxidative stress in obese and non-obese polycystic ovary syndrome among women attending infertility clinics in Karbala. Ahl al-Bayt Med Univ J. 2025;4(1):1–12.
[17] Hlail ZA, Alshukri AAM. Interleukin-10 levels in Iraqi women with polycystic ovary syndrome. Al-Kut Univ Coll J. 2025;Special Issue:1–8.