

Assessment of Reproductive Hormones, Thyroid Function, and Vitamin D Status in Women with Polycystic Ovary Syndrome in Baghdad City

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Abstract

Background: Polycystic ovarian syndrome (PCOS) is a common endocrine disorder affecting 8–13% of women of reproductive age. Anti-Müllerian hormone (AMH) has emerged as a potential diagnostic and prognostic biomarker due to its association with ovarian reserve and follicular dysfunction. This case-control study investigated the diagnostic value of AMH and related hormonal markers in polycystic ovarian syndrome (PCOS).

Methods: This case-control study included 80 women (40 diagnosed with PCOS and 40 healthy controls) recruited from three medical centers in Baghdad between September and December 2024. Serum levels of AMH, LH, FSH, prolactin, TSH, and vitamin D3 were measured. Statistical analysis included group comparisons, Pearson correlation, and ROC curve analysis.

Results: Women with PCOS had significantly higher BMI, LH levels, LH/FSH ratio, and AMH concentrations compared with controls ($p < 0.001$). Vitamin D3 levels were significantly lower in the PCOS group. No significant differences were observed in FSH, prolactin, or TSH levels. AMH demonstrated excellent diagnostic performance with high sensitivity and specificity.

Conclusion: Elevated AMH, along with increased LH/LH-FSH ratio and reduced vitamin D3 levels, characterizes women with PCOS. AMH appears to be a strong diagnostic biomarker and may reflect disease severity

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1. Introduction

Polycystic ovarian syndrome (PCOS) is a prevalent endocrine condition that affects 8-13% of reproductive-aged women. Its distinguishing features are anovulation, polycystic ovarian morphology (PCOM), and hyperandrogenism (HA) [1]. Some of the phenotypes linked with polycystic ovarian syndrome (PCOS) include hyperandrogenism with anovulation, hyperandrogenism with PCOS, oligo-ovulation with PCOS, and isolated hyperandrogenism. Granulosa cells in the anterior and minor ovarian follicles generate anti-Müllerian hormone (AMH). It measures ovarian reserve and function, and it was a beta-converting growth factor. It has a favorable correlation with polycystic ovarian syndrome (PCOS); women with PCOS have two to three times higher serum AMH levels [2, 3]. As a result, AMH may serve as a biomarker for PCOS diagnosis. Despite multiple studies and unanimity on AMH's relationship with PCOS, it is not regularly utilized as a diagnostic criterion. It is not yet apparent how this connects to the variability observed in the PCOS subgroup.

The most prevalent cause of ovulatory infertility and hyperandrogenism in young females is polycystic ovarian syndrome (PCOS). Women with this condition have an abnormally high number of small antral follicles, two to three times that

of normal ovaries [4]. The secretion gradually decreases as the antral follicles grow in size and completely stops when the follicles reach a diameter of approximately 8 mm or when follicular atresia occurs; in other words, as follicles develop and enlarge, their secretory activity declines, and once they either attain this critical size or undergo degenerative changes, secretion is no longer produced. AMH levels are age-dependent; they are seldom detectable in newborn females, peak during adolescence, and steadily diminish with age until menopause, when blood concentrations are undetectable [5].

There has been a significant increase in interest in the utilization of anti-müllerian hormone (AMH) and antral follicle count (AFC) to forecast patient responses to ovarian stimulation [6]. Anti-Müllerian hormone (AMH) has recently been found to be a diagnostic for polycystic ovarian syndrome (PCOS) [7].

2. Research Method

The study used a case-control design. Forty of the eighty samples were from healthy individuals. The inclusion criteria for PCOS subjects included females of reproductive age (18–40 years) who were diagnosed with PCOS according to the Rotterdam criteria (2003), fulfilling at least two of the following features: oligo-ovulation or anovulation manifested by irregular menstrual cycles, clinical and/or biochemical evidence of hyperandrogenism, and polycystic ovarian morphology on ultrasonography. While the control group was chosen based on healthy criteria and history for the samples. Blood samples were collected in the early follicular phase (days 2–5). Samples from the hospital were gathered. Women from the medical cities of Al-Elweya, Al-Hakeem, and Al-Imamain Al-Kadhimain were sampled for this study between September and December 2024. The Department of Chemistry and Biochemistry at Al-Nahrain University College of Medicine is where the practical work was completed. Before taking part, all women were informed about the study and provided signed informed consent. The ethical approval was obtained from Al-Nahrain University College of Medicine's Institutional Review Board (IRB), which approved this study. This study excluded patients with chronic hypertension, gestational hypertension, diabetes, smoking, multiple pregnancies, other-caused intrauterine growth restriction, heart failure, inflammatory disorders, infectious diseases, and endocrine diseases.

Collection of samples: Women who have PCOS had their blood drawn, and Healthy women (without PCOS and any diseases). Each woman took five milliliters of venous blood using a disposable syringe while seated. Without using an anticoagulant, blood is gradually drained into disposable test tubes. The blood was centrifuged at 1000 X g for 15 minutes after clotting for ten to fifteen minutes at 37°C. Until AMH, LH, FSH, TSH, and vitamin D3 were tested by using the CLIA technique manufactured by the Cobas ROCHE company (Germany). In addition, the serum was kept in Eppendorf tubes at -80°C.

3. Results

Clinical and biochemical characteristics were compared in the control and patient groups. Table 1 summarizes the comparison of clinical and biochemical characteristics between the patient and control groups. Age was comparable between the two groups, with no statistically significant difference observed. In contrast, body mass index was significantly higher in the patient group. Gonadotropin analysis showed no significant difference in FSH levels; however, LH levels and the LH/FSH ratio were markedly elevated in patients compared with controls. Prolactin concentrations did not differ significantly between groups. Vitamin D₃ levels were significantly lower in the patient group, whereas TSH levels were similar in both groups, with no significant difference. Notably, AMH levels were significantly increased in patients relative to the control group. Overall, statistically significant differences were identified for BMI, LH, LH/FSH ratio, vitamin D₃, and AMH, while age, FSH, prolactin, and TSH showed no significant variation between groups ($p < 0.05$).

Table 1. Shows a comparison of clinical and biochemical markers between the case and control groups.

Variables	groups	Mean	Std. Deviation	Std. Error Mean	P value
Age	control	30.6842	8.85722	2.03198	0.171
	Patient	34.0526	6.36373	1.45994	
BMI	control	25.6682	3.05328	0.70047	0.0001
	Patient	32.3838	3.82390	0.87726	
FSH	control	4.9368	0.58898	0.13512	0.131
	Patient	4.6158	0.68901	0.15807	
LH	Control	5.0526	0.43381	0.09952	0.0001
	Patient	7.7579	0.87134	0.19990	
LH/FSH ratio	control	1.0381	0.16274	0.03733	0.0001
	Patient	1.7127	0.28621	0.06566	
Prolactin	control	18.0511	3.27627	0.75163	0.240
	Patient	16.8474	2.91802	0.66944	
Vitamin D3	control	21.9937	2.27858	0.52274	0.0001

	Patient	13.3737	2.63920	0.60547	
TSH	control	1.9105	0.88995	0.20417	0.072
	Patient	1.4137	0.75440	0.17307	
AMH	control	1.2895	0.92996	0.21335	0.0001
	Patient	8.5158	1.48744	0.34124	

The comparison between the study group used a paired t-test with significant differences (p-value less than 0.05). BMI: Body mass index, FSH: Follicle-Stimulating Hormone, LH: Luteinizing hormone, TSH: Thyroid-stimulating hormone, AMH: Anti-Müllerian Hormone.

Pearson correlation coefficients were calculated for age, BMI, FSH, LH, the LH/FSH ratio, vitamin D₃, prolactin, TSH, and AMH. Correlation analysis (Table 2) demonstrated that age was not significantly associated with any of the studied parameters. In contrast, BMI showed significant associations with several hormonal markers, including positive correlations with LH, the LH/FSH ratio, and AMH, and a significant inverse relationship with vitamin D₃ levels. FSH was negatively correlated with the LH/FSH ratio. LH exhibited strong positive correlations with both the LH/FSH ratio and AMH, while showing a significant negative association with vitamin D₃. Similarly, the LH/FSH ratio correlated positively with AMH and negatively with vitamin D₃. Vitamin D₃ was positively associated with TSH but demonstrated significant inverse correlations with LH, the LH/FSH ratio, and AMH. No significant correlations were observed for prolactin with any studied variables. Overall, AMH displayed strong positive relationships with BMI, LH, and the LH/FSH ratio, alongside a pronounced negative association with vitamin D₃, while no additional significant correlations were detected.

Table 2. Pearson Correlation Coefficients among Age, BMI, Hormones, Vitamin D₃, and AMH.

Parameters	Related parameters	R	p-value
BMI	LH	0.682 **	0.0001
	LH/FSH ratio	-0.689 **	0.0001
	Vitamin D ₃	-0.586 **	0.0001
	AMH	0.673 **	0.0001
FSH	LH/FSH ratio	-0.627 **	0.0001
	LH/FSH ratio	0.870 **	0.0001
LH	Vitamin D ₃	-0.784 **	0.0001
	AMH	0.881 **	0.0001
LH/FSH ratio	Vitamin D ₃	-0.712 **	0.0001
	AMH	0.794 **	0.0001
Vitamin D₃	TSH	0.322 *	0.049
	AMH	-0.851 **	0.0001

The correlation was done by Spearman's correlation analysis

**Correlation is significant at the 0.01 level (1-tailed).

*Correlation is significant at the 0.05 level (1-tailed).

ROC curve analysis demonstrated that LH, AMH, vitamin D, and the LH/FSH ratio possess the strongest diagnostic performance, exhibiting excellent to near-perfect discriminatory ability. These markers showed very high accuracy with optimal sensitivity and specificity at their respective cut-off points, indicating a highly significant capacity to distinguish between groups. The LH/FSH ratio also displayed outstanding diagnostic efficiency, further supporting its clinical usefulness. In contrast, FSH, prolactin, and TSH showed limited diagnostic value, with comparatively lower discriminatory power, reduced sensitivity, and non-significant statistical associations. Overall, the findings emphasize that LH, AMH, vitamin D, and the LH/FSH ratio are robust and reliable biomarkers for identifying the studied condition, whereas FSH, prolactin, and TSH provide minimal diagnostic contribution.

Table 3 represents the AUC, Specificity, Sensitivity, and cut-off value for study parameters for patients compared to controls.

Variables	AUC	Specificity (%)	Sensitivity (%)	Cut-off	p-value
LH	1.0	100	100	6.20	0.0001
LH/FSH ratio	0.981	94.7	94.6	1.360	0.0001
AMH	1.0	100	100	3.770	0.0001
FSH	0.663	89.5	42.1	5.25	0.085
Prolactin	0.644	68.4	68.5	18.0	0.129
Vitamin D	1.0	100	100	18.265	0.0001
TSH	0.673	78.9	57.9	1.91	0.068

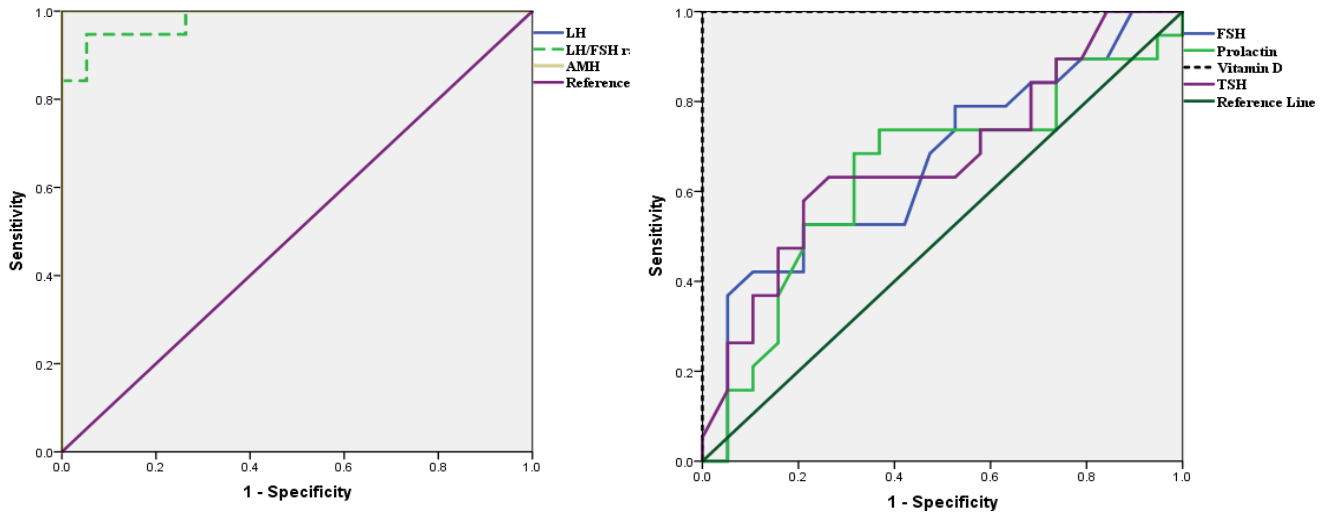


Figure 1. Showed ROC curve for study parameters

4. Discussion

Polycystic Ovary Syndrome (PCOS) is a complex endocrine disorder characterized by hormonal imbalances (like high androgens), irregular periods, infertility, and metabolic issues such as insulin resistance, often diagnosed using Rotterdam criteria (hyperandrogenism, ovarian cysts, irregular ovulation) [8]. In this study, the patient group had a greater BMI, LH, LH/FSH ratio, AMH, and vitamin D3 (D3) than the control group, but FSH, PRL, and TSH did not change. A rigorous comparison shows that these findings match some literature and differ in others [9-11]. Multiple studies indicate that women with polycystic ovarian syndrome (PCOS) commonly have increased AMH levels and vitamin D insufficiency. AMH elevation reflects increased antral follicle count and follicular arrest [12]. The observed inverse association between vitamin D3 and AMH suggests a possible modulatory role of vitamin D in ovarian endocrine function; however, causality cannot be inferred due to the cross-sectional design.

Vitamin D was inversely connected with AMH and hormonal/metabolic abnormalities in Iraqi women with PCOS, according to another study [13]. Vitamin D deficiency may modulate ovarian steroid genesis [14]. The negative link between BMI and vitamin D in our patient group aligns with a study of 311 PCOS patients that demonstrated a strong inverse correlation (lower vitamin D with greater BMI) [15]. Conflicting Results: our investigation demonstrated a considerably decreased vitamin D3 in the patient group; however, the relationship between vitamin D and AMH is inconsistent in the literature. A meta-analysis found that vitamin D administration could lower AMH in PCOS women but enhance it in non-PCOS women. The patient group exhibited higher AMH and decreased vitamin D, which is consistent with the “PCOS pattern” but does not support causality [16].

The increased AMH and LH levels (together with a higher LH/FSH ratio) in the cohort study indicate altered ovarian function or follicular dynamics, such as excessive follicular recruitment or hindered ovulation, characteristics frequently associated with PCOS or PCOS-like phenotypes. The markedly reduced vitamin D3 levels may indicate a metabolic or reproductive endocrine connection, as vitamin D is associated with ovarian physiology, folliculogenesis, and hormonal control

(e.g., AMH signaling). The elevated BMI in your patient cohort further substantiates metabolic dysregulation frequently associated with endocrine disorders [17].

The absence of a notable difference in FSH, PRL, and TSH indicates that not all hormonal regulatory axes are modified in the patient group relative to controls, potentially reflecting the condition's heterogeneity, sample size, or other confounding factors (e.g., sampling timing, lifestyle, or ethnic variations). The contradictory findings between vitamin D and AMH in the literature, together with the trend found in your study (low vitamin D, high AMH), contribute to the evidence indicating a relationship with PCOS phenotypes, albeit it remains observational. The potential for reverse causality (e.g., elevated AMH or modified ovarian function influencing vitamin D metabolism), confounding by BMI or other metabolic variables, or selection bias cannot be dismissed [18].

A comprehensive review and meta-analysis demonstrated a negative connection between vitamin D levels and LH/FSH ratio in women with normal BMI (Fisher's Z: -0.18; P = 0.041). This supports your discovery of a negative relationship between vitamin D3 and FSH/LH ratio. (15) A research study named "Key Predictors of Fertility" discovered a relationship between vitamin D and reproductive hormones (LH, FSH, and AMH) in women [19]. Different evidence and nuances: Many studies showed no relationship between vitamin D and AMH or LH, subgroup analysis demonstrated relevance in some individuals. The whole sample showed no significant association between vitamin D and AMH. Your strong negative correlation between vitamin D3 and AMH ($r=-0.851$) beats most previous findings [20].

The positive correlation you identified between BMI and AMH is intriguing: numerous studies indicate that elevated BMI is linked to reproductive failure and often diminished ovarian reserve, yet your findings imply that higher BMI correlates with increased AMH levels. This may indicate your particular patient demographic (perhaps the PCOS phenotype) in which AMH levels are raised rather than reduced. This study's findings support the hypothesis that in a population with endocrine or hormonal dysregulation, presumably characterized by PCOS or a PCOS-like phenotype, an elevated BMI is related to increased LH levels, a higher FSH/LH ratio, and raised AMH levels. The strong negative connection with vitamin D3 suggests that vitamin D deficiency may be linked to the current hormonal and metabolic disorders. The negative connection of vitamin D3 with LH, FSH/LH ratio, and AMH supports the idea that adequate vitamin D may play a regulatory or modulatory role in the endocrine axis that governs ovarian function [21].

5. Conclusion

This study demonstrates that women with PCOS exhibit significantly elevated AMH levels, increased LH and LH/FSH ratio, and reduced vitamin D3 concentrations compared with healthy controls. Among the evaluated biomarkers, AMH showed the strongest diagnostic performance, supporting its role as a reliable indicator of ovarian dysfunction in PCOS. These findings are consistent with current evidence and highlight the potential clinical value of AMH in PCOS assessment. Larger, multicenter studies incorporating phenotypic classification and multivariate analysis are recommended to validate these findings and explore their clinical implications.

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7. Declarations

7.1 Ethics approval and consent to participate

The ethical approval was obtained from Al-Nahrain University College of Medicine's Institutional Review Board (IRB), which approved this study.

7.2 Consent for publication

Not applicable.

7.3 Availability of Data and Materials

Data will be provided upon receiving a valid request.

7.4 Conflicts of interest

The authors declare that there is no conflict of interest

7.5 Funding

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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تقييم الهرمون المضاد لمولر AMH، الهرمونات التناسلية، البرولاكتين، فيتامين د3، وهرمون TSH كمؤشرات حيوية لدى النساء المصابات بمتلازمة تكيس المبايض في بغداد

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المستخلص:

تُعاني ما بين 8 إلى 13% من النساء في سن الإنجاب من متلازمة تكيس المبايض. وتُشخص هذه المتلازمة بانقطاع الإباضة، وتعدد تكيسات المبيض، وفرط الأندروجينية. يُمكن الآن تشخيص متلازمة تكيس المبايض أو التنبؤ بمآلها باستخدام هرمون مضاد المولر (AMH). في هذه الدراسة التي اعتمدت تصميم الحالة والشاهد، تم فحص 80 امرأة ضمن مجموعتين: 40 مصابة بمتلازمة تكيس المبايض و40 من النساء السليمات. هدفت الدراسة إلى تقييم مستويات هرمون AMH وعدد من الهرمونات الأخرى نظرًا لدورها المحتمل في تشخيص المتلازمة والتنبؤ بشدتها، خصوصًا مع ازدياد الأدلة التي تربط ارتفاع AMH باضطرابات الإباضة ومخزون المبيض. جُمعت العينات من مستشفيات مختلفة خلال الفترة من ايلول إلى كانون الاول 2024، وتم سحب ومعالجة عينات الدم وفق إجراءات مخبرية موحدة، ثم قياس مستويات AMH و LH و FSH و TSH وفيتامين D3.

أظهرت النتائج عدم وجود فرق معنوي في العمر بين المجموعتين، بينما كان مؤشر كتلة الجسم أعلى لدى المريضات. كما كان مستوى LH ونسبة LH/FSH مرتفعين بشكل واضح لدى المصابات، في حين بقيت مستويات FSH والبرولاكتين مشابهة بين المجموعتين. وسُجل انخفاض معنوي في مستوى فيتامين D3 لدى المريضات دون تغير يُذكر في TSH. وكان أبرز النتائج هو الارتفاع الكبير في مستوى AMH لدى المصابات مقارنة بالمجموعة الضابطة. وتُبرز هذه النتائج أن ارتفاع AMH يشكل مؤشرًا تشخيصيًا وتنبؤيًا قويًا لمتلازمة تكيس المبايض، إضافة إلى ارتباطه بمخزون المبيض وشدة المرض، بينما تُعد التغيرات في LH ونسبة LH/FSH ونقص فيتامين D3 عناصر داعمة في فهم خصائص المتلازمة وتأكيد تشخيصها.