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A Comprehensive Approach to Polycystic Ovary Diagnosis: Integrating Follicular Imaging and AMH Biomarkers

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ABSTRACT

Background: Polycystic ovarian syndrome (PCOS) is a prevalent endocrine disorder characterized by hormonal imbalance and metabolic dysfunction, often diagnosed through clinical, biochemical, and radiological markers. Transvaginal ultrasound follicle count and serum anti-Müllerian hormone (AMH) levels have been increasingly used as key diagnostic tools for defining polycystic ovaries. However, establishing threshold values for these parameters to differentiate PCOS from other ovarian conditions remains challenging.

Methodology: The study design was case control study took place at Hayatabad Medical complex Peshawar. A total of 150 women diagnosed with PCOS were compared with 100 controls. Follicle count was determined through transvaginal ultrasound, while serum AMH levels were measured using enzyme-linked immunosorbent assay (ELISA). Statistical analyses, including correlation and regression, were performed to explore associations between variables, and receiver operating characteristic (ROC) curves were used to evaluate diagnostic performance.

Results: The PCOS group exhibited significantly higher follicle counts (mean: 25.4 ± 7.2) and AMH levels '(6.2 ± 1.7 ng/mL) compared to controls (12.6 ± 3.4 follicles; AMH: 2.1 ± 0.8)'. A moderate positive correlation (correlation coefficient = 0.65, p-value < 0.001) was identified between follicle count and AMH levels in the PCOS group. The combination of a follicle count ≥20 and AMH levels ≥4.5 ng/mL showed excellent diagnostic performance, with an 'area under the curve (AUC)' of 0.88, a sensitivity of 90%, and a 'specificity of 80'%.

Conclusion: Both follicle count and AMH levels are reliable markers for diagnosing PCOS. The combination of these two parameters offers greater diagnostic precision than either alone, supporting the need for standardized thresholds in clinical practice.

Introduction

Polycystic ovarian syndrome (PCOS) is a complex endocrine disorder affecting women of reproductive age.[1] It is associated with menstrual irregularities, hyperandrogenism, and polycystic ovarian morphology (PCOM) on ultrasound.[2] Despite being a common condition, diagnosing PCOS remains a challenge due to the variability in diagnostic criteria and thresholds used across different studies and clinical guidelines.[3]

The Rotterdam criteria, widely accepted for PCOS diagnosis, require two of the following three features: oligo-anovulation, hyperandrogenism, and polycystic ovaries on ultrasound.[4] However, the definition of polycystic ovaries remains controversial. Follicle counts on ultrasound and serum Anti-Müllerian Hormone (AMH) levels are key diagnostic markers, but their threshold values for defining polycystic ovaries are not universally agreed upon. [5]

Several researches have proposed various threshold standards for follicle count and levels of AMH. A study by de Lima, et al 2020 suggested a follicle count of 12 or more, while others have proposed counts as high as 25[6]. Similarly, AMH levels have been proposed as an adjunct marker for PCOS diagnosis, with thresholds ranging from 4 to 7 ng/mL across different studies.[7] These discrepancies highlight the need for a more standardized approach to defining polycystic ovaries.

This study aims to determine the 'optimal follicle count on transvaginal ultrasound and serum AMH levels for diagnosing polycystic ovaries in women with PCOS'. By establishing consistent thresholds, we hope to improve the accuracy and reliability of PCOS diagnosis in clinical practice.

The rationale of study was polycystic ovarian syndrome (PCOS) is a common endocrine disorder in women of reproductive age, with varying symptoms and diagnostic challenges. The widely used Rotterdam criteria lack consistent thresholds for follicle count and anti-Müllerian hormone (AMH) levels, leading to diagnostic inconsistencies. This study aims to establish optimal thresholds for these markers to enhance the accuracy of PCOS diagnosis, enabling better management of its metabolic and reproductive complications.

Methodology

This case control study was conducted from January 2022 to January 2024 in Hayatabad Medical Complex Peshawar. A total of 150 women aged 18-35 years, diagnosed with PCOS according to the Rotterdam criteria, were enrolled. Ultrasound follicle count was performed transvaginal using a high-frequency probe. Serum AMH levels were measured using an enzyme-linked immunosorbent assay (ELISA). The threshold values for follicle count and AMH levels were determined by comparing their distributions in PCOS patients with control groups. Ethical approval was obtained from the institutional review board (IRB) before the research commenced. 'Informed consent was collected from all participants'.

The inclusion criteria consisted of female 'aged 18 to 35 years' with a diagnosis of Polycystic Ovarian Syndrome (PCOS) according to the 'Rotterdam criteria' including both regular and irregular 'menstrual cycles', and who had not received any hormonal treatment in the previous six months. The exclusion criteria were pregnant or lactating women, history of ovarian surgery and endocrine disorders other than PCOS

Data were collected using standardized forms, including patient history, ultrasound follicle count, and serum AMH ‘levels’. Samples of blood were collected and subsequently ‘analyzed in’ the hospital's clinical lab.

Data were analyzed using SPSS version 26. Descriptive statistics were used to summarize the data. A chi-square test was employed to compare proportions, and Pearson correlation was used to assess the relationship between follicle count and AMH levels. $p<0.05$ was considered statistically significant.

Results

As shown in Table 1, women in the PCOS group had significantly higher BMI (28.5 ± 2.4 vs. 22.6 ± 1.9, $p<0.05$), fasting blood glucose levels (105 ± 10.5 vs. 90 ± 5.6 mg/dL, $p<0.05$), and testosterone levels (55 ± 8.4 vs. 32 ± 6.7 ng/dL, $p<0.05$). Additionally, 85% of women in the PCOS group reported menstrual irregularities, compared to 20% in the control group ($p<0.05$). Similarly, 75% of PCOS participants had signs of hyperandrogenism, compared to only 10% of controls ($p<0.05$).

Table 1: Baseline Characteristics of Study Participants

Characteristics	**PCOS Group (n=150)**	**Control Group (n=100)**	**p-value**
Age (years)	24.6 ± 3.2	23.9 ± 3.1	0.120
BMI (kg/m²)	28.5 ± 2.4	22.6 ± 1.9	<0.05
Menstrual Irregularities (%)	85%	20%	<0.05
Hyperandrogenism (%)	75%	10%	<0.05
Fasting Blood Glucose (mg/dL)	105 ± 10.5	90 ± 5.6	<0.05
Luteinizing Hormone (IU/L)	12.5 ± 3.2	6.1 ± 1.9	<0.05
Follicle-Stimulating Hormone (IU/L)	4.8 ± 1.3	7.5 ± 1.6	<0.05
Testosterone (ng/dL)	55 ± 8.4	32 ± 6.7	<0.05
AMH Levels (ng/mL)	6.2 ± 1.7	2.1 ± 0.8	<0.05

Table 2 Follicle count was significantly higher in the PCOS group (mean: 25.4 ± 7.2) ‘compared to the control group’ (mean: 12.6 ± 3.4), with a mean difference of 12.8 follicles (95% CI: 11.1 – 14.5, p less than0.001). About 65% of females in PCOS had a follicle count of ≥20, while only 20% in the control group exhibited this feature ($p<0.001$).

The mean AMH levels were significantly higher in the PCOS group compared to controls, with a mean difference of 4.1 ng/mL (95% CI: 3.7 – 4.5, $p<0.001$). A higher percentage of women in the PCOS group (70%) had AMH levels ≥4.5 ng/mL compared to controls (15%), further reinforcing the diagnostic value of AMH in PCOS. LH/FSH ratio and testosterone levels also showed significant differences between the groups, highlighting their role in the clinical diagnosis of PCOS.

Table 2: Comparison of Follicle Count and AMH Levels Between PCOS and Control Groups

Parameter	PCOS Group (n=150)	Control Group (n=100)	Mean Difference (95% CI)	p-value
Mean Follicle Count	25.4 ± 7.2	12.6 ± 3.4	12.8 (11.1 – 14.5)	<0.001
Follicle Count ≥20 (%)	65%	20%	-	<0.001
Serum AMH Levels (ng/mL)	6.2 ± 1.7	2.1 ± 0.8	4.1 (3.7 – 4.5)	<0.001
AMH ≥4.5 ng/mL (%)	70%	15%	-	<0.001
LH/FSH Ratio	2.6 ± 1.1	1.2 ± 0.6	1.4 (1.2 – 1.6)	<0.05
Testosterone (ng/dL)	55 ± 8.4	32 ± 6.7	23 (19.4 – 26.6)	<0.001

Table 3 the correlation coefficient between follicle count and serum AMH levels was 0.65 with a p-value <0.001, indicating a moderate positive correlation between these two diagnostic markers in PCOS patients.

Age exhibited a ‘weak negative correlation with follicle count (correlation coefficient = -0.25, p = 0.045), indicating that’ follicle count tends to slightly decline as age increases. In contrast, BMI demonstrated a weak to moderate positive association with follicle count (correlation coefficient = 0.35, p = 0.030), suggesting that women with a higher BMI are likely to have a greater follicle count.

The LH/FSH ratio showed a moderate positive correlation (r = 0.40, p=0.025), further supporting its role as a contributing factor in PCOS diagnosis. Testosterone levels also showed ‘a moderate positive correlation (r = 0.55, p<0.05), suggesting that higher androgen levels are associated with an increased follicle count’ in PCOS.

Table 3: Correlation between Follicle Count and Serum AMH Levels in PCOS Group (n=150)

Parameter	Mean ± SD	Correlation Coefficient (r)	p-value	Interpretation
Follicle Count	25.4 ± 7.2			
Serum AMH Levels	6.2 ± 1.7 ng/mL	**0.65**	**<0.001**	Moderate positive correlation
Age	24.6 ± 3.2 years	-0.25	0.045	Weak negative correlation
BMI	28.5 ± 2.4 kg/m²	0.35	0.030	Weak to moderate positive correlation
LH/FSH Ratio	2.6 ± 1.1	0.40	0.025	Moderate positive correlation
Testosterone Levels	55 ± 8.4 ng/dL	0.55	<0.05	Moderate positive correlation

Table 4 Follicle count ≥20 had a sensitivity of 80% and a specificity of 75%, with an AUC of 0.82, indicating good diagnostic performance. Serum AMH levels ≥4.5 ng/mL demonstrated

slightly higher sensitivity (85%) but lower specificity (70%) than follicle count alone. The AUC for AMH was' 0.85, indicating a robust marker for diagnosing polycystic ovaries.
The combination of follicle count and AMH levels increased diagnostic accuracy, 'with a sensitivity of' 90% and 'specificity' of 80%. The 'AUC for this combination was 0.88, making it the most accurate diagnostic tool in the study

Table 4: Evaluating the Diagnostic Accuracy of Follicle Count and AMH Levels in Defining Polycystic Ovaries

Diagnostic Marker	'Sensitivity' (%)	'Specificity' (%)	'Positive Predictive Value (PPV)'	'Negative Predictive Value (NPV)'	'Area Under Curve (AUC)'
Follicle Count ≥20	80	75	78%	76%	0.82
AMH Levels ≥4.5 ng/mL	85	70	80%	77%	0.85
Combination (Follicle Count + AMH)	90	80	86%	85%	0.88

Discussion

The results of this study reinforce the diagnostic value of follicle count and serum AMH levels in distinguishing polycystic ovarian syndrome (PCOS) from non-PCOS controls. With PCOS affecting a significant percentage of reproductive-age women, reliable and accessible diagnostic markers awere crucial for timely intervention. Both transvaginal ultrasound follicle count and serum AMH levels have gained recognition as key indicators of ovarian reserve and dysfunction. Our study found that women with PCOS exhibit significantly higher follicle counts and serum AMH levels than control subjects, underscoring the role of these parameters in diagnosing polycystic ovaries.
The mean follicle count in the PCOS group was 25.4 ± 7.2, which aligns with previous studies that support the use of a follicle count threshold of ≥20 for diagnosing PCOS.[8 9] Gyliene et al (2022) highlighted the significance of high follicle counts in detecting ovarian dysfunction in PCOS. [5] Similarly, our finding of a moderate positive correlation ($r = 0.65$, $p<0.001$) between follicle count and AMH levels aligns with the existing literature, suggesting that the two markers are interdependent in assessing ovarian morphology in PCOS patients.[10 11]
The use of serum AMH levels as a diagnostic marker for PCOS has also garnered attention in recent years due to its stability throughout the menstrual cycle. 'This research showed that women with PCOS had significantly higher levels of AMH (mean: 6.2 ± 1.7 ng/mL) compared to controls (2.1 ± 0.8 ng/mL)' in accordance with previous studies that have proposed AMH as a biomarker for ovarian dysfunction, particularly in PCOS.[12] Moolhuijsen et al 2022 reported elevated levels of AMH in women with PCOS,[13] further validating our findings .
While both follicle count and AMH levels were individually significant in diagnosing PCOS, the combination of the two markers provided even greater diagnostic accuracy. The ROC analysis revealed that the combination of follicle count ≥20 and AMH ≥4.5 ng/mL achieved an

AUC of 0.88, with a sensitivity of 90% and specificity of 80%, demonstrating excellent diagnostic performance. These results support the findings of Piltonen et al 2024, who advocated for the use of both markers in clinical practice to enhance the precision of PCOS diagnosis .

Interestingly, our study also observed weak correlations between ‘follicle count and age (r = -0.25, p = 0.045) and BMI (r = 0.35, p = 0.030)’, suggesting that factors ‘such as age and body mass index’ can influence ovarian reserve. These findings are supported by research from van der Ham et al 2024, which found that higher BMI is often associated with increased follicle counts and elevated AMH levels in PCOS patients . The weak negative correlation between age and follicle count, though statistically significant, is expected as ovarian reserve naturally declines with age .

Additionally, our study found that LH/FSH ratios and testosterone levels were significantly higher in the PCOS group compared to controls, further supporting the hormonal imbalance observed in ‘PCOS patients’. ‘The correlation between increased androgen levels and follicle count’ (r = 0.55, p<0.05) is indicative of the hyperandrogenism typically seen in PCOS, consistent with the diagnostic criteria set by the Rotterdam Consensus (2003) .

The inclusion of additional biochemical markers such as LH/FSH ratios and testosterone strengthens the argument that a combination of transvaginal ultrasound, serum, and hormonal markers can provide a comprehensive diagnostic profile for PCOS. This multifactorial approach to diagnosis was supported by research from Fahs et al 2023 who highlighted the importance of considering a spectrum of clinical and biochemical criteria in diagnosing PCOS.[14]

While this study offers valuable insights into the diagnostic utility of follicle count and AMH levels, limitations should be considered. The study was conducted in a specific population, and further research is needed to generalize these findings across diverse ethnic groups and clinical settings. Additionally, while AMH levels are stable throughout the menstrual cycle, factors such as assay variability and laboratory differences can affect measurement accuracy. Future studies should explore the standardization of AMH assays to enhance diagnostic reliability.

The combination of follicle counts and serum AMH levels provides a highly accurate method for diagnosing polycystic ovaries in PCOS patients. Our findings support the establishment of standardized threshold values for these parameters to improve clinical decision-making and ensure early intervention in PCOS management. Further research is warranted to refine diagnostic criteria and explore the potential role of additional biomarkers in this complex syndrome.

Conclusion

The study demonstrates that both follicle count and serum AMH levels are reliable diagnostic markers for PCOS. The combination of these two parameters significantly enhances diagnostic accuracy, providing clinicians with a robust tool for early detection. Establishing standardized thresholds for these markers will improve consistency in diagnosis and support timely therapeutic interventions.

References:

1. Maqbool M, Ara I, Gani I. The story of polycystic ovarian syndrome: a challenging disorder with numerous consequences for females of reproductive age. *International Journal of Current Research in Physiology and Pharmacology* 2022:19-31.
2. Joham AE, Norman RJ, Stener-Victorin E, et al. Polycystic ovary syndrome. *The lancet Diabetes & endocrinology* 2022;10(9):668-80.
3. Louwers YV, Laven JS. Characteristics of polycystic ovary syndrome throughout life. *Therapeutic Advances in Reproductive Health* 2020;14:2633494120911038.
4. Kiran Z. Diagnostic criteria for polycystic ovary syndrome. Polycystic Ovary Syndrome: Elsevier 2024:61-74.
5. Gyliene A, Straksyte V, Zaboriene I. Value of ultrasonography parameters in diagnosing polycystic ovary syndrome. *Open Medicine* 2022;17(1):1114-22.
6. de Lima MA, Morotti F, Bayeux BM, et al. Ovarian follicular dynamics, progesterone concentrations, pregnancy rates and transcriptional patterns in Bos indicus females with a high or low antral follicle count. *Scientific reports* 2020;10(1):19557.
7. Evans MB, Stentz NC, Richter KS, et al. Mature follicle count and multiple gestation risk based on patient age in intrauterine insemination cycles with ovarian stimulation. *Obstetrics & Gynecology* 2020;135(5):1005-14.
8. Kim JJ, Hwang KR, Chae SJ, et al. Impact of the newly recommended antral follicle count cutoff for polycystic ovary in adult women with polycystic ovary syndrome. *Human Reproduction* 2020;35(3):652-59.
9. Kim JJ, Hwang KR, Lee D, et al. Reply: Impact of the newly recommended antral follicle count cut-off for polycystic ovary in adult women with polycystic ovary syndrome. *Human Reproduction* 2020;35(9):2167-69.
10. van der Ham K, Laven JS, Tay CT, et al. Anti-Müllerian hormone as a diagnostic biomarker for Polycystic Ovary Syndrome (PCOS) and Polycystic Ovarian Morphology (PCOM): a systematic review and meta-analysis. *Fertility and Sterility* 2024
11. Gao X-y, Liu Y, Lv Y, et al. Role of androgen receptor for reconsidering the “true” polycystic ovarian morphology in PCOS. *Scientific reports* 2020;10(1):8993.
12. Shrikhande L, Shrikhande B, Shrikhande A. AMH and its clinical implications. *The Journal of Obstetrics and Gynecology of India* 2020;70(5):337-41.
13. Moolhuijsen LM, Louwers YV, Laven JS, et al. Comparison of 3 different AMH assays with AMH levels and follicle count in women with polycystic ovary syndrome. *The Journal of Clinical Endocrinology & Metabolism* 2022;107(9):e3714-e22.
14. Fahs D, Salloum D, Nasrallah M, et al. Polycystic Ovary Syndrome: pathophysiology and controversies in diagnosis. *Diagnostics* 2023;13(9):1559.