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Original Research Article

Diagnostic utility of serum anti-Müllerian hormone in different phenotypes of polycystic ovary syndrome: a case-control study from a tertiary care centre

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ABSTRACT

Background: This study aimed to evaluate the diagnostic utility of serum Anti-mullerian hormone (AMH) in polycystic ovary syndrome (PCOS), to establish a cut off value, and to compare AMH levels across different PCOS phenotypes.
Methods: A case-control study was conducted in the Department of Obstetrics and Gynaecology at a tertiary care teaching hospital from July 2022 to June 2023. A total of 100 women aged 16-40 years were enrolled, including 50 PCOS cases diagnosed using the Rotterdam criteria and 50 age-matched controls with regular menstrual cycles. Participants underwent clinical evaluation, anthropometric assessment, transabdominal ultrasonography and serum AMH estimation using an enzyme-linked fluorescent assay. Receiver operating characteristic (ROC) curve analysis was performed to determine the optimal AMH cut-off for PCOS diagnosis.
Results: Mean serum AMH levels were significantly higher in women with PCOS compared to controls (7.10±2.70 ng/ml vs. 2.32±1.59 ng/ml; $p<0.001$). ROC analysis identified an optimal AMH cut-off of 4 ng/ml, yielding 90% sensitivity and 78% specificity, with an area under the curve of 0.895. Women with AMH ≥4 ng/ml had significantly increased odds of PCOS (odds ratio 31.9; 95% confidence interval: 10.2-99.9). Among phenotypes, AMH levels were highest in phenotype A and lowest in phenotype C.
Conclusions: Serum AMH is significantly elevated in PCOS and varies across phenotypes. It demonstrates excellent diagnostic performance and may serve as a useful adjunctive biomarker, particularly in settings where ultrasonography is limited.

Keywords: PCOS, Anti-mullerian hormone, Phenotypes, Rotterdam criteria, Biomarker

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting women of reproductive age, with a global prevalence ranging from 8-13% depending on diagnostic criteria and population studied.[1] It is a heterogeneous condition characterised by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology (PCOM), frequently associated with metabolic abnormalities such as insulin resistance, obesity, dyslipidaemia, and an increased risk of type 2 diabetes mellitus and cardiovascular disease.[2] These long-term complications underscore the importance of early and accurate diagnosis. In 2023 an International Evidence-Based Guideline was released with updated diagnostic pathway of PCOS.[4,11] It retained the underlying Rotterdam framework, but introduced a major change, by including Anti-mullerian hormone assay, which can be used instead

of ultrasound to document polycystic ovarian morphology in adults.

As per the Rotterdam criteria (2003), the presence of at least two of the following: oligo/anovulation (OA), clinical and/or biochemical hyperandrogenism (HA), and PCOM on ultrasonography, is required for the diagnosis of PCOS, after exclusion of related disorders.[3] Ovulatory dysfunction is characterized by irregular menstrual cycles, including oligomenorrhea or amenorrhea, reflecting infrequent or absent ovulation.[4] Clinical hyperandrogenism is identified by hirsutism, acne, or androgenic alopecia, while biochemical hyperandrogenism is defined by elevated circulating androgen levels.[5]

According to the 2023 PCOS diagnostic pathway, initially, pathologies such as thyroid disease, hyperprolactinemia, non-classic congenital adrenal hyperplasia, primary ovarian insufficiency and Cushing syndrome or androgen secreting tumours, should be ruled out. Then, the three core Rotterdam features, namely hyperandrogenism, ovarian dysfunction and ovarian morphology should be assessed. If hyperandrogenism and ovarian dysfunction are present, PCOS can be diagnosed. But if only one of these is present, then ovarian morphology criterion has to be assessed, either by ultrasound evidence of PCOM or elevated AMH. PCOM is based on increased ovarian volume (≥10 ml) or increased follicle number per ovary on ultrasound, with thresholds varying with advanced imaging technology. One of the major reasons for adding AMH in the diagnostic criteria was to reduce dependence on transvaginal ultrasound, making diagnosis less invasive and more accessible.

However, it has to be noted that AMH assay should not be used in adolescents. Ultrasound is also not recommended in adolescents, because high follicle counts and elevated AMH can occur normally during pubertal maturation. As per 2023 guideline, in adolescents, within 8 years post menarche, PCOS can be diagnosed only if both ovarian dysfunction and hyperandrogenism are present.

Based on the Rotterdam criteria, there are four different phenotypes in PCOS: phenotype A/classic PCOS (OA+HA+PCOM), phenotype B essential NIH criteria, (HA+OA), phenotype C/ovulatory PCOS (HA+PCOM), and phenotype D/non-hyperandrogenic PCOS (OA+PCOM). They differ in clinical presentation, metabolic risk, and reproductive outcomes. Classic phenotypes tend to demonstrate more severe endocrine and metabolic disturbances.[13-16]

Emerging studies have demonstrated variation in AMH levels across different PCOS phenotypes, suggesting its potential role in phenotypic stratification.[13] In the Indian population, data regarding phenotype-specific variations in AMH remain limited.[6,14] The present study was undertaken, in a tertiary care setting in Kerala State, India, primarily to evaluate the diagnostic utility of serum AMH in PCOS and to find a population specific cut off value. It also aimed to compare serum AMH levels across different PCOS phenotypes.

METHODS

Study design and setting

This is a Case Control study conducted in the Department of Obstetrics and Gynaecology at Ernakulam Medical Centre, Ernakulam district, Kerala State, India which is a tertiary care teaching hospital. Duration of study was from July 2022 to June 2023.

Sample size

The sample size was calculated based on the mean AMH level in PCOS patients (9.50±5.11) and in non-PCOS patients (3.53±1.95) as observed in an earlier publication by Gunasheela D et al Using these values, with a power of 90% and a confidence level of 95%, the minimum required sample size was calculated to be 20 participants per group using nMaster software.[14] To account for possible dropouts and to improve study power, 50 participants were included in each group, resulting in a total sample size of 100.

Participants

Women in the age group of 16-40 years who attended the gynaecology outpatient department, for various reasons like menstrual problems, hirsutism, fertility evaluation, general health checkup and infections during the study period formed the target population. Study and control groups were selected from this group, after applying the inclusion and exclusion criteria. To reduce the selection bias, 50 consecutive patients, who fitted in the criteria, were enrolled in each group. Written informed consent was obtained from all the participants prior to enrolment.

Inclusion criteria

Women aged 16-40 years, with oligo ovulation or hirsutism, who were willing to undergo AMH assay and who consented for the study were taken as the study group. Oligo- ovulation was defined as >35 days of cycle or <8 menstrual cycles per year. Control group was selected from the same age group-those with normal menstrual cycles, those willing to do AMH assay and who consented to the study.

Exclusion criteria

Women less than 16 years and above 40 years and those with endocrine disorders like Cushing syndrome, congenital adrenal hyperplasia, androgen secreting tumours, hypothyroidism and hyperprolactinemia were excluded from the study. Pregnant and lactating women, those with known malignancies and those with history of genital tuberculosis were also excluded from the study.

Study procedure

The study was commenced after due approval from the Institutional Ethics Committee and Scientific Research Committee. Baseline demographic details of both study and control groups were recorded using a predesigned proforma. A detailed clinical examination was done and details like the anthropometric measurements, BMI and clinical evidence of hyperandrogenism like hirsutism, acne or male pattern alopecia were noted on separate assessment sheets. Ferriman-Gallwey (FG) scoring system, which evaluates 9 body areas on a scale of 1 to 4 was used to score hirsutism. A total score more than 8 was considered clinically significant. The history and examination findings noted in the medical records were also accessed for data collection.

All these women were asked to undergo a transabdominal ultrasound of the pelvis as well as blood investigations like haemogram, blood sugar, thyroid function tests, serum prolactin and serum anti mullerian hormone level. The quantitative measurement of serum AMH was done by Enzyme linked fluorescent assay (ELFA)-automated test using Vitek immune diagnostic assay system (VIDAS).

Statistical analysis

Statistical analysis was performed using IBM SPSS version 20.0 software. All the data obtained from the medical records; proforma and assessment sheet was compiled and analysed to derive the results. The participants were categorised into different phenotypes as per Rotterdam criteria. Descriptive statistics of levels of serum AMH in different phenotypes of PCOS were computed. Categorical variables were expressed by frequency and percentage. To test the statistical significance of the association of categorical variables with respect to PCOS, chi-Square test was used and Odds ratio was used to measure the strength of association. Continuous variables were presented by mean and standard deviation.

To test the statistical significance of the difference in the mean of continuous variables such as age, BMI and AMH level between PCOS and non-PCOS patients, Student's t test was used for normally distributed data and Mann Whitney U test for skewed data. To find the cut-off value of AMH level for predicting PCOS, ROC curve analysis was used. Diagnostic measures such as sensitivity, specificity, positive and negative predictive value and accuracy were computed.

RESULTS

The demographic and anthropometric characteristics of participants in the PCOS and non-PCOS groups are summarized in Table 1. There was no statistically significant difference in mean age between the PCOS and non-PCOS groups (28.7±3.5 vs 30.2±4.5 years; p=0.08). Similarly, mean height did not differ significantly between the two groups (157.3±5.7 cm vs 158.8±6.2 cm; p=0.212). The mean weight was significantly higher in the PCOS group compared to the non-PCOS group (66.00±13.38 kg vs 61.04±10.51 kg; p=0.042). The median BMI was also significantly higher in the PCOS group (26.1 (23-30) kg/m²) compared to the non-PCOS group (23.8 (21.7-26.3) kg/m²; p=0.012). Similarly, the median waist–hip ratio was significantly higher among PCOS patients (0.80 (0.70-0.82)) compared to non-PCOS patients (0.78 (0.75-0.81); p<0.001).

Table 1: Demographic and anthropometric characteristics of study participants.

Variable	PCOS group	Non-PCOS group	P value
Age (years)	28.7±3.5	30.2±4.5	0.08
Height (cm)	157.3±5.7	158.8±6.2	0.212
Weight (kg)	66.00±13.38	61.04±10.51	0.042*
BMI (kg/m²)	26.1 (23-30)	23.8 (21.7-26.3)	0.012*
Waist-hip ratio	0.80 (0.70-0.82)	0.78 (0.75-0.81)	<0.001*

Data are presented as mean±standard deviation for normally distributed variables and median (interquartile range) for skewed variables. Group comparisons were performed using independent t-test or Mann-Whitney U test as appropriate. *p<0.05 considered statistically significant.

Table 2: Distribution of PCOS phenotypes and corresponding AMH levels.

Phenotype	Diagnostic features	N (%)	AMH (ng/ml), mean±SD
A (classic PCOS)	OA+HA+PCO	27 (54)	9.00±2.7
B (essential NIH criteria)	OA+HA	4 (8)	6.00±1.30
C (ovulatory PCOS)	HA+PCO	5 (10)	4.50±1.10
D (non-hyperandrogenic PCOS)	OA+PCO	14 (28)	5.00±3.60

Diagnosis and phenotypic classification were based on the Rotterdam criteria. Values are expressed as mean±standard deviation.

Among the PCOS cohort (n=50), ultrasound demonstrated a polycystic ovarian (PCO) morphology in the 45 patients (90%). Clinical features included hirsutism in 35 patients (70%) and oligomenorrhea in 46 patients (92%). Based on

the Rotterdam criteria, participants were classified into four phenotypes: phenotype A (Classic PCOS: OA+HA+PCO morphology), phenotype B (Essential NIH criteria OA+HA), phenotype C (ovulatory PCOS: HA+PCO) and phenotype D (non-hyperandrogenic PCOS: OA+PCO). Phenotype A was the most prevalent subtype. It also demonstrated the highest mean AMH levels (9.0±2.7 ng/ml), whereas phenotype C had the lowest mean AMH levels (4.51±1.10 ng/ml).

Table 3: Comparison of serum AMH levels between PCOS and non-PCOS groups.

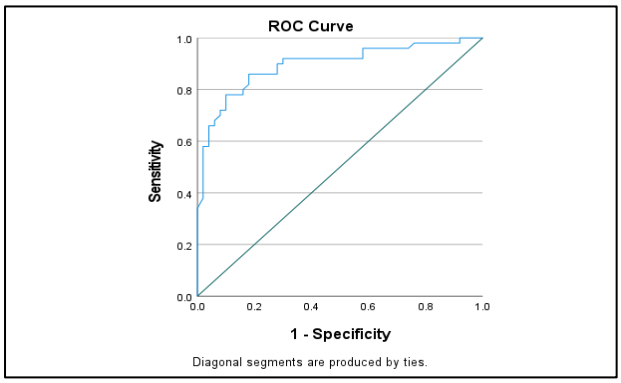
Parameters	PCOS (n=50)	Non-PCOS (n=50)	P value
Mean±SD (ng/ml)	7.10±2.70	2.32±1.59	<0.001
Median (IQR) (ng/ml)	7.7 (4-12)	2.3 (1.3-2.8)	<0.001

Statistical analysis was performed using independent t-test or Mann-Whitney U test as appropriate. p<0.05 was considered statistically significant.

Table 4: ROC curve analysis of AMH for prediction of PCOS.

Parameters	Value
AUC	0.895
Cut-off value (ng/ml)	4
Sensitivity (%)	90
Specificity (%)	78
Youden index	0.68
Odds ratio (95% CI)	31.9 (10.2-99.9)
P value	<0.001

Serum AMH levels were significantly higher among women with PCOS compared to non-PCOS controls. The mean AMH level in the PCOS group was 7.10±2.70 ng/ml, whereas it was 2.32±1.59 ng/ml in the control group (p<0.001).



The area under the curve (AUC)-0.895. Youden index-0.68.

Figure 1: ROC curve of serum AMH levels for prediction of PCOS.

The median (Interquartile Range-IQR) AMH levels were also higher in PCOS patients (7.7 (4-12) ng/ml) compared to controls (2.3 (1.3–2.8) ng/ml) and this difference was statistically significant (p<0.001) (Table 3). Receiver operating characteristic (ROC) curve analysis demonstrated good diagnostic performance of AMH in predicting PCOS, with an area under the curve (AUC) of 0.895 (Figure 1). An optimal cut-off value of 4 ng/ml yielded a sensitivity of 90% and specificity of 78% (Youden index=0.68).Women with AMH levels ≥4 ng/ml had significantly higher odds of having PCOS (Odds Ratio=31.9; 95% Confidence Interval (CI):10.2-99.9; p<0.001) (Table 4).

DISCUSSION

AMH is a glycoprotein secreted by granulosa cells of pre-antral and small antral follicles. Serum AMH levels correlate strongly with antral follicle count and reflect the pool of small follicles, which is typically increased in PCOS due to follicular arrest.[8] Unlike gonadotropins and steroid hormones, AMH exhibits minimal intra-cycle variability, making it a stable and convenient marker for clinical use.[9] However, variations in reported cut-off values across populations highlight the influence of ethnicity, assay variability and age-related differences.[12]

The 2023 International Evidence based guideline for PCOS, has allowed the use of AMH assay in adults, for ovarian morphology criterion, but has not given one single global cutoff value. So, the present study was undertaken to find a population based cut off value in a tertiary care centre.

This study demonstrated statistically significant elevation of serum AMH levels in women with PCOS compared to controls. This finding is consistent with previous studies that have shown increased AMH levels in PCOS due to an expanded pool of small antral follicles and impaired follicular maturation.[8,15] Hence AMH can be used as a reliable biomarker for PCOS.

Despite these promising findings, several limitations must be considered. One of the major challenges is assay variability, which affects comparability of AMH values across laboratories.[12] Additionally, factors such as age, BMI, and ethnicity influence AMH levels, necessitating population-specific reference ranges.[10,17]

The ROC curve analysis in this study revealed an optimal AMH cut-off of 4 ng/ml with high sensitivity (90%) and specificity (78%), and an AUC of 0.895, indicating good diagnostic performance. The strong association between AMH ≥4 ng/ml and PCOS (odds ratio 31.9) observed in this study further supports its diagnostic utility. So, in this population, if serum AMH value is above 4 ng/ml, and either hyperandrogenism or ovulatory dysfunction is present, PCOS can be diagnosed without ultrasound. Recent meta-analyses have reported comparable diagnostic accuracy, with pooled sensitivity and

specificity values supporting AMH as a useful adjunctive test in PCOS diagnosis.[6,10] Another important observation in this study is the variation in AMH levels among different PCOS phenotypes. Phenotype A exhibited the highest AMH levels, while phenotype C showed relatively lower levels. This aligns with earlier studies indicating that classic phenotypes, characterised by both hyperandrogenism and anovulation, have a greater follicular burden and consequently higher AMH levels. Recent studies have also demonstrated a significant correlation between elevated AMH levels and metabolic parameters, suggesting its potential role not only in diagnosis but also in risk stratification.[17] Milder phenotypes tend to exhibit less pronounced endocrine disturbances.[13,16]

Limitations

This study was aimed at establishing a population based cut off value for AMH to diagnose PCOS. But the relatively small sample size and single-centre design, limit its generalisability. Also, the use of transabdominal ultrasound instead of transvaginal ultrasound for PCOM assessment would have made the diagnosis less accurate. Another limitation of the study is that only clinical assessment, but not the biochemical assessment of hyperandrogenism was done.

CONCLUSION

This study contributes to the growing evidence supporting the incorporation of AMH into the diagnostic framework of PCOS. Serum AMH is a valuable, non-invasive biomarker for diagnosing PCOS and differentiating its phenotypes. Elevated serum AMH levels can be used as a strong predictor for PCOS among women of reproductive age, using age and population-specific cut-offs and standardized assays, alongside Rotterdam criteria. This makes diagnosis less invasive and more accessible.

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