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Diagnostic accuracy of serum AMH compared to ovarian ultrasound in PCOS detection

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Abstract
Background: Polycystic Ovary Syndrome (PCOS) is a complex endocrine disorder with diverse clinical manifestations and a high prevalence among women of reproductive age. Traditionally it is diagnosed using Rotterdam criteria, while recent 2023 guidelines propose the potential diagnostic use of serum Anti-Müllerian Hormone (AMH) and updated ultrasonographic parameters. This study aimed to assess the correlation between serum AMH levels and radiological markers of polycystic ovarian morphology (PCOM).
Methods: Our cross-sectional study was conducted on 100 women suspected to have PCOS. Serum AMH was estimated using chemiluminescent immunoassay. Transvaginal ultrasonography was performed by a single radiologist to assess ovarian volume (OV ≥10 cc), follicle number per ovary (FNPO ≥20), and follicle number per section (FNPS ≥10). Statistical analysis was performed using SPSS v26. ROC (receiver operating curve) analysis was used to assess the diagnostic performance of AMH in detecting PCOM.
Results: Mean serum AMH levels were significantly higher in participants showing positive PCOM criteria (mean AMH: 7.7±4.4 ng/ml). The highest correlation was found between AMH and FNPO. ROC analysis revealed that AMH had an AUC of 0.85, with a cut-off value of 4.45 ng/ml showing the best sensitivity and specificity for detecting PCOM.
Conclusion: Serum AMH levels are strongly correlated with ultrasonographic PCOM features and may serve as a reliable, non-invasive diagnostic marker for PCOS in line with the 2023 guideline updates.

Keywords: PCOS, anti-müllerian hormone, polycystic ovarian morphology, ultrasonography, diagnostic accuracy

Introduction
Polycystic Ovary Syndrome (PCOS) is a prevalent endocrine disorder, affecting approximately 10% of women of reproductive age. It is a heterogeneous condition characterized by ovulatory dysfunction, hyperandrogenism, and polycystic ovarian morphology (PCOM). Over the years, diagnostic criteria have evolved significantly-from the NIH guidelines in 1990 to the latest 2023 International Evidence-Based Guidelines. Traditionally Rotterdams criteria has been most commonly used for diagnosis of PCOS till date. Most recent 2023 guidelines emphasize objective diagnosis and reduce reliance on subjective clinical features. Notably, they have introduced refined ultrasound parameters, including follicle number per ovary (FNPO), follicle number per section (FNPS), and ovarian volume (OV), and for the first time, proposed serum Anti-Müllerian Hormone (AMH) as a potential diagnostic tool; but no cut off value has given for it [1].
AMH, a glycoprotein secreted by granulosa cells of pre-antral and small antral follicles, is found at elevated levels in women with PCOS due to an increased number of these follicles and disrupted folliculogenesis. In addition to reproductive implications, PCOS is also associated with metabolic abnormalities such as insulin resistance, type 2 diabetes mellitus, dyslipidaemia, and cardiovascular risk. These comorbidities further underline the need for early and accurate diagnosis. In this context, our study aims to investigate the correlation between serum AMH levels and the newly specified ultrasound-based PCOM criteria and and to determine an optimal AMH cut-off value for diagnosing PCOS.

Aim
To evaluate the correlation between serum Anti-Müllerian Hormone (AMH) levels and ultrasonographic parameters of polycystic ovarian morphology (PCOM), specifically ovarian

volume (OV), follicle number per ovary (FNPO), and follicle number per section (FNPS), in either ovary among women suspected to have PCOS.

Study Design

This study was cross sectional and observational designed to determine correlation of serum AMH levels with clinical, hormonal and ultrasonographic parameters among women suspected to have PCOS. A single Radiologist did ultrasonic assessment as per the latest guidelines.

Study population- Patients visiting Max smart super speciality hospital, Saket, New Delhi, with complaint of menstrual cycle abnormalities, acne, hair fall, hirsutism, weight gain; suspicious of PCOS.

Inclusion criteria

1. All the patients in the age group 18-38 years (reproductive group) suspected of PCOS and willing to be a part of the study will be included.
2. Patients will be suspected to have PCOS on basis of -

- Menstrual disorders - as oligo menorrhea (cycle length >35 days) or amenorrhoea (cycle length >12 weeks) or less than 8 cycles per year.
- Clinical hyperandrogenism (hirsutism -by FG score, presence of acne or seborrhoea).
- Excessive weight gain, Obesity and infertility

Exclusion criteria

1. The patients who are not willing to be a part of the study.
2. Patient on any hormonal contraceptive in last three month
3. Patient with any prior ovarian surgery
4. Hyperprolactinemia
5. Thyroid disorders
6. Late onset congenital adrenal hyperplasia, Cushing disease

Methodology

All those patients visiting the OPD, who were suspected to have PCOS and were willing to participate in study, were explained about study in detail in the language they best understand. All such cases were enrolled for examination after obtaining written and informed consent according to inclusion and exclusion criteria. A standard study proforma was used for documentation. Menstrual history was taken and patients were grouped as having normal or abnormal cycles. Signs of androgen excess (hirsutism, acne, hair fall) were noted. Hirsutism was evaluated by Ferriman gallway scoring chart and classified as mild (8-11), moderate (11-15) and severe (>15). Height was recorded in centimetres and weight in kilograms. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m2) and was categorized into four groups according to the Asian-Pacific cut off points: underweight (<18.5 kg/m2), normal weight (18.5-22.9 kg/m2), overweight (23-24.9 kg/m2), and obese (≥25 kg/m2).

In radiology - a Single radiologist did all transvaginal scans. The machine model used was Samsung WS 80A, with a probe frequency of 9-10 Mhz. PCOM Morphology- identified on USG by criteria: Ovarian volume (OV) ≥ 10 ml or Follicle number per section (FNPS) ≥ 10, and Follicle number per ovary (FNPO) ≥ 20 in at least one ovary was considered the threshold for PCOM.

Among blood investigations - serum Prolactin (ng/ml) and TSH (micro IU/ml) were done to rule out cases of hyperprolactinemia and thyroid disorders, which may cause confusion in diagnosis. Serum AMH (ng/ml) was estimated by chemiluminescent-immunoassay, with machine Beckman DXCi biochemical analyser. Same analyser was used for other investigation too. Serum total testosterone (ng/dl) was done to assess biochemical hyperandrogenism. As a part of assessment of metabolic syndrome - serum fasting insulin levels (micro IU/ml), 75 gm oral glucose test (fasting sugar and two hrs after 75 gm glucose mg/dl), and lipid profile (cholesterol and triglycerides) was done. Then calculation of HOMA IR [Homeostatic model assessment of insulin resistance (fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l) /22.5). In this article these values not included.

All the data was recorded in the proforma and analysed. AUROC (area under receiver operating curve) to determine the minimum cut off with the best sensitivity and specificity of AMH was applied in order to assess the diagnostic strength of AMH as a biomarker for PCOS.

Calculation of sample size

Based on the study by Woo *et al*., the area under the ROC curve (AUC) for serum AMH in diagnosing PCOS was reported to be 0.868. In their study, the disease prevalence was 87 out of 140 subjects (p = 0.62). Using this reference and applying the standard formula for estimating AUC with the given parameters, the minimum required sample size was calculated to be 55.

There were practical challenges in the target population, as many participants were adolescents or young women of reproductive age who were either not eligible for transvaginal ultrasound due to virginal status or had limited accessibility due to obesity. Additionally, a significant number had coexisting conditions such as hypothyroidism or hyperprolactinemia, which warranted exclusion. Hence, a sample size of 100 was chosen to maintain statistical power and accommodate these anticipated limitations.

Statistical Analysis

Data was recorded in Excel sheet and analyzed using SPSS version 26.0 (IBM). Student's t-test and ANOVA were used for group comparisons, with $p < 0.05$ considered statistically significant. Pearson's correlation coefficient (r) assessed the association between serum AMH levels and various clinical and radiological parameters.

Result

In our study, the mean age of all PCOS cases was 27.0±4.4 years and the BMI was 27.5±6.3 kg/m² where 64% patients were obese and 9% in overweight group, while 22%were in normal BMI group. The mean serum AMH levels in our study was 7.7 ng/ml ±4.4 ng/ml.

Further in 64% patients were with abnormal menstrual cycles, had a mean serum AMH level of 7.8±4.44ng/ml while normal menstrual cycle was seen in 36% with mean serum AMH level of 7.5±4.5ng/ml.

Clinical hyperandrogenism assessed by hirsuitism and presence of acne. Acne was present in 74% cases. Mean serum AMH level in cases with acne was 7.6±4.0ng/ml while those without acne have 8.3±5.6 ng/ml. Mean serum AMH values among hirsute groups - in mild hirsutism (FG score 8-11) it was 7.5±3.6ng/ml, moderate (FG score 11-15) it was 9.32±6.23ng/ml, severe (FG score >15) it was 6.83±2.6ng/ml respectively.

Biochemical hyperandrogenism was identified by serum Total Testosterone levels > 60 ng/dl. In our study, it was seen in 19% cases only. Mean serum AMH values in PCOS cases with raised testosterone levels (>60 ng/dl) was 9.3±6.2 ng/ml while those with lower testosterone values had mean serum AMH of 7.73±4.4 ng/dl.

A negative correlation was observed between mean serum AMH level and menstrual cycle pattern; and this correlation was not significant. A positive correlation was seen between serum AMH and clinical hyperandrogenism features as - Acne, hair fall and hirsutism. However, this correlation was significant. A Negative correlation was seen between total testosterone levels and AMH levels and it was not significant.

Table 1: Mean serum AMH values among radiological parameters for PCO Morphology and its significance.

Radiological Parameters	(n = cases)	AMH (ng/ml) Mean	AMH (ng/ml) SD	P value
Ovarian volume (OV) cc				
< 10	26	5.9	2.4	0.001
≥ 10	74	8.4	4.8	
Follicle number per ovary (FNPO)				
< 20	20	5.9	3.1	0.011
≥ 20	80	8.2	4.6	
Follicle number per section (FNPS)				
< 10	7	5.4	2.7	0.142
≥ 10	93	7.9	4.5	

In our study-observations were based on radiological parameters as per 2023 guidelines. Ovarian volume (OV) ≥10 cc was seen in 74% individuals and they had mean serum AMH of 8.4±4.8ng/ml. A significant difference was observed in mean serum AMH values between both groups of ovarian volume (p value 0.001).
FNPO (follicle number per ovary) ≥20 was seen in 80% cases and these had mean serum AMH of 8.2±4.6 ng/ml. A significant difference was observed in mean serum AMH values between both groups of FNPO (p value 0.011).
FNPS (follicle number per section) ≥10 was seen in 93% cases and these had mean serum AMH of 7.9±4.5 ng/ml. No significant difference was seen in mean serum AMH values between both groups of FNPS (p value 0.142).

Table 2: Correlation between radiological parameters of ovaries and mean serum AMH levels

Radiological parameters	AMH – Pearson's correlation	p value
OV ≥ 10 cc	0.374	<0.001
FNPO ≥ 20	0.230	0.021
FNPS ≥ 10	0.130	0.198

In our study, a positive correlation was observed between serum AMH values and all the three radiological parameters in ovaries (OV, FNPO, and FNPS).
There was a significant strong correlation (p value<0.05) was observed between serum AMH levels and two radiological parameters that is - ovarian volume (OV), follicle number per ovary (FNPO).
Receiver-operating characteristic (ROC) curves was constructed to evaluate the diagnostic test performance such as sensitivity, specificity of AMH and ultrasonographic ovarian parameters (OV & FNPO) for diagnosis of PCOS. Similar to previous criteria in our study also - the significant PCOM parameters identified were ovarian volume (OV≥10) and Follicle number per ovary (FNPO ≥20).

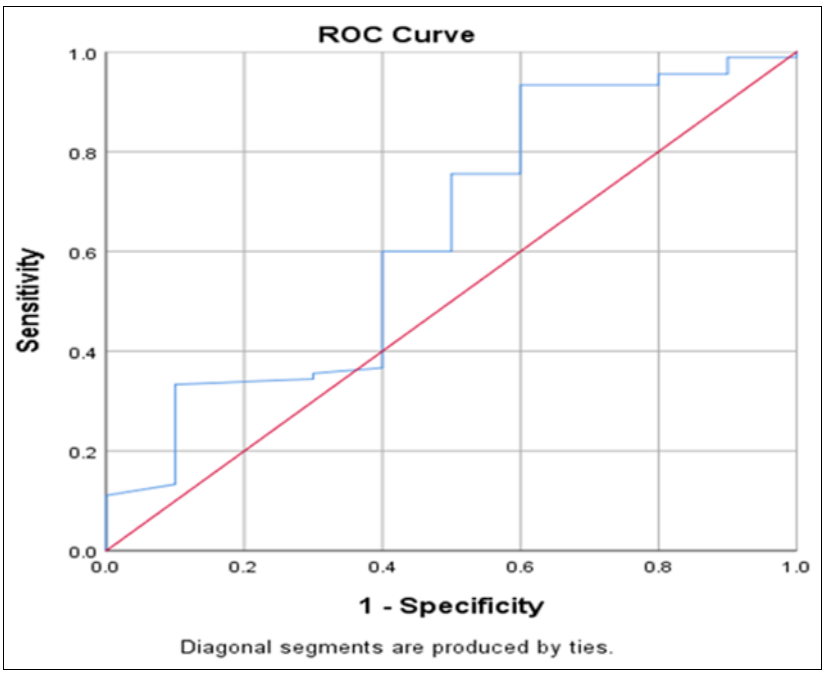

Fig 1: Receiver-operating characteristic (ROC) curve and area under the ROC curve of AMH.

AUC in our study was 0.633. Serum AMH value of 3.69ng/ml was identified as the best cutoff with sensitivity 93.3%.

Discussion

This cross-sectional observational study was conducted on 100 women of reproductive age suspected to have Polycystic Ovary Syndrome (PCOS) based on the Rotterdam criteria. There is absence of a single definitive diagnostic criterion and the considerable variation in PCOS prevalence depending on the criteria used. Hence, there is an ongoing need to identify reliable and objective diagnostic markers.
The 2023 international guidelines for PCOS have recognized the diagnostic potential of serum Anti-Müllerian Hormone (AMH), particularly in relation to polycystic ovarian morphology (PCOM), which is now defined using specific ultrasound parameters including ovarian volume, follicle number per ovary (FNPO), and follicle number per section (FNPS) [1].
Our study was aimed to evaluate the correlation between serum AMH levels and these revised morphological parameters, as well as its association with other clinical and metabolic features

of PCOS, such as irregular menstrual cycles, clinical hyperandrogenism (hirsutism, acne, hair fall), elevated total testosterone, family history of diabetes mellitus, insulin resistance, and dyslipidaemia. Although this study was conducted solely on PCOS cases without a control group, the findings showed a strong correlation between elevated serum AMH levels and PCOM features, aligning with results from previously published studies. These findings reinforce the emerging role of serum AMH as a valuable adjunct in the diagnosis and phenotypic assessment of PCOS

Polycystic ovarian morphology (PCOM)

In our study, PCOM was documented as per current 2023 guidelines by a single radiologist with Ultrasound findings in either ovary. This included -Ovarian volume (OV) ≥10 cc, FNPO (follicle number per ovary) ≥20, FNPS (follicle number per section) ≥10 in either ovary left or right. However, in most of studies PCOM was considered with ovarian ultrasonography showing follicle size of 2-9 mm and follicle count ≥12 in one or both ovaries with ovarian size 10 ml or more (one/both ovaries).

In our study, calculated mean serum AMH level was 7.7±4.4 ng/ml. Serum AMH values differed significantly in two parameters of PCOM that is - FNPO and OV. It was observed that mean serum AMH with positive radiological parameters had higher value.

Pandey *et al*. [2] in their study stated that PCOM can be replaced with serum AMH levels for PCOS diagnosis, the cut-off levels they stated was 4.7 ng/ml.

Halder *et al*. [3] in their study classified - PCOM types (type 1/ ovarian volume 11-15 ml and /or follicle count 12-15 of 2-9 mm size in one/both ovaries vs. type 2/ovarian volume 16-20 ml and/or follicle count 16-20 of 2-9 mm size in one/both ovaries). They found no significant differences in AMH values in these two specified groups. Median serum AMH value in their study was 8.5 ng/ml while cut off being 4.71ng/ml.

Moolhuijsen LM *et al*. [4] in their study found serum AMH levels correlate significantly with follicle count and mean serum AMH levels determined in this study ranged from 6.55 to 9.90 ng/ml.

In another study by Wongwananuruk *et al*. [5] serum AMH threshold of 4.7 ng/ml was seen, which was significantly and positively correlated with threshold values for PCOM (FNPO ≥15, FNPS ≥7 and OV ≥ 6.5 ml) for diagnosing PCOS.

In a study conducted by Aydogmus *et al*. [6] found serum AMH levels were significantly correlated with FNPO (presence of more than 12 follicles, 2-9 mm in diameter each, in an ovary) and serum AMH level cut-off value of 3.51 ng/ml, for PCOS diagnosis.

In another study by Matsuzaki T. *et al*. [7] strongest positive correlation was seen between ovarian volume per ovary and serum AMH levels. They also identified serum AMH level cut-off value of 7.33 ng/ml.

In a study by Taha *et al*. [8] mean serum, AMH levels in PCOS cases ranged from 6.19 ng/ml for mild cases to 7.49 mg/ml for moderate cases, and 12.83ng/ml for severe cases.

All the studies show a strong association of serum AMH levels with PCOS, however cut off are ranging from 2.8 to 10 ng/ml (Table 3). In our study, we found mean serum AMH value of 7.7±4.5 ng/ml among all PCOS cases.

Table 3: Threshold values of AMH reported in various studies.

Author, Year Reference	Population	PCOS Diagnosis Criteria	Study Design	No. of cases/controls	Cut-off (ng/ml)	Sensitivity%	Specificity%
Ahmed *et al*., 2019	Saudi	R	case-control	79/69	3.19	72	70
Saxena *et al*., 2017	India	R	case-control	45/45	3.44	77.8	68.9
Song DK *et al*., 2016	Korea	R	cross-sectional	270/220	10	71	93
Wiweko *et al*., 2014	Indonesia	R	case-control	71/71	4.45	76.1	74.6
Homburg *et al*., 2013	UK	R	case-control	90/90	6.7	60	98.2
Chao *et al*., 2012	Taiwan	R	case-control	45/59	3.5	74	79
Woo *et al*., 2012	Korea	R	cross-sectional	87/53	7.8	75.9	86.8
Ellertsen *et al*., 2012	Norway	R, AES	case-control	56^{R}, 44^{AEs} / 162	2.8	94.6	97.1
Lin *et al*., 2011	Taiwan	R	prospective	126/164	7.3	76	70
Dewailly *et al*., 2011	France	R	prospective	240/105	5.0	92	97
Li HWR *et al*., 2011	China	R	retrospective	124/26	5.9	79	96

Limitations

The primary limitation of this study is the absence of a control group, which restricts comparative analysis between PCOS and non-PCOS populations. Additionally, while the study aimed to evaluate the correlation between serum AMH and newly specified PCOM parameters (OV, FNPO, FNPS), the lack of longitudinal follow-up limits assessment of predictive value over time.

Strengths

A major strength of the study lies in its focus on the diagnostic utility of serum AMH as an alternative to ultrasound, particularly in settings where transvaginal imaging is limited. The use of a single radiologist and standardized ultrasound equipment minimized intra-observer variability. Furthermore, this study adds valuable data from an Indian population, where limited research exists on the association of serum AMH with clinical, hormonal, and metabolic features in PCOS, offering potential for more accessible diagnostic strategies.

Conclusion

Polycystic ovarian morphology may be present even in the absence of overt clinical symptoms or biochemical abnormalities, and in many cases, radiological findings can serve as an early indicator of PCOS. In our study, serum AMH levels showed a significant positive correlation with key ultrasonographic parameters, including ovarian volume (≥10 cc) and follicle number per ovary (≥20), as per the updated diagnostic criteria. These findings support the use of serum AMH as a reliable and specific biomarker for the diagnosis of PCOS. Additionally, serum AMH holds potential as a tool for monitoring treatment response, offering a non-invasive and objective alternative to ultrasound, especially in situations where imaging is limited or inconclusive.

Recommendation

Ours was a very small study just to see the correlation between serum AMH levels and PCO Morphology. However, serum AMH levels can be used as a single useful parameter in prediction of PCOS.

It can be useful in the clinical detection of PCO Morphology and the diagnosis of PCOS. A definite standardised internationally

approved cut off value of serum AMH must be determined. There is a need to do a multicentre large cohort study in a properly defined population of different age groups for determination of such a value.

Conflict of Interest: Not available.

Financial Support: Not available.

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