Evaluation of the Efficacy and Threshold of Serum Anti-mullerian Hormone (AMH) Levels for Diagnosis and Prediction of Polycystic Ovary Syndrome (PCOS) Among Indian Women.

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Research Article

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Abstract

**Purpose:** PCOS women exhibit higher levels of AMH and has been proposed to add value to diagnosis of PCOS incase ambiguity. However, variable cutoffs of AHM for PCOS prediction have been reported. This study was designed to determine diagnostic threshold of serum AMH levels and its correlation with clinical, hormonal and ultrasonographic parameters among women with PCOS.

**Materials:** In this prospective study, 113 women with PCOS as per Rotterdam criteria 2003 and 75 normo-ovulatory women were included. Clinical, biochemical, hormonal and sonographic assessment in addition to serum AMH levels were determined using standard methodology.

**Results:** Mean age was comparable (23.43±3.42 vs 24.21±3.18 years) between cases and controls. The mean number of menstrual cycles per year were lower while as mean BMI, FG score, and serum testosterone were higher in cases than controls (p<0.05). The mean serum AMH level was significantly higher in PCOS group (7.84±3.67 vs. 3.23 ±1.56 ng/mL) than controls. The serum AMH levels showed a positive correlation (p<0.05) with LH/FSH ratio (r = 0.206, p = 0.029), number of ovarian follicles (r=0.461) and volume,(r=0.521), but no correlation significant with age and BMI. As per receiver operating characteristic (ROC) curve, cut-off was worked out to be 3.76 ng/mL with 86.7% sensitivity and 62.7% specificity.

**Conclusion:** Serum AMH levels correlate positively with PCOM among PCOS women and may be a potent diagnostic marker of ovarian dysfunction either alone or in conjunction with other tools to ensure timely diagnosis and early treatment of the disorder.

Introduction

Polycystic ovary syndrome (PCOS) is a genetically intricate commonest endocrine disorder among reproductive aged women, [1], and is associated with diverse health issues such as menstrual disturbances, ovulatory dysfunction, infertility[2], and hyperandrogenism [3]. Women suffering from PCOS display an increased risk of metabolic aberrations[4], including insulin resistance (IR) [4], type 2 diabetes mellitus (T2DM) [5], dyslipidemia[6], obesity[7], cardiovascular disease (CVD) [8] and mitotic risks. PCOS also increase the risk for obstructive sleep apnea[9], depression[10] and non-alcoholic fatty liver disease (NAFLD)[11]. The prevalence of PCOS varies in accordance with origin of studies, age groups included, diagnostic criteria employed [12]. National Institutes of Health (NIH; 1990), Rotterdam (2003) and Androgen Excess and Polycystic Ovary Syndrome Society (AE-PCOS) criteria have been used by authors [13].

PCOS is characterized by an increased number of follicles at all growing stages particularly pre-antral and small antral follicles[14], which primarily produce AMH, owing to maturation arrest attributed to abnormal hormonal milieu. As a result, elevated serum AMH is 2-4 fold higher in women suffering from PCOS than in healthy women[15–17] which may partly be attributable to defects in apoptotic processes in maturing follicles [18]. Besides AMH production by granulosa cells in-vitro is also shown to be increased 75 fold higher in anovulatory PCOS and 20 fold more in normo-ovulatory PCOS in comparison to normal ovaries [19] and is positively correlated with serum androgens [20, 21].

Serum AMH assay is a superior marker of ovarian reserve [22], being non-invasive and stable from one menstrual cycle to another [23, 24]. AMH is proposed as a surrogate marker for PCOS diagnosis, although different cutoff values have been reported in various studies[25]. The meta-analysis of AMH in diagnosing symptomatic PCOS
women demonstrated 79.4% specificity and 82.8% sensitivity at cutoff value of 4.7 ng/mL[26]. This variation in cutoff values has been attributed to ethnicity, obesity, vitamin D deficiency, and methods of estimation [27–29]. Therefore, AMH is yet to be adopted as a part of standard diagnostic criteria for PCOS due to this variability and lack of international standard [30]. Data in our population is scant and hence the present study was undertaken to analyze serum AMH levels among Indian women with PCOS and to correlate it with clinical, hormonal and ultrasonographic parameters to evaluate the utility of AMH in the prediction of PCOS.

**Materials And Methods**

In this prospective study, women presenting between January 2017 to July 2018 with symptoms suggestive of PCOS such as oligomenorrhea and/or clinical hyperandrogenism and/or ultrasonography indicative of PCOM were informed about the study. Those who expressed desire and signed an informed consent were enrolled in the study. A total of 188 pre-menopausal women belonging to the age group of 18 to 35 years were recruited. The study was conducted as per Helsinki Declaration, and was approved by Institutional Ethics Committee.

**Study subjects**

**Cases:** A total of 113 cases (aged 18-35 years) with PCOS were enrolled in the current study. PCOS was defined when at least two of the three components were present, according to the Rotterdam criteria. Clinical hyperandrogenism (HA) was defined by a modified Ferriman and Gallwey (FG) score of greater than 8 with or without presence of acne and androgenic alopecia. Biochemical HA was defined as an elevation of serum total testosterone levels above 97.5 percentile limits, (i.e. >0.45 ng/mL or 2.1 nmol/L).

**Controls:** Age-matched healthy women (n=75) with regular menstrual cycles (cycle length of 25-35 days) with no evidence of hirsutism, acne, alopecia or endocrine dysfunction and PCOM on trans-abdominal ultrasonography were taken as controls. Subjects were recruited as a part of community outreach programs conducted by the department of Clinical Research periodically.

**Clinical assessment and anthropometric evaluation**

A pre-validated questionnaire was administered to all the subjects that included recording of medical facts such as menstrual cyclicity (age of menarche, cycle interval, cycle length), weight trajectory, symptoms of hyperandrogenism (unwanted male pattern hair growth, moderate to severe or treatment resistant acne vulgaris, androgenic alopecia), infertility etc. Oligomenorrhea was defined as inter-menstrual interval of >35 days or less than 8 cycles per year while as no menstrual cycle for >6 months was labeled as amenorrhea and >12 cycles per year was defined as polymenorrhea. Details of medications if any, including those of various health events such as clinically significant cardiovascular, liver, lung, neurologic, renal, musculoskeletal dysfunction etc. were recorded and women with these conditions were excluded. Other exclusions included non-classical adrenal hyperplasia (NCAH), Cushing’s syndrome, androgen secreting tumors, hyperprolactinemia and thyroid dysfunction.

Besides these, women with known medication history in last six months that included use of one or more drugs like insulin sensitizers, steroids, anti-androgens, oral contraceptives, anti-depressants, lipid-lowering agents, or other drugs known to effect glucose tolerance, insulin sensitivity etc. were also excluded from the study.

All the enrolled women underwent a brief general physical and systemic examination followed by detailed anthropometry - measurement of height (cms), weight (kgs), waist circumference (cms) with calculation of BMI.
(Kg/m²) using standardized scales (SECA-18, Hamburg, Germany) as a mean of three readings. Waist circumference was measured at the narrowest portion of the torso, approximately midway between the lower costal margin and iliac crest. Blood pressure (systolic and diastolic) mm of Hg was calculated from the mean of three readings using standard calibrated instrument (Omron 714, Kyoto Japan). Evaluation of hirsutism was done by using modified Ferriman-Gallwey (mFG) score by a single observer. The densities of terminal hair over 9 specific sites were scored from 0 to 4 and a total score was calculated. Acne vulgaris was graded as per standard guideline (0 = no acne; 1 = minor acne; 2 = minor acne on face only and 3 = severe acne on face, back and or chest) and androgenic alopecia was graded from I-III as per Ludwig scale as was acanthosis nigricans (1-3 as per severity).

**Laboratory Evaluation**

All the women slated for testing were asked to report after an overnight (10-12hr) fast at 8:00 hours on a day arranged in such a way that the samples for T4, TSH, LH, FSH, prolactin (PRL), serum total testosterone, cortisol (morning), 17-hydroxyprogesterone (17-OHP) and serum AMH were collected from days 2nd to 7th of spontaneous or medroxyprogesterone-induced menstrual cycle. Oral glucose tolerance test (OGTT) was performed using venous blood samples obtained at baseline (0 min), 60 minutes and 120 minutes after an oral load of 75gm anhydrous glucose dissolved in 200-300 ml of water. Aliquots for hemogram, LFT, KFT, calcium, phosphorous, uric acid and lipid profile were collected from the basal sample. ACTH-stimulated 17-OHP or overnight dexamethasone-suppressed cortisol test was done to rule out NCAH or Cushing's syndrome after the baseline, wherever necessary. Trans-abdominal and transvaginal ultrasonography (wherever appropriate) was performed using GE LOGIQP5 PRO USG (Chicago, USA) equipment to demonstrate any evidence of PCOM described in literature[31, 32] i.e. presence of 10 or more peripheral follicles each measuring 2-8mm in size with echogenic ovarian stroma and/or increased (>10 ml) ovarian volume in all subjects by a single observer following a common SOP.

**Assays**

Sample for PRL, TSH, T3, T4, LH, FSH and total testosterone were assayed using a commercial electrochemiluminescent immunoassay using (COBAS e-411, Roche Diagnostics, Indiana, USA). Serum AMH levels were assayed using the ELISA kits (Ansh Labs 445 Medical Centre Blvd, U.S.A). Plasma glucose and other parameters-uric acid, lipids, calcium, phosphorous, LFT, KFT etc. were estimated on a fully automated biochemistry analyzer (Respons®910, DiaSys, Holzheim, Germany). The inter and intra assay coefficients of variation wee according to manufacturer's protocol.

**Statistical Analysis**

SPSS version 26.0 was used to analyze the date and its was expressed as Mean±SD. Group differences were compared using the Chi-square test for categorical variables and the ‘t’ test for continuous variables. For comparing continuous variables ANOVA with f-test was used. Cut-off values of AMH levels as a predictor of the diagnosis of PCOS were analyzed using a Receiver Operating Characteristic (ROC) procedure. Pearson correlation was used to analyze the association of serum AMH levels with various clinical, hormonal and ultrasonographic parameters. The p-value < 0.05 was considered statistically significant.

**Results**

The baseline characteristics of the PCOS cases and the controls are summarized in Table 1 and 2 respectively. The mean age of cases and controls was comparable (23.43±3.42 years vs. 24.21±3.18 years) respectively (p=0.118).
Mean number of menstrual cycles per year for cases and controls was 7.16±3.55 vs. 11.55±4.22 (p=0.001) while their mFG scores were 13.60±4.43 vs. 4.21±2.18 respectively (p= 0.001). Mean BMI of cases and controls was 23.26±3.76 vs. 22.23±3.31 kg/m² (p= 0.048). Among PCOS group, 2.13% were underweight, 18.09% overweight and 7.45% were obese. As shown in Figure 1, Mild hirsutism (FG score 8 to 15) was seen in 52.2% and severe hirsutism (FG score of ≥16) in 17.7%, in PCOS group while as 29% of cases had grade 1, 18% grade 2 and 3% had grade 3 acne respectively. About 28%, 8% and 3% of cases had grade 1, 2 and 3 androgenic alopecia respectively. There was no acanthosis in 78% of the cases.

| Parameter                              | PCOS n=113 | Healthy Controls n=75 | P-value* |
|----------------------------------------|------------|-----------------------|----------|
| Age (years)                            | 23.43±3.428| 24.21±3.18            | 0.118    |
| Number of menstrual cycles/year       | 7.16±3.55  | 11.5±2.22             | 0.001    |
| Modified Ferriman Gallway score (mFG) | 13.60±4.43 | 4.21±2.18             | 0.001    |
| BMI (kg/m²)                            | 23.26±3.76 | 22.23±2.97            | 0.048    |
| Waist circumference (cm)               | 83.09±10.78| 73.16±11.06           | 0.002    |
| SBP (mmHg)                             | 113.88±10.40| 112.98±9.04          | 0.542    |
| DBP (mmHg)                             | 75.92±7.77 | 75.84±7.57            | 0.938    |
| TSH (mIU/L)                            | 3.45±1.63  | 3.40±1.20             | 0.800    |
| T3 (ng/ml)                             | 1.04±0.21  | 1.03±0.24             | 0.763    |
| T4 (mcg/dl)                            | 8.35±1.66  | 8.62±1.31             | 0.245    |
| LH (IU/L)                              | 8.53±4.23  | 4.60±1.55             | 0.001    |
| FSH (IU/L)                             | 5.17±1.99  | 5.81±1.97             | 0.061    |
| LH/FSH Ratio                           | 1.64±0.91  | 0.79±0.48             | 0.001    |
| Serum total Testosterone (ng/dl)       | 61.36±23.49| 30.76±12.67           | 0.001    |
| AMH (ng/ml)                            | 7.84±3.67  | 3.23±1.56             | 0.001    |

*p<0.05 is considered statistically significant
Table 2
Comparison of biochemical characteristics of PCOS women and healthy controls.

| Parameter                                      | PCOS n=113          | Healthy Controls n=75 | P-value* |
|------------------------------------------------|---------------------|-----------------------|----------|
| Plasma glucose- 0hr (mg/dL)                    | 88.41 ±11.19        | 82.73 ±10.90          | 0.001    |
| Post OGTT-1hr (mg/dL)                          | 99.75 ± 22.47       | 96.42 ±16.83          | 0.275    |
| Post OGTT-2hr (mg/dL)                          | 103.50 ±23.01       | 106.29 ±22.17         | 0.410    |
| Serum total cholesterol (mg/dL)                | 181.62 ±37.39       | 181.00 ±35.07         | 0.908    |
| Serum Triglycerides (mg/dL)                    | 155.25 ±63.04       | 134.42 ±56.698        | 0.022    |
| Serum High density lipoprotein (HDL) cholestrol (mg/dL) | 48.09 ±8.16        | 52.49 ±7.07           | 0.001    |
| Serum Low density lipoprotein (LDL) cholestrol (mg/dL) | 102.61 ±27.53      | 101.73 ±26.03         | 0.827    |
| Serum total Bilirubin (mg/dL)                  | 0.92 ±0.39          | 0.90 ±0.47            | 0.723    |
| Serum Aspartate transaminase (AST) (U/L)       | 30.07 ±14.38        | 28.41 ±12.82          | 0.421    |
| Serum Alanine transaminase (ALT) (U/L)         | 29.28 ±17.84        | 24.61 ±12.72          | 0.052    |
| Serum Alkaline Phosphatase (ALP) (U/L)         | 109.89 ±22.42       | 96.62 ±23.93          | 0.001    |
| Serum Total protein (g/dL)                     | 7.27 ±0.56          | 7.12 ±0.47            | 0.061    |
| Serum Albumin (g/dL)                           | 4.23 ±0.66          | 4.12 ±0.46            | 0.221    |
| Serum Urea (mg/dL)                             | 17.81 ± 5.85        | 20.92 ± 7.18          | 0.001    |
| Serum Creatinine (mg/dL)                       | 0.72 ±0.22          | 0.86 ±0.14            | 0.01     |
| Serum Uric acid (mg/dL)                        | 3.91 ±1.10          | 3.80 ± 1.11           | 0.511    |

*p<0.05 is considered statistically significant.

Among the biochemical parameters plasma glucose, serum HDL and serum SGPT were significantly different among the groups as expected (Table 2). Mean LH value of cases and controls was found to be 8.53 ± 4.23 vs 4.60 ±1.55 IU/ml significantly high (p=0.001) as were mean serum total testosterone levels (61.36 ± 23.49 vs. 30.76 ±12.67 ng/ml). The mean AMH level in cases was 7.84 ±3.67 ng/ml whereas it was 3.23 ±1.56 ng/ml in the control group (p= 0.01) (Table 3). The women with PCOS were grouped in four phenotypes as shown in Table 4. The most common PCOS phenotype in our study was phenotype A followed by D with AMH levels of 12.67±3.46 ng/ml and 11.87 ± 3.40 ng/ml respectively. The least common phenotype was B with serum AMH levels of 7.28 ± 1.60 ng/ml.
Table 3
Pearson’s correlation of serum AMH levels with clinical, hormonal and ultrasonographic parameters among PCOS women.

| Variable          | Correlation coefficient(r) | P-value |
|-------------------|----------------------------|---------|
| Age (in years)    | -0.076                     | 0.421   |
| FG score          | 0.014                      | 0.885   |
| BMI (kg/m²)       | -0.082                     | 0.385   |
| Waist (cm)        | 0.059                      | 0.535   |
| LH (IU/L)         | 0.148                      | 0.119   |
| FSH (IU/L)        | -0.148                     | 0.117   |
| LH/FSH Ratio      | 0.206                      | 0.029*  |
| Testosterone (ng/dl) | 0.097                    | 0.307   |
| No. of follicles  | 0.461                      | 0.001*  |
| Ovarian Volume    | 0.521                      | 0.001*  |

*p<0.05 is considered statistically significant.

Table 4
AMH levels among PCOS women based on phenotype

| Phenotype | OA | HA | PCO | Frequency | %    | AMH (ng/ml) |
|-----------|----|----|-----|-----------|------|-------------|
| A         | +  | +  | +   | 41        | 36.28| 12.67±3.46  |
| B         | +  | +  | -   | 17        | 15.04| 7.28 ± 1.60 |
| C         | -  | +  | +   | 21        | 18.59| 8.91 ± 3.21 |
| D         | +  | -  | +   | 34        | 30.09| 11.87 ± 3.40|

Abbreviations
HA: Hyperandrogenism
OA: Oligoanovolution
PCO: Polycystic ovary

For determining the AMH diagnostic cut-off, the ROC curve was used. A 3.76 ng/mL AMH cut-off level was determined with a sensitivity of 86.7% and a specificity of 62.7% with area under the curve equaling 0.875 (0.826-0.924). Among cases, AMH levels positively correlated with LH/FSH ratio (r = 0.206, p = 0.029), polycystic ovarian morphology (number of follicles r= 0.461, p=0.001; ovarian volume r= 0.521, p=0.001) whereas there was trend towards negative correlation of AMH with age (r= -0.076, p=0.421) and BMI (r= -0.082, p= 0.385). A positive non-
significant correlation was found with FG score (r= 0.014; p=0.885) and serum total testosterone (r=0.097;p=0.307) respectively. The scatter plots are depicted in Figure 2.

**Discussion**

There is no solitary criterion for the diagnosis of PCOS and the variability in the prevalence rates under different diagnostic criteria highlights an immense need for other modalities to help in positive diagnosis of PCOS[33] and reduce uncertainty. Even with the most technically advanced ultrasonography devices, it can be difficult to count antral follicles trans-abdominally in virgins or the obese. AMH has been shown to be an alternative marker for the follicle number and an index of ovulatory dysfunction due to its higher sensitivity and specificity[34, 35]. Our findings showed that compared to healthy controls, women with PCOS had significantly higher levels of serum AMH which was accompanied by higher levels of serum total testosterone levels. These results are in concordance with the previous reported studies[36]. Since AMH is produced by granulosa cells of both preantral and small antral follicles, increase substantially among PCOS women, so AMH increase in PCOS[37]. The ability of serum AMH level to serve as a marker for the diagnosis of PCOS has been reported in many studies with inconsistent threshold values[35, 38]. In this study, we report a threshold of 3.76 ng/mL with 86.7% sensitivity and 62.7% specificity in our study population. While as, earlier studies have reported a threshold ranging from 2.52 to 10 ng/mL[25, 34, 39, 40] as depicted in Table 5, Our results are consistent with the results by Sahmay et al.[41] who showed 80.7% sensitivity and 74.7% specificity for AMH cutoff value of 3.8 ng/mL. Our threshold value is slightly lower than that of reported by Iranian[42], Korean[39, 43], French [34] and Chinese observations[44]. Our suggestion for a cut-off value of serum AMH for the diagnosis of PCOS is more reliable, given that the sample size was larger than the previous studies. The inconsistency in serum AMH levels and lack of standard cut-off values may be explained due to differences in the sample size, inclusion criteria, different AMH assay and specified PCOS phenotypes among the studies[45]. Wongwananuruk et al suggested difference in BMI across the populations may contribute to the discrepancy between AMH cut offs observed across various studies [26], however another study reported BMI had no significant impact on AMH levels[45]. In this study, we found no significant correlation of AMH levels with BMI or waist circumference however serum LH was found to be high in lean PCOS patients as compared to obese PCOS patients and this was consistent with findings of the study conducted by Liou et al.[46]. We observed obese women with PCOS had significantly lower LH to FSH ratios than non-obese women with PCOS. A significant LH/FSH ratio difference between PCOS women and healthy controls was noted. We report AMH levels to be positively correlated with LH/FSH ratio.
| Author, Year | Population | PCOS Diagnosis Criteria | Study Design | No. of cases/controls | Cut-off (ng/ml) | Sensitivity % | Specificity % |
|--------------|------------|-------------------------|-------------|-----------------------|----------------|---------------|---------------|
| Ahmed et al, 2019 [29] | Saudi | R | case-control | 79/69 | 3.19 | 72 | 70 |
| Saxena et al, 2017 [51] | India | R | case-control | 45/45 | 3.44 | 77.8 | 68.9 |
| Song DK et al, 2016 [39] | Korea | R | cross-sectional | 270/220 | 10 | 71 | 93 |
| Wiweko et al, 2014 [36] | Indonesia | R | case-control | 71/71 | 4.45 | 76.1 | 74.6 |
| Homburg et al, 2013 [52] | UK | R | case-control | 90/90 | 6.7 | 60 | 98.2 |
| Chao et al, 2012 [53] | Taiwan | R | case-control | 45/59 | 3.5 | 74 | 79 |
| Woo et al, 2012 [54] | Korea | R | cross-sectional | 87/53 | 7.8 | 75.9 | 86.8 |
| Eliertsen et al, 2012 [55] | Norway | R, AES | case-control | 56<sup>R</sup>,44<sup>AES</sup>/162 | 2.8 | 94.6 | 97.1 |
| Lin et al, 2011 [56] | Taiwan | R | prospective | 126/164 | 7.3 | 76 | 70 |
| Dewailly et al, 2011 [25] | France | R | prospective | 240/105 | 5.0 | 92 | 97 |
| Li HWR et al, 2011 [57] | China | R | retrospective | 124/26 | 5.9 | 79 | 96 |
Serum AMH has been proposed to replace variable ultrasound assessment of ovarian follicle in diagnosis of PCO morphology as disrupted folliculogeneis leading to excess antral follicle accumulation underpins the higher serum AMH levels among PCOS women[47]. In the present study, we compared the AMH levels among PCOS women with different PCOS phenotypes. Serum AMH level was highest in Group A (OA+HA+PCO) who had PCO morphology on ultrasound and lowest in group B where no PCOM morphology was found. The results depict association of PCOM with AMH levels. Various studies have reported hyperandrogenism, the hallmark feature of PCOS [48], to increase AMH expression from granulosa cells and ovulatory dysfunction is also associated with AMH levels [49]. The current observations substantiate none of the findings which is in contrast to the study among Turkish women with PCOS [50].

The accuracy and sensitivity of AMH for PCOS was well established in our study and its higher values in serum reflected AMH as a potential marker of recruited non-growing follicles rather than a simple marker of ovarian reserve. Though sensitivity and specificity of AMH is low in absence of other diagnostic factors to make positive diagnosis of PCOS. Still it is a promising diagnostic tool for PCOS as an adjunct to existing Rotterdam criteria when it is used to replace PCOM.

**Conclusion**

The serum AMH level among patients with PCOS correlate positively with LH/FSH ratio and polycystic ovarian morphology (follicle number and ovarian volume). The combination of the serum AMH level with hyperandrogenism and/or oligo-amenorrhea markedly increases the diagnostic accuracy of PCOS and can be introduced as an objective and well-structured criterion for diagnosis of PCOS.

**Limitations**

All efforts were made to minimize limitations of the study as far as possible. However, enrolling of healthy volunteers as controls proved to be a minor challenge with the result only 75 volunteers participated in the study. The effect of this limitation on the study was minimized by applying appropriate statistical tests of significance. Besides, HOMA-IR, SHBG and DHEAS levels were not estimated in our study.

**Declarations**

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**Competing Interests:**

The authors have no relevant financial or non-financial interests to disclose.

**Author contribution:**

MAG: Project development;

SS: Data collection;

AR, TS, IAW: Data analysis;

AB, VV: Manuscript writing.

All authors have read and approved the final manuscript.

**Conflict of interest:**

The authors declare that they have no conflict of interest.

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**Figures**

![Figure 1](image)

**Figure 1**

Distribution of acne vulgaris, androgenic alopecia and acanthosis nigricans in women with PCOS
Figure 2

Scatter plots showing correlation of serum AMH levels with LH/FSH ratio (A), ovarian volume(B) and follicle number(C) in women with PCOS.
Figure 3

Receiver operating characteristic (ROC) curve of AMH for predicting PCOS among women