Research article

Diagnostic utility of serum anti-Müllerian hormone levels in south Indian women with polycystic ovarian syndrome

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ABSTRACT

Introduction and Aim: Polycystic ovarian syndrome (PCOS) is a prevalent endocrinopathy among women of reproductive age group, conventionally diagnosed using multiple clinical and biochemical tests. We aimed to evaluate the diagnostic utility of serum anti-Müllerian hormone (AMH) levels in PCOS.

Materials and Methods: The case control study involved 60 women of 18-40 years age, further grouped using Rotterdam criteria as cases with PCOS (n = 30) and controls without PCOS (n = 30). Study variables were compared between the groups using independent t-test. Correlation analyses were performed to predict the relationship of AMH with other PCOS determinants. Simple logistic regression and ROC analyses were used to determine the diagnostic utility of AMH.

Results: PCOS cases had increased levels of serum LH, testosterone, AMH, BMI, total antral follicular count (AFC), ovarian volume (OV) (p < 0.05*) than controls. Of all, serum AMH had the strongest association with PCOS (OR > 1, p < 0.05*) and showed considerable positive correlations with LH:FSH ratio, testosterone, AFC (r = 0.7768, p < 0.05*), OV (r = 0.7981, p < 0.05*). ROC analysis of AMH was significant (AUC = 0.966, p < 0.05*) with high sensitivity, specificity for cut points between 5.595 ng/mL and 5.90 ng/mL.

Conclusion: Elevated serum AMH levels were strongly associated with PCOS and correlated with its routine clinical determinants. Serum AMH estimation with cut-off between 5.595 ng/mL to 5.90 ng/mL, is proposed as a useful index for PCOS diagnosis.

Keywords: Anti-Müllerian hormone; Polycystic ovarian syndrome; Rotterdam criteria; Antral follicular count; Ovarian volume.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is the commonest endocrinopathy in women of reproductive age group, affecting around 5-15% women worldwide (1,2). It is regarded as a heterogeneous syndrome owing to its multifactorial and polygenic etiology, with pathogenic effects involving multiple reproductive, endocrinology and metabolic functions. Though initially considered as a disorder of primary ovarian dysfunction due to hormonal irregularities, recent evidence link insulin resistance to PCOS development (3).

PCOS is also a common cause of menstrual irregularity and female infertility (4). Other long-term consequences are obesity, insulin resistance, diabetes mellitus, dyslipidemia, cardiovascular disease, stroke, obstructive sleep apnea, virilization, mental depression, anxiety, cancers of endometrium, ovaries, breast. Early accurate diagnosis and management could reverse PCOS and prevent its complications.

Ovulatory dysfunction (OD), hyperandrogenism (HA), polycystic ovarian morphology (PCOM) forms the classical triad of signs seen in PCOS (5). The PCOS diagnostic criteria framed by different organizations were based on above clinical signs (6). As some of the cases were asymptomatic or presented with atypical clinical features, additional phenotypic identifications were recommended (7). Thus, the present PCOS diagnostic criteria requires multiple biochemical laboratory and radiology facilities, apart from skilled expert manpower in each field.

Anti-Müllerian hormone (AMH), previously known as Mullerian inhibiting substance (MIS) is a glycoprotein secreted by Sertoli cells of testes and granulosa cells of ovary. Its important functions are fetal sex differentiation, gonadal development, ovarian follicular growth, and development. As AMH levels declined with women’s age and corresponded with ovarian reserve and female fertility, researchers started exploring other diagnostic aspects of AMH (8,9). Few recent studies demonstrated higher AMH levels in PCOS cases (10,11).

We aimed at evaluating the diagnostic utility of serum AMH levels in our PCOS subjects, so that AMH could be used as a single biochemical marker for PCOS.
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Detection instead of the multitude of hormonal, physical and radiological tests used conventionally. Our results, if found significant, could greatly revolutionize PCOS diagnosis in the less resourcing rural healthcare settings.

MATERIALS AND METHODS

There were totally 60 participants in our prospective case control study. The study participants were apparently healthy women aged between 18 to 40 years, visiting the obstetrics and gynecology OPD of our tertiary care hospital. The study was approved by the Institutional Ethics Committee. Informed consent was initially obtained from all the study participants. The participants were divided into two groups as controls without PCOS (n = 30) and cases of PCOS (n = 30).

The control women had regular menstrual cycles of 25 to 35 days duration with 3 to 8 days of menstruation and had delivered at least one live born child. Whereas the cases included women newly diagnosed with PCOS based on Rotterdam criteria, which required presence of any two of the three following PCOS features such as oligo-anovulation (OA), hyperandrogenism (HA), polycystic ovarian morphology (PCOM) (6). Women who had undertaken recent medical, hormonal or surgical treatments for reproductive diseases were excluded from the study. Post-menopausal women, those with other endocrinopathies like thyroid disorders, Cushing’s disease, congenital adrenal hyperplasia, hyperprolactinemia, ovarian tumors, autoimmune diseases were also excluded from study.

Basic medical and menstrual history was collected from all participants. Their height and weight were recorded for BMI calculation from the formula BMI (Kg/m²) = (body weight in Kg)/(height in metre)² (12). About 5mL of venous blood was collected from both cases and controls on their third day of menstrual cycle and sent to the clinical laboratory for hormonal analyses, which included estimations of serum free T3, free T4, TSH, FSH, LH, total testosterone, estradiol, prolactin and AMH levels by enzyme immunoassay technique using standardized and quality control checked assay kits.

Physical examination was conducted to detect hirsutism using Ferriman-Gallwey scores over 8 (6). Then the participants were subjected to trans-vaginal ultrasonography (TVUS) using 6.5 MHz transducer by an experienced sonologist, to determine ovarian volume (OV), antral follicular size and count (AFC). In case of unmarried participants, trans-abdominal ultrasonography was performed instead of TVUS. OV was calculated from the formula OV = 0.5 x (length x width x thickness of ovary). An average of OVs of both ovaries were computed to obtain total OV of the subjects. PCOM was considered in presence of either an increased OV of above 10 cm³ or an increased AFC of ≥ 12 follicles having diameter of 2-9 mm visualized in one or more ovaries (6).

Statistical analysis

Study data were entered in Microsoft Excel and analyzed using statistical software SPSS 20.0. Continuous variables were expressed as mean and standard deviation (SD). Unpaired t-test was used to compare the variables between the cases and control groups. Associations of serum AMH levels, other study variables with the occurrence of PCOS in the study participants were detected by simple logistic regression analysis. Pearson and Spearman’s correlation analyses were employed to determine correlations of serum AMH levels with other study variables. Receiver operating characteristic (ROC) curve was plotted for serum AMH levels to determine its utility for PCOS diagnosis in the study population. Statistical significance was considered at the level of p-value < 0.05.

RESULTS

The study participants (n = 60) were women between 18 to 40 years of age, with a mean age of 28.63 ± 5.64 years. They were grouped as controls without PCOS (n = 30) and cases with PCOS (n = 30) for further study comparisons using independent t-test. The groups did not vary much with respect to participants’ age distribution (Table 1).

The cases showed remarkably elevated levels of BMI, serum LH and testosterone compared to the healthy controls (p < 0.05*). But they did not vary much with respect to their serum FSH and estradiol levels. The cases exhibited more than three-fold increase in measured ultrasonography PCOM findings of AFC and OV (p < 0.05*). Similar increments in serum AMH levels were noted in PCOS cases than controls (p < 0.05*) (Table 1).

Association of study parameters with PCOS in the participants were determined by simple logistic regression analysis. Serum FSH levels did not significantly influence PCOS occurrence in our subjects. But other study variables like serum LH, testosterone and AMH levels demonstrated stronger associations with PCOS (OR > 1, p < 0.05*). Of all associated variables, serum AMH levels had the greatest association with PCOS in our participants (OR > 1, p < 0.05*) (Table 2).
Table 1: Comparison of study parameters between cases and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n = 30)</th>
<th>Cases (n = 30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.77 ± 5.76</td>
<td>28.50 ± 5.63</td>
<td>0.857a</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>25.52 ± 5.51</td>
<td>28.46 ± 4.78</td>
<td>0.031**a</td>
</tr>
<tr>
<td>Serum FSH (mIU/mL)</td>
<td>5.54 ± 2.78</td>
<td>5.60 ± 1.99</td>
<td>0.925a</td>
</tr>
<tr>
<td>Serum LH (mIU/mL)</td>
<td>7.39 ± 4.10</td>
<td>10.60 ± 3.24</td>
<td>0.001**a</td>
</tr>
<tr>
<td>Serum Testosterone (ng/mL)</td>
<td>49.94 ± 13.21</td>
<td>73.02 ± 19.43</td>
<td>&lt; 0.001**a</td>
</tr>
<tr>
<td>Serum Estradiol (pg/mL)</td>
<td>73.70 ± 12.50</td>
<td>71.18 ± 10.88</td>
<td>0.409a</td>
</tr>
<tr>
<td>Total AFC</td>
<td>8.20 ± 1.62</td>
<td>30.20 ± 3.36</td>
<td>&lt; 0.001**a</td>
</tr>
<tr>
<td>OV (cm³)</td>
<td>5.14 ± 0.87</td>
<td>15.13 ± 2.67</td>
<td>&lt; 0.001**a</td>
</tr>
<tr>
<td>Serum AMH (ng/mL)</td>
<td>3.65 ± 1.46</td>
<td>11.28 ± 5.29</td>
<td>&lt; 0.001**a</td>
</tr>
</tbody>
</table>

a - Independent t test, * - Significant p value < 0.05

BMI - Body mass index, FSH - Follicle stimulating hormone, LH - Luteinizing hormone, AFC - Antral follicular count, OV - Ovarian volume, AMH - Anti müllerian hormone

Table 2: Association of study variables with PCOS in the study population (n = 60)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum FSH (mIU/mL)</td>
<td>1.010</td>
<td>1.080 - 1.475</td>
<td>0.923a</td>
</tr>
<tr>
<td>Serum LH (mIU/mL)</td>
<td>1.262</td>
<td>0.817 - 1.250</td>
<td>0.003**a</td>
</tr>
<tr>
<td>Serum Testosterone (ng/mL)</td>
<td>1.102</td>
<td>1.046 - 1.161</td>
<td>&lt; 0.001**a</td>
</tr>
<tr>
<td>Serum AMH (ng/mL)</td>
<td>485.11</td>
<td>241.32 - 756.40</td>
<td>&lt; 0.001**a</td>
</tr>
</tbody>
</table>

a - Simple logistic regression, * - Significant p value < 0.05, OR - Odds ratio, CI - Confidence interval

FSH - Follicle stimulating hormone, LH - Luteinizing hormone, AMH - Anti müllerian hormone

Table 3: Correlation of study variables with serum AMH levels in the study population (n = 60)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation coefficient (r)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-0.306a</td>
<td>0.0174*</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>0.2285a</td>
<td>0.0791</td>
</tr>
<tr>
<td>Serum LH:FSH</td>
<td>0.4084b</td>
<td>0.0012*</td>
</tr>
<tr>
<td>Serum testosterone (ng/mL)</td>
<td>0.3178 a</td>
<td>0.0133*</td>
</tr>
<tr>
<td>Total AFC</td>
<td>0.7768a</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>OV (cm³)</td>
<td>0.7981a</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

a - Pearson correlation, b - Spearman’s correlation, * - Significant p value < 0.05

BMI - Body mass index, FSH - Follicle stimulating hormone, LH - Luteinizing hormone, AFC - Antral follicular count, OV - Ovarian volume

Correlation analysis between serum AMH and BMI levels were insignificant, while participants’ age showed a negative correlation with AMH (r = -0.306, p < 0.05*). Serum testosterone levels (r = 0.3178, p < 0.05*) and LH:FSH ratio (r = 0.4084, p < 0.05*) depicted positive correlations with AMH. Utmost positive correlations were noticed between AMH and the PCOM signs of total AFC (r = 0.7768, p < 0.05*), OV (r = 0.7981, p < 0.05*) in our participants (Table 3).

The serum AMH levels had a significant ROC curve for identification of PCOS cases in our study population (AUC = 0.966, p < 0.05*) (Fig. 1). The serum AMH cut-off points for PCOS were determined form the data of ROC analysis. Serum AMH levels above 5.5 ng/mL showed greater levels of diagnostic accuracy in terms of sensitivity and specificity to detect PCOS in our participants (Table 4).
Moreover, Of and 1) to the PCOM testosterone mechanism, to LH:FSH una cardiovascula with affirm The South higher obese variables were comparable between cases and controls, as they were age and gender matched apart from their equal participant share. Almost 76.67% cases were obese when compared to the much fewer obesity rates of 43.33% in controls. Lim et al., deduced a pooled obesity prevalence of 49% in PCOS cases (13). The far higher obesity prevalence rates in our participants could be due to their categorization based on WHO South Asian BMI cut-off points (12).

The elevated BMI levels of PCOS cases than controls, affirm the linking of dyslipemia and central obesity with PCOS (Table 1). The underlying hyperinsulinemia could have led to the above findings (3). Such obese PCOS women also have increased risk of complications like insulin resistance, diabetes mellitus, cardiovascular disease, obstructive sleep apnea.

The significantly raised serum LH levels with unchanged serum FSH levels and a resultant hiked LH:FSH ratio in our PCOS cases, explain the underlying pathophysiology of a dysfunctional hypothalamic-pituitary-ovarian axis. These effects lead to abnormal expression of enzymes required for gonadal steroid hormone synthesis, resulting in hyperandrogenism (14). Substantiating the above mechanism, our cases showed higher serum testosterone levels and lower serum estradiol levels than the controls (Table 1).

The higher AFC and OV in our PCOS cases are the PCOM signs depicting ovarian follicular arrest, causing the anovulatory cycles and subfertility in them. Though the deranged LH:FSH and hyperinsulinism contribute to the follicular arrest, recent evidence connects AMH to the same, thus reasoning for the observed three-fold raise in serum AMH levels in our PCOS cases and its significant positive correlations with AFC, OV (Table 1) (10,11,15). Inverse relation between AMH levels and participants’ age could be attributable to the former’s decline with age (Table 3; 15).

Of all, serum AMH had the strongest associations with PCOS occurrence in our participants (Table 2). Moreover, correlation results of increasing AMH levels along with raising LH:FSH ratio and testosterone levels, show the definitive role of AMH in the PCOS pathology (11,15-17). Owing to these results, further diagnostic utility of serum AMH levels in PCOS were determined from an ROC analysis, which confirmed its potential usefulness from the significant AUC results (Fig.1).

AMH levels between 5.595 ng/mL to 5.90 ng/mL showed highest diagnostic sensitivity and specificity, making them ideal cut-off points to identify PCOS (Table 4). Similarly, the study by Mahajan N et al., conducted in the northern part of India, claimed that serum AMH above 5.03 ng/mL could be used for PCOS diagnosis (18). Varying from the above results, Ahmed N et al., suggested a lowered AMH cutoff point of >3.19 ng/mL to be useful for PCOS diagnosis in Saudi women (19).

These geographical and ethnic variations in serum AMH cut-off levels pose restrictions to its application in the PCOS diagnosis globally, thus calling for future multi-centric studies involving multi-ethnic populations to formulate a consensus in the universal AMH cut points for PCOS diagnosis. Lack of affordable and standardized assay procedure is another limiting factor for AMH utility in PCOS diagnosis in less resourceful healthcare settings of developing nations.

Still AMH could potentially replace the current PCOS diagnostic criteria, as the latter involve multiple test requirements and some of them such as the PCOM are more prone for subjective variabilities (20,21). Unlike other PCOS diagnostic tests, AMH remains unaffected by the menstrual cyclical changes, making it quantifiable in PCOS women with irregular cycles as well (22). Other advantages of AMH estimation are that it can be used in cases with asymptomatic phenotypes and in obese patients with difficult ultrasonography too (23,24). Thus, AMH could be used with ease for PCOS diagnosis if approved and standardized for the intended purpose.

CONCLUSION

Serum AMH levels were strongly associated with PCOS and remained highly elevated in PCOS cases. AMH had significant positive correlation with conventional determinants of the disease like serum LH:FSH ratio, serum testosterone, AFC and OV.

Table 4: Cut off points of serum AMH levels to detect PCOS in the study population (n = 60)

<table>
<thead>
<tr>
<th>AMH cut-off levels (ng/mL)</th>
<th>Sensitivity % ¹</th>
<th>Specificity % ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.545</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>5.595</td>
<td>100</td>
<td>96.7</td>
</tr>
<tr>
<td>5.70</td>
<td>96.7</td>
<td>96.7</td>
</tr>
<tr>
<td>5.90</td>
<td>96.7</td>
<td>100</td>
</tr>
<tr>
<td>6.40</td>
<td>93.3</td>
<td>100</td>
</tr>
<tr>
<td>7.00</td>
<td>83.3</td>
<td>100</td>
</tr>
</tbody>
</table>

¹ - Receiver operating characteristic (ROC) curve
AMH - Anti Müllerian hormone

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Serum AMH levels ranging between cut-off limits of 5.595 ng/mL to 5.90 ng/mL is proposed as a useful index for PCOS diagnosis.

CONFLICT OF INTEREST
The authors declare no conflicts of interest.

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