ORIGINAL RESEARCH

Diagnostic utility of anti mullerian hormone (AMH) for polycystic ovarian syndrome (PCOS) in Indian women

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ABSTRACT
Objectives: Polycystic ovarian syndrome (PCOS) is most frequent among women of reproductive age and causes anovulatory infertility. Serum Anti Mullerian hormone (AMH) level is known to be elevated in women with polycystic ovarian syndrome (PCOS), it to be used as a diagnostic marker for PCOS**. Aim:** This study has been done to determine whether the measurement of serum AMH can be used to diagnose PCOS and as a tool to predict the prognosis of PCOS. **Methods:** This was a prospective case control study, comprised of 80 women diagnosed with PCOS using Rotterdam criteria and 80 non PCOS controls. Clinical data were collected including history, oligomenorrhea, hirsutism, examination included BMI and blood investigations including serum FSH, LH, TSH, prolactin, estradiol and serum AMH level. **Results:** There is no significant difference was seen in age, BMI and socio-economic status among PCOS cases and non PCOS group ($p>0.05$). Mean level of AMH in PCOS cases and controls was 8.73 and 5.67 respectively, AMH was significantly higher in women with PCOS than non PCOS women ($p<0.05$). Serum LH and LH/FSH ratio was significantly higher in the PCOS group than in the control group ($p < 0.001$), whereas serum FSH, estradiol and prolactin level not significantly differ in PCOS and non PCOS group ($p>0.05$). **Conclusion:** AMH level can be used as diagnostic and prognostic modalities in PCOS. AMH as an independent marker could not effectively diagnose PCOS

Keywords: AMH, PCOS, LH, LH/FSH, Rotterdam criteria

INTRODUCTION

Polycystic ovary syndrome is the most common endocrine disorder in women of reproductive age, with an estimated prevalence of 8–13% [1]. It is a heterogeneous disorder with multiple different phenotypes and presents with wide spectrum of clinical features and delayed sequale like type 2 diabetes mellitus, cardiovascular diseases, metabolic syndrome and endometrial cancer which are preventable [2]. It is caused by imbalance of sex hormones which ultimately leads to menstrual irregularities, infertility, anovulation and other metabolic disturbances [3]. Additionally, patients with PCOS have been reported to express multiple metabolic manifestations including insulin resistance, obesity and dyslipidemia that still manifest even post-menopause [4]. The disorder can be morphological (polycystic ovaries) or predominantly biochemical (hyperandrogenaemia). Hyperandrogenism, a clinical hallmark of PCOS, can cause inhibition of follicular development, microcysts in the ovaries, anovulation, and menstrual changes [5]. Anti-Mullerian Hormone (AMH) is a glycoprotein belonging to the transforming growth factor (TGF-β) family which is produced by the granulosa cells exclusively in women. In addition to its important embryonic role in sex determination via suppression of Mullerian system and hence paving the way for male internal genitalia AMH has also been considered responsible for the ovarian dysfunction in PCOS, and also seem to play an important role in folliculogenesis [6-8]. Presently, the Rotterdam 2003 criteria have been the benchmark for PCOS diagnosis. According to the Rotterdam criteria, PCOS is normally diagnosed by

the appearance of at a minimum two of the following three criteria: 1-Oligo and Anovulation, 2-Hyperandrogenism 3-Polycystic Ovaries.

Women with PCOS are noted to have higher levels of Anti-Mullerian hormone. Serum AMH levels are significantly higher in normogonadotropic anovulatory women, especially those with polycystic ovarian morphology compared to age-matched normoovulatory premenopausal women [10]. The controversy regarding the diagnosis of PCOS still continues due to the complexity of presentation. Feature which should be considered essential for its diagnosis is still a dilemma. Due to the limitations associated with existing Rotterdam criteria, new tool AMH can be used as a potential objective, quantitative and biological diagnostic marker for PCOS [11-12].

This study will also help to understand the pattern and utilization of serum AMH levels as a diagnostic factor along with other Rotterdam criteria among PCOS women.

AIMS & OBJECTIVES

The objectives of this study were to evaluate the diagnostic utility of serum AMH levels for the PCOS detection in our study population and compare the level of AMH and other hormones between women with, and without PCOS

MATERIALS & METHODS:

This was a prospective case–control study, conducted in the collaboration of Department of Obstetrics and Gynaecology with department of biochemistry in central India. A total of 160 subjects (80 cases and 80 controls), those attended outpatients department of our hospital during the study period were enrolled this study. Cases were women who met the diagnostic criteria for PCOS. The controls were healthy women having at least one healthy child. Case and control women were matched for age.

INCLUSION CRITERIA

- Women aged 18–45 years
- Patients diagnosed as PCOS (By Rotterdam Criteria)
- Participants were not taking any medication known to affect sex hormones or metabolism three months before participating in the study
- Should not be subjected to any surgical procedure in the reproductive system
- Participants who provided written informed consent for the study

EXCLUSION CRITERIA

- Women aged <18 or >45 years
- Women with moderate to severe endometriosis, a previous history of surgery, and preexisting diabetes mellitus
- Subjects who received hormonal therapy within three months of the study
- Participants who not provided written informed consent for the study

Diagnosis of PCOS was established based on Rotterdam 2003 consensus, which is the finding of 2 out of the 3 following criteria:

- Oligo and/or anovulation;
- Hyperandrogenism, defined as hirsutism or minor signs such as acne, seboborrhea; and
- Criteria for polycystic ovary by ultrasound examination (minimum of 12 follicles with 2-9 mm diameters in each ovary and/or increasing ovarian volume with a minimum size of 10 mm3).

The control group consisted of women without endometriosis, cysts, or other ovarian gynecological disorders; had regular menstrual cycles (26-35 days); did not have endocrine abnormalities and had morphologically normal ovaries according to ultrasound.

Baseline information on age, marriage duration, fertility status, menstrual cycle length, and previous history of treatment, socio-demographic data and body mass index (BMI) were measured. A detailed general examination was done for identification of PCOS.

The biochemical evaluation included serum follicle-stimulating hormone (FSH, mlU/ml), luteinizing hormone (LH, IU/L), prolactin (ng/ml), anti-Müllerian hormone (AMH, ng/ml), and thyroid-stimulating hormone (TSH, IU/L) during the follicular phase (1–5 days) of the menstrual cycle. Serum LH, FSH, and prolactin were measured using automated chemiluminescent immunoassays. Serum AMH was measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions

STATISTICAL ANALYSIS

Statistical analysis was done using statistical package for social sciences (SPSS) version 21.0.The values are expressed in Mean±SD. Statistical significance of differences between PCOS and non PCOS were carried out by unpaired t-test or non-parametric test. The level of significance was considered as $p<0.05$.

RESULTS

In this study 80 patients of age group 18 to 45 years with PCOS (based on Rotterdam criteria) and 80 subjects of same age group as controls (non PCOS) were enrolled and analysed.

Majority of the patients were 26-35 years age group, mean age was 29.23 years. Most of the PCOS patients (35%) were overweight whereas 38.7% non PCOS participants was normal weight. Majority of them belong to middle socio-economic class. There is no significant difference between PCOS and non PCOS group in respect to age, BMI and socio-economic class ($p>0.05$).

Table 1: Socio-demographic characteristics of PCOS group and non-PCOS group

Socio-demographic variable		PCOS group	Non-PCOS group	P value
Age (years)	18-25	19 (23.8%)	17 (21.2%)	0.860
	26-35	37 (46.2%)	36 (45%)	
	36-45	24 (30%)	27 (33.8%)	
Mean age ± SD: 29.23 ± 5.54 years				
BMI (kg/m^2)	Underweight (< 18.5)	6 (7.5%)	10 (12.5%)	0.326
	Normal (18.5–24.9)	26 (32.5%)	31 (38.7%)	
	Overweight (25-29.9)	28 (35%)	27 (33.8%)	
	Obese (>30)	20 (25%)	12 (15%)	
Mean BMI (kg/m2)		29.59	28.37	
Socio-economic class	Lower	16 (20%)	23 (28.8%)	0.383
	Middle	41 (51.2%)	39 (48.7%)	
	Upper	23 (28.8%)	18 (22.5%)	

The AMH levels in the various age groups in PCOS patients and controls are listed in Table 2. AMH level was significantly higher among PCOS group than that in the non PCOS group ($p<0.05$)

Table 2: AMH Levels according to the age among PCOS and non PCOS group

Age (years)	Frequency (N)		AMH (ng/ml) Mean±SD		P value
	PCOS	Non PCOS	PCOS	Non PCOS	
18-25	19	17	9.06±4.64	5.94±3.72	<0.01
26-35	37	36	8.87±4.49	6.21±3.21	<0.01
36-45	24	27	8.23±4.28	5.13±3.14	<0.01

The mean AMH level was higher in PCOS patients (cases) 8.73 ± 4.43 ng/mL compared to 5.67 ± 3.12 ng/mL in controls, this difference was found to be statistically significant ($p < 0.001$).

The mean LH, TSH and LH/FSH ratio were significantly higher among PCOS group as compared to non PCOS group, whereas mean levels of FSH, Estradiol, and prolactin were not significant differ in patients with PCOS compared to controls.

Table 3: Comparison of AMH and hormonal levels in PCOS and non-PCOS group

Hormone	PCOS	Non-PCOS	P value
AMH (ng/mL)	8.73 ± 4.43	5.67 ± 3.12	< 0.001
LH (mIU/mL)	10.35 ± 3.65	7.41 ± 4.09	< 0.001
FSH (mIU/mL)	5.72 ± 2.28	5.34 ± 1.78	0.241
LH/FSH	1.53 ± 0.86	0.88 ± 0.12	< 0.001
Estradiol (pmol/L)	73.77 ± 11.43	71.23 ± 10.53	0.145
Prolactin (ng/mL)	14.02 ± 3.43	15.34 ± 4.21	0.031
TSH (mIU/L)	2.65 ± 0.98	2.31 ± 0.67	0.011

DISCUSSION

PCOS is one of the leading causes of female infertility and the most common endocrine disorder among women of reproductive age. Despite many decades of extensive research, the exact etiology and pathogenesis of this complex disorder remain hidden, thus diagnosis of PCOS requires an objective and quantitative criteria to help clinicians to diagnose and treat patients suffering from this complex endocrine disorder [13].

In our study, there was no statistical difference between mean age of PCOS cases and non PCOS controls, similar findings were observed in previous studies: Sahmay S et al [14] and Iliodromiti S et al [15], However, Koninger A, et al [16] reported different age profiles of PCOS cases and controls in their study.

In the present study, majority of the PCOS patients were overweight whereas majority of the healthy controls was normal weight, but no statistically BMI difference was observed in PCOS group and non PCOS group, this was in agreement with the Saxena et al [17] and Wiweko B, et al [18]. Thus, a healthy and controlled lifestyle with a maintained BMI of 18.5 to 24 kg/m2 can improve the ovulatory cycles of PCOS women or at the very least decrease the severity of the disease.

In our study, AMH levels increased at first in all age groups, reaching the highest level in the 18–25-year-old and then decreasing among old women, and the same changes were seen in the control group. AMH were significantly higher among all age group of PCOS cases as compared to non PCOS control, accordance to Sivanandy, et al [19] and Yu Ran et al [20].

Current study found that PCOS women had an increased menstrual cycle length with the elevation in serum AMH levels and had a greater risk of menstrual disturbance. The increase in menstrual cycle length

with AMH elevation was concurrent with previous studies [21].

The present study investigated the serum level of AMH as a diagnostic marker for PCOS and showed that the serum level of AMH in women with PCOS were significantly higher than the levels in women without PCOS ($p<0.05$), this finding has consistently been reported in numerous studies like: Pandey U, et al [22], Villarroel C, et al [23], Mohammad H, et al [24] and Bhattacharya et al [25]. Factors which have been recently described to influence absolute AMH concentrations include weight, Vitamin D status, smoking, polymorphisms of AMH and its receptor, and genetic variations.

In our study, LH levels were significantly higher in the PCOS group than in the healthy group ($p<0.001$). The LH/FSH ratio was almost double in the PCOS group compared to controls group, our results are comparable with the Naveetha et al [26] and Kumar A et al [27], this was 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.

Present study observed no significant change in serum FSH, Estradiol and prolactin level between PCOS and control group, our findings correlate with the Sengupta S et al [28] and Saadia Z, et al [29]. Follicular development is hampered by low FSH levels, whereas ovarian androgen production is enhanced by high LH levels.

CONCLUSION

Our study helped establish biological reference intervals for AMH, specific for different age groups in Indian women. Serum AMH levels were strongly associated with PCOS and remained highly elevated in PCOS cases. An accurate and appropriate PCOS diagnosis is essential, as it has long-term implications on women's health. An elevated serum AMH level can be used as a strong predictor to reflect the certainty of PCOS diagnosis among women of reproductive age

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