



## Original Research Article

## Age specific references for anti-mullerian hormone and use as a potential diagnostic marker of PCOS in an Indian population

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## ABSTRACT

**Introduction and Aim:** Polycystic ovary syndrome (PCOS) is the most common endocrine and metabolic disorder of reproductive age characterized by anovulatory infertility and diagnosed by Rotterdam criteria. The International evidence based guidelines for PCOS, confirms that raised levels of anti-Müllerian hormone (AMH) in patients with PCOS but lack of cutoff values in different age groups is limited. Hence the present study was aimed to determine a cutoff value of AMH in different age groups that could facilitate potential diagnostic marker of PCOS Chennai, India.

**Materials and Methods:** A total of 800 women aged 20 to 40 years, including 139 (17.3%) PCOS patients and 160 eumenorrheic non-hirsute control women were prospectively enrolled in this study between May 2020 and July 2021. The Rotterdam criteria were used for diagnosis of PCOS. The cut-off levels of AMH in different age categories were estimated, using the Bayesian method. Mann-Whitney and Chi-square statistical analysis were done for the variables.

**Result:** Out of 139 PCOS patients, 38/139 (27.3%) aged 20-25 years, 35/139 (25.1%) aged 26-30 years, 32/139 (23%) aged 31-35 years and 34 (24.4%) aged 36-40 years. Mean AMH concentrations in women with PCOS was higher  $5.24 \pm 3.61$  ng/mL in comparison to controls  $2.51 \pm 1.74$  ng/mL. The cutoff levels of PCOS in the age categories of 20-25, 26-30, 31-35 and 36-40 years were 5.6 (95% CI: 5.20–6.18), 5.3 (95% CI: 5.14–4.28), 4.03 (95% CI: 3.55–4.42) and 3.52 (95% CI: 3.72–3.27), respectively.

**Conclusion:** Serum AMH concentration  $>3.52$  ng/mL could be used as cutoff value as a potential diagnostic marker of PCOS in all age group. Further, studies needed with more PCOS patients to identify the reference intervals of serum AMH according to age groups and can be used an additional marker for PCOS diagnosis.

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## 1. Introduction

Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disease that is prevalent in 6%–13% of women of childbearing age.<sup>1</sup> PCOS is a clinically manifested as menstrual thinning, hemorrhoids, hairiness, obesity and infertility. It is also characterized by abnormal levels of reproductive hormones, which can lead to anovulatory, infertile and menstrual disorders.<sup>2</sup> Because of the large individual differences between

women with PCOS, there are no exact effects of clinical treatment and diagnosis.<sup>3</sup> After several years, there is international consensus that the diagnosis of PCOS should follow the Rotterdam criteria, which requires at least two of the following three criteria after the exclusion of other differential diagnoses: oligo-anovulation; hyperandrogenism; and polycystic ovarian morphology (PCOM).<sup>4</sup>

In females, Anti-Müllerian hormone (AMH) has been proposed as a marker of ovarian aging and reserve. AMH plays a role in regulating ovarian activity and it is secreted by the granulosa cells of the pre-antral and antral follicles

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up to 6 mm in diameter.<sup>5</sup> Its highest concentrations are in small antral follicles and are very low or undetectable in follicles >10 mm. Raised levels of AMH in PCOS may be because of an increased pre-antral follicle count and/or increased follicular secretion.<sup>6</sup> The international evidence-based guidelines for PCOS 2018 acknowledge the fact that raised levels of AMH have been reported in patients with PCOS but the lack of standardization of AMH assays as well as variation in age groups and ethnicity have prevented any agreement about a threshold value to define PCOS.<sup>7</sup>

There is accumulating data suggesting that AMH could present as a biochemical marker for PCOS. The aim of the present study was to explore the age-related diagnostic value of AMH when detecting PCOS in Indian women. The hypothesis of the present study is that AMH can be used as a potential indicator of PCOS and that it can be used together with other indicators to improve detection.

## 2. Materials and Methods

### 2.1. Patient recruitment and sample collection

This is a retrospective study that was conducted on women who consulted Institute of Reproductive Medicine, Madras Medical Mission Hospital, Chennai, India, between May 2020 – July 2021. Human ethical clearance was obtained and the ethics committee waived the requirement for informed consent. A total of 800 women who consulted, we selected 150 (17.3%) women diagnosed with PCOS from among the outpatients for our retrospective analysis. In addition, 160 other participants were selected as healthy controls after exclusion of other endocrine and gynecological diseases. PCOS was diagnosed in the 139 aged 20-40 years based on the Rotterdam criteria: oligo and/or anovulation (OA); clinical and/or biochemical signs of hyperandrogenism (HA); and presence of polycystic ovaries (PCOs), defined as the presence of  $\geq 12$  follicles measuring 2–9 mm in diameter in each ovary and/or increased ovarian volume ( $>10$  ml).<sup>8</sup> OA was defined as a cycle length of more than 35 days or amenorrhea. Biochemical HA was defined as circulating total testosterone levels above the 95th percentile (0.51 ng/mL). Clinical HA was defined as the presence of hirsutism, acne, and/or alopecia. Hirsutism was defined as the presence of hirsutism, as evidenced by an m-FG score  $\geq 8$ .<sup>9</sup> The exclusion criteria were history of endocrine disorders or use of medications that could affect the function of the hypothalamic-pituitary-gonadal. Age range of  $< 20$  or  $>40$  years, Ovarian / Adrenal tumors, History of hysterectomy, oophorectomy or ovarian surgeries. None of the participants were taking any form of steroid drugs before their samples were collected. Peripheral blood samples (5 ml) were obtained on days 2-5 of the menstrual cycle. The sample was collected in a serum separator tube and allowed to clot for 30 min before centrifugation at  $1000 \times g$  for 15

min. All peripheral blood samples were processed within 2 h of collection.

### 2.2. Examination and evaluations

The examination and hormonal and ultrasound evaluations were performed in the early follicular phase (between days 2 and 5) of a spontaneous cycle or after a progesterone challenge test. During the clinical work-up, the menstrual history of all patients was recorded in detail. Oligo-anovulation was defined as an average cycle length of over 35 days and included women with frank amenorrhea ( $>90$  days). Clinical hyperandrogenism was defined by the presence of hirsutism and/or acne located in more than two body areas.

All ultrasound examinations were performed with according to a standardized protocol. After determining the longest medial axis of the ovary, the highest possible magnification was used to examine the ovaries. For each ovary, volume measurement ( $H \times L \times W \times 0.52$ ) and the total number of antral follicles of the entire ovary, from one margin to the other in longitudinal cross-sections.<sup>9</sup> Serum levels of TSH, free T4, prolactin, estradiol, follicle-stimulating hormone, luteinizing hormone (LH), and testosterone were measured with ELISA and fasting glucose levels were measured. Levels of serum AMH were measured by ELISA in all patients.

### 2.3. Statistical analysis

For data with normal distribution and homogeneity of variance, an independent-sample t test was used to compare differences between two groups, and one-way ANOVA was performed if there were three or more means. For non-normally distributed data, differences between groups were evaluated with the non-parametric Mann-Whitney U-test. All statistical analyses were performed by means of Graph Pad Prism version 5.0.

## 3. Results

The data of 800 infertile women were evaluated, of which 299 women were included in the study based on the inclusion and exclusion criteria. Out of these women, 139 (17.3%) were diagnosed with PCOS according to the Rotterdam criteria and 661 were non-PCOS of which 160 were controls. Table 1 shows the characteristics of the PCOS and non-PCOS groups. The women's age, body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters), and duration of married life were similar in the PCOS and non-PCOS groups. Menstrual irregularities, ovarian volume, and FNPO were significantly higher in the PCOS group than the non-PCOS group ( $P < 0.001$ , respectively). The mean serum levels of LH were significantly higher in the PCOS group than in the non-PCOS group ( $P < 0.001$ ). The mean level of serum AMH

**Table 1:** Main clinical and hormone features in women with PCOS and non PCOS<sup>a</sup>

S.No	Characteristics	PCOS (n = 139)	Non PCOS (521)	P value
1.	Age (Years)	26.3±3.42	28.36±4.58	0.052
2.	BMI (kg/m <sup>2</sup> )	26.2±4.35	23.64±4.21	0.546
3.	Time married (years)	5.7±3.1	6.0±3.9	0.052
4.	Menstrual irregularities	69 (66.2)	88 (17.1)	<0.001
5.	AFC > 12	102 (65)	47 (8)	<0.001
6.	FSH (IU/L)	6.12±1.52	7.06±2.05	0.005
7.	AMH (ng/mL)	7.25±5.37	2.54±1.78	<0.001

**Table 2:** PCOS in women at the age group of 20-25 years

S. No	Parameter	AMH ≥ 5.6 ng/ML (n = 38)	Control ≤ 2.51± 1.74 ng/ML (n = 40)	P value
1.	Age (Years)	25.52±2.1	24.3±2.1	0.406
2.	BMI (kg/m <sup>2</sup> )	24.03±3.6	23.3±3.8	0.167
3.	Time married (years)	5.4±3.2	4.9±2.1	0.081
4.	Menstrual irregularities	28(56.2)	08(13.8)	<0.001
5.	FNPO (AFC)> 12	25(84.6)	04 (23.2)	<0.001
6.	HA	31(74.3)	05(10.8)	<0.001

**Table 3:** PCOS in women at the age group of 26-30 years

S. No	Parameter	AMH ≥ 5.3 ng/ML (n = 35)	Control ≤ 2.51± 1.74 ng/ML (n = 40)	P value
1.	Age (Years)	21.52±1.11	20.3±1.09	0.303
2.	BMI (kg/m <sup>2</sup> )	23.09±3.1	22.3±3.2	0.152
3.	Time married (years)	4.8±2.8	3.9±1.7	0.072
4.	Menstrual irregularities	19(54.2)	08(12.8)	<0.001
5.	FNPO (AFC)> 12	28(83.6)	04(21.2)	<0.001
6.	HA	22(71.3)	07(9.8)	<0.001

**Table 4:** PCOS in women at the age group of 31-35 years

S. No	Parameter	AMH ≥ 4.03 ng/ML (n = 32)	Control ≤ 2.51± 1.74 ng/ML (n = 40)	P value
1.	Age (Years)	29.2±2.4	27.7±2.9	0.061
2.	BMI (kg/m <sup>2</sup> )	24.1±4.8	24.1±4.1	0.102
3.	Time married (years)	6.8±3.1	6.8±3.9	0.213
4.	Menstrual irregularities	18(51.6)	09(20.8)	<0.001
5.	FNPO (AFC)> 12	20(63.7)	04(8.7)	<0.001
6.	HA	24(40.1)	06(5.6)	<0.001

**Table 5:** PCOS in women at the age group of 36-40 years

S. No	Parameter	AMH ≥ 3.52 ng/ML (n = 34)	Control ≤ 2.51± 1.74 ng/ML (n = 40)	P value
1.	Age (Years)	31.2±3.4	29.7±2.7	0.086
2.	BMI (kg/m <sup>2</sup> )	28.1±5.8	25.3±5.3	0.100
3.	Time married (years)	8.2±4.9	8.1±4.3	0.312
4.	Menstrual irregularities	17(22.3)	06(25.6)	<0.001
5.	FNPO (AFC)> 12	14(27.4)	04(19.7)	<0.001
6.	HA	20(42.4)	07(6.8)	<0.001

**Table 6:** Overall values for AMH

Age groups	PCOS	AMH (ng/mL)	Sensitivity	Specificity
Total (no=800)	139(17.3)	≥3.52	78	64
20-25	38(27.2)	≥5.6	62	75
26-30	35(25.4)	≥5.3	67	74
31-35	32(23)	≥4.03	80	70
36-40	34(24)	≥3.52	85	69

was also significantly higher in the PCOS group than in the non-PCOS group (7.25 ng/mL Vs 3.19 g/mL;  $P < 0.001$ ). Out of 139 PCOS patients, 38/139 (27.3%) aged 20-25 years, 35/139 (25.1%) aged 26-30 years, 32/139 (23%) aged 31-35 years and 34 (24.4%) aged 36-40 years. Mean AMH concentrations in women with PCOS was higher  $5.24 \pm 3.61$  ng/mL in comparison to controls  $2.51 \pm 1.74$  ng/mL. The cutoff levels of PCOS in the age categories of 20-25, 26-30, 31-35 and 36-40 years were 5.6 (95% CI: 5.20–6.18), 5.3 (95% CI: 5.14–4.28), 4.03 (95% CI: 3.55–4.42) and 3.52 (95% CI: 3.72–3.27), respectively. Table 2 shows women with levels of AMH over 5.46 ng/mL had a significantly higher prevalence of menstrual irregularities and antral follicle count (AFC) compared to women with a level of AMH at 5.46 ng/mL less in the 20–25 years age group. Table 3 shows women with levels of AMH over 5.3 ng/mL had a significantly higher prevalence of menstrual irregularities and antral follicle count (AFC) compared to women with a level of AMH at 5.3 ng/mL less in the 26-30 years age group. Table 4 shows women with levels of AMH over 4.03 ng/mL had a significantly higher prevalence of menstrual irregularities and antral follicle count (AFC) compared to women with a level of AMH at 4.03 ng/mL less in the 31-35 years age group. Table 5 shows women with levels of AMH over 3.52 ng/mL had a significantly higher prevalence of menstrual irregularities, antral follicle count (AFC), and PCOS compared to women with a level of AMH at 3.52 ng/mL less in the 36-40 years age group. Table 6 shows overall age specific AMH cut off values for PCOS patients with specificity & sensitivity.

#### 4. Discussion

The majority of infertile women in the present study had menstrual irregularities, hirsutism, and psychological and sexual issues, and 139 women had PCOS. There was no statistically significant difference between mean age and BMI in the cases of PCOS and controls in the present study, and the same has been observed in previous studies.<sup>10,11</sup> The present study evaluated levels of serum AMH as a potential diagnostic marker of PCOS in different age groups and showed significantly higher levels of AMH in the patients with PCOS than in the controls.

Wiweko et al. reported that AMH levels are higher in PCOS when hyperandrogenism is present,<sup>12</sup> which is different from our findings. Our finding showed that

compared to healthy controls, all the women with PCOS, even those who did not have PCOM or HA, had significantly higher AMH levels. This is probably because the AMH level in patients with PCOS is not only related to increase in the follicle pool but also increase in the production per follicle.<sup>13</sup>

There are only a few published studies in the literature targeting age-related AMH cut-off for the diagnosis of PCOS. Levels of serum AMH decrease with advancing women's age with and without PCOS. In the present study, a significant decline in the level of serum AMH was seen after the age of 30 to 40 years so the women were divided into four groups. Yue et al.<sup>14</sup> determined the cut-off levels of AMH at 8.16 ng/mL and 5.89 ng/mL for the age groups 20-29 years and 30-39 years, respectively. The mean level of serum AMH in that study was higher in both patients with PCOS and in healthy controls ( $6.2 \pm 2.5$  ng/mL). This can be due to ethnic variations and different AMH assays. Whereas us in our study documented cutoff AMH value for different age groups. The use of levels of serum AMH in the diagnosis of PCOS are recommended, along with the Rotterdam criteria, to increase diagnostic accuracy, to undermine misdiagnosis, in conditions where it is not feasible to do ultrasonography, where there is a lack of standard diagnostic criteria for hyperandrogenism, and whenever the diagnosis is difficult, especially in obese and overweight women in whom menstrual irregularities and PCOM are common findings.

#### 5. Conclusion

Our results show that AMH may have potential as a marker of PCOS. The cutoff levels of PCOS in the age categories of 20-25, 26-30, 31-35 and 36-40 years were 5.6 (95% CI: 5.20–6.18), 5.3 (95% CI: 5.14–4.28), 4.03 (95% CI: 3.55–4.42) and 3.52 (95% CI: 3.72–3.27), respectively. Serum AMH concentration  $>3.52$  ng/mL could be used as cutoff value as a potential diagnostic marker of PCOS in all age group. Further studies needed with more PCOS patients to identify the reference intervals of serum AMH according to age groups and can be used an additional marker for PCOS diagnosis.

#### 6. Abbreviation

BMI, Body mass index; FSH, Follicle-stimulating hormone; AMH, Anti-mullerian hormone; AFC, antral follicle count.

Values are given as number (percentage) or mean  $\pm$  SD.

## 7. Source of Funding

None.

## 8. Conflict of Interest

The authors declare that there are no conflicts of interest.

## 9. Ethical Approval and Consent for Publication

Ethical approval was obtained from the Institute.

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## References

1. Speroff L. Clinical gynecologic endocrinology and infertility. vol. 12. United States: Lippincott Williams; 2005. p. 485–513.
2. Bako AU, Morad S, Atiomo WA. Polycystic ovary syndrome: An overview. *Rev Gynecol Pract.* 2005;5:115–22.
3. Ran Y, Yi Q, Li C. The Relationship of Anti-Mullerian Hormone in Polycystic Ovary Syndrome Patients with Different Subgroups. *Diabetes Metab Syndr Obes.* 2021;14:1419–24.
4. Tehrani FR, Rahmati M, Mahboobifard F, Firouzi F, Hashemi N, Azizi F. Age-specific cut-off levels of anti-Müllerian hormone can be used as diagnostic markers for polycystic ovary syndrome. *Reprod Biol Endocrinol.* 2021;19(76). doi:10.1186/s12958-021-00755-8.
5. Visser JA, DeJong FH, Laven JSE, Themmen APN. Anti-Müllerian hormone: a new marker for ovarian function. *Reproduction.* 2006;131(1):1–9.
6. Pigny P, Merlen E, Robert Y, Cortet-Rudelli C, Decanter C, Jonard S, et al. Elevated serum level of anti-mullerian hormone in patients with polycystic ovary syndrome: relationship to the ovarian follicle excess and to the follicular arrest. *J Clin Endocrinol Metab.* 2003;88(12):5957–62.
7. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Hum Reprod.* 2018;33(9):1602–18.
8. Dewailly D, Catteau-Jonard S, Reyss AC, Leroy M, Pigny P. Oligoanovulation with polycystic ovaries but not overt hyperandrogenism. *J Clin Endocrinol Metab.* 2006;91(10):3922–7.
9. Pellatt L, Hanna L, Brincat M, Galea R, Brain H, Whitehead S, et al. Granulosa cell production of anti-Müllerian hormone is increased in polycystic ovaries. *J Clin Endocrinol Metab.* 2007;92(1):240–5.
10. Sahmay S, Atakul N, Aydogan B, Aydin Y, Imamoglu M, Seyisoglu H. Elevated serum levels of anti-Müllerian hormone can be introduced as a new diagnostic marker for polycystic ovary syndrome. *Acta Obstet Gynecol Scand.* 2013;92(12):1369–74.
11. Woo HY, Kim KH, Rhee EJ, Park H, Lee MK. Differences of the association of anti-Müllerian hormone with clinical or biochemical characteristics between women with and without polycystic ovary syndrome. *Endocr J.* 2012;59(9):781–90.
12. Wiweko B, Maidarti M, Priangga MD, Shafira N, Fernando D, Sumapraja K, et al. Anti-mullerian hormone as a diagnostic and prognostic tool for PCOS patients. *J Assist Reprod Genet.* 2014;31(10):1311–6.
13. Perkins NJ, Schisterman EF. The Inconsistency of “Optimal” Cut-points Using Two ROC Based Criteria. *Am J Epidemiol.* 2006;163(7):670–5.
14. Yue CY, Lu LKY, Li M, Zhang QL, Ying CM. Threshold value of anti-Mullerian hormone for the diagnosis of polycystic ovary syndrome in Chinese women. *PLoS One.* 2018;13(8):e0203129. doi:10.1371/journal.pone.0203129.

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