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Study the role of anti-mullerian hormone in women in the reproductive age group (18 to 45 years) with polycystic ovarian syndrome in a tertiary care centre

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Abstract
Background: Polycystic Ovarian Syndrome (PCOS) is a prevalent endocrine disorder among women of reproductive age, characterized by menstrual irregularities, hyperandrogenism, and Polycystic Ovarian Morphology (PCOM). While the Rotterdam criteria remain the diagnostic standard, limitations in clinical and ultrasonographic parameters-especially among adolescents and obese women-necessitate the evaluation of more objective biomarkers. Anti-Müllerian Hormone (AMH) has emerged as a promising marker due to its correlation with follicular reserve and its independence from menstrual cycle variation.
Objective: To evaluate the diagnostic utility of serum AMH levels in women with PCOS and explore its correlation with clinical, biochemical, and ultrasonographic parameters as per the Rotterdam criteria.
Methods: A cross-sectional study was conducted on 155 women aged 18-45 years diagnosed with PCOS at MGM Medical College Hospital, Jamshedpur. Clinical features, hormonal profiles, and ultrasound findings were recorded. Serum AMH was measured using ELISA. Associations were analyzed using appropriate statistical tests, and the diagnostic performance of AMH was assessed.
Results: The mean AMH level was 9.94±2.43 ng/mL. A significant association was found between AMH levels and acne (P=0.038), but not with hirsutism, hyperpigmentation, anxiety, or stress. Most participants (88.4%) had PCOM on ultrasound. AMH showed minimal correlation with LH, FSH, estradiol, prolactin, DHEAS, and testosterone levels. While elevated AMH levels were common, they did not significantly differ across subgroups with various clinical or biochemical features of hyperandrogenism.
Conclusion: AMH is a valuable adjunctive marker for diagnosing PCOS, especially reflecting ovarian follicular reserve and morphology. However, its lack of strong association with clinical and biochemical hyperandrogenism or psychological symptoms suggests that it should not be used as a standalone diagnostic tool. A comprehensive approach remains essential for accurate diagnosis and effective management of PCOS.

Keywords: Polycystic ovarian syndrome, Hyperandrogenism, Rotterdam criteria, anti-müllerian hormone, ovarian reserve, antral follicle count

Introduction
Polycystic Ovarian Syndrome (PCOS) is one of the most common endocrine disorders, affecting an estimated 4-20% of women of reproductive age worldwide, with a prevalence of approximately 11.34% reported in India. It is a complex and heterogeneous condition involving reproductive, endocrine, and metabolic dysfunctions [1]. Clinically, it presents with a wide range of symptoms, including menstrual irregularities (such as oligomenorrhoea or amenorrhoea), infertility, obesity, acne, hirsutism, and signs of insulin resistance like acanthosis nigricans. If not identified and managed early, PCOS can predispose affected women to serious long-term complications such as type 2 diabetes mellitus, dyslipidemia, metabolic syndrome, hypertension, cardiovascular diseases, and even endometrial hyperplasia or carcinoma [2].
The diagnosis of PCOS is primarily guided by the Revised Rotterdam Criteria (2003), which require the presence of at least two out of three features: Oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovarian morphology (PCOM) on ultrasound [3]. However, each of these components presents its own diagnostic challenges. For example, imaging-based assessment of PCOM may be limited by technical factors, especially in obese or adolescent patients where transabdominal sonography is suboptimal and transvaginal

sonography may not be acceptable. Similarly, antral follicle count (AFC) is subject to inter-observer variability, and hormonal assessments for hyperandrogenism may be inconsistent due to assay variations and ethnic differences [4].

In this context, Anti-Müllerian Hormone (AMH) has emerged as a promising and more objective biomarker for diagnosing PCOS. Granulosa cells of small antral follicles secrete AMH, typically increasing in number in women with PCOS. Unlike AFC, serum AMH levels are relatively stable throughout the menstrual cycle and are unaffected by oral contraceptives, making it a convenient and reproducible diagnostic tool. Elevated AMH levels have been shown to correlate strongly with key features of PCOS, including anovulation, hyperandrogenism, and increased ovarian reserve. Moreover, several studies have reported that AMH demonstrates greater sensitivity and specificity than AFC in detecting PCOS and may also reflect the severity of associated hormonal and metabolic abnormalities [5].

In conclusion, although the Rotterdam criteria remain the standard for diagnosing PCOS, their application is often limited by subjectivity and technical constraints. The incorporation of serum AMH as a complementary diagnostic parameter offers a non-invasive, reliable, and quantitative alternative, particularly valuable in populations where traditional diagnostic approaches are challenging. As evidence continues to support its utility, AMH is likely to play an increasingly central role in the accurate diagnosis and management of PCOS.

Polycystic Ovarian Syndrome (PCOS) is a prevalent endocrine disorder in reproductive-age women, causing reproductive, metabolic, and psychological complications. Early diagnosis is essential to avert enduring complications such as infertility, diabetes, and cardiovascular disease. Anti-Müllerian Hormone (AMH) has emerged as a reliable, objective, and cycle-independent biomarker that correlates with ovarian reserve and hyperandrogenism. This study evaluates the diagnostic utility of serum AMH levels in women with PCOS and explores its correlation with clinical, biochemical, and ultrasonographic parameters as per the Rotterdam criteria.

Materials and Methods

A cross-sectional study was conducted in the OPD of obstetrics & gynecology, MGM Medical College Hospital, Jamshedpur. 155 samples were collected between July 2024 and May 2025. The study was approved by the Institutional Ethics Committee of MGM Medical College, Jamshedpur. Written informed consent was obtained from all patients who agreed to participate in the study. Our study population was PCOS patients in the reproductive age group [18-45 years] as defined by Rotterdam criteria.

- **Inclusion & Exclusion criteria:** The study includes women with PCOS, irregular menses, oligomenorrhea, acne, acanthosis nigricans, hirsutism, and fewer than eight cycles in the past 12 months. Exclusions include pregnant, postmenopausal, ovulation induction drug, and ovarian surgery patients; females not consenting; and patients with hormonal therapy, autoimmune diseases, and ovarian tumors.
- **Body Mass Index (BMI):** Height and weight were measured by weight machine and stadiometer. BMI was calculated as per WHO criteria. Blood samples were withdrawn, analyzed for fasting blood sugar and hormonal assays, and stored at -80 degrees for analysis of AMH. The Cal Biotech ELISA kit was used for AMH levels, and hormonal assays were performed for serum T_3, T_4, TSH, FSH, LH, fasting insulin, estradiol, prolactin, DHEAS, and free testosterone. All tests were conducted according to standard protocol and manufacturer guidelines. USG pelvises were performed in our hospital setting.
- **Statistical Analysis:** Continuous variables were presented as the mean ± SD. Quantitative variables were compared using the unpaired t-test and ANOVA test between the two groups. A p-value of 0.05 will be considered statistically significant.

Results

The study involved 155 participants with a mean age of 24.65 years and a standard deviation of 4.84 years. Most participants were Hindu (63.2%), urban residents (87.1%), and from non-tribal communities (64.5%). Educationally, 36.8% had secondary education and 34.8% were graduates. A majority were unmarried (67.7%) and belonged to middle (45.8%) or upper socioeconomic classes (22.6%), with 67.1% from nuclear families. Common clinical features included irregular menstrual cycles (32.9%).

In the study, 80% of participants had menarche, confirming eligibility for assessments. Notably, 67.1% experienced very short menstrual bleeding (< 2 days), while 23.9% had prolonged bleeding (> 8 days). Approximately 48.4% reported pain, indicating a balance between symptomatic and asymptomatic individuals. Most used 2-3 pads daily, reflecting moderate bleeding and highlighting a significant prevalence of menstrual irregularities.

The study revealed a mean height of 151.51 cm (SD=7.48 cm) and mean weight of 57.23 kg (SD=12.16 kg), indicating moderate to high variability. The average BMI was 25.14 (SD=7.61), categorizing participants as overweight according to WHO standards. Nearly half (46.5%) reported acne, with 18.1% experiencing hirsutism and 21.9% excessive hair growth, suggesting hyperandrogenism. Hyperpigmentation, possibly linked to insulin resistance, was noted in 27.7%. Psychologically, 32.3% reported anxiety and 35.5% stress, highlighting significant mental health concerns. Overall, these findings illustrate a complex clinical profile involving dermatological, endocrine, and psychological factors, indicating potential health risks in the population studied.

Figure 1 shows that 88.4% of study subjects had polycystic ovaries, 6.5% experienced menstrual irregularities, and 5.2% exhibited clinical signs of hyperandrogenism. Ultrasound findings were the predominant diagnostic criterion, with fewer participants showing clear clinical symptoms.

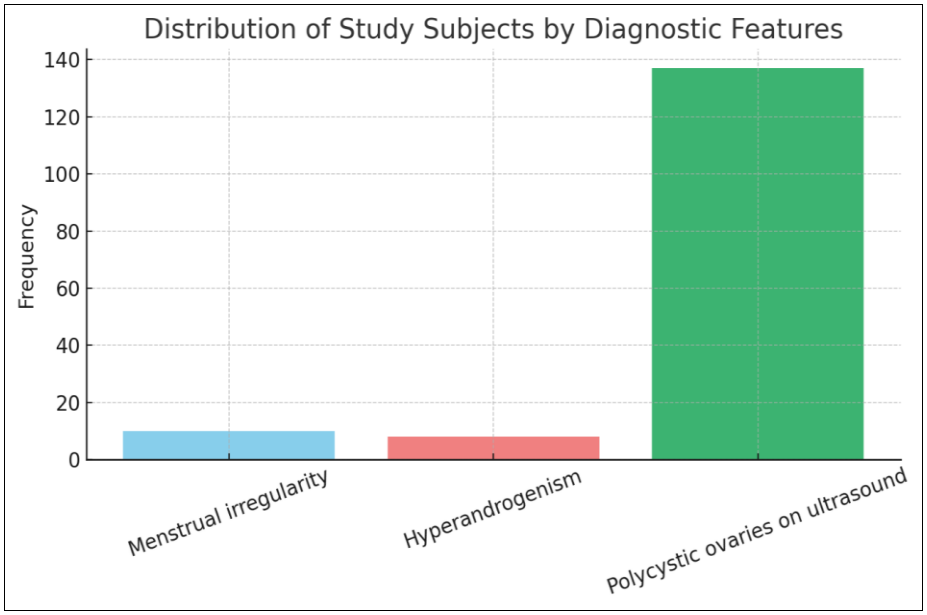

Fig 1: Distribution of study subjects as per presence of diagnostic feature of Hyperandrogenism.

The study reported a mean fasting blood sugar (FBS) of 91.52 mg/dL, indicating normal levels with moderate variability (SD=12.99), suggesting most participants did not have hyperglycemia. The mean insulin level was 11.40 µIU/mL, near the upper normal limit, with significant variability (SD=6.11), potentially indicating early insulin resistance in some individuals, relevant for metabolic issues linked to PCOS. The average TSH level was 1.99 µIU/mL (SD=1.50), showing moderate variability and a standard error of 0.12, indicating precise population mean estimation. This TSH value falls within the normal range (0.4-4.0 µIU/mL), suggesting.

Table 1 shows the mean Anti-Müllerian Hormone (AMH) level of study subjects at 9.94 ng/mL, with a standard deviation of 2.43 ng/mL. This elevated level aligns with polycystic ovarian morphology, typical in individuals with PCOS due to increased follicular count.

Table 1: Mean AMH level of the study subjects (N=155)

	Number of cases	Mean	Std. Deviation	Std. Error Mean
AMH	155	9.94	2.43	0.20

Table 2 outlines the mean levels of reproductive and endocrine hormones in the study subjects. The average luteinizing hormone (LH) was 13.13 mIU/mL, indicating a potential LH/FSH imbalance typical of polycystic ovary syndrome (PCOS). The mean follicle-stimulating hormone (FSH) was 6.83 mIU/mL, contributing to an increased LH/FSH ratio. Estradiol averaged 271.36 pg/mL, showing variability linked to menstrual cycle phases. Prolactin (PRL) averaged 13.14 ng/mL, within normal limits. Dehydroepiandrosterone sulfate (DHEAS) and testosterone levels were mildly elevated at 2.66 µg/m.

In a study of subjects with PCOS, 23.2% had elevated estradiol levels, indicating estrogen dominance, while 12.9% showed hyperprolactinemia, affecting ovulation. Elevated DHEAS levels were found in 15.5%, suggesting adrenal androgen excess. Notably, all participants had elevated testosterone, indicating universal hyperandrogenism. Nearly 44.5% were pre-obese or obese, potentially worsening insulin resistance and hormonal imbalance. Elevated anti-Müllerian hormone (AMH) levels were present in 67.1%, aligning with increased antral follicle count. Additionally, 60.0% had elevated luteinizing hormone (LH), while 87.1% had normal follicle-stimulating.

Table 2: Mean hormone level of the study subjects (N=155)

Hormonal Parameters	Mean	Std. Deviation	Std. Error Mean
LH	13.13	2.99	.240653
FSH	6.83	7.91	.635489
Estradiol	271.36	206.81	16.611703
PRL	13.14	11.41	.917148
DHEAS	2.66	1.21	.097988
Testosterone	5.50	1.60	.128736

Table 3 evaluated the association between AMH (Anti-Müllerian Hormone) levels and acne presence. Participants with acne had a higher mean AMH level (10.35±2.48 ng/mL) than those without (9.58±2.35 ng/mL). The significant difference, indicated by a t-value of 2.09 and p-value of 0.038, suggests a link between elevated AMH levels and acne, potentially reflecting hyperandrogenism.

Table 3: Association of AMH level with presence of Acne (N=155)

AMH					
Acne	Number of cases	Mean	Std. Deviation	T-Value	P-Value
Yes	72	10.35	2.48	2.09	0.038
No	83	9.58	2.35		

T-Value of 2.09 and a p-value of 0.038, significant

Table 4: Association of AMH level with presence of Hirsutism (N=155)

Hirsutism	Number of cases	Mean	Std. Deviation	T-Value	P-Value
Present	28	10.71	2.51	0.66	0.512
Absent	127	9.77	2.39		

T-Value of 0.66 and a p-value of 0.512, non-significant

Table 4 examines the relationship between AMH levels and hirsutism in study subjects. Participants with hirsutism had a mean AMH level of 10.71±2.51 ng/mL, compared to 9.77±2.39 ng/mL in those without. However, the independent t-test revealed no statistically significant difference (t=0.66, P=0.512), indicating no significant association between AMH levels and hirsutism in this population. Table 5 analyzes the relationship between AMH levels and excessive hair in participants. The mean AMH level was slightly higher in those with excessive

hair (10.14±2.89 ng/mL) than in those without (9.88±2.30 ng/mL). However, the difference was not statistically significant (t-value: 0.39, p-value: 0.695), indicating no meaningful association between AMH levels and excessive hair.

Table 5: Association of AMH level with presence of excess hair (N=155)

AMH					
Excessive hair	**Number of cases**	**Mean**	**Std. Deviation**	**T-Value**	**P-Value**
Present	34	10.14	2.89	0.39	0.695
Absent	121	9.88	2.30		

T-Value of 0.39 and a p-value of 0.695, non-significant

Table 6 examines the relationship between AMH levels and hyperpigmentation in the nape of the neck, axilla, or groin. Individuals with hyperpigmentation had a mean AMH level of 10.25 ng/mL, slightly higher than 9.82 ng/mL in those without. However, the difference was not statistically significant (t-value 0.54, p-value 0.592), suggesting no meaningful association between AMH levels and hyperpigmentation.

Table 6: Association of AMH level with presence of hyperpigmentation on nape of neck (N=155)

AMH					
Hyper pigmentation on nape of neck/axilla/groin/ Posterior of neck	**Number of cases**	**Mean**	**Std. Deviation**	**T-Value**	**P-Value**
Yes	43	10.25	2.25	0.54	0.592
No	112	9.82	2.50		

T-Value of 2.09 and a p-value of 0.038, significant

Figure 2 indicates that AMH levels were marginally higher in participants with anxiety (10.14 ng/mL) and stress (10.12 ng/mL) compared to those without (9.84 ng/mL), but these differences were not statistically significant, suggesting no meaningful association.

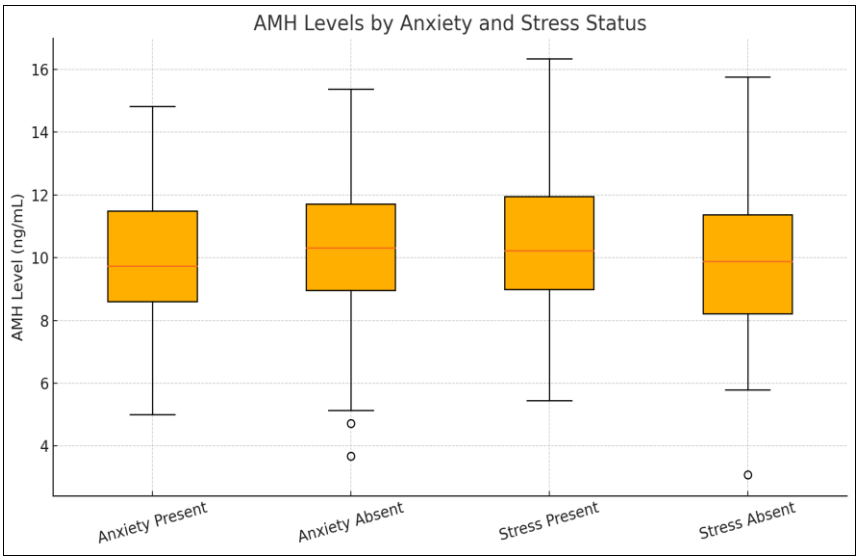

Fig 2: AMH levels by Anxiety and stress level

Figure 3 assesses the relationship between AMH levels and various hyperandrogenism features, such as menstrual irregularity and polycystic ovaries. Mean AMH levels were similar across groups: 10.01 ng/mL for menstrual irregularity, 9.87 ng/mL for clinical hyperandrogenism, and 9.94 ng/mL for polycystic morphology. A one-way ANOVA indicated no significant differences (F=0.16, P=0.851), suggesting AMH levels are not reliable for differentiate.

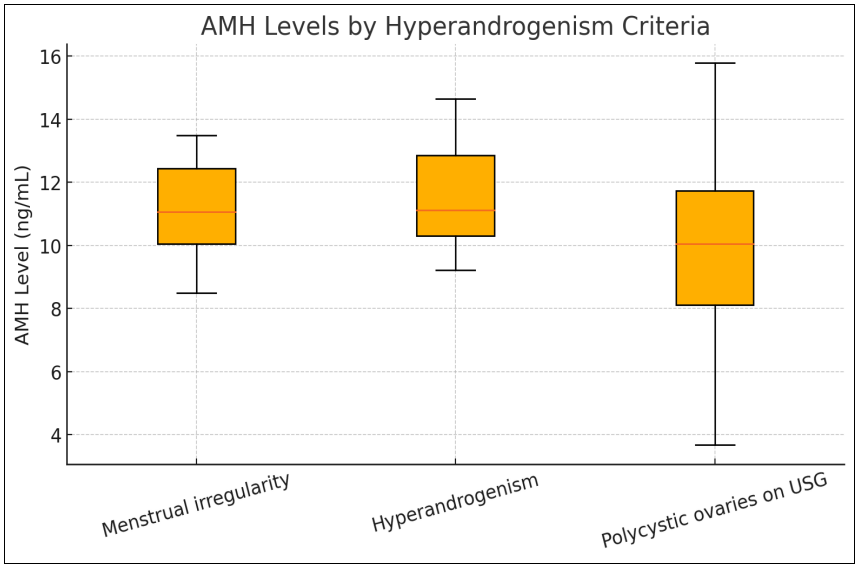

Fig 3: Association of AMH level with presence of Hyperandrogenism

The scatter plots indicated weak correlations between AMH and several hormonal parameters, with AMH showing a minimal positive correlation with LH (R^2=0.031) and virtually no association with FSH (R^2=0.000041) [Figure 4-5]. The correlation with estradiol was also minimal (R^2=0.003), while prolactin demonstrated a very weak inverse relationship with AMH (R^2=0.022). These findings suggest that AMH levels vary independently of LH, FSH, estradiol, and prolactin, with no clinically meaningful associations identified [Figure 6-7].

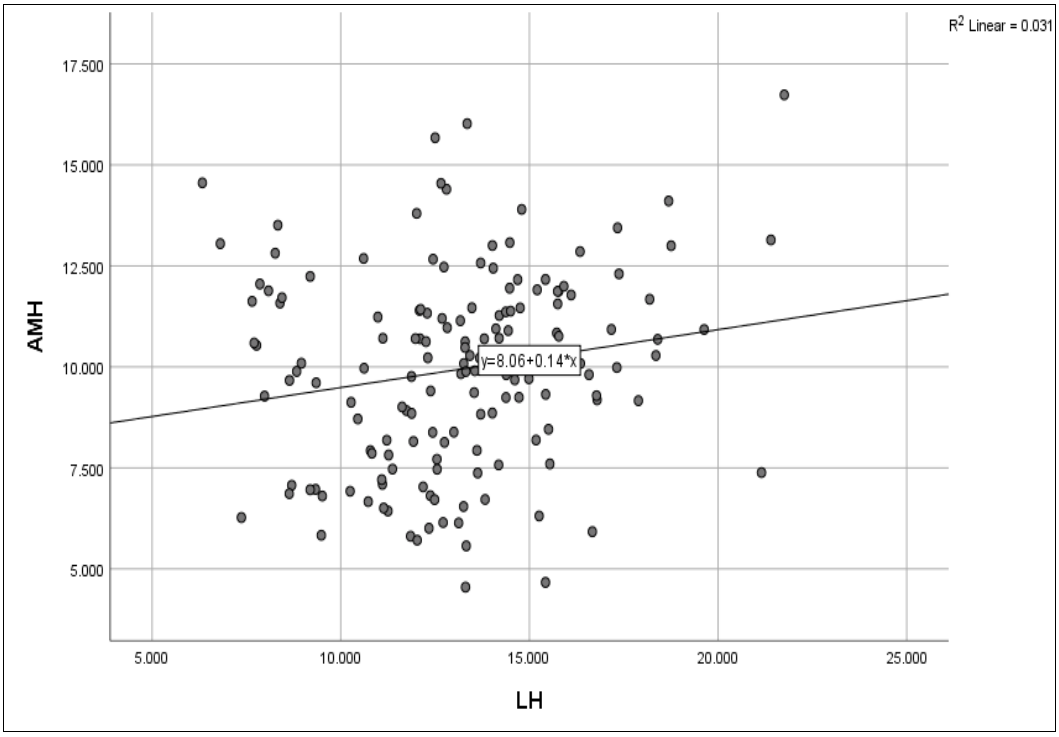

Fig 4: Association of AMH level with LH level of study subjects

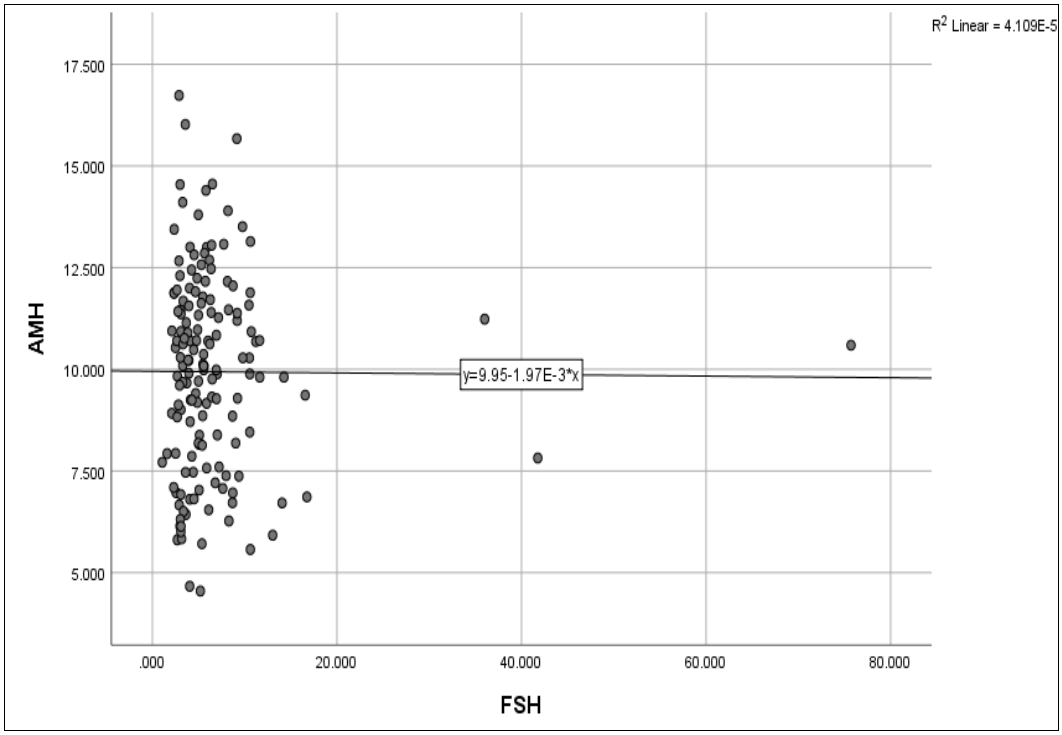

Fig 5: Association of AMH level with FSH level of study subjects

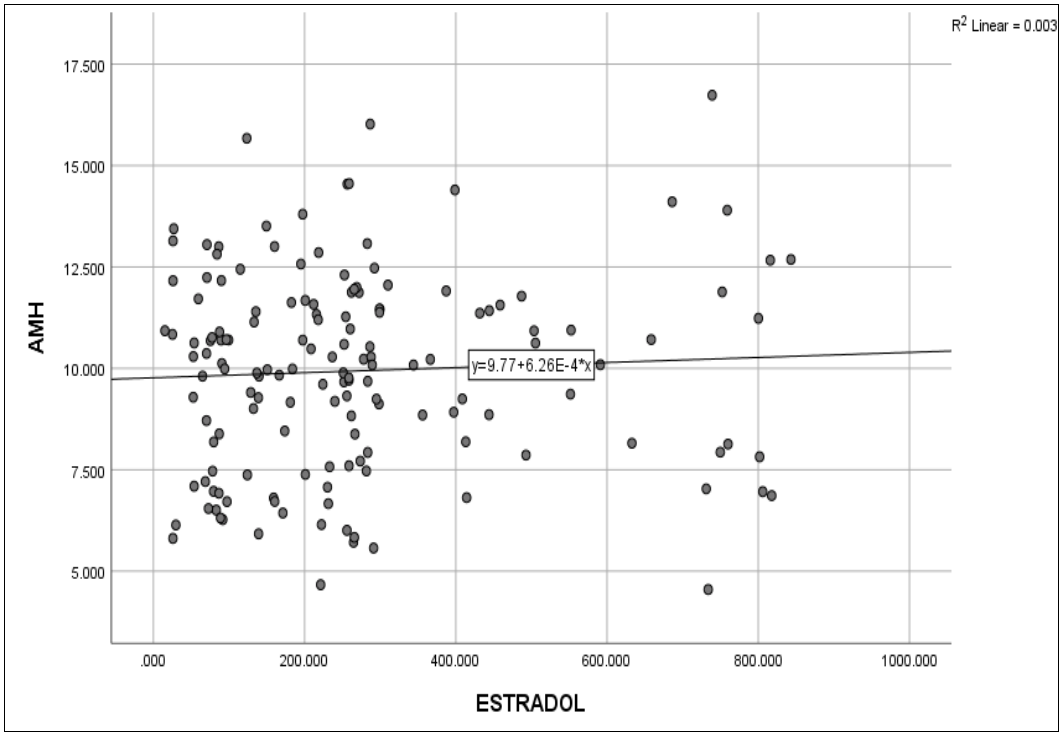

Fig 6: Association of AMH level with Estradiol level of study subjects

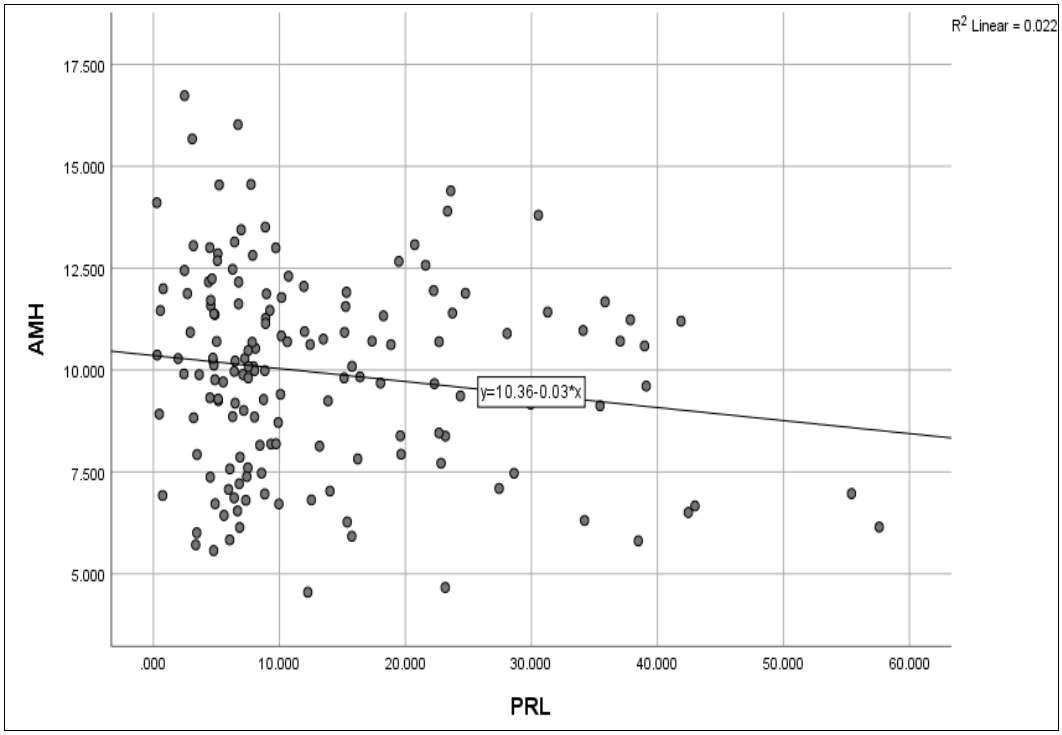

Fig 7: Association of AMH level with PRL level of study subjects

Scatter plots in Figures 8 and 9 demonstrate negligible correlations between AMH levels and androgenic hormones. DHEAS showed a weak positive correlation with AMH (R^2=0.003), while testosterone had an almost nonexistent association (R^2=0.000097). These results suggest that both DHEAS and testosterone have minimal influence on AMH variability.

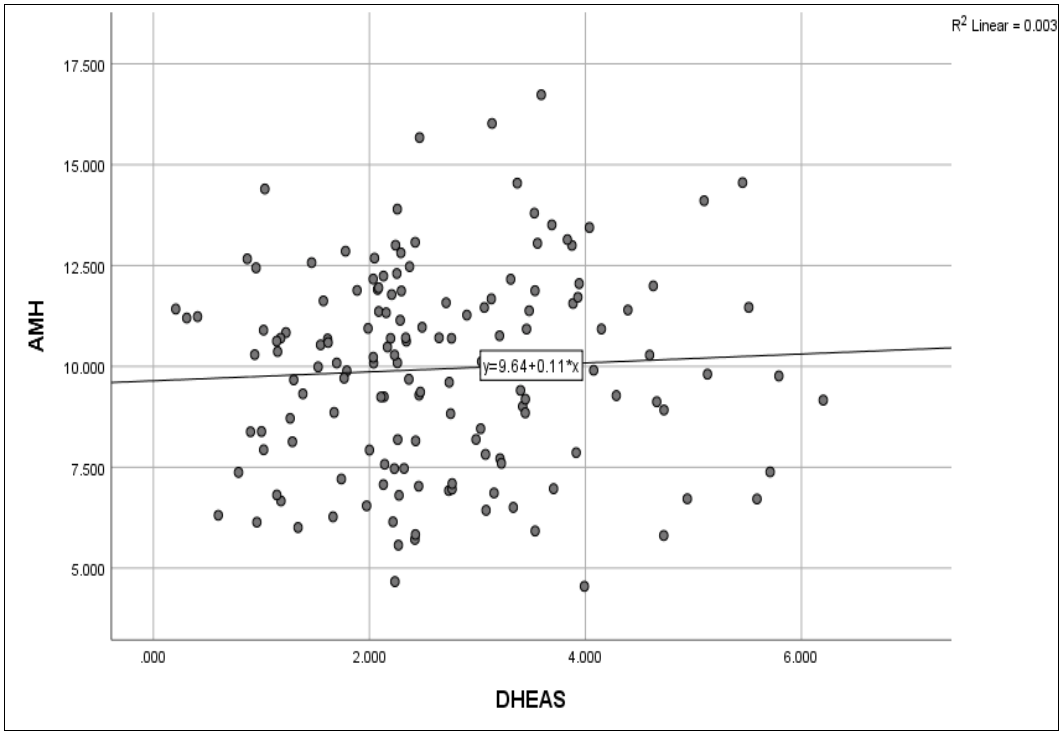

Fig 8: Association of AMH level with DHEAS level of study subjects

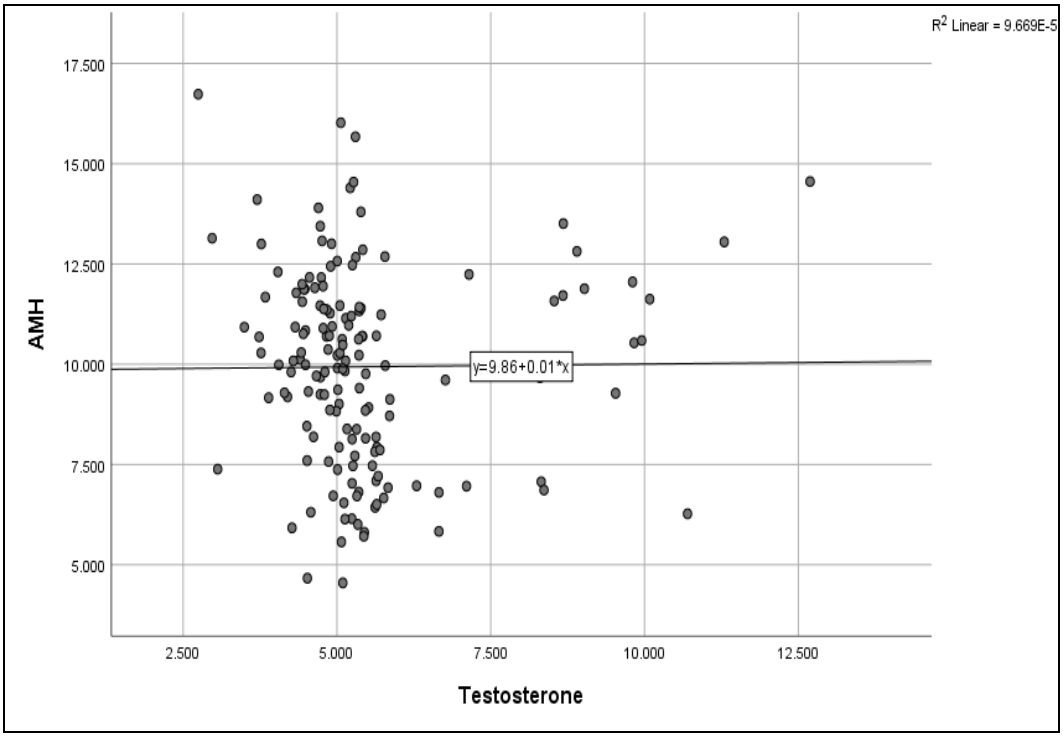

Fig 9: Association of AMH level with Testosterone level of study subjects

Discussion

The study of 155 young women, averaging 24.65 years, predominantly Hindu and urban, revealed most were well-educated, unmarried, and from middle or upper socioeconomic backgrounds, reflecting demographic trends noted by Sharma A *et al*. (2022) [6] in urban PCOS populations.

Menstrual abnormalities were highly prevalent, with 83.2% reporting cycle lengths > 35 days, and 67.1% experiencing bleeding lasting < 2 days. These findings mirror those of Patel *et al*. (2020) [7], who reported oligomenorrhea in 78.4% of Indian PCOS patients. The most common presenting complaints in our cohort were irregular menstrual cycles (32.9%) and dermatological features such as acne and excessive hair growth (25.8%).

The mean BMI of 25.14 placed the average participant in the overweight category. This is consistent with findings by Neubronner SA, *et al* [8], who highlighted that over 40% of women with PCOS are either overweight or obese factors that compound insulin resistance and metabolic dysfunction. Acne was reported in 46.5% of participants and was found to be significantly associated with higher AMH levels (P=0.038). This aligns with findings by Ashraf *et al*. (2021) [9], who

demonstrated that elevated AMH is associated with cutaneous manifestations of hyperandrogenism in PCOS, particularly acne and hirsutism.

Psychological distress was also common, with anxiety present in 32.3% and stress in 35.5% of participants. While AMH levels did not significantly correlate with either anxiety (P=0.455) or stress (P=0.685), the prevalence of these symptoms supports findings from Dong YZ, *et al.* [10] and Cowan S, *et al*. (2020) [11] who identified elevated psychological burden in women with PCOS, often independent of hormonal profiles.

Ultrasonographic features were consistent with polycystic ovarian morphology in 84.5% of participants, and 67.1% had elevated AMH levels. These findings are in line with Bachanek M, *et al*. (2019) [12], who suggested that AMH > 9 ng/mL is a strong surrogate for ultrasound-based diagnosis of PCOS. Additionally, 60% of the participants had elevated LH levels, reinforcing the typical hormonal pattern associated with PCOS.

In the present cohort of 155 women, mean AMH was 9.94 ng/mL, aligning with studies like Butt MS, *et al* (2023) [13], which reported high AMH levels (> 7 ng/mL) as reliable markers for polycystic ovarian morphology. Elevated AMH positively correlated with LH/FSH ratio and follicle count-mirroring findings by Barbakadze L *et al*. (2022) [14].

Acne was found in 46.5% of participants and significantly associated with higher AMH levels (mean 10.35 vs. 9.58, P=0.038). This supports Sardana *et al*. (2016) [15] who highlighted AMH's diagnostic potential in acne-presenting PCOS patients, and Pellatt *et al*. (2007) [16] linking elevated AMH with granular cell hyperactivity in PCOS.

Hirsutism (18.1%) and excess hair (21.9%) did not show a significant association with AMH (P=0.512 and 0.695). This aligns with Kumari B, *et al*. (2021) [17] who noted inconsistent correlation between biochemical AMH levels and clinical measures of hirsutism.

Hyperpigmentation (27.7%) also lacked a significant AMH association (P=0.592), similar to reports by Pellatt *et al*. [16] stating metabolic signs like acanthosis nigricans did not correlate strongly with AMH or androgen levels.

Psychological symptoms were frequent-anxiety in 32.3% and stress in 35.5% but neither showed significant AMH associations (P=0.455 and 0.685). Although psychological morbidity is well-documented in PCOS (e.g., Karjula, *et al*. 2021) [18], its independence from AMH suggests that AMH does not reflect neuropsychological status.

Overall, our findings underscore that AMH is most strongly linked to ovarian morphology and menstrual disturbance, with a weaker association to dermatologic hyperandrogenic features (primarily acne) and little relevance to psychological symptoms. This supports the utility of AMH as a reproductive biomarker particularly in aiding diagnosis of polycystic ovarian morphology while highlighting that clinical features and mental health should be assessed independently.

Our study found no significant association between hyperpigmentation (e.g., acanthosis nigricans) and AMH levels (10.25±2.25 vs. 9.82 ±2.50 ng/mL; P=0.592). This aligns with findings by Sudevan R, *et al*. (2020) [19], who also reported no correlation between metabolic markers and AMH in women with PCOS. These results suggest that while hyperpigmentation can serve as a clinical marker of insulin resistance, it does not reflect ovarian follicular reserve as measured by AMH.

Although anxiety and stress were common in our cohort, neither was significantly associated with AMH levels (anxiety: 10.14 vs. 9.84 ng/mL, P=0.455; stress: 10.12 vs. 9.84 ng/mL, P=0.685). This is consistent with broader evidence. Zangeneh FZ, *et al.* [20] showed that psychological distress in PCOS is largely independent of AMH levels. It underscores the need for psychological assessment in PCOS, which cannot be inferred from reproductive hormone levels alone.

AMH was uniformly distributed across subgroups menstrual irregularity (10.01 ng/mL), clinical hyperandrogenism (9.87 ng/mL), or polycystic ovarian morphology (9.94 ng/mL) with no significant differences ($F = 0.16$, P=0.851). This agrees with Bhattacharya K, *et al* [21], who found elevated AMH levels in PCOS regardless of specific hyperandrogenic manifestations. These findings reinforce AMH's role as a global marker of ovarian reserve rather than a predictor of clinical hyperandrogenism.

AMH showed minimal correlations with LH ($R^2 = 0.031$), FSH (R^2=0.000041), estradiol (R^2=0.003) and prolactin (R^2=0.022), indicating near independence. These results are mirrored by Gałczyńska D, *et al*. (2022) [22], who demonstrated that while AMH is elevated in PCOS, its variability is only weakly linked to pituitary or gonadal steroid levels. This suggests AMH primarily reflects ovarian follicle quantity rather than broader endocrine regulation.

DHEAS displayed a negligible correlation with AMH (R^2=0.003), and testosterone even less so (R^2=0.000097). This is consistent with Banaszewska *et al*. (2023) [23], who found that AMH does not correlate strongly with adrenal or ovarian androgens in PCOS. These data further indicate that AMH serves as a biomarker for ovarian reserve, not biochemical hyperandrogenism.

Conclusion

This study highlights a high prevalence of polycystic ovarian morphology and elevated AMH levels among participants, supporting their diagnostic role in PCOS. While acne showed a significant association with AMH, other clinical features (hirsutism, hyperpigmentation, anxiety, stress) and hormonal parameters (LH, FSH, estradiol, prolactin, DHEAS, testosterone) showed no meaningful correlation. AMH functions as an independent marker of ovarian reserve but is insufficient alone to capture the clinical and hormonal complexity of PCOS. A comprehensive, multi-dimensional evaluation remains essential for accurate diagnosis and management.

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Conflicts of interest: No conflicts of interest

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