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Original Research Article

Relationship between anti-Mullerian hormone and ovarian response after using letrozole in subfertile polycystic ovary syndrome patients

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is the most common cause of anovulation in reproductive-age women. Anti-Mullerian hormone (AMH) was higher in PCOS women due to increased antral follicles. This study examined the relationship between Anti-mullerian hormone and ovarian response after letrozole in sub-fertile PCOS patients.
Methods: This cross-sectional study was conducted in the Department of Obstetrics and Gynaecology, BIRDEM General Hospital, Dhaka, Bangladesh, from April 2022 to September 2023. 116 sub-fertile PCOS patients receiving Letrozole were purposively selected. Patients underwent transvaginal ultrasound (TVS) and were divided into two groups by follicle size: non-responder group (<16 mm) and responder group (≥16 mm). Data was analyzed using statistical package for the social sciences (SPSS) version 26.0.
Result: Most women were aged 21-30 years, with a mean age of group I at 30.2±3.7 and group II at 29.9±5.1. Most were primary subfertile; group I 35(65.5%) and group II 41(70.7%). Mean serum FSH was higher in group II, 9.28±5.6, than in group I, 7.19±3.64 ($p<0.001$). Mean AMH was higher in group II, 5.82±1.4, than in group I, 5.2±1.8 ($p=0.054$) but statistically non-significant. ROC curve showed AMH cut-off value of 5.12 with 62.1% sensitivity and 65.5% specificity. Patients with AMH <5.12 ng/ml had 3.1 times more chance of ovarian response versus those with AMH >5.1 ng/ml (OR=3.1; 95% CI (1.5-6.6), $p=0.003$).
Conclusion: Elevated serum AMH level is a risk factor for poor ovarian response in PCOS, which may require adjusting the letrozole dosage.

Keywords: Polycystic ovary syndrome, Anti-Mullerian hormone, Letrozole, FSH, Subfertility

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder that affects approximately 20% of women of reproductive age worldwide and is responsible for nearly 80% of cases of anovulatory infertility.[1] The syndrome is clinically defined by ovulatory dysfunction, hyperandrogenism, and polycystic ovarian morphology, with variable presentations across populations.[2] In addition to reproductive disturbances, PCOS is associated with metabolic complications, including insulin resistance,

type 2 diabetes, and cardiovascular disease.[3] These complexities make PCOS a challenging condition to manage, particularly in women seeking fertility treatment.

Ovulation induction is the mainstay of treatment for infertility in PCOS. Historically, clomiphene citrate was used as first-line therapy, but recent evidence indicates that letrozole, a third-generation aromatase inhibitor, is superior in achieving higher ovulation and live birth rates.[4,5] Despite its growing use, the ovarian response to letrozole is inconsistent, with a significant proportion of women showing inadequate follicular development. Identifying biomarkers that predict ovarian responsiveness is essential to optimize treatment strategies and reducing unnecessary exposure to ineffective therapy.

Anti-Müllerian hormone (AMH), secreted by granulosa cells of pre-antral and small antral follicles, has been widely investigated as a marker of ovarian reserve.[6] AMH reflects the number of small follicles, declines with age, and is relatively stable across the menstrual cycle, making it a reliable biomarker compared to gonadotropins such as follicle-stimulating hormone (FSH).[7] In PCOS, AMH levels are often markedly elevated, reflecting both the increased number of small follicles and heightened secretion per follicle.[8,9] Elevated AMH has been implicated in the follicular arrest characteristic of PCOS, potentially through reduced follicular sensitivity to FSH and inhibition of follicle selection.[10]

Previous studies have shown that AMH correlates strongly with ovarian response during assisted reproductive technology (ART) cycles.[11] However, its predictive value for ovulation induction with oral agents such as letrozole is less clear. Some studies suggest that higher AMH predicts poor response to induction agents, while others report weak or inconsistent associations.[12] This variability underscores the need for population-specific evidence, as AMH cut-off values may differ across ethnic and clinical contexts. In South Asian women, where PCOS prevalence is high and fertility challenges are common, data on the relationship between AMH and ovarian response to letrozole are scarce. A better understanding of this association could allow clinicians to personalize induction protocols, avoid unnecessary delays in treatment, and improve success rates.

The present study was conducted to determine the relationship between AMH levels and ovarian response to letrozole in subfertile women with PCOS. Specifically, it aimed to establish whether higher serum AMH is associated with poor responsiveness, to identify potential cut-off values for prediction, and to evaluate AMH as a clinical tool in guiding fertility management.

METHODS

This cross-sectional analytical study was conducted at the Center for Assisted Reproduction (CARE), Department of Obstetrics and Gynaecology, BIRDEM-II General Hospital, Dhaka, Bangladesh. The study was carried out over 18 months, from April 2022 to September 2023. A total of 116 women with PCOS who presented with subfertility and underwent letrozole induction were enrolled.

Study population

The study population comprised women of reproductive age diagnosed with PCOS according to the Rotterdam criteria (2003), which requires the presence of at least two of the following: oligo- or anovulation, clinical or biochemical features of hyperandrogenism, and polycystic ovarian morphology on ultrasound. After letrozole induction, participants were divided into two groups based on their ovarian response, as assessed by transvaginal ultrasound, group I (responders) included women who developed at least one dominant follicle measuring ≥16 mm on day 12/13 of the cycle and group II (non-responders) included women whose follicles remained <16 mm despite induction with letrozole.

Selection criteria

Inclusion criteria

Inclusion criteria included women aged 18–35 years, diagnosed with PCOS according to Rotterdam criteria, having history of subfertility (inability to conceive despite ≥12 months of unprotected intercourse) and willingness to undergo ovulation induction with letrozole.

Exclusion criteria

Women with thyroid dysfunction or hyperprolactinemia, having history of premature ovarian insufficiency and previous ovarian surgery were excluded.

Data collection and study procedure

At recruitment, baseline demographic information and anthropometric measurements were recorded using a structured case record form. A detailed clinical history was obtained, including duration of subfertility, obstetric background, and family history.

All participants underwent baseline transvaginal sonography (TVS) on day 2 of the menstrual cycle to document ovarian morphology and exclude other pelvic pathology. On the same day, blood samples were collected after overnight fasting for measurement of serum AMH, FSH, and LH levels. Hormone assays were performed in the institutional laboratory using the chemiluminescent microparticle immunoassay (CMIA) method, ensuring standardized testing conditions.

Ovulation induction was carried out with letrozole at a fixed dose of 5 mg daily from cycle days 2–6. Patients were instructed to return for follicular monitoring by TVS on days 12 or 13 of the cycle. Follicular size was measured,

and women were categorized as responders or non-responders based on follicle growth criteria described above. For quality assurance, ultrasound assessments were performed by a single experienced sonologist to minimize inter-observer variability.

Data collection was carefully cross-checked against hospital records and laboratory reports to ensure accuracy and completeness. The final dataset included demographic information, type of subfertility, baseline serum AMH, FSH, and LH levels, and ovarian response status.

Ethical considerations

Ethical clearance was obtained from the Institutional Review Board of BIRDEM, Dhaka. Informed consent was obtained from the study populations. The welfare and interests of participants were prioritized to safeguard their health throughout the research. Given that the study involved only 5 ml of blood from participants, complications were extremely unlikely. However, any discomfort, pain, weakness, or dizziness experienced by participants was promptly addressed with reassurance and analgesics. Participants could withdraw from the study at any point without consequence. Confidentiality was strictly maintained throughout the study.

Statistical analysis

All data were entered and analyzed using statistical package for the social sciences (SPSS) version 26 (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as mean±standard deviation (SD), while categorical variables were expressed as frequencies and percentages. Independent sample t-tests were applied to compare mean hormone levels between responders and non-responders. Chi-square or Fisher's exact tests were used for categorical comparisons. Receiver operating characteristic (ROC) curve analysis was performed to determine the predictive accuracy of serum AMH, with sensitivity, specificity, and optimal cut-off values calculated. Odds ratios (OR) with 95% confidence intervals (CI) were estimated to quantify the association between AMH levels and ovarian response. A $p<0.05$ was considered statistically significant.

RESULTS

A total of 116 subfertile women with PCOS were included in the study, of whom 58 were classified as responders and 58 as non-responders to letrozole. The following tables and figures present the distribution of baseline characteristics and hormonal parameters, as well as the predictive performance of AMH for ovarian response.

Table 1 presents the age distribution of participants according to ovarian response. The majority of women in both groups were between 21 and 30 years of age (53.4% of responders and 55.2% of non-responders). The mean age was comparable between groups (30.2±3.7 years versus 29.9±5.1 years, p=0.743).

Table 1: Comparison of the participants according to age between the two groups.

Age (in years)	Group I (n=58)	Group II (n=58)	P value
Up to 20	0 (0.0)	2 (3.4)	0.334
21-30	31 (53.4)	32 (55.2)	
>30	27 (46.6)	24 (41.4)	
Mean±SD	30.2±3.7	29.9±5.1	0.743

Table 2 shows the distribution of subfertility type among responders and non-responders. Primary subfertility was the most common in both groups, reported in 65.5% of responders and 70.7% of non-responders. Secondary subfertility was present in 34.5% and 29.3% of participants, respectively. The difference between groups was not statistically significant (p=0.550).

Table 2: Comparison of the participants according to the type of subfertility between the two groups.

Type of subfertility	Group I (n=58)	Group II (n=58)	P value
Primary	38 (65.5)	41 (70.7)	0.55
Secondary	20 (34.5)	17 (29.3)	

Table 3 compares baseline serum hormone levels between responders and non-responders. Mean serum FSH was significantly higher in non-responders (9.28±5.60 IU/l) than in responders (7.19±3.64 IU/l, p<0.001). Serum LH levels were slightly higher in non-responders (11.49±6.51 IU/l) compared to responders (9.41±9.05 IU/l), although this difference did not reach statistical significance (p=0.102). Mean serum AMH was also higher among non-responders (5.82±1.40 ng/ml) compared with responders (5.20±1.80 ng/ml), with borderline statistical significance (p=0.054).

Table 3: Comparison of the participants according to Serum FSH, LH and AMH levels by two groups.

Parameter (mean±SD)	Group I (n=58)	Group II (n=58)	P value
Serum FSH	7.19±3.64	9.28±5.60	<0.001
Serum LH	9.41±9.05	11.49±6.51	0.102
Serum AMH	5.20±1.8	5.82±1.40	0.054

The ROC for the association of serum AMH level with ovarian response is shown in Figure 1. The area under the curve was 0.638 (0.537–0.739), with a standard error of 0.052 and a significance level of 0.010, categorizing it as a fair test for the association of ovarian response.

Table 4 summarizes the diagnostic performance of serum AMH at various cut-off values for predicting ovarian response. An AMH threshold of 5.12 ng/ml provided the

best balance between sensitivity (62.1%) and specificity (65.5%), with a Youden index of 0.276.

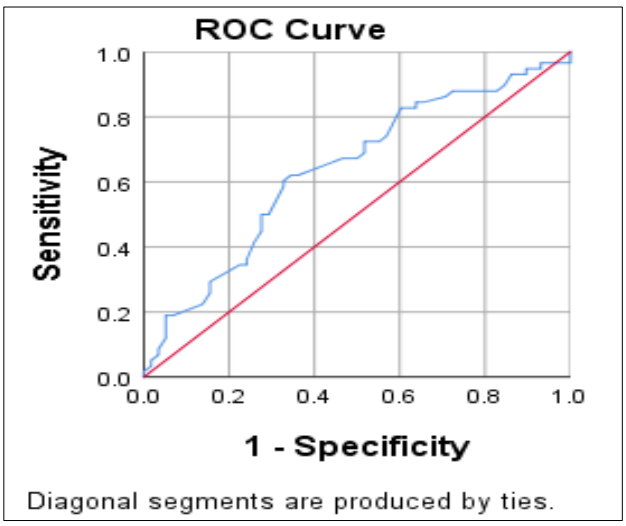

Figure 1: Receiver operating characteristic (ROC) curves of serum AMH level for the association of ovarian response (area=0.638, SE=0.052, asymptomatic significance=0.010, 95% CI: lower bound=0.537, upper bound=0.739).

Table 4: Different sensitivity and specificity at different scores according to the Serum AMH levels (coordinates of the ROC).

Score	Sensitivity	Specificity	1-specificty	YI
3.74	0.138	0.948	0.052	0.086
4.85	0.448	0.724	0.276	0.172
4.94	0.483	0.724	0.276	0.207
4.99	0.5	0.724	0.276	0.224
5	0.5	0.707	0.293	0.207
5.01	0.586	0.672	0.328	0.258
5.06	0.603	0.672	0.328	0.275
5.12	0.621	0.655	0.345	0.276
5.22	0.621	0.638	0.362	0.259
5.4	0.638	0.603	0.397	0.241
5.55	0.655	0.569	0.431	0.224
5.62	0.672	0.534	0.466	0.206
5.67	0.672	0.517	0.483	0.189
5.7	0.672	0.5	0.5	0.172

Patients with serum AMH level ≥5.12 ng/ml had 3.1 times more chance to have poor ovarian response compared to those with serum AMH level <5.12 ng/ml (p=0.003; OR=3.1; 95% CI=1.5-6.6) (Table 5).

Table 5: Odds ratios (OR) and 95% confidence intervals (CI) for PCOS according to serum AMH level (With a cut-off value of 5.12 ng/ml).

Serum AMH level (ng/ml)	Group II (n=58)	Group I (n=58)	P value	OR (95% CI)
≥5.12 (high level)	38 (65.5)	22 (37.9)	0.003	3.1 (1.5-6.6)
<5.12 (normal level)	20 (34.5)	36 (62.1)		

DISCUSSION

This study evaluated the relationship between serum AMH levels and ovarian response to letrozole in subfertile women with PCOS. The findings demonstrated that women with higher serum AMH concentrations had significantly poorer ovarian response. The ROC curve identified an AMH cut-off value of 5.12 ng/ml, above which the likelihood of non-response increased, with an odds ratio of 3.1. Although mean AMH levels were higher in non-responders compared to responders, the difference did not reach conventional statistical significance, likely due to the modest sample size. Nonetheless, the cut-off analysis and odds ratio clearly highlighted the predictive value of elevated AMH for poor responsiveness to letrozole.

These results align with the biological role of AMH in folliculogenesis. AMH is secreted by granulosa cells of pre-antral and small antral follicles and functions to inhibit primordial follicle recruitment and reduce follicular sensitivity to FSH.[10] In PCOS, both the number of small follicles and AMH production per follicle are increased, contributing to follicular arrest and anovulation.[8,9] Elevated AMH may therefore impair the development of a dominant follicle in response to exogenous induction, explaining the reduced responsiveness observed in this study.

The ROC-derived cut-off value of 5.12 ng/ml for AMH is consistent with earlier reports. Torres et al found that women with serum AMH above 4.53 ng/ml had significantly higher rates of letrozole resistance, with comparable sensitivity and specificity.[13] Similarly, Bhide and Homburg in a meta-analysis identified a cut-off around 4.7 ng/ml for predicting ovarian response in PCOS, reinforcing the clinical relevance of the present study.[14] Although the exact threshold may vary across populations due to methodological and demographic differences, the consistent trend supports the use of AMH as a reliable predictor of responsiveness.

The present study also showed that mean serum FSH was significantly higher among non-responders, corroborating earlier work by Ashrafi et al., who reported elevated basal FSH as a marker of insufficient ovarian response during stimulation.[15] This indicates that AMH and FSH may have complementary roles: AMH reflecting ovarian reserve and follicular environment, and FSH indicating functional

ovarian capacity. The combined interpretation of both markers could refine individualized treatment decisions.

Interestingly, mean serum LH did not differ significantly between groups, despite the well-documented role of hypersecretion of LH in PCOS. Prior studies have reported mixed results regarding LH as a predictor of ovarian response.[16] It appears that although LH contributes to the pathophysiology of PCOS, its value as a standalone biomarker for responsiveness to letrozole is limited compared to AMH.

Age and type of subfertility showed no significant influence on ovarian responsiveness in this study. This finding is consistent with reports that in PCOS, ovarian reserve remains relatively preserved even at older reproductive ages, due to the high follicular pool.[17] Similarly, the reproductive history of primary or secondary subfertility may not significantly alter ovarian responsiveness, as the underlying mechanism of follicular arrest is common across both groups.

From a clinical standpoint, these results underscore the importance of incorporating AMH measurement into pre-treatment assessment for PCOS women undergoing letrozole induction. Identifying patients with elevated AMH levels can guide clinicians to anticipate poor response and consider alternative strategies. For instance, adjusting letrozole dosage, combining with gonadotropins, or moving earlier to second-line treatments may improve outcomes. This approach would save time, reduce emotional distress, and enhance cost-effectiveness in fertility management.

The findings also carry implications for personalized medicine. While demographic factors and BMI may provide some context, hormonal biomarkers such as AMH and FSH are more powerful in predicting response. Integrating these biomarkers into clinical protocols can optimize ovulation induction strategies and support individualized care.

Limitations

This study had some limitations as well. The study was performed in a single tertiary hospital; therefore, the outcome of the study may not entirely reflect the exact status of the broader population. Study sample was limited. Patients were selected who are taking only a fixed dose (5 mg) letrozole which does not reflect the entire phenomena. Therefore, the study findings cannot be generalized to the entire population.

CONCLUSION

This study demonstrated that elevated serum AMH levels are strongly associated with poor ovarian response to letrozole in subfertile PCOS patients. An AMH cut-off value of 5.12 ng/ml predicted reduced responsiveness with reasonable sensitivity and specificity, and patients above this threshold had a threefold higher risk of non-response. Serum FSH was also significantly higher in non-responders, suggesting complementary predictive value. Incorporating AMH into clinical evaluation can help personalize treatment strategies and optimize outcomes in women with PCOS undergoing ovulation induction.

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