Original Article

Correlation of Anti-Müllerian Hormone (AMH) with Antral Follicular Count (AFC)

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Abstract

Objective: To determine the correlation between serum Anti-Müllerian Hormone levels and antral follicular count in infertile women.
Methodology: The study was a cross-sectional analytical research done in the Department of Gynaecology and obstetrics, PAEC general hospital, Islamabad between June and August 2020. Non-probability consecutive sampling was used to sample 103 infertile women aged 20-40 years. The level of serum AMH, FSH, LH, and estradiol was measured in the early follicular phase (Day 23). To evaluate bilateral AFC and ovarian volume, transvaginal ultrasound was done. The SPSS version 26 was used when analysing data. The KolmogorovSmirnov test was used to test whether there is normalcy and Pearson correlation coefficient. The p-value of 0.05 was taken as significant.
Results: The mean serum AMH level was 4.04 ± 1.95 ng/mL, and the mean total AFC was 19.69 ± 6.68. No statistically significant correlation was observed between serum AMH and total AFC (r = 0.044, p = 0.658). Serum AMH also showed no significant association with ovarian volume or age.
Conclusion: Serum AMH did not show a significant correlation with antral follicular count in this cohort. Assessment of ovarian reserve should therefore incorporate both hormonal and ultrasonographic parameters.
Keywords: Anti-Müllerian hormone, Antral follicular count, Ovarian reserve, Infertility

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Introduction

Infertility is a major issue of worldwide health with an estimated prevalence rate of 10-15 percent amongst couples of reproductive age.[1] Ovarian reserve assessment is significant in the assessment and treatment of infertile women, especially in forecasting the reproductive potential and response to fertility treatment. The amount and quality of remaining primordial follicles is represented by ovarian reserve and decreases with age.[2, 3]

Anti-Mullerian hormone (AMH) released by the granulosa cells of pre-antral and small antral follicles has developed as one of the reliable endocrine predictors of ovarian reserve.[4] Its comparative stability in terms of its cycles and ease of measurement has seen it gain popularity in the practice of infertility. Another well-known marker, which is also deemed to be a direct measure of the count of recruitment-eligible follicles and which offers instant visualization of ovarian reserve, is antral follicular count (AFC), determined through transvaginal ultrasound.[5, 6]

Despite the fact that both AMH and AFC are used as predictors of the ovarian follicular pool, the correlation between the two variables has been erratic across studies. A number of studies have indicated the existence of a high positive relationship between AMH and AFC and others have shown weak, inconsistent, or discordant relationships. These discrepancies could be attributed to factors like assay variability, reliance on ultrasound operators, ethnic diversity, increase in age of the study population, and clinical heterogeneity of study population. [7, 8]

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The use of a single ovarian reserve marker can result in poor assessment and counseling in clinical practice. The association between AMH and AFC in individual populations is thus critical to maximize the infertility assessment and management. The objective of this study was to determine the correlation between serum Anti-Müllerian Hormone levels and antral follicular count among infertile women attending a tertiary care hospital.

Methodology

This cross-sectional analytical research was carried out in the Department of Gynaecology and obstetrics, PAEC General hospital, Islamabad based between June 2020-August 2020. Non-probability consecutive sampling was used to enroll women of reproductive age that had infertility. The Institutional Ethical Review Committee of the PAEC General Hospital, Islamabad Ref no PDGI-IRB (DMe) RCD06-016 provided ethical approval of the study. This study received the full board meeting which took place on 03 August 2020, and the assigned ethical approval reference number was PGHI-IRB (DMe)-RCD-06-016. Written informed consent was obtained from all participants prior to enrollment.

The WHO sample size calculator of correlation studies was used to calculate the sample size. Based on the literature published before, an expected correlation coefficient (r) of 0.30 was assumed.[9] At a 95% confidence level, power of 80 of a study with a two-tailed test, the required sample size was seen to be 85 participants. In order to correct the potential non-response and incompleteness of data, an extra 20 percent was included in the sample, and the final sample size was 103 women.

The study included women of primary or secondary infertility, between 20-40 years. To minimize the confounding factors in ovarian reserve evaluation, women with polycystic ovarian syndrome, endometriosis, who had undergone ovarian surgery or hormonal medication in the last three months were excluded.

Demographic and clinical information, such as age, body mass index, infertility duration and type, and regularity of menstrual cycles were captured on a structured proforma after receiving informed written consent. Hormonal and ultrasound assessment was performed on all participants in the first three days of the follicular phase (Day 2-3) of menstruation cycle in order to minimize the physiological variance.

Measurements of serum Anti-Mullerian Hormone, follicle stimulating hormone, luteinizing hormone and estradiol in venous blood samples were done under aseptic conditions. Hormonal testing was done by the use of standardized laboratory tests as per institutional laboratory procedures.

The transvaginal ultrasound was also conducted at the same phase of menstrual period with the help of the high-resolution transvaginal probe. The number of antral follicles (2-10 mm in diameter, each ovary) was counted and the total antral follicular count was obtained by adding both bilateral counts. The ovarian volume of each of the two ovaries was taken, and the mean ovarian volume was taken.

Validity was ensured by using strict inclusion and exclusion criteria, as well as, all measurements were determined by using standard definitions and protocols. To increase internal validity, hormonal and ultrasound tests were timed at the identical menstrual cycle.

The consistency of the study was also achieved through the consistency of ultrasound equipment and consistent methods of measurement. Measurements of ultrasound were made by qualified clinicians and inter-observer error reduced by triangulating measurements on a small group of subjects, and disagreements adjudged by consensus.

The analysis and entry of data were performed on Statistical Package of Social Sciences (SPSS) version 26. Continuous variables had their means in form of mean ± standard deviation and categorical ones in form of frequencies and percentages. Explore function in the SPSS was used to test the normality of continuous variables using the Kolmogorov-Smirnov test and the graphical technique. Due to the normal distribution of the data, Pearson correlation coefficient was employed to identify the correlation between serum AMH levels and the ovarian reserve parameters. A p-value of <0.05 was deemed to be significant.

Results

This study included 103 women in total. Participants had a mean age of 31.67 ± 4.76 years of age with mean body mass index (BMI) of 25.08 ± 4.13 kg/m^2. On average, infertility was present for 5.66 years. Each one of them was married. 49.5% of women had primary infertility while 50.5% suffered from secondary infertility. 53.4% of respondents had regular cycles whereas 46.6% of respondents had irregular cycles.

e serum AMH, FSH, LH and estradiol levels of the study subjects showed mean reference ranges to be normal for the early follicular phase. The study population has a well-preserved overall endocrine function of the hypothalamus-pituitary-ovarian axis. These findings support the suitability of the participants for evaluating ovarian reserve parameters. (Table I)

Table I: Hormonal Profile of Study Participants. (n = 103)

Hormonal Parameter	Mean ± SD	Min	Max	Reference Range*
Serum AMH (ng/mL)	4.04 ± 1.95	0.64	7.80	1.0 – 6.8
FSH (IU/L)	6.78 ± 1.87	4.00	9.90	3.0 – 10.0
LH (IU/L)	7.06 ± 2.40	3.20	10.90	2.0 – 12.0
Estradiol (pg/mL)	58.14 ± 18.77	30.30	89.70	30 – 100

**Reference ranges based on early follicular phase (Day 2–3).*

The mean total antral follicular count found was 19.69 ± 6.68. The average right ovarian volume was 6.67 ± 1.34 cm^3 while the mean left ovarian volume was 6.72 ± 1.32 cm^3. (Table II)

Table II: Ultrasound Parameters of Ovarian Reserve (n = 103)

Ultrasound Parameter	Mean ± SD	Min	Max
Right ovarian AFC	10.04 ± 4.72	2	18
Left ovarian AFC	9.65 ± 4.89	2	18
Total AFC	19.69 ± 6.68	6	34
Right ovarian volume (cm^3)	6.67 ± 1.34	4.50	8.98
Left ovarian volume (cm^3)	6.72 ± 1.32	4.53	8.99
Mean ovarian volume (cm^3)	6.70 ± 0.89	5.17	8.84

There was no statistically significant correlation between serum AMH levels, total antral follicular count, and mean ovarian volume. Similarly, age was not significantly correlated either to serum AMH levels or antral follicular count. According to our results, it was suggested serum AMH was not a good predictor of ultrasound markers of ovarian reserve. (Table III)

Table III: Correlation of Serum AMH with Ovarian Reserve Parameters (n = 103)

Variables	Correlation coefficient (r)	p-value	Interpretation
AMH vs Total AFC	**0.044**	0.658	Weak, not significant
AMH vs Mean ovarian volume	-0.082	0.411	Weak, not significant
Age vs AMH	-0.001	0.994	No correlation
Age vs Total AFC	0.134	0.178	Weak, not significant

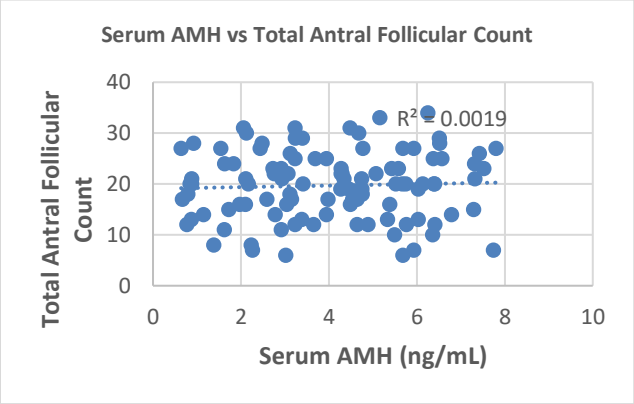

Figure 1. Scatter plot showing the relationship between serum Anti-Müllerian Hormone (AMH) levels and total antral follicular count. Each point represents an individual participant.

Discussion

statistically significant relationship with total AFC (r = 0.044, p = 0.658) and had no statistically significant relationship with mean ovarian volume. In spite of the fact that AMH and AFC are common indicators of ovarian reserve, recent studies indicate that the strength of association may differ significantly depending on population, type of assays, ultrasound procedure, inclusion criteria, and age and infertility phenotype distributions. Other studies in other areas also have found ambiguous results with moderate to strong positive correlation to weak and inconsistent correlations, in which local population characteristics and measurements factors can determine the AMH-AFC relationship.[9]

A positive relationship has been identified between AMH and AFC in several recent original studies in infertile women and /or ART populations, which substantiates the classical idea that AMH tends to monitor the antral follicle pool. Indicatively, cross-sectional and infertility clinic-based studies have demonstrated statistically significant AMH-AFC relationships, and ART cohorts have also demonstrated both AMH and AFC to be associated with ovarian response (e.g. oocyte yield), with clinical outcomes, such as pregnancy, perhaps still inconsistent.[10, 11]

Conversely, the recent literature is increasingly pointing to the fact that the AMH-AFC relationship may be weakened or non-significant relative to analytical and technical considerations especially assay-assay variability in AMH measures, operator bias in AFC counts, and the effects of clinical heterogeneity. Analytical performance measurement studies are even

in which both measures have been extensively applied in practice.[12] and in which the reproducibility of measurement in the real world points out that variations in the method of measurement can alter the observed relationship and its clinical inference.[13]

Our non-significant results might thus be attributed to a number of plausible causes: (i) biological variability in AMH secretion among women with comparable follicle counts, (ii) ultrasound-related variability in AFC measure (in particular, when follicles cluster together, when image quality is not uniform), and (iii) a comparatively broad infertility mix in which the underlying causes (other than the major confounders we excluded) might have a direct effect on AMH irrespective of the level of AFC. Notably, recent histological validation experiments continue to find AMH and AFC to be significant biological indicators of the primordial follicle pool[14, 15], but clinical correlation strength in typical infertility clinical practice may not always be strong, as would be predicted by the lack of an evident linear trend in our scatter plot.[16, 17]

Clinically, the findings indicate that AMH cannot be adopted as a substitute surrogate of AFC in this group; instead, assessment of ovarian reserve should be multimodal, including clinical profile, ultrasound, and hormonal analysis. Future research in comparable Pakistani infertility populations would be well advised to include bigger multi-center groups, standardized AMH assays, structured protocols of AFC counting (including image archiving) and stratified results on age categories and infertility phenotype to elucidate whether particular subgroups demonstrate a more robust AMH-AFC fit.[18-20]

Limitations: This study has limitations. Causal inferences are restricted by the cross-sectional design, and single-center sampling can decrease their external validity. Despite the standardizations of measurements to the early follicular phase and the reduction of inter-observer variance, AFC is operator-dependent by nature and AMH values can change based on the assay platform that can affect observed associations. Also, unmeasured clinical variables (e.g. minor endocrine differences, lifestyle, or subclinical ovarian pathology) can also help explain residual variability in AMH and AFC.

Recommendations: It is suggested that when assessing the ovarian reserve in the infertility practice, a combination of AMH-based, AFC-based and other appropriate clinical parameters should be applied instead of using one specific marker. Future studies could employ standardized AMH assays and established protocols of AFC measurement and sub-analysis by age group and infertility diagnosis to obtain multi center Pakistani studies using larger samples. The reproducibility can be further enhanced by the incorporation of repeat AFC measurements, recording ultrasound images to ensure quality assurance, and the true biological relationships can be made clear.

Conclusion

In this study of infertile women assessed during the early follicular phase, serum AMH did not demonstrate a significant correlation with total AFC or ovarian volume, and age was not significantly associated with AMH or AFC. These findings indicate that AMH alone may not reliably predict ultrasound-based ovarian reserve markers in this setting. A comprehensive assessment combining hormonal and ultrasonographic parameters is recommended for clinical evaluation of ovarian reserve.

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