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RESEARCH ARTICLE

A STUDY TO CORRELATE SERUM ANTI MULLERIAN HORMONE, BASAL FOLLICLE STIMULATING HORMONE AND ANTRAL FOLLICLE COUNT IN PRIMARY INFERTILITY AS A MEASURE OF OVARIAN RESERVE

Dr. E. Ramadevi, Dr. P. Neeraja, Dr. B. Sweethi and Dr. A. Vandana
Department Of Obstetrics And Gynaecology, Caims, Karimnagar.

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Abstract

Objectives: This study aims to find the correlation between anti mullerian hormone (AMH) and follicle stimulating hormone (FSH) and to observe the above mentioned hormones' relation with antral follicle count (AFC) in patients with primary infertility.

Methodology: This is a cross-sectional correlation study in which 60 patients with primary infertility meeting inclusion criteria, attending infertility clinic in Chalmeda Ananda Rao Institute Of Medical Sciences, Karimnagar, between October 2020and April 2022 were enrolled by simple random sampling. Detailed menstrual, obstetric, coital and medical history was obtained. On the third day of the spontaneous cycle, all patients were investigated with a transvaginal scan to assess the number of antral follicles and a fasting venous blood sample was obtained for the measurement of serum AMH and serum basal FSH level.

Results: Basal serum FSH shows a moderately strong negative correlation with antral follicle count (AFC) (r=0.65; p=<0.001); and a strong negative correlation with anti mullerian hormone (AMH) (r=0.69 and p=<0.001). However, the strongest correlation between a biochemical marker and biophysical marker of ovarian reserve is between anti mullerian hormone (AMH) and antral follicle count (AFC) with a very strong positive correlation with a correlation co-efficient r=0.89 (p=<0.001).

Conclusion: Serum AMH best correlates with the antral follicle count. Antral follicle counts although an efficient test to detect ovarian reserve is uncomfortable for the patient as it has to be done during menstrual flow. Serum AMH with minimal intracycle and intercycle variation is a more convenient marker to assess ovarian reserve while it maintains the accuracy of AFC.

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Introduction:-

Infertility refers to the inability of a woman to become pregnant after having unprotected coitus for a specified amount of time, usually, a year(1). One of the most important factors contributing to infertility is the quantity and quality of the ovarian reserve. Traditionally, age, follicle stimulating hormone (FSH), estradiol (E2) levels and antral

Corresponding Author:- Dr. A. Vandana
Address:- Department Of Obstetrics And Gynaecology, Caims, Karimnagar.

follicle count (AFC) by ultrasound investigation at the early follicular phase have been used for evaluation of ovarian reserve.

For years the levels of FSH and E2 were considered to be determining biochemical markers for assessment of low ovarian reserve. However, it has been found that the FSH level is above the norm only in cases when the ovary function is largely decreased (2). Later stage identification of the AFC is considered to be more reliable in assessment of the ovarian reserve.Follicle count can be determined easily with the help of high resolution sonographic systems (3-5). Although, there are well-known difficulties in obtaining correct AFC such as high inter-observer differences and anatomical variations. It has been suggested that AFC predicts poor response much better than basal FSH (4). Thus, by some investigators AFC is considered as the first choice test (3,6).

Recently, identification of anti-Mullerian hormone (AMH) levels became important in assessment of ovarian reserve. AMH, also known as Mullerian-inhibiting substance, is a dimeric glycoprotein that belongs to the transforming growth factor – β family (7, 8). In reproductive-aged women AMH is expressed by small antral follicles. It is manifested by granulosa cells of the ovary (9). In the ovary AMH inhibits initial primordial follicle recruitment and decreases the sensitivity of preantral and small antral follicles to FSH (10). In comparison with other ovarian reserve assessment tests AMH is characterized by a number of advantages. AMH levels are stable throughout the menstrual cycle and therefore can be measured at any day of the cycle (8, 11). AMH levels are not affected by other hormonal variations, including the use of oral contraceptives (12). However, a recent study by Bentzen et al. has indicated that ovarian reserve markers are lower in women who use sex steroids for contraception. Thus, AMH concentration and AFC may not retain their accuracy as predictors of ovarian reserve in women who use hormonal contraception (13). AMH is not detected in women until puberty and reaches its highest levels at age 24.5 years (14). With increasing age, the number and quality of oocytes decline. Accordingly, the AMH level also declines and is lowest at menopause; later, it is not detected at all (12).

This study aims to find a correlation between biochemical (hormonal) markers of ovarian reserve such as serum AMH, and basal serum FSH with the biophysical marker of ovarian reserve i.e antral follicle count to better assess the patients with primary infertility.

Materials And Methods:-

This is a cross-sectional correlation study involving 60 patients with primary infertility attending the infertility clinic in Chalmeda Ananda Rao Institute Of Medical Sciences, Karimnagar, between October 2020and April 2022. The selection was done by simple random sampling.

Inclusion Criteria:
-Age group of 25-40 years
-With regular menstrual cycles of 21-35 days
-With BMI of <25kg/m^2

Exclusion Criteria:
-Patients with PCOS,
-Abnormal uterine bleeding,
-Evidence of endocrine disorders and
-Current or past diseases affecting the ovaries

The patients were evaluated on an outpatient basis after obtaining detailed menstrual, obstetric, coital and medical history. On the third day of the spontaneous cycle, all patients were investigated with a transvaginal scan by the same investigator to assess the number of antral follicles. All follicles measuring 2-10mm size were counted in both ovaries and "Antral Follicle Count" was obtained. On the same day, a fasting venous blood sample was obtained for the measurement of serum AMH and serum basal FSH level. Serum levels of AMH were determined by enzyme linked immune sorbent assay. FSH measurement was done by standard techniques in the laboratory (Beckman coulter by chemi-luminescence immune assay). The correlation between parameters was analysed.

Statistical analysis:
Descriptive analysis of variable data is expressed as mean and standard deviation (SD). Statistical analysis was done using SPSS software. The relationship between two different continuous variables was assessed by Pearson

correlation. The Fisher r to z-test is used to determine if the coefficient of correlation (r) is significantly different from 0. A p value of less than 0.05 was considered as statistically significant.

Results:-

In the 60 patients involved in the study group 28 were below 30yrs of age and 32 were above 30 s of age, with mean age of 31.2kg and with themean BMI of 22.2kg/m^2 (table 1).

Table 1:- Average age and BMI of study participants (N=60).

Features	Mean (SD)
Age in years	31.2(3.9)
BMI in Kg/m^2	22.2 (1.8)

The average mean hormonal levels and standard deviation of the hormonal levels in our study population is as mentioned in table - 2. The mean and SD of anti mullerian hormone level in patients above 30 years of age was 2.49ng/mL and 1.4ng/mL which was significantly less (p - 0.001) than in patients ≤30 years with a mean hormonal level of 3.82ng/mL and a SD of 1.5ng/mL respectively. It moderately strong negative correlation with age, thus indicating a gradual fall in AMH with advancing age (table 3).

Table 2:- Average hormone levels, ovarian volume and antral follicle count of study participants (N=60).

Hormones	Age group in mean (SD)		P value#
	≤30 years	≥30 years	
AMH(ng/mL)	3.82 (1.5)	2.49 (1.4)	0.001*
FSH(mIU/mL)	8.18 (3.2)	11.26 (3.7)	0.002*
LH(mIU/mL)	6.39 (3.6)	6.25(2.2)	0.88
Prolactin(ng/mL)	1.46(4.9)	12.11(3.5)	0.03*
Antral follicular count	11.28(4.2)	8.73(3.6)	0.01*
Ovarian volume (cc)	6.94(1.3)	7.44(1.5)	0.20

Note: # p value based on independent sample t test, * statistically significant (p<0.05)

The mean and SD of follicle stimulating hormone level inpatients above 30years of age was 11.26mIU/mL and 3.7mIU/mL which was significantly more (p - 0.002) than in patients ≤30years with a mean hormonal level of 8.18mIU/mL and a SD of 3.2mIU/mL respectively. It moderately strong positive correlation with age, thus indicating a rise in FSH with advancing age (table 3).

Table 3:- Correlation between various hormones related to infertility with age.

Correlating factors	Correlation factor (r)	Direction	P value
AMH vs Age	0.66	Negative	<0.001*
AFC vs Age	0.55	Negative	<0.001*
FSH vs Age	0.57	Positive	<0.001*

Note: P value based on Pearson correlation, * Statistically significant (p<0.05)

The antral follicle count on day 3 of menstrual cycle showed a significant fall in number with the advancing age. The mean (SD) of the two groups being 11.28(4.2) and 8.73(3.6) respectively. The decrease in AFC is statistically significant with the p value of 0.01. It moderately strong negative correlation with age, thus indicates a decrease in ovarian reserve with advancing age (table 3).

Further, results obtained from 60 patients with primary infertility were correlated for AMH, AFC, and basal FSH by Pearson correlation and correlation coefficient represented by "r". Serum AMH showed a strong negative correlation with serum basal FSH, with a correlation coefficient (r) of 0.68 and a p-value of <0.001. AFC showed a strong negative correlation with basal serum FSH, with a correlation coefficient (r) of 0.66 and a p-value of <0.001. AMH shows a very strong positive correlation with AFC, with a correlation coefficient (r) of 0.88 and a p-value of <0.001(table 4) thus being strongest of the correlation between the biochemical and biophysical markers of ovarian reserve.

Table 4:- Correlation of various factors indicating ovarian reserve along with strength of correlation and direction.

Correlating factors	Correlation coefficient	Direction and strength of correlation
AMH vs FSH	0.68	Strong negative
AFC vs AMH	0.88	Very strong positive
AFC vs FSH	0.66	Strong negative

Discussion:-

Correlating factors	Correlation coefficient		
	Ludmila Barbakadze et al 2015	Bala et al 2014	Present study
AMH vs FSH	0.41	0.448	0.68
AFC vs AMH	0.71	0.641	0.88
AFC vs FSH	0.48	0.174	0.66

FSH and AMH are individually, widely used to assess functional ovarian reserve. At younger ages, abnormally elevated FSH levels have lower significance in the presence of good AMH levels, whereas in older women, especially those older than age 42years, AMH loses specificity in the presence of still decent FSH levels(15). Hence we tried to find the correlation between serum FSH and AMH on the second day of the menstrual cycle to better understand the hormonal interplay involved in maintaining fertility. Our study showed a moderately strong negative correlation between the two hormones. Previous studies done by Bala et al in 2014 and Ludmila Barbakadze et al in 2015 also showed a moderately strong negative correlation between the two hormones. However, we achieved a much tighter negative correlation compared to the above two studies(16,17). Correlating serum basal FSH, one of the prominent biochemical (hormonal) marker of ovarian reserve with antral follicle count being the biophysical (measured by TVS) marker of ovarian reserve, we found a moderately strong negative correlation between the two. This result obtained in our study goes along with the study conducted by Ludmila Barbakadze et al in 2015 who also obtained a moderately strong correlation between the two ovarian reserve variables(18)However study conducted by Göksedef et al, only found a moderate negative correlation between FSH and AFC and Bala et al, did not find a statistically significant correlation between the two variables(17,19). AFC is one of the best biophysical markers of ovarian reserve, measured by transvaginal ultrasonography. It is considered the "test of the first choice" by some investigators for the assessment of ovarian reserve.(3,6) AMH, on the other hand, is a potential new test for the assessment of ovarian reserve. With its minimal intra and intercycle variation, it acts as one of the prominent biochemical (hormonal) tools in the assessment of ovarian reserve. Thus the correlation between these two parameters can help in better assessment of patients with infertility. In our study, we found a very strong positive correlation between the two markers. The results obtained in the study conducted by Ludmila Barbakadze et al is in support of our study, where the correlation between the two markers was also a very strong positive one(18). However studies conducted by Göksedef et al and Bala et al, derived a moderately strong positive correlation between these two novel markers of ovarian reserve. Finally, in our study, we found that the AMH and AFC had a much tighter correlation when compared to the correlation between other markers of ovarian reserve. Serum AMH best correlates with antral follicle count and with its minimal intracycle and intercycle variation can be considered the best biochemical marker to assess changes occurring in ovarian function over time (i.e. reproductive aging).

Conclusion:-

In our study serum AMH best correlated with antral follicle count. Antral follicle count although an efficient test to detect ovarian reserve is uncomfortable for the patient as it has to be done during menstrual flow. Serum AMH with minimal intracycle and intercycle variation is a more convenient and accurate marker to assess ovarian reserve.

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