Original Research Paper

Gynaecology

CORRELATION OF SERUM ANTI-MULLERIAN HORMONE WITH ANTRAL FOLLICLE COUNT IN WOMEN WITH INFERTILITY

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ABSTRACT

Aim- To find out the correlation between AMH and AFC in women with Infertility.

Materials and Method- This study was conducted at AVBRH, Sawangi on 100 women fulfilling the inclusion criteria between the age group of 20-45 years undergoing IVF. On day 2 of menstrual cycle, AMH was measured by ELISA kit and AFC was calculated using with 7.5 MHz Transvaginal USG probe to find correlation between these two biomarkers.

Result- The mean AMH calculated was 3.53±2.46 ng/ml while the mean AFC estimated was 8.29±3.06. There was a significant negative correlation of age with AMH (r = -0.287; p-value=0.004) and AFC (r= -0.363; p-value=0.0001). There was a significant positive correlation found between AMH and AFC(r= 0.816; p-value-0.0001).

Conclusion- There is a positive correlation between serum AMH and AFC.

KEYWORDS : Anti-mullerian Hormone, Antral Follicle Count, Ovarian Reserve,

INTRODUCTION

Infertility is defined as the inability of a woman to conceive even after 1 year of regular unprotected intercourse or after 6 months in women with > 35 years of age.

Approximately 10% of the married couples in India are facing this problem. For this both male and female factors are equally responsible. Apart from other basic investigations assessment of ovarian reserve is also important to determine the strategy for treatment of female infertility.[1]

Initially, follicle stimulating hormone and Estradiol were used to assess ovarian reserve.[1] Later on, after the identification of AFC,it was 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.[2-4] It has been suggested that AFC can predict poor ovarian response much better than basal FSH.[3] Thus, by some investigators AFC is considered as the first choice test.[2,5]

Recently, identification of (AMH) levels has become important in assessment of ovarian reserve. AMH is also known as Mullerian-inhibiting substance which is a dimeric glycoprotein that belongs to the transforming growth factor – ß family.[6, 7] In reproductive-aged women AMH is released by small antral follicles. It is released by granulosa cells of the ovary.[8] In the ovary AMH inhibits initial primordial follicle recruitment and decreases the sensitivity of pre-antral and small antral follicles to FSH.[9]

LOW	NORMAL RANGE	HIGH
<1 ng /ml	1.0-4.0 ng/ml	>4 ng/ml

Fig-1: Normal AMH levels according to ages

Age	Unit
Younger than 24 months	Less than 5 ng/mL
24 months to 12 years	Less than 10 ng/mL
13–45 years	1 to 10 ng/mL
More than 45 years	Less than 1 ng/mL

AFC -The antral follicle count is the number of antral follicles measuring 2-10 mm in both ovaries taken together, if not else specified. It can be determined by transvaginal USG.AFC is used in routine to monitor the ovarian responses.

NORMAL RANGE OF AFC :-

Depending on the number of antral follicles, a woman is considered to have an adequate ovarian reserve if the count is between 6-10. Low ovarian reserve, if the count is less than 6 and high ovarian reserve if the count is greater than 12. Follicular size appropriate for AFC is between 2 to 10 mm.[10]

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 levels and AFC may not retain their accuracy as predictors of ovarian reserve in women who use hormonal contraceptives.[11]

This study is to identify the correlation between current tests which are used in ovarian reserve assessment i.e. AMH and AFC in different age groups of infertile woman and to determine whether they are correlated in terms of number of oocytes retrieved in order to select an appropriate strategy for the initial stages of infertility treatment.

OVARIAN RESERVE- Formulation of ovulation induction protocol is an important aspect of fertility management. Determination of ovarian reserve gives some idea about prediction of successful outcome. The total number of oocytes and their quality at any given time present in the ovary is known as the ovarian reserve.[20] Ovarian reserve varies according to genetic variability and environmental factors which leads to differences in follicle density amongst individuals and also the number of oocytes at birth.[21]This is due to the different mitotic rates of germ cells in the fetal ovary and also by different rates of follicle depletion .

Fig-2: Number of oocytes with advancing age.

AGE	PHYSIOLOGICAL	NUMBER OF
3-6 weeks of gestation	Endoderm of yolk sac	10,000
8 weeks	Proliferation by mitosis	6,00,000
8-20 weeks	Mitosis, meiosis, atresia	6-7,000,000
20-40 weeks	80% loss	1-2,000,000
Birth to puberty	Loss to atresia	3,00,000
Reproductive years	Ovulation	400-500

In the present study, we have taken serum AMH and AFC to assess the ovarian reserve and their correlation with each other.

MATERIALS AND METHOD :-

This study was conducted at Acharaya Vinoba Bhave Rural Hospital, Sawangi (Meghe), Wardha from July 2016 to June 2018 on women seeking treatment for infertility. It is a prospective observational study. In this study the patients were recruited from Infertility OPD. 100 women were selected for study who met inclusion and exclusion criteria for the study. The inclusion criteria-willing to give consent, with both the ovaries intact, up to 45 years of age. The exclusion criteria-Abnormal uterine bleeding with hormonal disorders like deranged levels of Androstendione, Testosterone, Prolactin , difficult visualization of ovaries through Transvaginal USG or any previous ovarian surgery. The study was approved by Institutional Ethical Committee.

After taking consent, detailed history was taken and on Day-2 of menstrual cycle measurement of serum AMH levels were performed using the MIS/AMH Robonik enzyme-linked immunosorbent assay (ELISA) kit in machine. On same day i.e. Day 2, these selected patients went through a transvaginal scan by (N.I) using a Hitachi Aloka f-31 USG MACHINE.A 7.5MHZ transvaginal ultrasound probe was used to assess the number of antral follicles, measuring 2–6 mm. The correlation of Day 2 AMH and AFC was calculated. Statistical analysis was done by using descriptive and inferential statistics using Pearson's correlation coefficient, student's unpaired T-test and software used in the analysis were SPSS 22.0 version and Graph Pad Prism 6.0 version and p<0.05 is considered as level of significance. The Fisher r to z-test was used to determine the coefficient of correlation (r) which is significantly different from zero. A p < 0.05 was considered as statistically significant.

OBSERVATIONS AND RESULTS

A total of 100 women fulfilling the criteria were taken in this study and S. AMH was assessed on Day 2 of menses along with AFC.

TABLE 1: Distribution of women according to age:-

Age Group(years)	No. of women	Percentage (%)
20-25	18	18
26-30	44	44
31-35	25	25
36-40	10	10
41-45	3	3
Total	100	100
Mean±SD	29.87±4.82 (22-45 years)	

TABLE 2: Distribution of women according to BMI (kg/m2):-

BMI(kg/m2)	No of women	Percentage (%)
Less than 18.5	2	2
18.5-24.9	87	87
25-29.9	11	11
More than 30	0	0
Total	100	100
Mean ± SD	22.67±1.72(18.23-28.04 kg/m2)	

TABLE 3: Distribution of women according to type of Infertility:-

Type of infertility	No of women	Percentage(%)
Primary	83	83
Secondary	17	17
Total	100	100

TABLE 4: Distribution of women according to AMH level :-

AMH level (ng/ml)	No of women	Percentage (%)
0 to 3	51	51
3.1 to 6	37	37
6.1 to 9	7	7
9.1 to 12	3	3
12.1 to 15	2	2
Total	100	100
Mean ± SD	3.53±2.46(0.20-12.94)	

TABLE 5: Distribution of women according to Antral Follicle Count:-

AFC	No of women	Percentage (%)
0 to 5	14	14
6 to 10	63	63
11 to 15	22	22
16 to 20	1	1
Total	100	100
Mean±SD	8.29±3.06(1-17)	

TABLE 6: Correlation of age with AMH and AFC level :-

Parameters	Correlation 'r'	p-value
Age	-	-
AMH	-0.287	0.004,S
AFC	-0.363	0.0001,S

TABLE 7: Correlation between AMH level and AFC :-

Ovarian Reserve markers	Mean	Std. Deviation	N	Correlation 'r'	p-value
AMH	3.53	2.46	100	0.816	0.0001,S
AFC	8.29	3.06	100		

Table 7. shows correlation of AMH level with AFC in which mean is 3.53 for AMH and 8.29 for AFC. While SD are for AMH 2.46 & AFC 3.06. Their Correlation 'r' is 0.816 & p-value is 0.0001 which is <0.05, therefore it is significant in this study. In this study AMH is positively correlated with AFC .

RESULTS :- The distribution of women according to their age. 44% of women belonged to 26-30 years of age, while mean age with SD in this group is 29.87±4.82 years. Primary infertility around 83% of total patients attending infertility OPD. 17% belonged to secondary infertility. According to BMI 87% of total patients fall within normal range in our study, 2 % were underweight and 11% of women were obese. The mean BMI calculated was 22.67±1.72 kg/m^2 which was taken within the range of 18.23-28.04 kg/m^2. In this study we found 51% patients had AMH less than 3 ng/ml while the mean with SD was 3.53±2.46 ng/ml. In this study 63% patients had AFC 6-10 with mean and SD of 8.29±3.06 which means the average AFC in these patients was with in normal range. The correlation of age with AMH and AFC in this study was evaluated and the p-value & correlation 'r' for AMH and AFC was found respectively as p=0.004;r= -0.287 and p=0.0001; r= -0.363 of which p-value was significant as < 0.005 depicting there is a negative correlation of age with AMH and AFC. The correlation between serum AMH and AFC in this correlation, the p- value is 0.0001 while correlation 'r' evaluated here is 0.816 which is positive hence there is a positive correlation between Serum AMH and AFC.

DISCUSSION - The results of this study shows mean age with SD in this study was 29.57±4.99 years According to BMI 87% of total patients fall within normal range in our study, 2 % were underweight and 11% of women were obese. The mean with SD calculated was 22.67±1.72 kg/m^2 **.** In our study maximum women were of primary infertility around 83% of total patients while 17% belonged to secondary infertility . In this study we found 51% patients had AMH less than 3 ng/ml and the mean with SD was 3.53±2.46 ng/ml which can be compared with the study done by there is another study done by Verma et al[12] in 2016 in which Mean AMH with SD at different age 20-25,26-30, 31-35 and >35 years were 2.81±2.31, 3.55±3.50 3.52±3.39 and 1.94±1.86 which is comparable to present study. In this study 63% patients had AFC 6-10 with mean and SD of 8.29±3.06 which means the average AFC in these patients was with in normal range which can be compared with another study done by Study done by Himabindu et al in 2013 [13] taking 56 patients between 25 to 42 years found mean AFC with SD as mean AFC was 8.57 ± 5.16 which is comparable to this study. The correlation of age with AMH and AFC was found. The p-value for AMH and AFC as 0.004 and 0.0001 The correlation value 'r' for AMH and AFC was -0.287 and -0.363 out of which there is a negative correlation of age with AMH and AFC. The mean with SD is 3.53±2.46 for AMH and 8.29 ± 3.06 for AFC. Their Correlation 'r' is 0.816 & p-value is 0.0001.p-value<0.05 is considered as significant in this study. Therefore AMH is positively correlated with AFC. Another study done by Bala et al [14] in 2014 also found that there was a positive correlation between serum AMH and AFC as the p-value and correlation 'r' were <0.001 and 0.641 which shows their positive correlation. Another study done by Hansen et al [15] in 2011 came to the conclusion that there was a positive correlation between AMH and AFC. The p-value and correlation 'r' in this study were<0001 and 0.75 which shows their positive correlation. As all the above studies and the results obtained in present study, it was concluded that there is a positive correlation between serum AMH and AFC.

CONCLUSION- There is a positive correlation between Serum AMH and AFC in women with infertility . Both AMH and AFC decrease with advancing age.

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