Original Article

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Anti-Mullerian Hormone: A Marker of Ovarian Reserve and its Association with Polycystic Ovarian Syndrome

Obstetrics and Gynaecology Section

ANIL KUMAR VERMA[1], SARITA RAJBHAR[2], JYOTI MISHRA[3], MAYANK GUPTA[4], MRATUNJAI SHARMA[5], GEETA DESHMUKH6, WAHID ALI[7]

ABSTRACT

Introduction: Anti-Mullerian Hormone (AMH) is a useful endocrine marker for assessing the ovarian reserve. AMH serum level reflects the number of follicles that have made the transition from the primordial pool into the growing follicle pool, and it is not controlled by gonadotropins.

Aim: The present study was conducted to correlate serum AMH levels with Polycystic Ovarian Syndrome (PCOS) and type of treatment protocol.

Materials and Methods: Serum AMH levels were performed in the early follicular phase (on 2nd day of menstrual cycle) both in infertile females including PCOS and control women. The results were analyzed in relation to age, Body Mass Index (BMI), ovarian volume, serum Follicle Stimulating Hormone (FSH) levels, Antral Follicle Count (AFC), type of treatment protocols and also in association with PCOS patients. The serum levels of AMH were measured in all the participants on 2nd day of menstrual cycle using ultra sensitive Enzyme Linked Immunosorbent Assay (ELISA).

Results: The plasma AMH levels were significantly higher in women with polycystic ovarian syndrome. The significant association was seen between FSH and AFC with AMH. However, no significant association was observed between AMH levels with age, BMI, ovarian volume and type of treatment protocols.

Conclusion: The serum AMH measurement was significantly higher in PCOS patients. No association with type of treatment protocol was obtained.

Keywords: Follicle stimulating hormone, Infertility, polycystic ovarian syndrome

INTRODUCTION

Infertility clinics have been facing the challenge to determine the degree of ovarian reserve so that treatment can be implemented effectively and wisely. There are several methods of evaluating ovarian reserve (size of ovarian follicle pool and remaining time left to conceive) such as elevated serum Follicle Stimulating Hormone (FSH), low ovarian volume, an Antral Follicle Count (AFC) Anti-Mullerian Hormone (AMH) of <5 per ovary, low inhibin B levels and serum AMH levels [1]. AMH, a member of the transforming growth factor β family is a novel marker for predicting ovarian response. It has an inhibitory effect on the primordial follicular recruitment in the ovary and on the responsiveness of the growing follicles to the FSH; thus it is important in patients with polycystic ovary syndrome [2,3]. AMH serum levels are not controlled by gonadotropins [4,5]. During infancy AMH levels increase, whereas during adolescence, a plateau until the age of 25 year is observed. From the age of 25 year onward, the serum AMH levels correlate inversely with age, implying that AMH is applicable as a marker of ovarian reserve only in women of 25-year-old and older. Thus the total number of ovarian follicles is determined early in life, and the depletion of this pool leads to reproductive senescence [6,7]. The serum AMH levels are not controlled by hypothalamic-pituitary-gonadal axis which makes it a useful marker in diagnosing conditions such as Polycystic Ovarian Syndrome (PCOS) and premature ovarian failure [8]. Furthermore serum AMH could also indicate the presence of underlying PCOS in those cases in whom TVS is not possible because of either non acceptance or some psycho-social issue [9].

We conducted this study for assessing the effect of serum AMH levels on infertility treatment outcome and compared our findings with some of the studies previously done from India and other countries.

MATERIALS AND METHODS

This cross-sectional study was conducted in Department of Obstetrics and Gynaecology, King George's Medical University (KGMU), Lucknow, for one year (May 2012-April 2013).

Inclusion Criteria

a. All female patients (n=150) who visited infertility clinics of Obstetrics and Gynaecology Department were considered as cases.

b. Twenty uniparous or multiparous females in reproductive age group with no complaints of infertility were considered as controls.

Exclusion Criteria

c. All the females who did not give consent for participation in study.

d. Those patients who were lost for follow-up (n=10).

Serum levels of AMH were measured in all the participants on 2nd day of menstrual cycle using ultra sensitive enzyme linked immunosorbent assay AMH Gen II ELISA Kit *(Beckman Coulter)*. These cases were followed up for the period of one year. The occurrence of conception was confirmed either by ultrasonography or by urine pregnancy card test. We extracted the patient's sociodemographic data, clinical history (e.g., hirsutism, oligomenorrhea, and amenorrhea), anthropometric measurements and other relevant data from information given by patients in clinical questionnaire form, case sheet notes.

STATISTICAL ANALYSIS

SPSS 16 software (SPSS Inc. Chicago IL) was used for data collection and analysis, t-test, Mann-Whitney U-test was done for

quantity data. The 2 test or Fisher-exact tests were used for the categorical or dichotomous variables. Pearson test for correlation was used. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

A total of 170 females (150 cases and 20 controls) were enrolled in the study (only 20 controls could be obtained limited by the study time line). Out of which 10 cases were excluded as they were lost for follow-up, 140 cases and 20 controls were included in the study. The mean age range ± S.D. (Standard Division) was 29.37 ± 4.76 years in cases group and 29.30 ± 4.35 years in control group. There were 102(72.9%) cases of primary infertility and 38 (27.1%) cases of secondary infertility. Twenty two (15.7%) cases had polycystic ovarian syndrome. All the cases underwent infertility treatment as per guidelines of infertility unit of our hospital. The ovulation induction was performed in 122 cases while in 18 cases who did not conceive after two cycles of ovulation induction underwent intrauterine insemination was done [Table/Fig-1].

The mean AMH was maximum in age group 26-30yr (3.55±3.50ng /ml) and minimum in women more than 35year of age (1.94±1.86 ng/ml). However, this association was not found to be statistically significant (p-value= 0.615). AMH levels were found to be significantly higher in the cases diagnosed as polycystic ovarian syndrome (6.90±5.09ng/ml) in comparison non PCOD cases of infertility (1.41±0.90ng/ml) and controls (2.15±1.86ng/ml). These findings were statistically significant (p<0.001). An inverse correlation was obtained between basal FSH value and serum AMH levels which was statistically significant (p=0.018). The mean AMH levels were found to be higher (7.1±3.73ng/ml) in cases with basal FSH value <5.56 mIU/ml. The AFC was available in 26 of our cases. There were significantly higher AMH levels (6.90±5.09) in cases with AFC count >3 (p=0.011). The ovarian volume was available in 102 cases. Though the mean AMH level (3.58±3.49 ng/ml) were higher among those with ovarian volume ≤ 7cc however, data was not found to be statistically significant (p=0.886). AMH levels in underweight (n=12), normal weight (n=92), overweight (n=28) and obese (n=8) were in the range of (1.47±1.24), (3.33±3.36), (3.95±2.77), (1.85±0.62) ng/ml respectively. Hence no linear association was obtained in between serum AMH levels and body mass index (BMI) with p-value= 0.312. No significant correlation of AMH level with type of infertility; primary (3.54±3.31 ng/ml) and secondary (2.33±2.13ng/ml) was found (p-value≥ 0.144). Sixteen cases with plasma AMH levels of (3.06±1.41ng/ml) conceived while 124 cases with the levels (3.23±3.23ng/ml) did not conceive in the study period of one year [Table/Fig-2].

S. No.	Treatment Protocol	No. of cases
1.	Ovulation induction only	122 (87.1%)
2.	Ovulation induction + intrauterine insemination	18 (12.9%)

[Table/Fig-1]: Distribution of cases according to type of treatment protocol.

Parameters	Groups	N	Mean AMH ± S.D. (ng /ml)	p-value
Age (Years)	20-25	36	2.81±2.31	0.615
	26-30	54	3.55±3.50	
	31-35	38	3.52±3.39	
	>35	12	1.94±1.86	
BMI	Underweight	12	1.47±1.24	0.312
	Normal weight	92	3.33±3.36	
	Overweight	28	3.95±2.77	
	Obese	8	1.85±0.62	
Type of infertility	Primary	102	3.54±3.31	0.144
	Secondary	38	2.33±2.13	
Type of treatment protocol	Ovulation induction only	122	3.29±3.15	0.582
	Ovulation induction + intrauterine insemination	18	2.67±2.49	
Ovarian Volume	≤ 7cc	52	3.58±3.49	0.886
	≥7cc	50	3.45±3.20	
FSH(IU/ml)	≤ 5.56	22	7.18±3.73	0.018
	≥5.56	20	3.50±2.61	
AFC	≤ 3	16	1.41±0.90	0.011
	≥3	10	6.90±5.09	
PCOD	Absent	108	1.41±0.90	<0.001
	Present	22	6.90±5.09	
Outcome in follow-up of one year study period	Conceived	16	3.06± 1.41	0.887
	Not Conceived	124	3.23±3.23	

[Table/Fig-2]: Association of AMH with various parameters
Footnotes: N-Number of cases

DISCUSSION

The serum AMH levels gradually decreases from reproductive age group to the undetectable level at menopause. In our study the mean serum AMH level was maximum in age group upto 26-30 years and decreased levels were seen in higher age group. The study conducted by Sergio Parco et al., showed inverse correlation between AMH levels and age [8]. Similarly, two other independent studies conducted by Franks et al., and Rotterdam et al., showed that from 25 years onwards, AMH level gradually declined to undetectable level at menopause [10,11]. In our study, serum AMH levels declined after 30 years.

AMH levels were found to be significantly higher in the cases diagnosed as polycystic ovarian syndrome in comparison to other participants of our study (p<0.001). These findings were similar to the study by Sergio parco et al., who reported very high AMH levels (10.0±2.28 ng/ml) in PCOS patients [8]. Dumont et al., suggested that adolescents with PCOS have higher serum AMH levels [12]. Psaikumar et al., also found that serum AMH level are three-four fold higher in PCOS patients and it is a marker of recruited non growing follicles [13]. Zadehmodarres S et al., concluded that AMH with cut off level of 3.15 ng/ml with sensitivity 70.37% and specificity 77.36% could use for early diagnosis of PCOS patients [14].

In our study, mean AMH levels were found to be significantly higher in cases with basal FSH value <5.56 mIU/ml (p=0.018).These findings were in agreement to studies conducted by Sergio parco et al., and Fanchin R et al., who also observed inverse correlation between serum AMH and basal FSH levels [8,15].

The AFC was available in 26 of our cases. There were significantly higher AMH levels (6.90±5.09) in cases with AFC count >3(p=0.011). Kunt C et al., included AFC of 180 patients and found direct correlation between AFC and serum AMH levels. The poor responders had significantly lower AFC [16]. Similarly Nardo LG et al., also suggested direct correlation between AMH and AFC. The findings of these studies were consistent with findings of our study [17].

In our study, the ovarian volume was available in 102 cases. Though the mean AMH level (3.58±3.49 ng/ml) were higher among those with ovarian volume ≤ 7cc however, data was not found to be statistically significant (p=0.886). No studies determining the correlation between AMH levels and ovarian volume have been found in English literature, so far.

In our study no linear association was obtained in between serum AMH levels and Body Mass Index (BMI). The study conducted by Buyuk et al., showed that low serum AMH levels in overweight and obese women [18].

In our study no significant correlation of AMH level with type of infertility (primary /secondary) was found (p-value 0.144). Sixteen cases with plasma AMH levels of (3.06±1.41 ng/ml) conceived while 124 cases with the levels (3.23±3.23ng/ml) did not conceive in the study period of one year. These findings were not statistically significant (p= 0.887).

LIMITATION

The follow-up period was only one year. The cases were not followed further. The other limitation was that we had only 20 controls.

CONCLUSION

The plasma AMH levels were significantly higher in women with PCOS. The significant association was seen between FSH and AFC with AMH. However, no significant association was observed between AMH levels with age, BMI, ovarian volume and type of treatment protocols. Thus, the serum AMH measurement can be used as a marker of ovarian dysfunction such as primary ovarian insufficiency and PCOS.

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PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Pathology, School of Medical Sciences and Research, Sharda Hospital, Greater Noida, Uttar Pradesh, India.
2. Senior Resident, Department of Obstetrics and Gynaecology, PGIMER and Dr Ram Manohar Lohia Hospital, New Delhi, India.
3. Assistant Professor, Department of Pathology, School of Medical Sciences and Research, Sharda Hospital, Greater Noida, Uttar Pradesh, India.
4. Senior Resident, Department of Radiology, PGIMER and Dr Ram Manohar Lohia Hospital, New Delhi, India.
5. Assistant Professor, Department of Pathology, School of Medical Sciences and Research, Sharda Hospital, Greater Noida, Uttar Pradesh, India.
6. Professor, Department of Pathology, School of Medical Sciences and Research, Sharda Hospital, Greater Noida, Uttar Pradesh, India.
7. Associate Professor, Department of Pathology, King George's Medical University, Lucknow, Uttar Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Jyoti Mishra,
Department of Pathology, School of Medical Sciences and Research, Greater Noida-201306, U.P., India.
E-mail: drm714@gmail.com

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