The Relationship of AMH Level with Ovarian Response in PCOS Patients

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| Abstract | | Original Research Article |

Introduction: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of women of reproductive age. Anti-Mullerian hormone (AMH) has a glycoprotein dimer structure and is a member of the transforming growth factor-β (TGF-β) family. AMH is produced by the granulosa cells surrounding preantral and antral follicles and has an important role in the development and maturation of follicles. Several studies have suggested that AMH serum levels may be a marker for polycystic ovary syndrome (PCOS). Serum AMH has also demonstrated its utility in the treatment of infertility. ***Objective:*** To assess relationship of AMH level with ovarian response in Polycystic Ovary Syndrome (PCOS) patients. ***Methods:*** This is a cross sectional comparative study which was held at Sylhet women's Medical college from 2018 to 2019. ***Result:*** In this study 45 respondent had participated. For statistical analysis of this study AMH divided by two sub groups Group A (<8) and Group B (8<) and study found that 50.7% respondent had AMH which is Group A (<8). This study also revealed the negative correlation between AMH and follicle size (r= -0.288). On other hand AMH Group have positive correlation between LH (r = 0.238. Besides follicle size have positive correlation with ET (r=0.044) which is statistically significant. ***Conclusion:*** From this study it is easily understandable that AMH plays an important role to predict ovarian response to ovulation education in Polycystic Ovary Syndrome (PCOS) patients.
Keywords: Polycystic ovary syndrome (PCOS), Anti-Mullerian hormone (AMH), Ovarian response.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is among the primary causes of infertility due to anovulation, with a prevalence rate of 4%-6% in women of reproductive age.

PCOS is almost certainly a genetic condition but the precise causes of hyperandrogenism and anovulation, are still under investigations. Anti-Mullerian hormone (AMH) has a glycoprotein dimer structure and is a member of the transforming growth factor-β (TGF-β) family. AMH is produced by the granulosa cells surrounding preantral and antral follicles and has an important role in the development and maturation of follicles [1, 2].

Several studies have suggested that AMH serum levels may be a marker for polycystic ovary syndrome (PCOS). The level of AMH circulating in the blood is not affected by the menstrual cycle nor altered during the use of oral contraceptives, therefore it can be used as a potential biochemical marker for PCO or PCOS. Serum AMH has also demonstrated its utility in the treatment of infertility. But the absence of an international standard for serum AMH assay and the inability to define thresholds makes application of serum AMH more difficult. AMH has been predominantly known for its role in male sexual differentiation. In women, AMH expression is restricted to one cell type: the granulosa cells of the ovary. It starts around the 25th week of gestation continuing until menopause.

Serum AMH levels in women with PCOS are 2- to 3-fold higher than in ovulatory women with normal ovaries, which corresponds to the 2- to 3-fold increase in the number of small follicles seen in PCOS. The increased AMH has been hypothesized may reduce follicle sensitivity to FSH and estradiol production, thus preventing follicle selection, resulting in follicle arrest at the small antral phase with the failure of dominance [3, 4].

Normal AMH level is over 1 ng/ml. Though 2 to 4 ng/ml is considered normal but more than 3 ng/ml is an indicator for PCOS. A serum level of AMH >5 ng/ml is suggested to be the most sensitive and specific diagnostic marker for PCOS. Below 1 ng/ml is considered low. In between 5 to 7ng/ml is high normal [5].

A linear relationship between the serum AMH level and ovarian response is well-known in fully stimulated IVF cycles in normo ovulatory women. This suggests increased serum AMH levels in PCOS would also reflect an intrinsic dysregulation of the granulosa cells, in which AMH, itself, could be involved since an over expression of the AMH receptor type II (AMHRII) has also been demonstrated. The cause of such high production of AMH in antral follicles from PCO is currently unknown, but there is evidence to support a role played by androgens. Indeed, a positive correlation between serum androgen and AMH levels has been reported and the over production of androgens could be an intrinsic defect of thecal cells in PCOS Studies demonstrated contradictory results concerning AMH regulation by gonadotropins. For some authors, gonadotropins (especially FSH) inhibit serum AMH production in vivo in normal ovaries. On the contrary, others demonstrated a stimulating effect of FSH on AMH expression in normal ovaries as well as in PCOS. The recent finding that E2 inhibits AMH expression could reconcile those different results. FSH may directly stimulate AMH in small antral follicles, as long as they do not express aromatase. But in larger follicles, by increasing E2 Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of women of reproductive age [5-7].

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has also been demonstrated. The cause of such high production of AMH in antral follicles from PCO is currently unknown, but there is evidence to support a role played by androgens. Indeed, a positive correlation between serum androgen and AMH levels has been reported and the over production of androgens could be an intrinsic defect of thecal cells in PCOS [9, 10].

In this study our main goal is to evaluate the relationship of AMH level with ovarian response in PCOS patients.

OBJECTIVE
To assess the relation of AMH level with ovarian response to ovulation induction with Clomiphene Citrate in Polycystic Ovary Syndrome (PCOS) patients.

METHOD AND MATERIALS
Study design: Cross sectional comparative study.

Study period and place: This study was carried out at Sylhet women's Medical college from 2018 to 2019.

Study Population
Diagnosis of PCOS was done according to 2003, Rotterdam Revised diagnostic criteria. Study participants was selected from the patients attending the Reproductive Endocrinology and Infertility OPD clinic at Sylhet women's Medical college. Those PCOS women with AMH level <8 ng/dl will be in group-A and AMH level $\geq$ 8 ng/dl will be in group-B.

Sampling Method: Purposive sampling

Variables
Independent Variable
- Age
- Education
- BMI
- Serum AMH level

Dependent or outcome variable
Ovarian response (follicle size & ET)
Confounding variables, if applicable: LH level
Sample size measurement: Sample size and the statistical basis of it

Sample size determination: To determine the sample size, Wilcoxon Test (Normal Distribution) was followed:

Sample size was determined by the following formula:
Sample size is determined by

$$n = \frac{(u+v)^2(\sigma_1^2 + \sigma_0^2)}{(\mu_1 - \mu_0)^2}$$

u=1.96 (95% level type 1 error)

v=0.42 (80% level type 2 error)

σ_1=1.97 [σ_1=SD of one group: from previous study]

σ_0=3.49 [σ_0=SD of other group: from previous study]

μ_1=5.34 [μ_1=mean of one group: from previous study]

μ_0=7.81 [μ_0=mean of other group: from previous study]

Mean (μ_1 & μ_0) and standard deviation (σ_1 & σ_0)

So, sample size=20.66=21 (for each group)

So ultimate sample size = 42.Upto 10% patients may be drop out. So, we have taken 50 subjects reasonably.

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INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria

* Diagnosed case of PCOS patients according to Rotterdam criteria.
* Age 18 to 35 years.
* Has given consent to participate in the study.
* BMI less than 30 KG/M^2
* Normal semen parameter
* At least three follow up visit will be needed, one visit for AMH assay, one for ovulation induction and one for folliculometry.

Exclusion Criteria

* Age more than 35 years
* Bilateral tubal block
* Endometriosis
* Male infertility (azoospermia, oligozoospermia)
* Hyperprolactinemia
* Uncontrolled hypothyroidism
* Uncontrolled DM
* Uncontrolled Hypertension

Data analysis: A semi-structured, pre-tested and modified questionnaire designed was used to collect the information. All the data were entered and analyzed by using Statistical Package for Social Science (SPSS-23).

RESULTS

In Table-1 showed the age distribution of the patients. The mean age of the patients is 28.200 and minimum and maximum age range was 21 and 38 (respectively). The following table is given below in detail:

Table-1: Distribution of age of the patients (N=45)

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
Age in year	45	21.0	38.0	28.200	3.4351
Valid N (listwise)	45				

In Table-2 shows distribution of the study populations by demographic characteristic where in both group most of the patients belong to 25 to 29 years age group. Also, maximum patients coming from urban area. The following table is given below in detail:

Table-2: Distribution of the study populations by demographic characteristic (n=45)

Social Demographic Characteristics	Group A (<8)		Group B (8<)		P Value
	N	(%)	N	(%)	
Age (in Years)					
20-24	4	11.8%	1	9.1%	0.843
25-29	17	50.0%	7	63.6%	
30-34	12	35.3%	3	27.3%	
35-39	1	2.9%	0	0.0%	
Total	34	100.0%	11	100.0%	
Occupation					
Student	3	9.7%	1	9.1%	0.724
Housewife	22	71%	9	81.8%	
Service	6	19.4%	1	9.1%	
Total	31	100%	11	100%	
Residence					
Urban	24	75%	9	81.8%	0.644
Rural	8	25%	2	18.2%	
Total	32	100%	11	100%	

Ns=not significant

P value reached from unpaired t test in quantitative data

P value reached from Chi-square test

Group A= PCOS patients whose AMH level is <8

Group B=PCOS patients whose AMH level is >8

In Figure-1 shows frequency distribution of group AMH where all PCOS women 50.7% population had AMH level below <8 ng/ml belong to Group A and 15.9% population belong to Group B (≥8ng/ml)). The total mean of AMH is 6.56 ng/ml. Mean SD was 5.87. The following figure is given below in detail:
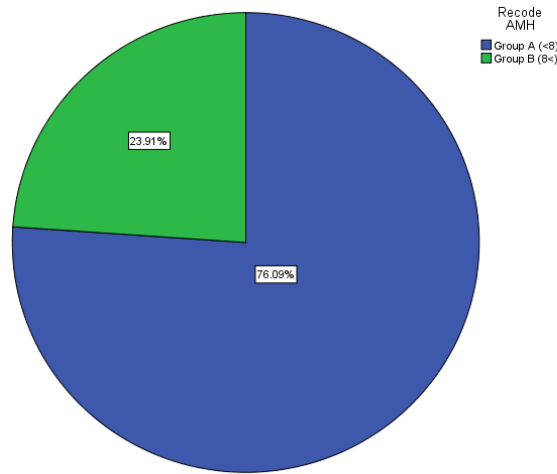

Fig-1: Frequency distribution of group AMH

Table-3 shows the distribution of the study populations by clinical characteristics was observed that more than half 55.6% in group A and 63.6% in group B populations had primary subfertility. According to BMI level more than 74.2% in group A and 60% in group B were overweight. In Group A 19.4% were obese and 30% were obese in Group B. According to hirsutism FG score more than 70 % had more than 60.T he difference was not ($p>0.05$) statistically significant.

Table-3: Distribution of the study populations by clinical characteristics (n=45)

Clinical Characteristics	Group A (<8)		Group B (8<)		P Value
	N	(%)	N	(%)	
Types of Infertility					
Primary	15	55.6%	7	63.6%	0.647
Secondary	12	44.4%	4	36.4%	
Total	27	100%	11	100%	
BMI (Body Mass Index kg/m^2)					
Normal Range (18.5-24.9kg/m^2)	2	6.5%	1	10%	0..692
Overweight (25-29.9kg/m^2)	23	74.2%	6	60%	
Obese (>30 kg/m$^{2)}$)	6	19.4%	3	30%	
Total	29	100%	10	100%	
Modified FG Score					
>6	7	29.2%	3	30%	0.961
6>	17	70.8%	7	70%	
Total	24	100%	10	100%	

In Table-4 shows correlation between BMI and follicle. BMI and follicle size are positively correlated but not significance, here P>0.05. The following table is given below in detail:

Table-4: Correlation between BMI and follicle

		BMI	Follicle Size
BMI	Pearson Correlation	1	.027
	Sig. (2-tailed)		.867
	N	41	41
Follicle Size	Pearson Correlation	.027	1
	Sig. (2-tailed)	.867	
	N	41	46

BMI and follicle size are positively correlated but not significance, here P>0.05.

In Table-5 shows regression analysis between Serum LH, Serum AMH, BMI where R denotes the correlation between predicted and observed serum LH. In our case, R = 0.390. Since this is a positive correlation, our model predicts serum LH rather precisely. R square indicates the proportion of variance in Serum LH that can be "explained" by our two predictors. Here R square is 0.152. The adjusted r-square estimates the population R square for our model and thus gives a more realistic indication of its predictive power. Here adjusted R square is 0.106.

Table-5: Regression analysis between Serum LH, Serum AMH, BMI

Model Summary				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.390[a]	.152	.106	4.74844
a. Predictors: (Constant), Serum AMH, BMI				

In Table-6 shows distribution of frequency of Follicle Size with respect to AMH Group. Where mean follicle size in Group A was 19.5 where as in Group B it was 28.3. The following table is given below in detail:

Table-6: Distribution of frequency of Follicle Size with respect to AMH Group.

AMH Group	Follicle Size		
	N	Mean	Stdv.
Group A (<8)	35	19.5	3.00
Group B (8<)	12	28.3	3.60

In Table-7 shows correlation between AMH and follicle size. Correlation of AMH with itself (r=1), and the number of nonmissing observations for height (n=46). Correlation of AMH and Follicle Size (r= -0.288), based on n=46 observations with pairwise nonmissing values. AMH and Follicle Size have a statistically significant linear relationship (r= -0.288, $p \leq 0.05$). The direction of the relationship is negative meaning that these variables tend to decrease when serum AMH increase. The following table is given below in detail:

Table-7: Correlation between AMH and Follicle size.

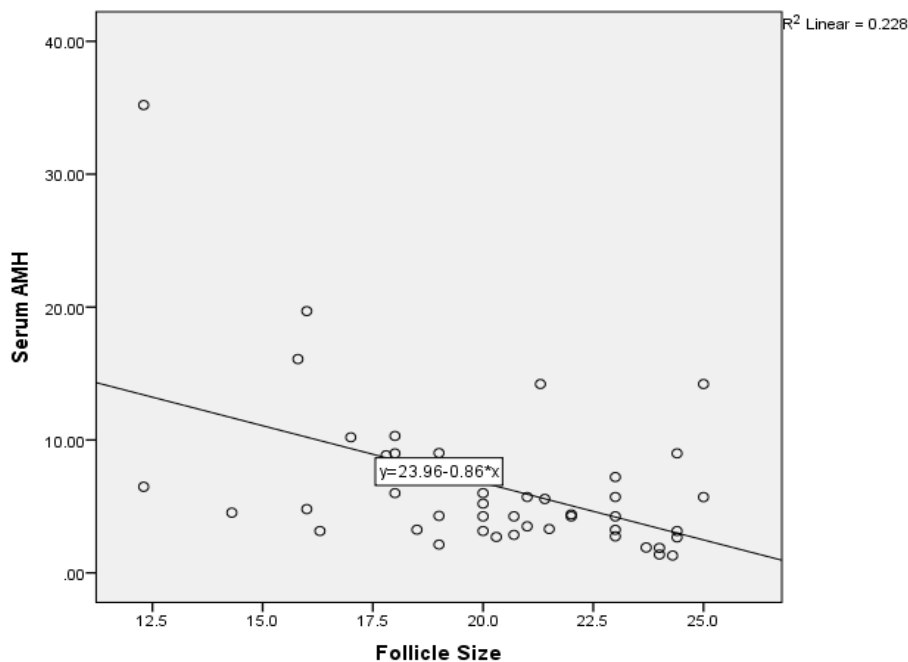| Correlations			
		AMH	Follicle Size
AMH	Pearson Correlation	1	-.288
	Sig. (2-tailed)		.052
	N	46	46
Follicle Size	Pearson Correlation	-.288	1
	Sig. (2-tailed)	.052	
	N	46	46

Fig-2: Scatter plot of Serum AMH and Follicle Size

In Table-8 shows correlation of AMH with itself (r=1), and the number of nonmissing observations (n=46). Correlation of AMH and LH (r=0.238), based on n=46 observations with pairwise nonmissing values.

AMH and LH have not a statistically significant linear relationship (r= 0.238, p ≥0.05). The following table is given below in detail:

Table-8: Correlation between AMH and LH

Correlations			
		Serum AMH	Serum LH
Serum AMH	Pearson Correlation	1	.238
	Sig. (2-tailed)		.116
	N	46	45
Serum LH	Pearson Correlation	.238	1
	Sig. (2-tailed)	.116	
	N	45	45

DISCUSSION

In this study, information has been collected from 46 patients. Here all the respondents was female. The mean age between 28.200± 3.4351 years and age range between 21 to 38 year. Maximum 47.8% respondents were coming from Urban area. Besides maximum 44.9% respondents were belonging with housewife.

More than half of the patients belong to age group 25 to 29 years. In group A 50% and in Group B 63.6%. Then second highest age group is 30 to 34 years, 35.3% in Group A and 27.3% in Group B. More than two third patients were housewife 71% in Group A and 81.8% in group B. more than two third patients 75% in Group A and 81.8% in Group B. the difference was not significant (p> 0.05) between two groups. BMI and follicle size are positively correlated but not significance, here P>0.05.

The AMH is more sensitive and specific than follicle count as it reflects both preantral and small antral follicles. Similar findings Recovery was assessed at low, medium, and high concentrations for each internal standard. The mixture at each concentration was added to the FF matrix prior to the extraction or instrumental analysis. A recovery experiment was conducted in triplicate for quality control (QC) purposes. Recoveries were calculated using the peak area ratios of the standards spiked before extraction to the standard spiked before instrumental analysis. The results indicated that the recoveries ranged from 86.4% to 113.7% for the six internal standards at low, medium, and high concentrations. Therefore, the recovery rate of the detection method used was satisfactory [10].

Demonstrated results also shows the distribution of the study populations by clinical characteristics was observed that more than half 55.6% in group A and 63.6% in group B populations had primary subfertility. According to BMI level more than 74.2% in group A and 60% in group B were overweight. In Group A 19.4% were obese and 30% were obese in Group B. According to hirsutism FG

score more than 70 % had more than 60.The difference was not (p>0.05) statistically significant. This result correlate to one study, in university of Nottingham UK did a study at fertility unit, Derby. It was a T a prospective cohort observational study included 60 anuovulatory women with PCOS received ovulation induction with CC between November2009 to March 2011.Primary study outcome was ovarian response, secondary was pregnancy.35, (58%) ovulated during ist cycle of CC. This number was 48 (80%) when dose raised upto maximum (150 mg/d). Of the 187 cycle Serum AMH concentrations were significantly (P .001) lower in responders (achieving ovulation) vs. non-responders (mean SEM, 2.5 0.1 vs 5.8 0.7 ng/mL, respectively) [11].

From regression analysis R denotes the correlation between predicted and observed serum LH. In our case, R = 0.390. Since this is a positive correlation, our model predicts serum LH rather precisely. R square indicates the proportion of variance in Serum LH that can be "explained" by our two predictors. Here R square is 0.152. The adjusted r-square estimates the population R square for our model and thus gives a more realistic indication of its predictive power. Here adjusted R square is 0.106. Similarly, serum AMH concentrations were significantly (P .046) lower in pregnant (3.0 0.4 ng/mL) vs nonpregnant patients (4.4 0.5 ng/mL). There was a significant (P .02) gradient increase of serum AMH levels with the increasing dose of CC required to achieve ovulation. The receiver-operating characteristic curve showed AMH to be a useful predictor of no ovulation (area under the curve, 0.809;.001) with a useful cutoff level of 3.4 ng/mL. Ovulation and pregnancy rates were significantly higher (97%, P .001, and 46%, P .034) in patients with low AMH (3.4 ng/mL) vs women with AMH 3.4 ng/mL or greater (48% and 19%).

According to correlation table of AMH with itself (r=1), and the number of nonmissing observations for height (n=46). Correlation of AMH and Follicle Size (r= -0.288), based on n=46 observations with pairwise nonmissing values. AMH and Follicle Size have a

statistically significant linear relationship (r= -0.288, p ≤0.05). The direction of the relationship is positive meaning that these variables tend to increase together. Similarly study conducted by one study in 2018 did this retrospective study included 100 patients were admitted to Ministry of Health Etlik Zubeyde Hanim Woman's Health Teaching and Research Hospital. All patients diagnosed with unexplained infertility had ovulation induction using CC followed by intrauterine insemination. The patients who developed at least one follicle of >16 mm in diameter were considered as a positive ovarian response and had intrauterine insemination failed to develop at least a follicole and the patients who developed at least one follicle of >16 mm in diameter were considered as a positive ovarian response [12].

CONCLUSION

From this study it is easily understandable that AMH plays an important role to predict ovarian response to ovulation education in Polycystic Ovary Syndrome (PCOS) patients.

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