Original Research Article

Correlation of anti-müllerian hormones in diagnosed polycystic ovarian syndrome in tertiary care hospital

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Abstract

Background: Polycystic ovarian syndrome (PCOS) is a common problem causing menstrual irregularity and infertility among women of fertile age. Increased level of anti-müllerian hormone (AMH) is currently thought to be an important marker for PCOS. 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. Anti-Mullerian hormone (AMH) is a glycoprotein hormone secreted by the granulosa cells of the antral and preantral follicles. AMH level decreases throughout the reproductive period and becomes undetectable at the time of menopause. **Aim Of The Study:** To Correlate Anti-Müllerian Hormones in Diagnosed Polycystic Ovarian Syndrome. **Methods:** This cross-sectional study included 50 PCOS patients and 50 healthy women of fertile age as controls who are attending the outpatient Department of Madha medical college and hospital Chennai. PCOD was diagnosed on the basis of the Rotterdam 2003 criteria. AMH (ng/ml) was measured by enzyme-linked immunosorbent assay (ELISA).The other hormones (follicle-stimulating hormone, FSH; luteinizing hormone, LH; Testosterone) by immunochemiluminometric assay. **Results:** AMH was significantly higher in PCOS (9.21 ± 0.50ng/ml vs. 4.40 ± 0.41 ng/ml, M ± SE; $p<0.001$) than that of healthy controls. AMH showed inverse relationship with FSH, though not statistically significant (mIU/ml, $r = -0.129$; $p = 0.253$), and BMI (kg/m2, $r = -0.046$; $p = 0.686$) whereas positive relationship with testosterone (ng/dl, $r = 0.146$; $p = 0.197$) and LH (mIU/ml, $r = 0.102$; $p = 0.368$).With cut -off value of 3.5 ng/ml for AMH, sensitivity, and specificity of AMH was found to be 67% and 78.33% respectively. **Conclusions:** AMH level is significantly increased in PCOS.PCOS women of fertile age have higher AMH level than that of healthy control subjects. It can be considered as an important zero marker for the diagnosis of PCOS.
Key Words: Anti-Mullerian Hormone, Polycystic Ovary Syndrome, Menopause, Rotterdam Criteria

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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.Anti-Mullerian hormone (AMH) is a glycoprotein hormone secreted by the granulosa cells of the antral and preantral follicles.[1] AMH level decreases throughout the reproductive period and becomes undetectable at the time of menopause, however, an increased level of AMH can be observed in females suffering from PCOS, which indicates the presence of a larger number of antral follicles in such women.[2]

METHODS

This cross-sectional study included 50 PCOS patients and 50 healthy women of fertile age as controls who are attending the outpatient Department of Madha medical

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college and hospital Chennai. This sample size was calculated based on a standard deviation of 2.06, d = 0.5, α = 0.05, and β = 0.8. Rotterdam criteria were used to select these patients using a convenient sampling technique. The PCOS was diagnosed when ≥2 through the following three criteria: oligomenorrhea or amenorrhea, clinical hyperandrogenism or hyperandrogenemia, and polycystic ovaries on ultrasonography. All the patients were infertile (i.e., lack of pregnancy after one year of unprotected intercourse) and with PCOS. The inclusion criteria were women with previously diagnosed PCOS according to Rotterdam criteria, aged between 20-35 years, and having a body mass index (BMI) between 18-30 kg/m2. The exclusion criteria encompassed women with infertility of any other etiology, exposed to the cytotoxic drug, pelvic radiation therapy, or suffering from renal or liver diseases.

BIOCHEMICAL ASSAYS:

AMH was estimated by single measurements by an enzyme-linked immunosorbent assay, AMH GEN II ELISA kit (Beckman Coulter, Inc. USA) whereas other hormones (follicle-stimulating hormone, luteinizing hormone, Testosterone) by immune chemiluminometric assay. Values of AMH were presented as nanograms per milliliters (conversion factor to pmol/l = ng/ml × 7.1). AMH was calculated using kc3 biographs with help of the standard supplied with the kit. QC (quality control) was used in each assay run to assess the precisions of the assay. Intraassay CV (coefficient of variance) was 3.4 to 5.4% and interassay CV 4.0 to 5.6% for AMH assay[1,3,7]

STATISTICAL ANALYSIS

AMH levels were expressed as the mean ± (SE). Student's t-test for continuous variables and Chi-Square test for discrete variables were used. Correlation among variables was assessed by using Pearson's correlation test. Multiple regressions were done to see the impact of independent factors over AMH. P values ≤ 0.05 were considered statistically significant.

Table 1: Characteristics of the studied PCOS patients and control(n=100)

VARIABLES	PCOS (n = 50)	Controls(n = 50)	p
Age (mean ±SD, year)	23.7±4.8	26.3±2.9	<0.001
BMI (mean ±SD, kg/m^2)	26.7±4.5	21.7±2.8	<0.001
Menstrual disturbance	69	1	<0.001
Family history of PCOS	6	2	0.276
*Infertility			
Primary	14/43 (32.6)	0/34	<0.001
Secondary	8/43 (18.6)	0/34	
MR/Abortion	12/43 (27.9)	10/34 (29.4)	NS

Table- 1 Shows Baseline characteristics of subjects (as seen in Table-I) revealed statistically higher BMI (26.7 ± 4.5 vs. 21.7 ± 2.8, kg/m2; p <0.001) in PCOS than that of control. Menstrual irregularity (86.3% vs. 1.3%; p<0.001) and infertility rate (p<0.001) were also significantly higher in the PCOS.

Table 2: Basal serum amh levels in control women and in women with pcos

Group of subjects	Controls (n = 50) AMH ng/ml)±(SE)	PCOS (n = 50) AMH (ng/ml)±(SE)	p-value
1.Whole group	4.40 ± 0.41	9.21 ± 0.50	<0.001
2. Age group(years) n(control, PCOS)			
23 – 27 (49,32)	4.52 ± 0.54	9.91 ± 0.71	<0.001
28 – 31 (25,7)	4.22 ± 0.68	8.28 ± 1.51	<0.011

Table-2 shows AMH level was significantly higher (9.21 ± 0.50 vs. 4.40 ± 0.41, ng/ml; p<0.001) in the PCOS patients than that of controls. However, when compared according to age-group, this was significantly different between PCOS and controls in the age group 23-27 years (9.91 ± 0.71 vs. 4.52 ± 0.54, ng/ml; p<0.001) and age-group 28-31 years (8.28 ± 1.51 vs. 4.22 ± 0.68, ng/ml; p<0.011).

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Table 3: Sensitivity and specificity of AMH for the diagnosis of PCOS holding cut-off as 3.5 ng/ml (CHAO *ET AL.*, 2011)

Group(s)	Anti-mullerian hormone (ng/ml)		Total
	≥ 3.5 ng/ml	<3.5 ng/ml	
PCOS	30	20	50
Control	33	27	50
Total	**63**	**37**	**100**

Table-3 shows the subgroups of PCOS and control subjects divided on the basis of a cut-off value of AMH at 3.5ng/ml. AMH ≥ 3.5ng/ml was considered as positive in the diagnosis of PCOS. Thus 30out of 50in the PCOS patients and 33 out of 50 controls could be labeled as positive. The calculated sensitivity was found to be 67% and specificity 78.33%. PCOS**:** polycystic ovarian syndrome-Sensitivity = true positive / (all positive) × 100= 67/ (67+33) × 100= 67 %. Specificity= true negative / (all negative) × 100= 47/ (47+13) × 100= 78.33%.

DISCUSSION

In the present study, it was seen that the serum AMH level is higher in women with PCOS and amenorrhoea, compared to those with oligomenorrhoea. The mean value of AMH in those cases with oligomenorrhoea is 8.92 ng/ml (range 3.45 – 18.36) with a standard deviation of 4.2 and in those with amenorrhoea is 15.69 ng/ml (range 7.56 – 20.36) with a standard deviation of 3.5. [7]The mean of AMH level differed significantly between those presenting with oligomenorrhoea or amenorrhoea (p-value < 0.0001) Polycystic Ovary Syndrome (PCOS) is one of the most common endocrinological problems in women.[8] In addition to chronic oligo-anovulation, the main features of the PCOS include elevated levels of circulating androgens and/or clinical hyperandrogenism, polycystic ovary morphology, altered gonadotropin secretion, insulin resistance and/or compensatory hyperinsulinemia often associated with obesity.[9]Women affected by PCOS also show a higher risk of type 2 diabetes, dyslipidemia, hypertension and cardiovascular disease Differences in the association of anti-Müllerian hormone with clinical or biochemical characteristics between women with and without polycystic ovarian syndrome.[10] The sensitivity and specificity of AMH for detecting PCOS in patients aged 18-35 years were calculated to be 67% and 78.33% respectively, using an AMH cut-off value of 3.5 ng/ml as followed in another study. Mean AMH differed significantly between PCOS subjects and healthy controls. Increased serum AMH concentrations in PCOS patients have been explained by the increased number of small ovarian follicles responsible for AMH secretion.[11] In the ovary AMH is produced from granulosa cells of pre-antral and small antral follicles. From experimental data, mainly obtained in rodents, the proposed functions of AMH are 1) inhibition of the initial recruitment of primordial follicles, through a paracrine effect and 2) inhibition of aromatase activity in granulosa cells, thus reducing the production of estradiol (E2). [12] Pathophysiology of PCOS has been known to be multifactorial. Anovulation and/or oligo-ovulation are the main underlying cause of infertility. Altered LH: FSH ratio, hyperandrogenemia, and hyperinsulinemia as well as insulin resistance – all had been thought to be linked to the probable cause of anovulatory cycles. But in the past decade much attention had been concentrated on AMH in context of PCOS. Several factors have been reported to be associated with AMH secretion.[13] A negative correlation was observed between FSH and AMH levels in some studies. Low dose recombinant FSH therapy in PCOS patients decreased serum AMH levels, suggesting the negative role of AMH in aromatase expression during dominant follicle selection. Increasing serum FSH will cause a shift of small antral follicles to larger ones, expressing less AMH, thus a decline in AMH and allowing dominance of follicle to occur.[14] It has been observed that AMH serum levels significantly and inversely correlate to FSH levels in healthy women.[15] Apropos with the above facts, in the present study a negative relationship was observed between FSH and AMH though not significant statistically. Follicles from AMH knockout mice have been shown to be more sensitive to FSH than those from the wild type. [16]This further suggests that the inhibiting effect of AMH on aromatase activity acts through a decrease in granulosa cell sensitivity to FSH. The balance between the opposite effects of AMH and FSH on aromatase activity might be crucial for the cohort at the time of the selection process for dominant follicle. [17] Wilkes S *et al* used a cut-off value of 3.5 ng/ml of AMH in discrimination of PCOS from control and observed sensitivity and specificity on its basis as 74% and 79 %. A negative relationship was seen between age and AMH level by regression analysis but found to be nonsignificant. The age-related decline in AMH level among control women is supported by other studies whereupon negative correlation between age and AMH has been reported.[18] As because AMH levels correlate with the number of early antral follicles which might represent the size of the resting follicle pool, AMH may constitute a marker for ovarian aging.[19,20]

CONCLUSION

It may be concluded that PCOS has significantly higher serum AMH than healthy women during the reproductive period. Age-related decline of AMH occurs in healthy women as well as in PCOS women. This is indicative of ovarian aging. Observed relatively higher AMH levels in the healthy control group may reflect the ethnic variation. The sensitivity and specificity of AMH for diagnosing PCOS were calculated to be 67% and 78% respectively holding a cut-off value of AMH at 3.5 ng/ml. Thus, AMH seems to be an important zero marker in the diagnosis of PCOS irrespective of other characteristics of PCOS. This difference of associations might suggest a loss of multi-factorial control for AMH production in PCOS, and which might contribute to the pathogenesis of PCOS. Further investigation is needed to elucidate the role of AMH and the regulation mechanism of AMH production

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