Role of Anti Mullerian Hormone (AMH) in diagnosis of Polycystic Ovarian Syndrome (PCOS) in Indian women.

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Abstract

Introduction: Polycystic ovarian morphology (PCOM) obtained by transvaginal ultrasound is an integral component of Rotterdam criteria most widely used for diagnosis of PCOS. Transvaginal ultrasound may not be widely available and possible in sexually inactive unmarried women in India. Anti-Mullerian hormone (AMH) is a surrogate marker of PCOM.

Aim: To assess diagnostic power of serum AMH for diagnosis of PCOS and to analyse if serum AMH can replace PCOM in Rotterdam criteria and co-relation of AMH with hyperandrogenaemia.

Methods: A study was done in SSH BHU, various PCOS parameters were used in diagnosis. Serum AMH and Radiology were used. By Transvaginal Sonography single observer obtained dimensions for ovarian volume and the maximum number of follicles in one section. AMH levels were estimated using commercially available Gen-II ELISA assay.

Result: Biochemical evaluation was done in the Department of Bio Chemistry IMS BHU. Serum AMH was estimated using commercially available ultra-sensitive anti-mullerian hormone Gen-II enzyme linked immunosorbent assay (ELISA, Beckman Coulter, CA) with lower limit of detectability (LoD) of 0.08 ng/ml, lower limit of quantification (LoQ) of 0.17 mg/ml and intra-assay coefficient of variation of 5.8%. The unit of measurement is ng/mL (1ng/mL=7.14 pmol/L).

Conclusion: In this study, it was demonstrated that AMH levels were significantly higher in PCOS than in controls. AMH as an independent marker could not effectively diagnose PCOS. However, AMH levels as an adjunct to existing Rotterdam criteria for diagnosis of PCOS had good diagnostic potential.

Keywords: Endocrinology, PCOS, Antimullerian hormone.

Introduction

The clinical features of PCOS are a spectrum of disorder with a heterogeneous collection of signs and symptoms from mild to severe disturbance of metabolic functions and reproductive endocrine. The Polycystic Ovarian Syndrome pathophysiology appears to be polygenic and multifactorial. PCOS is diagnosed based on the presence of any two of the following three criteria according to Rotterdam's (2003) are: Oligo and Anovulation, Hyperandrogenism, Polycystic Ovaries. PCOS is prevalent in young Reproductive age group where the distribution 20-30% [1, 2]. Presence of Insulin Resistance, Dyslipidaemia and Central Obesity which might lead to the complications of Diabetes and Cardiovascular disease in PCOS women. Ovarian reserves are assessed by Biochemical parameter and Ultrasound parameter. AMH level decreases steadily with increasing age from 24 to 50 years of age [3]. AMH concentration in the serum is directly related to the antral follicle count and is a better indicator of ovarian reserve when compared to FSH and Estradiol level [4]. The strong involvement of AMH in the pathophysiology of PCOS has opened a wide discussion about whether AMH could be involved in facilitating the diagnosis of PCOS, as a more sensitive and specific marker than follicle count in ultrasonographic examination.

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***Received**: 09-Jan-2023, Manuscript No. AAGGS-23-86198; **Editor assigned**: 12-Jan-2023, PreQC No. AAGGS-23-86198(PQ); **Reviewed**: 20-Jan2022, QC No. AAGGS-22-82603; **Published**: 30-Jan-2023, DOI:10.35841/2591-7994-7.1.131*

***Citation:** Pandey U. Role of Anti Mullerian Hormone (AMH) in diagnosis of Polycystic Ovarian Syndrome (PCOS) in Indian women. Gynecol Reprod Endocrinol. 2023;7(1):131*

Methods

Period of the study: Women 18 to 40 years old without history of other diseases at their visit between November 2020 to October 2022. Control women were selected from general population with healthy history and have at least one baby. About 5ml blood sample were drawn on day three of the cycle or progesterone induced cycle. AMH, E2, FSH, LH, Ft3, Ft4, TSH, PRL and Total testosterone analysis were carried out at the central clinical laboratory.

The inclusion criteria: Menstrual cycle- regular (length of the cycle 25-35 days, 3-8 days duration of Menstruation. Medications or hormone should be avoided for 3 months. She should not be subjected to any surgical procedure in the reproductive system and patients diagnosed as PCOS (By Rotterdam Criteria).

Exclusion criteria: Post-Menopausal, Thyroid dysfunction, Cushing syndrome, Congenital adrenal hyperplasia, Ovarian tumour, Autoimmune disease.

Ethical considerations: The researcher obtained the necessary approval to conduct the study from The Institute ethical committee, IMS BHU. Women were explained about the purpose of this study and an informed written consent was obtained, confidentiality about their results was assured. Their participation was optional.

Parameters used in this study:

1. Body Mass Index

2. Hirsutism

3. Ultrasonography

4. Hormone assays: Antimullerian hormone assay, Testosterone assay, Estradiol assay, Follicle Stimulating Hormone assay, Luteinizing Hormone assay, Prolactin assay.

Results

On correlating AMH level with various study parameters in PCOS patients, there were significant negative correlation was observed between AMH level with LH and FSH level ($p<0.001$ and $p<0.001$) and positive correlation was observed with T3 level ($p=0.014$) (Table 1, 2). No significant correlation was observed between AMH level with age, BMI, FBS, T4, TSH, testosterone, prolactin, AFC, ovary volume and estrogen. On correlating AMH level with various study parameters in control group, there were no significant correlation was observed between AMH level and various study parameters. Based on the current study findings and ROC data table, the cut-off for AMH level between 4.70 units seems appropriate in delineating PCOS subjects and control subjects as it has high level of sensitivity and specificity (Table 3).

Discussion

PCOS is one of the leading causes for female sub fertility and the most common endocrine disorder among women of reproductive age (Table 4) [5].

AMH is a promising marker for diagnosis of PCOS, as its concentration is stable throughout the menstrual cycle and is not affected by fluctuations of other reproductive hormones [5]. The present study aimed to assess diagnostic power of serum AMH for diagnosis of PCOS and analyse if serum AMH can replace PCOM in Rotterdam Criteria and also analyse correlation of AMH with hyperandrogenaemia (Table 5).

In the present study, there was no statistical difference between mean age of PCOS cases and controls this finding correlates with the study of Singh et al (2020), in which no statistically significant difference was found between mean age of PCOS group and control group [6]. In the present study, AMH level was found to be significantly higher in PCOS group as compared to control group, with median AMH levels of 7.1686 ± 2.73776 ng/ml in PCOS group being almost twice as high of 3.8034 ± 0.63199 ng/ml in control group ($p<0.001$). A study reported the highest AMH in women presenting with all three Rotterdam criteria and with 80% prevalence of PCOS in women with AMH>11 ng/ml (Lin et al, 2011). PCOS was observed in 97% women with AMH higher than 10 ng/ml in a study (Tal et al, 2014) (Table 6).

In the present study, the best diagnostic potential of AMH was found at cut-off of 4.70 ng/ml with sensitivity and specificity

Table 1: *Comparison of mean BMI between PCOS and control group.*

Group	N	BMI (kg/m^2) Mean±SD	p-value
PCOS group	170	28.28 ± 4.96	<0.001
Control group	170	24.81 ± 5.14	

Note: The mean BMI in PCOS group was 28.28±4.96 kg/m2 and 24.81 ± 5.14 kg/m2 in control group. The mean BMI was significantly high in PCOS group as compared to control group ($p<0.001$).

Table 2: *Comparison of presence of acne / hirsutism between cases and control group.*

Acne / hirsuitism	Group			
	PCOS (n=170)		Control (n=170)	
	No.	%	No.	%
Present	100	58.8	99	58.2
Absent	70	41.2	71	41.8
Total	170	100	170	100

$\chi^2=0.012$; $p=0.912$

Note: In PCOS group 100 (58.8%) patients had presence of acne / hirsuitism and in control group, 99 (58.2%) patients had presence of acne / hirsuitism which showed no significant association ($p=0.912$).

Table 3: *Comparison of mean T3, T4 and TSH levels between PCOS and control group.*

	Group		p-value
	PCOS Group (n=170)	Control Group (n=170)	
T3	1.9109±.81328	2.3917±.57532	<0.001
T4	1.2772±0.36250	1.4565±1.03718	0.034
TSH	2.4975±0.97980	2.6814±0.71749	0.049

Note: The mean T3 level was 1.9109±.81328 in PCOS group and 2.3917 ± 0.57532 in control group, the mean T4 level was 1.2772 ± 0.36250 in PCOS group and 1.4565 ± 1.03718 in control group and the mean TSH level was 2.4975 ± 0.97980 in PCOS group and 2.6814 ± 0.71749 in control group. In PCOS group, the mean T3, T4 and TSH levels was significantly low as compared to control group (p<0.001, p=0.034 and p=0.049 respectively).

Table 4: *Comparison of mean AMH level between PCOS and control group***.**

Group	N	Mean±SD	p-value
PCOS	170	7.1686±2.73776	<0.001
Control	170	3.8034±0.63199	

Note: The mean AMH level was 7.1686±2.73776 in PCOS group and 3.8034±0.63199 in control group. In PCOS group, the mean AMH level was significantly high as compared to control group (p<0.001).

Table 5*: Correlation between AMH and various study parameters in PCOS group.*

	AMH	
	r-value	p-value
Age	-0.023	0.770
BMI	-0.006	0.942
Fasting blood sugar	-0.022	0.776
LH	-0.417	<0.001
FSH	-0.350	<0.001
T3	0.188	0.014
T4	-0.015	0.841
TSH	0.055	0.478
Testosterone	-0.008	0.920
Prolactin	0.059	0.446
AFC right ovary	-0.010	0.900
AFC left ovary	0.097	0.207
Right ovary volume	0.046	0.550
Left ovary volume	0.144	0.062
Estrogen	0.057	0.460

Note: On correlating AMH level with various study parameters in PCOS patients, there were significant negative correlation was observed between AMH level with LH and FSH level (p<0.001 and p<0.001) and positive correlation was observed with T3 level (p=0.014). No significant correlation was observed between AMH level with age, BMI, FBS, T4, TSH, testosterone, prolactin, AFC, ovary volume and estrogen.

Table 6: *Area under the Curve.*

Area under the curve	Cut-off	p-value	Sensitivity	Specificity	Asymptotic 95% Confidence Interval	
					Lower Bound	Upper Bound
0.973	4.70	.000	90.0%	90.60%	0.958	0.988

Note: The above table shows that the best cut-off value of AMH level was 4.70 with a sensitivity of 90% and specificity of 90.60% and p-value was statistically significant. Based on the current study findings and ROC data table, the cut-off for AMH level between 4.70 units seems appropriate in delineating PCOS subjects and control subjects as it has high level of sensitivity and specificity.

Table 7: *Logistic regression of various factors with PCOS (n=170).*

Independent variables	Odds Ratio (95% CI) for PCOS	*p* value
Age in years (continuous)	0.992 (0.906 – 1.085)	0.854
LH (mIU/ml)	1.262 (1.080 – 1.475)	0.003
FSH (mIU/ml)	1.010 (0.817 to 1.250)	0.923
Testosterone levels(ng/ml)	1.102 (1.046 to 1.161)	<0.001
AMH levels(ng/ml)	485.11 (241.32 to 756.40)	<0.001

Logistic regression of various factors with PCOS shows that:

1. From the above table it is clear that the variable which has maximum influence on the occurrence of PCOS is the serum anti-mullerian hormone levels.
2. The odds ratio for age and FSH levels were not significant and hence cannot be accounted as an association with the occurrence of PCOS

Elevated serum levels of LH, testosterone and AMH were found statistically significant predictors of PCOS, with AMH levels demonstrating high odds for developing the disease.

Citation: *Pandey U. Role of Anti Mullerian Hormone (AMH) in diagnosis of Polycystic Ovarian Syndrome (PCOS) in Indian women. Gynecol Reprod Endocrinol. 2023;7(1):131*

of 90% and of 90.60%, respectively. Similar cut-off of AMH of 4.90 ng/ml with a higher sensitivity and specificity of 92% and 97%, respectively, was reported by Dewailly et al (2011) [7]. Hence, they concluded that AMH not only reflects AFC but also the degree of hyperandrogenism making AMH a better marker than follicle numbers per ovary (Table 7).

Conclusion

The mean BMI in PCOS group was 28.28 ± 4.96 kg/m^2 and 24.81 ± 5.14 kg/m^2 in control group. The mean BMI was significantly high in PCOS group as compared to control group ($p<0.001$). The mean FBS level was 109.09 ± 12.74 in PCOS group and 105.71±8.48 in control group. In PCOS group, the mean FBS level was significantly high as compared to control group ($p=0.004$). The mean T3, T4 and TSH levels was significantly low as compared to control group ($p<0.001$, $p=0.034$ and $p=0.049$ respectively). The mean testosterone level was significantly high and the mean prolactin level was significantly low in PCOS group as compared to control group ($p<0.001$ and <0.001 respectively).

The mean BMI in PCOS group was 28.28 ± 4.96 kg/m^2 and 24.81 ± 5.14 kg/m^2 in control group. The mean BMI was significantly high in PCOS group as compared to control group ($p<0.001$). The mean right and left AFC were significantly high in PCOS group as compared to control group ($p<0.001$ and <0.001 respectively).

PCOS is a complex and common gynecological condition and PCOM used currently in Rotterdam criteria is highly subjective and poorly reproducible. Though sensitivity and specificity of AMH alone is low and no single cut-off of AMH is diagnostic, still it is a promising diagnostic tool for PCOS as an adjunct to existing Rotterdam criteria especially when it is used to replace PCOM. Additional advantages of AMH as diagnostic tool are that it is biological, objective, quantitative marker not affected by day of menses or OCP intake. So, in future, more studies should be undertaken to validate AMH' role as diagnostic tool for PCOS.

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