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Original Research Article

Role of anti mullerian hormone (AMH) in diagnosis of polycystic ovarian syndrome (PCOS) in Indian women

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ABSTRACT

Background: Transvaginal ultrasound is an important part of the Rotterdam criteria, which are commonly used to diagnose polycystic ovary syndrome (PCOS). Specifically, the presence of polycystic ovarian morphology (PCOM) is a key factor in the criteria. Another useful indicator of PCOM is the Anti-Mullerian hormone (AMH) level.

Aim: The objective is to evaluate the diagnostic accuracy of serum Anti-Mullerian hormone (AMH) in identifying polycystic ovary syndrome (PCOS) and determine whether it can be used as a substitute for polycystic ovarian morphology (PCOM) in the Rotterdam criteria. Additionally, we aim to investigate the relationship between AMH levels and hyperandrogenism in PCOS patients.

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

Result: Biochemical evaluation will be done in the Department of Bio-Chemistry IMS BHU. The Anti-Mullerian hormone (AMH) serum levels will be measured using a commercially available ultra-sensitive Gen-II enzyme-linked immunosorbent assay (ELISA) kit from Beckman Coulter, CA. The ELISA has a lower limit of detectability (LoD) of 0.08 ng/ml, a lower limit of quantification (LoQ) of 0.17 mg/ml, and an intra-assay coefficient of variation of 5.8%. The unit of measurement is ng/mL (1ng/mL=7.14 pmol/L).

Conclusion: The study showed that Anti-Mullerian hormone (AMH) levels were markedly higher in individuals with polycystic ovary syndrome (PCOS) than in controls. While AMH alone was not a reliable diagnostic marker for PCOS, the findings suggested that incorporating AMH levels as an additional factor in the existing Rotterdam criteria could improve the accuracy of PCOS diagnosis. Therefore, AMH levels have the potential as a useful adjunct marker for the diagnosis of PCOS.

1. Introduction

Polycystic ovary syndrome (PCOS) is a complex disorder that manifests a spectrum of clinical features. These features can vary in severity and can affect metabolic functions as well as reproductive endocrine functions. The symptoms of PCOS are heterogeneous and can range from mild to severe.

The pathophysiology of polycystic ovary syndrome (PCOS) is believed to be polygenic and multifactorial, meaning that it involves the interaction of multiple genetic and environmental factors. This complexity makes it challenging to pinpoint a single cause for PCOS, but research suggests that it may involve abnormal hormone levels, insulin resistance, and chronic low-grade

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inflammation. The exact mechanisms underlying PCOS pathophysiology are still being studied and remain a subject of ongoing research.

According to the Rotterdam criteria of 2003, polycystic ovarian syndrome (PCOS) is normally diagnosed by the appearance of at a minimum two of the following three criteria:

1. Oligo and Anovulation
2. Hyperandrogenism
3. Polycystic Ovaries

PCOS is a common disorder among young women of reproductive age, with a prevalence of around 20-30%. Women with PCOS often have insulin resistance, dyslipidemia, and central obesity, which can increase their risk of developing diabetes and cardiovascular disease.[1,2] Ovarian reserves can be assessed using biochemical and ultrasound parameters, with anti-mullerian hormone (AMH) being a more reliable indicator of ovarian reserve than FSH and oestradiol levels.[3] AMH levels decrease steadily with age, from 24 to 50 years old.[4] AMH levels are directly related to the antral follicle count and are a better marker for ovarian reserve than FSH and oestradiol levels. The involvement of AMH in the pathophysiology of PCOS has led to discussions about its potential use as a more sensitive and specific marker for diagnosis, particularly as a replacement or adjunct to ultrasonography for the detection of polycystic ovaries. In healthy, reproductive-aged women the ovaries and the adrenal cortex share the bulk of the steroid biosynthesis pathways, with relatively equal contributions to the circulating levels of testosterone and androstenedione. The ovaries and the adrenal cortex both secrete more androstenedione than testosterone, but ~50% of circulating testosterone is derived from the peripheral metabolism of and rostenedione.[5–9] Within the ovary, the theca interna layer within the ovarian follicle produces androgens, whilst within the adrenal cortex; it is the zona fasciculate responsible for synthesis.[10]

2. Materials and Methods

2.1. Period of the study

Women between the ages of 18 to 40, who had no history of any other diseases, were included in the study during their visit between November 2020 to October 2022. The control group consisted of women from the general population who had a healthy history and had given birth to at least one baby. Blood samples of approximately 5ml were collected on day three of the menstrual cycle or a progesterone-induced cycle. The samples were sent to the central clinical laboratory for analysis of AMH, E2, FSH, LH, Ft3, Ft4, TSH, PRL, and total testosterone.

2.2. Inclusion criteria

Women who have regular menstrual cycles (with a cycle length of 25–35 days and a duration of 3–8 days), have not taken hormonal drugs for at least 3 months, and have not had any reproductive system surgeries are eligible for the study. Individuals with PCOS (By Rotterdam Criteria).

2.3. Exclusion criteria

Women who are post-menopausal, have thyroid issues, Cushing syndrome, congenital adrenal hyperplasia, ovarian tumours, or autoimmune conditions are excluded from the study.

2.4. Ethical considerations

It is important to obtain ethical approval and informed consent before conducting a research study to ensure that the rights and welfare of the participants are protected. It is also essential to maintain confidentiality to protect their privacy and personal information.

2.5. Parameters used in this study

1. Body Mass Index
2. Hirsutism
3. Ultrasonography
4. Hormone assays
5. Anti-Mullerian hormone assay
6. Testosterone assay
7. Oestradiol assay
8. Follicle Stimulating Hormone assay
9. Luteinizing Hormone assay
10. Prolactin assay

3. Results

On correlating AMH level with various study parameters in PCOS patients, there was significant negative correlation was observed between AMH level with LH and FSH level ($p<0.001$ and $p<0.001$), and a 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 estragon.

The Table 2 shows that the best cut-off value of AMH level was 4.70 with a sensitivity of 90% and specificity of 90.60% and the 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 a high level of sensitivity and specificity.

Logistic regression of various factors with PCOS shows that.

Table 1: Correlation between AMH and various study parameters in the 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

Table 2: ROC curve of AMH levels (ng/ml) in PCOS

The 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

Area under the curve

Table 3: 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

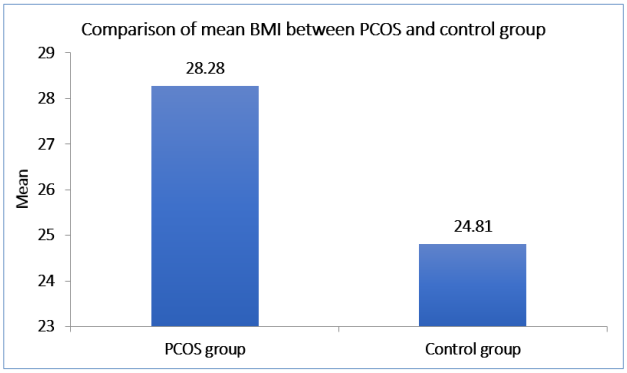

Fig. 1: The mean BMI in the PCOS group was 28.28±4.96 kg/m^2 and 24.81 ± 5.14 kg/m^2 in the control group. The mean BMI was significantly high in the PCOS group as compared to the control group (p<0.001)

1. From the Table 3 show the variable which has the 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
3. Elevated serum levels of LH, testosterone, and AMH were found statistically significant predictors of PCOS, with AMH levels demonstrating high odds of developing the disease.

4. Discussion

Polycystic ovary syndrome is a set of symptoms related to a hormonal imbalance that can range from mild to severe and it has reproductive, endocrine, and metabolic implications.[5] PCOS is one of the leading causes of female subfertility and the most common endocrine disorder among women of reproductive age. Despite many decades of extensive research, the exact etiology and pathogenesis of this complex disorder remain hidden. The use of ultrasound to diagnose PCOS can be problematic; the interpretation of ultrasound results may demand subjective judgments and is subjected to interobserver variability.[11]

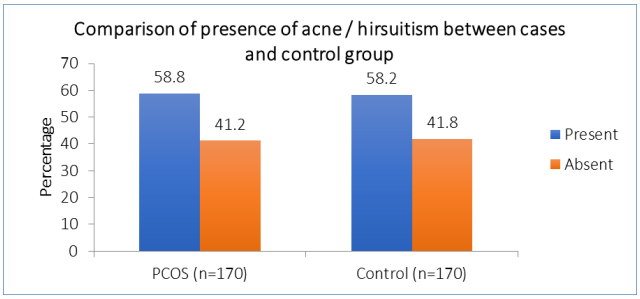

Fig. 2: In the PCOS group 100 (58.8%) patients had the presence of acne/hirsutism and in the control group, 99 (58.2%) patients had the presence of acne/hirsutism which showed no significant association (p=0.912)

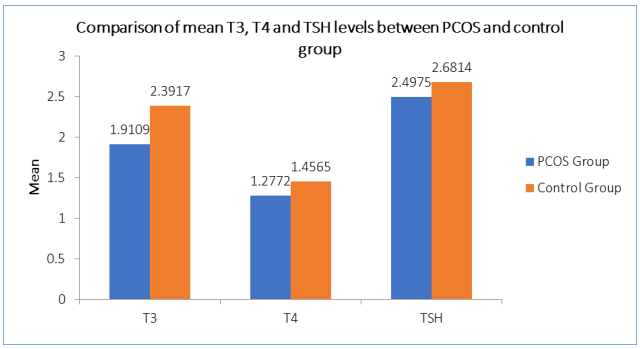

Fig. 3: The mean T3 level was 1.9109±.81328 in PCOS group and 2.3917±.57532 in the control group, the mean T4 level was 1.2772±0.36250 in PCOS group and 1.4565±1.03718 in the control group and the mean TSH level was 2.4975±0.97980 in PCOS group and 2.6814±0.71749 in the control group. In the PCOS group, the mean T3, T4, and TSH levels were significantly low as compared to the control group (p<0.001, p=0.034, and p=0.049 respectively).

Moreover, most of the target population is teenagers and women of reproductive age, and they may be unavailable for transvaginal ultrasound evaluation because of their virginal status or obesity.[12] In addition to unclear diagnostic criteria, the presence of different phenotypes with PCOS further complicates the diagnosis. The technical advances in imaging have led to an artificial increase in PCOM resulting in confusion over its use as diagnostic criteria. The diagnosis of PCOS requires objectives and quantitative criteria to help clinicians to diagnose and treat patients suffering from this complex endocrine disorder. AMH is a promising marker for this condition, as its concentration is stable throughout the menstrual cycle and is not affected by fluctuations of other reproductive hormones.[5,13]

Although AMH serum levels are used as a predictive marker of ovarian response during IVF, there are conflicting reports of its predictive value for folliculogenesis in ovulation induction with clomiphene citrate.[5]

Measuring AMH levels allows for the further investigation of PCOS and its clinical implications. The present study aims to assess the diagnostic power of serum AMH for the diagnosis of PCOS and analyze if serum AMH can replace PCOM in Rotterdam Criteria and also analyze the correlation of AMH with hyperandrogenaemia. In the present study, there was no statistical difference between the mean age of PCOS cases and controls this finding correlates with the study of Singh et al.,[14] in which no statistically significant difference was found between the mean age of the PCOS group and control group.

A similar finding was also observed by Ahmed et al.[15] Saxena et al.,[16] and Sahmay et al[17] however, a different result was found in a study by Wiweko et al[18] Johnstone et al.[19] and Hsu, M. I.,[20] in which the average age of PCOS patients were significantly younger than non-PCOS patients, they concluded that the proportion of women with PCO decreased with age. In our study the mean age of menarche was also comparable and statistically not significant between PCOS and the control group. In the present study, the mean BMI in the PCOS group was significantly high in PCOS group as compared to the control group.

PCOS tends to affect women of reproductive age, but it can also occur in younger or older women. It would be important to investigate further whether age is a significant factor in the development of PCOS or if it has any impact on the diagnostic power of serum AMH. AMH levels were found to be substantially higher in the PCOS group compared to the control group in the current study; the median AMH levels in the PCOS group were 7.1686 ±2.73776 ng/ml, which was nearly twice as high as the 3.8034± 0.63199 ng/ml in the control group (p <0.001). According to a study, women who meet all three of the Rotterdam criteria and have an AMH level of at least 11 ng/ml have an 80% chance of having PCOS.[7] In a study, 97% of women with AMH levels greater than 10 ng/ml had PCOS.[8]

In the present study, a cut-off of 4.70 ng/ml with sensitivity and specificity of 90% and 90.60%, was found as the best diagnostic potential of AMH. A 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.[9] Hence, they concluded that AMH is a more accurate predictor than follicle counts per ovary because it not only indicates AFC but also the level of hyperandrogenism.

5. Conclusion

The current Rotterdam criteria, PCOM, are used to diagnose PCOS, a complex and widespread gynecological disorder. PCOM is extremely subjective and has poor reproducibility.

Despite the fact that its sensitivity and specificity on their own are weak and no single cut-off of AMH is diagnostic, AMH is a promising method of diagnosis for PCOS as an

addition to the current Rotterdam criteria. This is especially the case when it is used to replace PCOM. The fact that AMH is a biological, objective, quantitative marker that is unaffected by the day of menstruation or OCP ingestion gives it further advantages as a diagnostic tool. Therefore, more research should be done in the future to support its use as a PCOS diagnostic tool.

6. Source of Funding

None.

7. Conflict of Interest

None.

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