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Determination of Anti-Mullerian hormone status as an additional biomarker for diagnosis of polycystic ovary syndrome

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Abstract: ***Background:*** Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in adolescents and women of reproductive age and AMH (Anti-Mullerian Hormone) is an important hormonal indicator of PCOS. ***Methods:*** This was a cross-sectional type of comparative study on 80 reproductive women attending in the OPD of Obstetrics and Gynecology, Rajshahi medical college Hospital, Rajshahi over a period of 1 year from July 2021 to June 2022. Among them 40 PCOS women were selected in group A and 40 age matched healthy women were also recruited in group B for comparison. History of oligomenorrhoea, hirsutism, BMI and serum AMH level were estimated using the auto analyzer machine. ***Results:*** Mean AMH of PCOS and healthy women were 9.89 ± 3.84 mg/dl and 1.36 ± 1.05 mg/dl, respectively. In the study, the common phenotype of PCOS was phenotype C (40%) but highest mean level of AMH was found in phenotype B which was 12.7 ± 6.6 ng/ml. AMH was nine times higher in women with PCOS than healthy women and it was statistically highly significant ($p < 0.001$). Maximum diagnostic potential for PCOS was at cut-off 2.55 ng/ml with sensitivity 90% and specificity 85%. ***Conclusions:*** As a diagnostic tool, AMH is highly sensitive and specific in case of PCOS.

Keywords: Polycystic ovary syndrome, Anti-Mullerian Hormone.

Original Research Article

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Article at a glance:

Study Purpose: *The purpose of this study was to determine whether the measurement of serum AMH could be used as an additional diagnostic tool for PCOS or not.*

Key findings: *The ROC curve of Anti-Mullerian hormone denotes that Anti-Mullerian hormone was a highly sensitive and specific additional biomarker for diagnosis of PCOS.*

Newer findings: *AMH level could be used as diagnostic and prognostic tool for PCOS.*

Abbreviations: *BMI: Body mass index, ERC: Ethical Review Committee, IR: Insulin resistance, OPD: Outpatient department, PCOS: Polycystic ovary syndrome and ROC: Receiver Operating Curve.*

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a chronic, complex and common endocrine disorder observed in women of reproductive age and it also affects the adolescents. The estimated prevalence of PCOS is reported to be 8.7% according to NIH criteria, rising to 17.8% by Rotterdam criteria and 12% using the AE-PCOS definition.[1] Till date research, the a etiopathogenesis of PCOS is still unclear, diagnostic criteria is evolving, management is complex and newer therapeutic options are being explored every day. It is important to remember that it is a syndrome more to prevent than to treat. There is a recent rise in PCOS cases in urban area due to westernization, modernization, stress and lifestyle changes. The aetiology of PCOS remains unclear and it is likely

to be multifactorial. While insulin resistance (IR) and hyperandrogenism are the two key hormonal disturbances of PCOS, other contributing factors are obesity, genetic inheritance, lifestyle and environment changes.[2] The risk factors of PCOS include irregular menstruation, family history of infertility and diabetes, mother's irregular menstruation, unpleasant mood and lack of physical exercise.[3] It can present with wide spectrum of clinical features and delayed sequelae like type 2 diabetes mellitus, cardiovascular diseases, metabolic syndrome and endometrial cancer which are preventable.

Ultrasonography is a most popular diagnostic tool of PCOS. But it has subjective variation and may lead to over diagnosis. Due to complexity of this disorder and various controversies, different diagnostic criteria have been proposed over last three decades. Due to limitations of Rotterdam criteria, a new tool AMH can be used as a diagnostic marker for PCOS.[4] Anti-Mullerian hormone is produced by the granulosa cells surrounding the preantral and antral follicles and has an important role in the development and maturation of follicles. Several studies have suggested that serum AMH levels may be a marker for polycystic ovary syndrome (PCOS). When the ovarian volume is more than 10 cm3 or the presence of 12 follicles with a diameter of 2–9 mm, it may correlate with the level of serum AMH.[5] 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 biological marker for PCOS. To the best of our knowledge a very few studies have been addressed this problem in the context of Bangladesh. Moreover, the present study might be facilitated the clinicians and gynecologists to update their knowledge regarding diagnostic tools of PCOS.

METHODS

This was a cross-sectional type of comparative study on 80 women of reproductive age in the Department of Obstetrics and Gynecology, Rajshahi Medical College, Rajshahi from July 2021 to June 2022 to determine the role of AMH as an additional biomarker for the diagnosis of PCOS. Approval of the Ethical Review Committee (ERC) was obtained prior to the commencement of the study and a purposive sampling technique was used. A questionnaire was developed for the study by consulting with the guide and reviewing the previous published literature. Prior to data collection, respondents were briefed about the purpose of the study and their informed written consent was taken. After taking informed written consent, complete history taking and physical examination were done and recorded in preformed data sheet. Blood samples were obtained from median cubital vein in antecubital fossa making the subject to sit comfortably in a chair on day 2-3 of menses or after withdrawal bleeding. Through a sterile DISPOVAN syringe under sterile precautions, about three milliliters of blood was collected in EDTA coated vaccutainers.

The sample was then analyzed for the serum AMH using auto-analyzing machine. Qualitative variables were described by frequency and percentage while quantitative variables were described by mean and standard deviation. Comparison of serum Anti-Mullerian hormone (AMH) between PCOS and healthy women was done by Unpaired t-test and cut-off values of AMH as a predictor of the diagnosis of PCOS was done by Receiver Operating Curve (ROC) procedure. Data processing and analysis were done via Statistical Package for the Social Sciences (SPSS) software, version 24.0. The statistical significance was evaluated as appropriate probability level $p < 0.05$ for all tests.

RESULTS

In both PCOS and healthy women groups, 25-35 years women were predominant higher. The mean ages of the PCOS and healthy women were 26.03 ± 4.65 years and 30.80 ± 5.84 years, respectively. There was statistically significant difference between the PCOS and healthy women in terms of age ($p < 0.001$) (Table 1).

Table 1: Distribution of the women according to their age (n=40 in each group)

Age (Years)	PCOS group	Healthy women group	p-value#
	Frequency (%)		
< 25 years	17 (42.50%)	5 (12.50%)	< 0.001
25-35 years	22 (55.00%)	29 (72.50%)	
> 35 years	1 (2.50%)	6 (15.00%)	
Total	40 (100.00%)	40 (100.00%)	

Data were analyzed by independent sample t test.

Married respondents were predominant in both PCOS and healthy women groups and it were 82.50% and 85.00%, respectively (Figure 1).

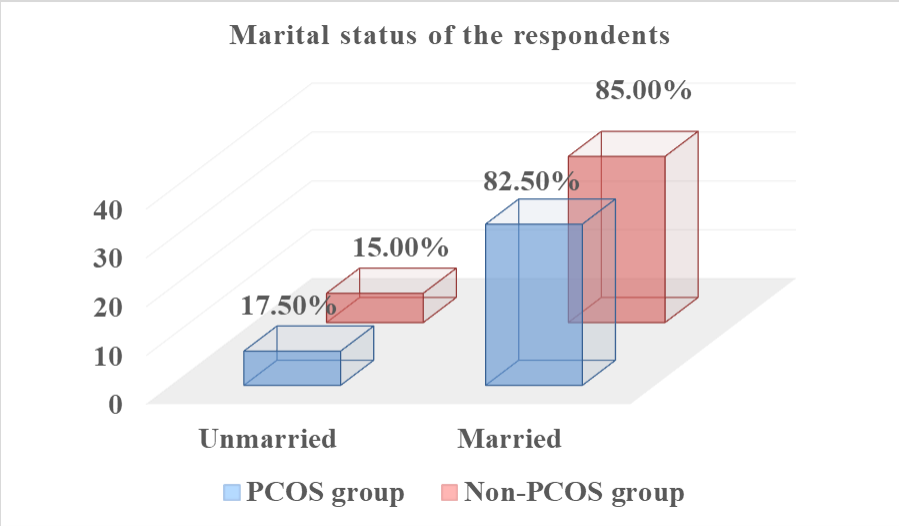

Figure 1: Marital status of the respondents (n=40 in each group).

In both groups, predominant respondents were overweight. The mean BMI of the PCOS women was 28.37 ± 1.92 kg/m² and healthy women was 27.63 ± 2.17 kg/m². There was no statistically significant difference between the PCOS and healthy women in terms of BMI (p > 0.05) (Table 2).

Table 2: Distribution of the respondents by their BMI (n = 40 in each group)

BMI (kg/m²)	PCOS group	Healthy women group	p-value#
	Frequency (%)		
< 18.5 kg/m² (Underweight)	0 (0%)	0 (0%)	> 0.05
18.5 to 24.9 kg/m² (Normal)	0 (0%)	2 (5.00%)	
25.0 to 29.9 kg/m² (Overweight)	35 (87.50%)	34 (85.00%)	
≥ 30.0 kg/m² (Obese)	5 (12.50%)	4 (10.00%)	
Total	40 (100.00%)	40 (100.00%)	

Data were analyzed by independent sample t test.

Phenotypic distribution of the PCOS women revealed that two-fifth (40%) of the respondents had phenotype C (HA+PCO), less than one-third (30%) had phenotype A (OH+HA+PCO), more than one-fifth (22.50%) had phenotype D (OA+PCO) and only less than one-tenth (7.50%) had phenotype B (HA+OA) (Figure 2).

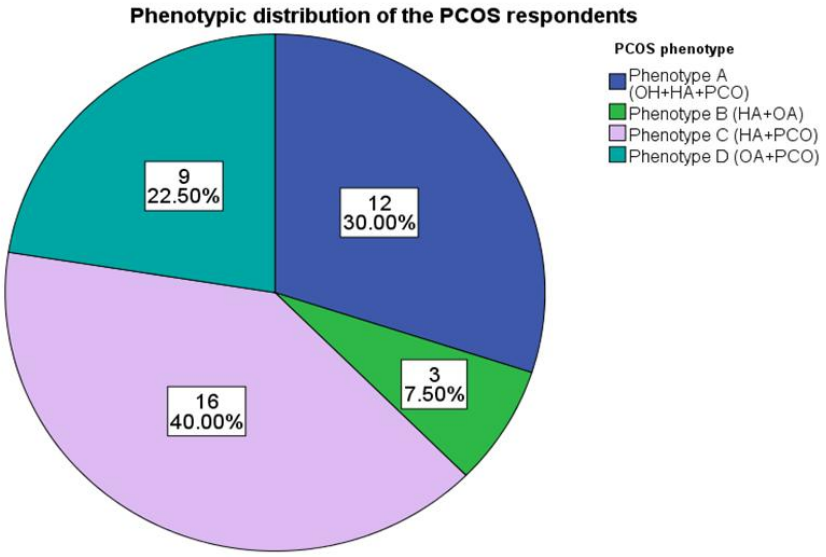

Figure 2: Phenotypic distribution of the PCOS respondents (n=40)

(N.B.- Only PCOS group was considered here)

There was no statistically significant difference among the phenotype of PCOS in terms of serum AMH level ($p > 0.05$) (Table 3).

Table 3: Relationship of the phenotype of PCOS with serum AMH level of the respondents (n=40)

Phenotype	OA	HA	PCO	Frequency (%)	Serum AMH (ng/ml) (mean ± SD)	p value
A	+	+	+	12 (30%)	8.9 ± 2.6	> 0.05
B	+	+	-	3 (7.5%)	12.7 ± 6.6	
C	-	+	+	16 (40%)	9.06 ± 3.09	
D	+	-	+	9 (22.5%)	11.75 ± 4.86	

(N.B.- Only PCOS group was considered here)

AMH level was significantly increased in PCOS group women than healthy women group ($p < 0.001$) (Table 04).

Table 4: Comparison of serum Anti-Mullerian hormone (AMH) between PCOS and healthy women group (n=40 in each group)

	Group		t-	p-
Serum AMH	PCOS group (n = 30)	Healthy women group (n = 30)	value	value
mean ± SD (mg/dl)	9.89 ± 3.84	1.36 ± 0.88	13.57	< 0.001
Range (mg/dl)	5.01 to 20.60	0.05 to 3.98		

The best cut-off value for high sensitivity without much compromise with specificity was 2.55 ng/ml with an area under the curve being 0.952 [(95% CI = 0.906-0.997), $p < 0.001$]. The area under the curve indicates that 90% of the PCOS could be correctly diagnosed with AMH 2.55 or more in PCOS patients. So, using an AMH concentration of 2.55 ng/ml, the sensitivity would be 90% and the specificity 85% (Figure 3 and Table 5).

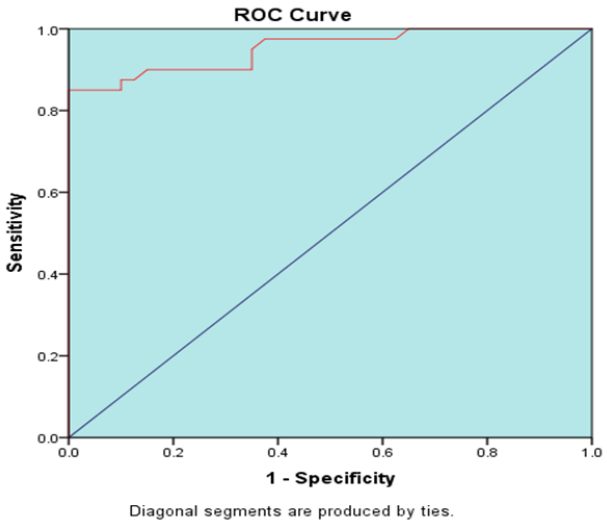

Figure 3: Receiver operating characteristic (ROC) curve of AMH with respect to PCOS diagnosis

Table 5: ROC curve analysis of Anti-Mullerian hormone for diagnosis of PCOS

ROC curve analysis for Anti-Mullerian hormone	
Area under the ROC curve	0.952
Standard error	0.23
95% Confidence interval	0.906-0.997
p value	< 0.001
Cut off value	2.55 ng/ml
Sensitivity	90%
Specificity	85%

DISCUSSION AND CONCLUSIONS

Women with PCOS exhibit a wide spectrum of clinical and biochemical characteristics. Though not all patients have the typical clinical syndrome, PCOS patients usually present with symptoms of anovulation, hyperandrogenemia and/or obesity. The anovulation may result in dysfunctional uterine bleeding or may be undetected because of cyclic menses whereas the hyperandrogenism may present as acne rather than as hirsutism or be cryptic with no cutaneous manifestations. The aim of the study was to determine whether the measurement of serum AMH could be used as an additional diagnostic tool for PCOS or not. In this study, the mean age of PCOS women (26.03 ± 4.65 years) was lower than healthy women (30.80 ± 5.84 years). These findings were consistent with the studies done by Romualdi *et al*. [6] and Jovanovic *et al*.[7] who both reported that the proportion of women with PCOS decreased with age. This can be caused by a decrease in the number of antral follicles throughout the reproductive years that occurs in normal women, a phenomenon that also applies to patients with PCOS. Murphy *et al*.[8] also reported that half of the women diagnosed with PCOS an average age of 30 years, no longer exhibited these phenotypes 8 years later. But the findings were not consistent with the study findings of Singh *et al*.[4] where age was similar in both groups. In the present study, the mean BMI of PCOS women was 28.37±1.92 kg/m^2 and healthy women was 27.63±2.17 kg/m^2 which revealed that BMI of both groups were close to each other. This was in agreement with previous studies of Wiweko *et al*.[5] and Aliyev[9] in which BMI was comparable between both the groups and no significant differences were found among the two groups. Lim *et al*.[10] reported that obesity prevalence in PCOS was lower in Asian women than Caucasian women.

In the study serum AMH level in PCOS women (9.89 ± 3.84 mg/dl) was higher as compared to the healthy women (1.36 ± 1.05 mg/dl) and it was statistically highly significant (p < 0.001). These findings have consistently been reported in numerous studies Mulders11, Sopher *et al*.[12], Yue *et al*.[13], Bhide *et al*.[14], Rudnicka *et al*.15 and Pigny *et*

al.[16]. This increase is due to increased synthesis and secretion of AMH by polycystic ovaries. Pellat *et al*.[17] reported that AMH production increases approximately 75 times higher in each polycystic ovarian granulosa cell. These findings were supported by Catteau-Jonard *et al*.[18] who found increased mRNA expression of AMH in ovarian granulosa cells. Elevated serum AMH levels in PCOS patients may also be caused by disturbances in folliculogenesis resulting in the accumulation of excessive pre-antral and small antral follicles. Cessation of antral follicle development toward the dominant follicle is due to suppression of aromatase activity by AMH and by lower follicle sensitivity to FSH.[19].

Measurement of serum AMH levels as a diagnostic modality of PCOS turned out to have a high sensitivity (90%) and specificity (85%) in the current study. The AUC of the serum AMH assay in PCOS patients reached a value of 0.952 (95 % CI 0.906 – 0.997). Optimal specificity and sensitivity were achieved at the cut-off level of 2.55 ng/mL. Results for AMH may vary depending on the assay used. Pigny *et al*.[16] found that a satisfactory specificity of 92% but a low sensitivity of 67% with an AMH cut-off of 8.4 ng/mL and a mean serum AMH of 11.42 ng/mL. Both the mean AMH and AMH cut-off values were higher than the values in the present study possibly due to their small patient population.

Lin *et al*.[20] reported that serum AMH levels were elevated in adolescent young adult Chinese women with PCOS but the serum AMH measurements offered a relatively poor diagnostic power with a sensitivity of 61.7% and a specificity of 70% at a cut-off of 8 ng/mL which were not consistent with our findings. They suggested that the low specificity and sensitivity in their study was attributable to the lower prevalence of hyperandrogenism, obesity and insulin resistance in their cohort owing to racial differences. Hart *et al*.[21] found the most effective cut-off value of AMH to be 4.2 ng/mL (30 lmol/L) which were slightly higher from our study findings. On applying receiver operating characteristic curve (ROC curve) analysis Singh *et al*.[4] reported that area under the curve was 0.98 (95% CI 0.929 to 0.998) and p value < 0.001. Maximum diagnostic potency of AMH alone for PCOS was at cut-off of 4.22 ng/ml with sensitivity of 92% (95% CI 80.8 to 97.8) and specificity of 100% (95% CI 92.9 to 100). These findings were close to our study findings.

The results of the present study were compatible with previous results only in terms of an elevated serum AMH level in women with PCOS. Both mean serum AMH levels and suggested cut-off values for AMH were inconsistent among the studies, probably because of differences in sample size, sample selection criteria and specified PCOS phenotypes among the studies. Such difference in the cut off value might be because of different types of diagnostic system laboratories being used.

The main advantages of this study were homogeneity of study population with respect to geographic origin and no infertility treatment were associated here. However, this could also be considered as a limitation of the study as these women might not necessarily be representative sample of the general population. Furthermore, the ultrasound ovarian examination and the assessment of hirsutism were performed by the same physician, thus eliminating inter-observer bias.

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Authors' contributions

IS, RA: Concept and design, data acquisition, interpretation, drafting and final approval. IS, SAS and NNP: Data acquisition, interpretation, drafting, final approval and agree to be accountable for all aspects of the work.

Declarations

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Conflict of interest

Authors declared no conflict of interest.

Ethical approval

Ethical approval of the study was obtained from the Ethical Review Committee, Rajshahi

Medical College, Rajshahi and informed consent was taken from all participants. Methodology of the study was carried out following the relevant ethical guidelines and regulations.

Consent for publication: Taken.

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