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Role of antimullarian hormone in the diagnosis of sonographically inconclusive polycystic ovary syndrome

Hurjahan Banu, Md Shahed Morshed, SadiqaTuqan, Nazma Akhtar, MA Hasanat

Article Info

Department of Endocrinology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh (HB, MAH); Department of Emergency, Kurmitola General Hospital, Dhaka, Bangladesh (MSM); Department of Endocrinology, Square Hospital, Dhaka, Bangladesh (ST); Department of Medicine, Shahid Tajuddin Ahmad Medical College, Gazipur, Bangladesh (NA)

For Correspondence:
Dr. Hurjahan Banu
Email: dr.hurjahan_banu@yahoo.com

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Abstract

Antimullarian hormone (AMH) is found to be a vital tool for the diagnosis of polycystic ovary syndrome (PCOS). AMH may help in the diagnosis of sonographically inconclusive cases of PCOS. This study measured the AMH level in PCOS to assess its impacts on the diagnosis of the syndrome. This cross-sectional study included 160 newly diagnosed females with PCOS who were diagnosed using a modified revised Rotterdam criteria. Fasting blood was collected to measure AMH by enzyme-linked immunosorbent assay and other hormones [total testosterone, luteinizing hormone and follicle-stimulating hormone] were measured by chemiluminescent microparticle immunoassay. Ovarian USG was done in the follicular phase of the menstrual cycle. Serum AMH≥ 3.5 ng/mL and ovarian volume >10 mL was considered as a combined marker of polycystic ovary (PCO). USG-PCO criteria could detect 84.38% PCO, whereas AMH-PCO criteria 67.5%. There was a lack of agreement between USG-PCO and AMH-PCO criteria [κ=-0.004] in PCOS. AMH-PCO criteria identified 68% of PCO patients undiagnosed by USG-PCO criteria [17/25]. Age [β=-0.172, p=0.040], systolic [β =-0.213, p=0.037] and diastolic blood pressure [β=0.301, p=0.004] had significant predictive associations with AMH by linear regression. AMH had a fair discriminating index for combined-PCO [AUC=0.824] in PCOS patients. In conclusion, AMH assessment can help detect PCOS patients who are inconclusively diagnosed by USG-PCO criteria.

Introduction

Polycystic ovary Syndrome (PCOS) is a prevalent, diverse female reproductive endocrinopathy associated with severe reproductive, cutaneous, metabolic, and cardiovascular morbidity. The updated Rotterdam criteria are routinely used to diagnose PCOS, and an international evidence-based guideline for the evaluation and treatment of PCOS was published in 2018.[1,2] A unique ovarian appearance (polycystic ovary, PCO) detected by ultrasonography (USG) is one of the features of PCOS. The ovarian volume (OV) threshold (>10 ml) has remained the same however the follicle number per ovary (FNPO) criteria has altered from 12 to 25 as technology has advanced (transducer frequency of 4-8 MHz).[1] Furthermore, USG is operator-dependent, and transvaginal USG is not appropriate for many females, particularly in our social setting. It's also less specific up to eight years of gynecological age; thus, it's not included in the adolescent PCOS diagnostic criteria.[1] Without PCO, many individuals report ovulatory failure or hyperandrogenism symptoms. Furthermore, there might be a variety of PCOS phenotypes.[3] In these situations, PCOS diagnosis may be delayed or undetected. As a consequence, a time-demanding alternate test is required.

Even in adolescent PCOS, serum antimullarian hormone (AMH), a glycoprotein released mainly through granulosa cells of tiny preantral follicles, is a potential predictor of PCO and correlates substantially with FNPO.[4] AMH may be able to replace PCO with greater sensitivity and specificity, according to recent findings.[5] It may be done at any time throughout the menstrual cycle. It's also utilized as an ovarian reserve marker, and it's linked to various PCOS symptoms and severity.[6] The worldwide evidence-based recommendation, however, does not

advocate it as a substitute for PCO. This is because blood AMH levels are affected by various parameters, including age, race, and body mass index (BMI). Consequently, AMH cannot be utilized as a universal marker of PCO since it may be measured using a variety of assay techniques with differing cut-offs.[1] As a result, population- and assay-specific AMH may be a viable alternative. AMH was discovered to be a marker of PCOS in a previous Bangladeshi research.[7] However, there has been no research into employing AMH in conjunction with sonographic ovarian appearance as a diagnostic marker in the Bangladeshi population as far our best knowledge. The goal of this research was to explore whether AMH might aid in the diagnosis of PCOS in situations when the USG-PCO test was negative.

Methods

This cross-sectional research included 160 persons with PCOS who were in reproductive age and had a gynecological age of more than eight years [age (years): 23.0 (19.0, 26.0); BMI (kg/m^2): 26.03 (22.78, 28.80), median (IQR)] at BSMMU's Department of Endocrinology. To diagnose PCOS, we employed a modified updated 2003 Rotterdam criteria (two of the following three: oligo/anovulation (OA), clinical and/or biochemical evidence of hyperandrogenism (HA), and PCO by USG and/or AMH, as well as the exclusion of other disorders).[5] The BSMMU institutional review board gave its approval to the research protocol. Each participant signed an informed written permission form. Physical exams and pertinent reproductive history were obtained [height, weight, blood pressure (BP), hirsutism, acne and acanthosis nigricans]. Obesity was defined as a BMI of 25 kg/m^2 or more, computed from height and weight.[8] The modified Ferriman-Gallwey (mFG) score was employed to measure hirsutism, with a score of 8 considered significant.[9] Total testosterone (TT), luteinising hormone (LH), follicle-stimulating hormone (FSH), and AMH were all measured in fasted blood. AMH was quantified using the AMH GEN II ELISA kit (Beckman Coulter, Inc. USA), which had an intra-assay coefficient of variability of 3.4–5.4% and an inter-assay coefficient of variability of 4.0–5.6%. As a marker of AMH-PCO, we chose a serum AMH cut-off of 3.5 ng/mL.[7] Chemiluminescent microparticle immunoassay was used to test other hormones. Hyperandrogenemia and an altered ratio were defined as TT >46 ng/dl and LH/FSH ratio (LFR) >2.0, respectively.[10,11] USG of the ovaries was performed via trans-abdominal or trans-vaginal route depending on marital status during the follicular phase of the menstrual cycle. USG-PCO was defined as any OV more than 10 ml.[1]

The statistical analysis was carried out using version 23.0 of the statistical program for the social sciences (SPSS). As applicable, data were reported as median (interquartile range, IQR) or frequency (percentages, %). Cohen's kappa (κ) test was used to examine the agreement between USG-PCO and AMH-PCO. Kruskal Wallis one way ANOVA with post hoc Dunn's test were done for comparison of more than two groups. Multivariate linear regression analysis was done to see the predicted relationships between various factors and AMH. AMH was studied using a receiver operating characteristic (ROC) curve to determine whether it might be used as a marker for combined PCO. Statistical significance was defined as a P value of less than 0.05.

Results

Figure-1 depicts the influence of AMH and OV as PCO indicators. In 61 (38.1%) instances, USG and AMH disagreed, and in 8 (5.0%) cases, both could not detect PCO. PCO was found in 27 (16.9%) more cases by USG than by AMH. AMH discovered 17 of the 25 instances of PCO that were not reported by USG (68 per cent). Because Cohen's kappa value was less than zero, there was no agreement between USG-PCO and AMH-PCO criteria.

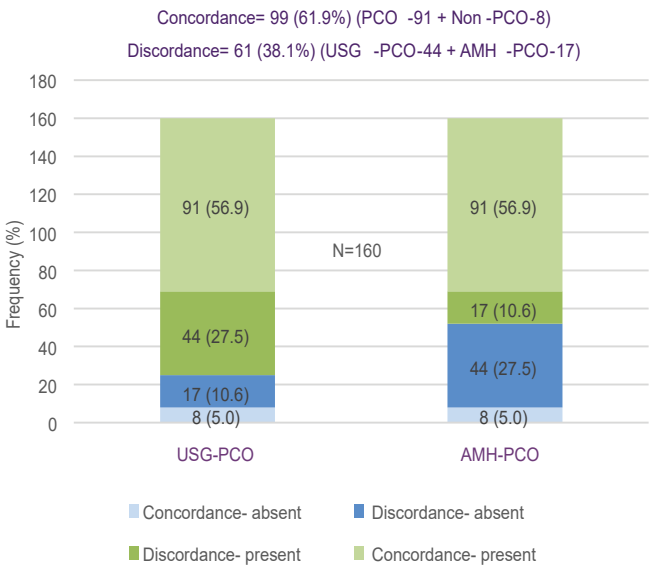

Figure-1. Comparison of USG-PCO and AMH-PCO criteria

Table-I illustrates the research population's characteristics based on a combination of USG and AMH PCO criteria. Except for systolic BP, which was substantially higher in the only USG-PCO group than the both-negative PCO group [post hoc adjusted p=0.036]. Patients with USG-PCO had AMH that was statistically equivalent to those without [5.82 (2.45, 10.4) vs. 8.90 (3.0, 12.1), p=0.308].

Table-I

Characteristics of the study population (N=160)

Variables	Both PCO (n=76)	Only USG-PCO (n=59)	OnlyAMH-PCO (n=16)	No PCO (n=9)	p
Age, years	23.0 (19.0, 25.75)	23.0 (20.0, 26.0)	21.0 (18.25, 25.75)	20.0 (18.0, 29.25)	0.474
BMI, kg/m^2	23.03 (25.85, 28.77)	26.57 (22.0, 29.0)	25.34 (24.22, 29.78)	25.50 (22.36, 27.75)	0.917
WC, cm	83.0 (78.0, 91.50)	86.0 (80.0, 96.0)	83.50 (78.50, 90.0)	86.0 (82.0, 93.50)	0.664
Systolic BP, mm-Hg	105.0 (100.0, 117.50)	110.0 (100.0, 120.0)	110.0 (100.0, 120.0)	100.0 (92.50, 110.0)	**0.029**
Diastolic BP, mm-Hg	80.0 (70.0, 80.0)	80.0 (70.0, 80.0)	80.0 (70.0, 87.0)	70.0 (65.0, 80.0)	0.140
Modified F-G score	9.0 (6.0, 14.0)	9.0 (7.0, 12.0)	10.50 (8.0, 13.0)	11.0 (5.50, 13.50)	0.908
Total testosterone, ng/dL	45.95 (23.0, 84.42)	73.20 (44.60, 94.50)	43.75 (33.45, 71.73)	58.10 (40.25, 90.20)	0.053
LH/FSH ratio	1.15 (0.72, 1.99)	1.43 (0.64, 2.15)	1.83 (1.01, 2.65)	1.86 (0.84, 3.01)	0.268

Data were expressed in median (Inter-quartile range). Kruskal Wallis One-way ANOVA with pairwise comparison by Dunn's test was done

Table-II

Multivariate linear regression analysis of serum AMH (dependent variable) with clinical and hormone profile

Independent variables	β	p
Age, years	-0.172	**0.040**
BMI, kg/m^2	0.227	0.174
WC, cm	-0.145	0.374
Systolic BP, mm-Hg	-0.213	**0.037**
Diastolic BP, mm-Hg	0.301	**0.004**
mFG score	0.023	0.776
LH/FSH ratio	-0.005	0.946
Total testosterone, ng/dL	-0.041	0.620
Constant	–	0.098

β (standardised regression co-efficient)

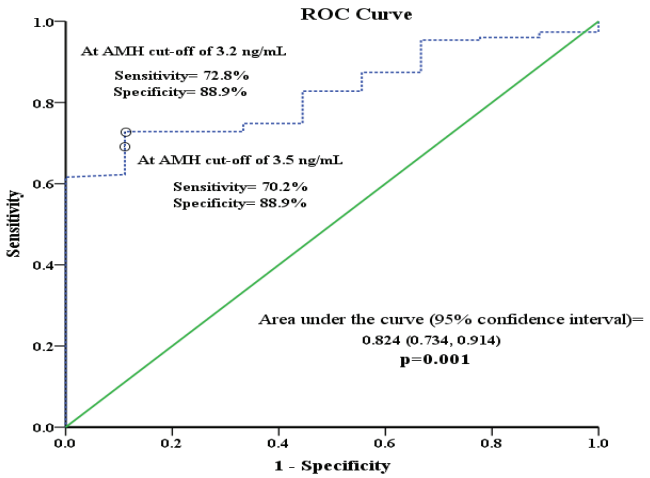

Figure-2. ROC curve analysis of serum AMH as a marker of combined PCO

Age, systolic BP (SBP), and diastolic BP (DBP) all demonstrated significant predictive relationships with AMH [(**β**, p): age (-0.172, 0.040); SBP (-0.213, 0.037); DBP (0.301, 0.004)] (Table-II).

AMH may be deemed a reasonable marker for combined-PCO, according to a ROC curve study (area under the curve, AUC= 0.824, p= 0.001). The sensitivity was 70.2 per cent, and the specificity was 88.9 per cent when using a cut-off of 3.5 ng/ml. The sensitivity rose slightly to 72.8 per cent with a lower cut-off of 3.2 ng/mL, but the specificity remained the same (Figure-2).

Discussion

This research aimed to investigate whether a combination of elevated blood AMH and USG-PCO may help to detect more PCOS patients. In 17 of the 25 patients where USG failed to identify PCO, serum AMH was shown to see it. However, when it came to detect PCO, USG-OV criteria outperformed AMH. Our findings are comparable to those of prior research.[12]

We found concordance of USG and AMH in 61.9% of instances. This is a lower figure than in earlier research.[5,13] Elevated AMH and PCO by USG are thought to be two

different manifestations of PCOS. While AMH is linked to a brain phenotype (higher LH pulse frequency), PCO by USG is connected to metabolic phenotype.[5] As PCOS is a diverse disorder, combining these two may help identify more patients.

Several studies have already confirmed AMH cut-offs for the diagnosis of PCO in various categories. In a cohort analysis, Carmina et al. (2016) discovered a cut-off value of >4.7 ng/mL with a sensitivity of 79% and a specificity of 96%. (AUC: 0.952).[12] In the more recently published APHRODITE research, an AMH cut-off of 3.2 ng/mL resulted in a sensitivity of 88.6% (95% CI: 85.3–91.3) and specificity of 84.6 per cent (95% CI: 81.1–87.7) for PCO diagnosis.[14] ROC curve analysis demonstrated that AMH was a good marker for combined-PCO (AUC= 0.824) with a sensitivity of 70.2 per cent and specificity of 88.9% when using a cut-off of 3.5 ng/mL in our research. When the cut-off level is adjusted to 3.2 ng/ml, the sensitivity rose to 72.8 per cent, but the specificity remains the same. The AMH cut-off established by Carmina et al. (2016) was designed for PCOS diagnosis rather than PCO diagnosis.[12] This explains why the AMH cut-off in this research was higher than ours (4.7 vs. 3.2, ng/mL). AMH readings have also been shown to vary amongst various immunoassay techniques.[15] This shows that in ordinary clinical practice, AMH values should be interpreted according to the test.

According to recent recommendations, women with PCOS still remain undiagnosed, and the USG criterion could not be employed as one of the diagnostic criteria in the teenage group. The AMH test used in this research has a low sensitivity for detecting PCO but a reasonable specificity for ruling out false positives.[16] Because patients often report symptoms indicative of PCOS (e.g., HA or monthly menstrual irregularity), using a sensitive and specific assay to determine PCO might help doctors identify PCOS more promptly.

The FNPO was found superior than OV or cross-sectional follicle count in some studies.[17,18] OV might be different depending on the phenotypes of PCOS.[19] We could not include FNPO due to use of different routes and machines to count them. However, OV strongly correlates with FNPO and it also correlates with AMH.[20,21] According to Li et al. (2012), AMH readings are much lower in PCOS patients with normal androgen levels, suggesting that AMH is only relevant for predicting PCOS in those with hyperandrogenism.[22] Comparing the hyperandrogenic and normoandrogenic females with PCOS, Köninger et al. (2014) discovered that AMH was superior to androgens and equivalent to antral follicle numbers in hyperandrogenic women.[23] In our investigation, testosterone had no predictive relationship with AMH, while the USG-PCO group had considerably higher testosterone levels. In our investigation, we discovered that the patient's age might predict AMH levels and a substantial negative connection, comparable to Ran et al.'s (2021) results, who found similar alterations in a healthy control group.[24] However, another research found comparable alterations in healthy young females but no link in PCOS people.[25] We also discovered a link between blood pressure and AMH. A recent study found a strong link between AMH and metabolic syndrome, but not with BP.[26] However, that study excluded patients with obesity. Our study's principal limitations were the limited number of patients and the use of only OV criteria assessed by different routes and sonologists.

Conclusions

AMH is not as useful as USG in diagnosing PCOS in all individuals suspected of having PCOS; however, combining AMH with USG-OV may give an extra advantage in diagnosing more cases. AMH is also an expensive and inaccessible test. So, based on the findings of this research, we advocate testing AMH to diagnose PCOS when the USG of the ovary is not indicative of PCOS in a patient who meets just one of the other two criteria.

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Conflict of interest

None of the authors has any conflict of interest to declare.

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