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Serum Anti-Mullerian Hormone (AMH) Levels are Effective in Predicting the Diagnosis of Four Polycystic Ovarian Syndrome (PCOS) Phenotypes

Justin Armstrong, MD., Christina Cortes, M.D, Kristina Hawkins, M.D and Abdelmoneim Younis, PhD*

Department of Obstetrics and Gynecology, & Women's Care Fertility & Reproductive Medicine, Atrium-Health Navicent, Mercer University School of Medicine. Macon, GA, United States, 31210.

***Correspondence:**
Abdelmoneim Younis, DVM. PhD, Women's Care Fertility & Reproductive Medicine, Atrium Health Navicent, Department of OB/GYN, Mercer School of Medicine. 4075 Elnora Dr. Macon, GA 31210; USA, Tel: 7708265257; Fax: 478-757-7887.

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ABSTRACT

Purpose: *Women with PCOS have higher levels of AMH than matched controls; however, the feasibility of using elevated serum AMH value as a criterion, in the diagnosis of PCOS, is still debatable. The goal of this study was to examine a population of women with elevated AMH (>5.0 ng/mL) and evaluate whether high serum AMH value can be predictive of four different clinical PCOS phenotypes (phenotype A (AOM, amenorrhea/oligomenorrhea + HA, hyperandrogenism + PCO, polycystic ovaries); Phenotype B: AOM + HA; Phenotype C: HA + PCO; and phenotype D: AOM + PCO, as defined by the Rotterdam criteria.*

Methods: *This retrospective study included 227 women with one or more diagnoses of PCOS (ICD-9 256.4, ICD-10 E28.2) and 103 women without PCOS. All serum AMH levels were measured using Beckman Access-2 automated chemiluminescence assay and the age, BMI and AMH levels were analyzed using univariate analysis of covariance. Received operator curves were used to determine the AMH thresholds for predicting PCOS features and phenotypes*

Results: *Mean serum AMH levels were 9.96, 6.84, 6.43, 6.03, and 1.98 ng/ml in women with PCOS phenotype A, B, C, D, and control respectively. 101 (44.5%) patients were oligo/amenorrheic PCOS, 98 (43.2%) were hyperandrogenic PCOS, and 103 (45.4%) were PCO. Women with all three PCOS features had a significantly higher mean serum AMH compared to those with less of these features. The area under the curve (AUC) estimates of AMH showed high value ranging from 0.76 (95% CI, 0.71-0.81) in AOM group to 0.82 (95% CI, 0.79-0.88) in the PCO group.*

Conclusion: *This study confirms the diagnostic opportunity of AMH test for discriminating between patients with PCOS phenotype and controls. High AMH accurately predicted PCOS in 92% (209 out of 227) patients diagnosed with PCOS. AMH value can predict PCOS in 78% women with oligo/amenorrheic PCOS, 77% with hyperandrogenic PCOS, and 79% with PCO. In keeping with the view that women with PCOS have a variety of phenotypic presentation that can be challenging to diagnose, using AMH test in combination with oligo/amenorrhea or hyperandrogenism offers a non-invasive objective tool to screen patients with clinical features of PCOS.*

Keywords
AMH, PCOS Phenotypes, Diagnostic criteria.

Introduction

Polycystic ovarian syndrome (PCOS) currently affects up to 10% of reproductive-age women and is increasing in prevalence [1]. PCOS is also the most common cause of anovulation and hyperandrogenism in young women and represents 80% of anovulatory infertility cases [2,3]. Despite the increasing prevalence and the significant metabolic side effects, a recent study reported that over thirty-five percent of patients required over two years and three to four health professionals before they received their PCOS diagnosis. While PCOS can present with varying symptoms and severity in women, a thorough understanding of disease pathophysiology and a standardized diagnostic procedure must be adopted to diagnose PCOS early in presentation and minimize patient mortality and morbidity [4]. PCOS is associated with increased androgens and gonadotrophin-releasing hormone (GnRH) pulse generations [5]. Higher frequency GnRH pulses cause a high luteinizing hormone to follicle-stimulating hormone (LH:FSH) ratio (mostly due to high LH instead of low FSH) which has many implications in PCOS symptomatology. Increased LH causes a further increase in androgen secretion [6]. Hyperandrogenism causes hirsutism and acne, phenotypic traits characteristic of PCOS [7]. Androgens also cause an increased number of small antral follicles, which contain granulosa cells that release AMH. Normally, AMH decreases FSH receptor expression and ovarian aromatase expression, protecting small follicles from premature aromatase expression [5,8]. When AMH levels are high, this protective process goes awry and inhibits the development of a dominant follicle, causing follicular arrest and anovulation. This explains the observation of increased primordial and primary follicles in women with PCOS. Interestingly, these follicles have lower rates of atresia. As a result, premature depletion of follicular pools is typically not observed in PCOS [5]. Typical symptoms of PCOS include hirsutism and acne, and anovulation or oligo-ovulation. Women with PCOS have a higher risk of infertility, cancer, metabolic disease (obesity, diabetes mellitus, and dyslipidemia), cardiovascular disease, and obstructive sleep apnea [7,9]. Recent studies show an increased risk of endometrial cancer in women with PCOS, specifically in premenopausal women with PCOS [10]. There is also a significant psychiatric burden of a PCOS diagnosis. The broad symptoms associated with PCOS make the diagnosis challenging as patients may see a variety of different health professionals for what they believe is a combination of unrelated health problems. Many of the PCOS comorbidities such as metabolic disease and infertility are best treated if caught early, making early detection essential to improve long-term health [13]. A process of exclusion currently achieves diagnosis of PCOS. According to the Rotterdam consensus, patients must have two of the three following criteria: hyperandrogenism (clinical and/or biochemical), ovulatory dysfunction (irregular menses), or polycystic ovarian morphological features (twelve or more antral follicles that are 2 to 9 mm in diameter in either ovary, an ovarian volume that is greater than 10 ml in either ovary, or both) [7]. Antral follicle count (AFC) is obtained through a vaginal ultrasound, which has many shortcomings and can lead to an inconsistent diagnosis of PCOS [7]. First, ultrasound technology is evolving. More advanced ultrasound equipment and software allow more follicles to be detected. This may result in changes to the definition of and numbers of follicles required for the diagnosis of polycystic ovarian morphological features. Additionally, different ultrasound techniques and equipment lead to variations in follicle counts between providers and locations. Ovarian follicles are common in adolescent girls undergoing puberty. Potentially increased AFCs along with irregular ovulation and acne that typically presents during puberty further complicates the diagnosis of PCOS in pubertal girls [13].

While not a comprehensive list, the previously mentioned shortcomings provide insight into why another diagnostic tool would be beneficial for providers and patients. A potential diagnostic tool for PCOS is serum AMH. Several studies have shown that high serum levels of AMH is indicative of PCOS [2,14-16]. However, current PCOS guidelines recommend against using AMH as a diagnostic tool [17].

Therefore, more research is needed to investigate whether serum AMH is an effective alternative for diagnosis of PCOS. Furthermore, data have shown the AMH/AFC ratio is higher in PCOS phenotypes with anovulation [12], and AMH production by follicles is higher in PCOS patients when they are anovulatory [13]. This suggests that serum AMH assay may provide an assessment of the severity of ovulatory dysfunction and screening for PCOS phenotypes. The objective of this study was to investigates a population of women with elevated AMH and evaluate its relationship with clinical, biochemical, and sonographic features of PCOS. The aim was to attempt to promote AMH value utility in PCOS diagnosis using Rotterdam ESHRE/ASRM criteria by providing new evidence for high serum AMH as a diagnostic tool.

Materials & Methods

This retrospective study was conducted in women seen at a university-affiliated reproductive medicine institute for evaluation and treatment of infertility between. Data of records of infertility workup including serum hormone levels and ultrasonography were obtained from electronic medical system of patients screened for diagnoses of PCOS using the 2003 Rotterdam ESHRE/ASRM criteria between January 2015 and December 2020. Exclusions were those women with Cushing's syndrome, adrenal cortical hyperplasia, thyroid dysfunction, hypothalamic/pituitary amenorrhea, and age <18 or >41 years. Data from patients with clinical and/or biochemical evidence of hyperandrogenism (HA), evidence of oligo-anovulation (AOM) and ultra-sonographic evidence of a polycystic ovary (PCO) were analyzed.

Evidence of oligo-anovulation (AOM, amenorrhea or oligomenorrhea) was defined as 0–8 menses per year and/or menstrual cycle intervals >35 days. Ultra-sonographic evidence of a polycystic ovary were based on the new PCO guideline (19)

in which an ovary must contained ≥20 antral follicle count. All AFC were measured during the follicular phase (days 1–5) of the menstrual cycle by an experienced REI physician using GE Voluson S6 ultrasound system (USA) with a 3D capable probe. The serum AMH levels were measured using Beckman Access-2 automated chemiluminescent immunoassays. The clinical and biochemical features of hyperandrogenism (defined as circulating total testosterone levels above the 95th percentile and/or presence of hirsutism, acne, and/or alopecia) were collected and analyzed. To effectively evaluate the diagnostic effectiveness of serum AMH in PCOS patients, we categorized study population into the four PCOS phenotype subgroups: phenotypes A (Classic, AOM+HA+PCO), phenotype B (AOM + HA), phenotype C(HA + PCO) and phenotype D(AOM+ PCO).

Statistical analysis was performed using SPSS software (version 25.0, SPSS Inc., Chicago, IL). Continuous data are presented as mean and standard deviation. Student's t-test and ANOVA were carried out when appropriate. The $\chi 2$ test was used for categorical data with usual correction for small samples when appropriate. Received operator curves (ROC) were used to defined appropriate AMH thresholds for predicting women with the four PCOS phenotypes. Youden Index approach was used to determine the optimal cutoff value of AMH to diagnose PCOS, which is the maximum sensitivity-(1-specificity), and the shortest distance on the ROC between the optimal sensitivity and specificity ($[1 - \text{sensitivity}]^2 + [1 - \text{specificity}]^2$).

Results

In this study, 1344 women were screened for one or more diagnoses of PCOS (ICD-9 256.4, ICD-10 E28.2), AMH information was found in only 227 women of those 76 (33.5%) were identified with classic PCOS (phenotype A), 48 (21.1%) phenotype B, 50 (22.0%) phenotype C, and 53 (23.3%) phenotype D. The age, BMI, measured AMH levels, and AUC values of the four PCOS phenotype subgroups and control patients are shown in Table 1.

The age and BMI did not differ significantly between controls and the four PCOS phenotype subgroups, whereas as AMH levels and AUC values were significantly ($P<0.001$) higher in-patient with PCOS compared to controls. There was a significant ($p<0.01$) difference between severe PCOS (phenotype A) patients and the mild PCOS (Phenotype B, C, &D).

Table 2 shows the mean AMH values and ROC curves analysis for AMH as a predictor of PCOS women with AOM, HA, PCO. In this cohort, 101 of 227 (44.5%) patients were oligo/amenorrheic PCOS, 98 of 227 (43.2%) were hyperandrogenic PCOS, and 103 of 227 (45.4%) were PCO (Table 2). Women with PCO had higher mean serum AMH level (8.27 ± 2.3 ng/ml) compared to women with either hyperandrogenism (6.21 ± 2.9) or oligo/amenorrhea (6.04 ± 2.8, $p<0.01$). ROC curve analysis for AMH as a predictor of PCOS show that AMH value can accurately predict PCOS in 78% (177 out of the 227) patients diagnosed with AOM, 77% (174 of 227) with HA, and 79% (179 or 227) with PCO.

Table 1. Age, BMI, Mean Serum AMH levels values discriminating the four PCOS phenotype subgroups and Control.

Parameter	Phenotype A (PCOM+AOM+HA) n=76	Phenotype B (AOM+HA) n=48	Phenotype C (PCOM+HA) n=50	Phenotype D (AOM + PCOM) n=53	Normo-ovulatory (Control) n=103
Age (yr: mean ±SD)	29.6 ± 4.2	30.4 ± 4.5	31.7 ±4.2	30.6 ± 4.2	33.9 ± 4.6
BMI mean ±SD	34.4 ± 8.5	32.8 ± 8.5	29.6 ± 7.5	32.1 ± 9.9	31.1 ± 7.2
Mean serum AMH, ng/ml (95% CI)	9.96 (9.32-10.59)*	6.84 (6.43-7.24)**	6.43 (6.03-6.89)**	6.03 (5.66-6.40)**	1.98 (1.72 -2.24)

PCOM=presence of polycystic ovaries; OAM=oligo/amenorrhea; HA=clinical and/or biochemical signs of hyperandrogenism. CL= Confidence Interval for Mean. Univariate ANOVA, *p-value for phenotype A vs B, C, or D =<0.001; p value B vs D, B vs C, C vs D NS at p>0.05.

Table 2. Comparison of mean serum AMH values in women presenting with oligo/amenorrhea, hyperandrogenism, or polycystic ovary; and the ROC curve analysis for the AMH as a predictor of PCOS.

PCOS Feature	Mean AMH (ng/ml) ±SD	AUC (95% CI)	Threshold AMH value (ng/ml)	Specificity (%)	Sensitivity (%)
AOM (N=101)	6.21 ± 2.9	0.76 (0.71-0.81)	5.58	80%	74%
HA (N=98)	6.04 ± 2.8	0.77 (0.72-0.82)	5.58	81%	75%
PCOM (N=103)	8.27 ± 2.3*	0.83 (0.79-0.88)*	7.58*	82%	79%

AMH=Anti-Mullerian Hormone; AUC=area under the curve; PCOM=presence of polycystic ovaries; OAM=oligo/amenorrhea; HA=clinical and/or biochemical signs of hyperandrogenism. *P-value <0.01.

The area under the curve was 0.76 (95% CI 0.71-0.81) for AMH as a predictor of AOM with cut-off level of AMH of 5.58ng/ml. In the hyperandrogenism group, the AUC was 0.77 (95% CI,0.72-0.82) with cut-off level of AMH of 5.58ng/ml. The AUC in the PCO group was 0.83 (95% CI, 0.79-0.88) with cut-off level of AMH of 7.2ng/ml which slightly higher ($p<0.055$) than those of AOM or HA.

Discussion

PCOS is the most common endocrine disorder in women of reproductive age and the leading cause of HA, and AOM causing infertility. Diagnosis is complicated by a wide range of phenotypes and consists of confusing women's testosterone blood test levels for assessing hyperandrogenism and the invasive vaginal ultrasound AFC that may not be feasible or reliable in certain patients. Furthermore, ever-advancing technology can make some PCO data collected from vaginal ultrasounds unreliable and outdated. Advanced ultrasound machines and software place a significant financial burden on medical facilities as well as patients and may not be feasible for rural or underserved areas. Serum AMH levels are inexpensive compared to an ultrasound, lessening the financial burden of a diagnosis for both provider and patient. With the availability and adoption of automated immunoassay systems by major diagnostic laboratories and the development of a universal standard reference range for its values, AMH levels will be accurate across all patient populations and will remove the variable present in vaginal ultrasounds due to equipment and software advances.

Data from the present study reports the diagnostic opportunity of AMH test for discriminating between patients with PCOS phenotype and controls. High AMH accurately predict PCOS in 92% (209 out of the 227) patients diagnosed with the four different clinical PCOS phenotypes, conversely, when applied to the control population, it accurately predicted the absence of PCOS in 100% (103 out of 103) patients. AMH test was both sensitive and specific for predicting a diagnosis of PCOS by Rotterdam criteria. Additionally, our data reports that high AMH value can accurately predict PCOS in 78% (177 out of 227) patients with menstrual disturbance, 77% (174 of 227) of women with clinical or biochemical signs of hyperandrogenism, and 79% (179 or 227) of patient with confirmed PCO.

In agreement with our findings, a few other studies [13-18] have shown positive correlation between high AMH with the PCOS phenotypes and suggested that AMH could represent a useful marker for the diagnosis of PCOS. However, challenges to the use of AMH to diagnose PCOS include lack of standardized optimal AMH assays and little consensus on appropriate reference range stratified by age that can associate thresholds with clinical features of PCOS [19]. Moreover, a study compared the performance of five commercial AMH assays in the diagnosis of PCOS and found that whilst the assays offered similar performance, newer automated assays reported 23–30% lower values than manual assays [20]. The serum AMH levels in this study was measured in-house using a Beckman Access-2 automated chemiluminescent immunoassays which was validated and compared with a known major diagnostic laboratory AMS assay using different automated chemiluminescent immunoassays system [21]. With the availability and adoption of automated immunoassay systems by laboratories and the development of a universal standard reference range stratified by age, we believe it is high time to use serum AMH levels as a diagnostic screening tool for identifying PCOS. The strength of our study lies in the fact that women were screened for features of PCOS by a board-certified reproductive endocrinologist with experience in the accurate assessment of AFC and ovarian morphology, thus reducing inter-observer bias. Limitations of our data include retrospective selection of women seeking fertility treatment between 18-40 years of age. Consequently, women over 40 years of age, with low AMH were not included in the present study. Furthermore, it was not possible to address whether higher values of AMH over the reporting limit of the assay would confer even greater indication of severe PCOS.

Conclusion

Our data showed that AMH is a strong predictor of PCOS clinical phenotypes, and its value is both sensitive and specific for diagnosis of mild or severe PCOS patients. Our data, in keeping with the view that women with PCOS have a variety of phenotypic presentation that can be challenging to diagnose in rural underserved area, AMH test in women with oligo/amenorrhea or hyperandrogenism is the best approach.

Authors' contributions

1. JA: Data collection and drafting the manuscript
2. CC: Data collection, and drafting the manuscript
3. KH: Conception and interpretation the data and review of manuscript.
4. AY: Principal investigator, data analysis, critical revision, and edition of the manuscript.

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