

Anti-Mullerian Hormone: An Indicator for the Severity of Polycystic Ovarian Syndrome

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ABSTRACT

Background: Polycystic ovarian syndrome (PCOS) is a common endocrinopathy that accompanied with long term complications. *Purpose* Features of PCOS including sonographic aspects, androgens, luteinizing hormone (LH) and lutenizing and follicular stimulating hormones ratio LH/FSH ratio as well as Anti-Mullerian Hormone (AMH) were evaluated according to their diagnostic potency in detecting different degrees of PCOS severity.

Objective: The aim of this study to assess the possible role of AMH as the diagnostic marker for different degrees of PCOS.

Materials and Methods: In this cross-sectional study, a total of 150 women aged 18–46 years Patients were consecutively included as they presented in our clinic. 50 patients were diagnosed with sever PCOS (based on Rotterdam criteria consensus), 50 patients were diagnosed with mild PCOS, and 50 women served as controls. In day 2-4 of cycle, transvaginal sonography was performed and serum hormonal level of AMH, LH, FSH, testosterone. PCOS patients fulfilling all Rotterdam criteria were defined as having severe PCOS ($n = 50$), while patients showing oligo-/amenorrhoea and polycystic ovaries but without hyperandrogenemia were defined as having mild PCOS ($n = 50$). And control group ($n = 50$). All patients were treated at the University Hospital of El-Hussein, Al-azhar university and at Ain Shams General Hospital Cairo Egypt.

Results: The strongest group difference between controls and severe PCOS patients was observed for AMH showing an age-adjusted odds ratio of 2.56 [95 % confidence interval (CI) 2.00–3.27; $p < 0.0001$]. Age-adjusted receiver operating characteristic analysis showed that the area under the curve (AUC) of 0.88 (95 % CI: 0.80–0.95) for AMH and 0.94 (95 % CI 0.88–0.98) for antral follicle count did not differ significantly in their ability to discriminate between severe PCOS patients and controls. AMH showed higher AUC estimates than androgens, ovarian volume, LH and LH/FSH ratio and an AUC of 0.80 (95 % CI: 0.65–0.91) for detecting mild PCOS.

Conclusions: this study comparing the diagnostic potency of AMH, sonographic aspects, androgens and LH/FSH ratio according to different PCOS subgroups while accounting for the age-dependency of AMH. In cases where vaginal scans are not feasible or in patients without hyperandrogenemia AMH may be used as a surrogate parameter in PCOS diagnosis, superior to androgens and gonadotropins.

Keywords: diagnosis of PCO, PCO and antimulleian hormone, diagnosis of PCO phenotypes.

INTRODUCTION

Polycystic ovary syndrome (PCOS), which is the most common endocrine disorder in reproductive-aged women, affects 6% to 10% of premenopausal women⁽¹⁾. The diagnosis of PCOS is based on a combination of clinical, biochemical, and ultrasound criteria, and the main diagnostic criteria of PCOS are polycystic ovarian morphology (PCOM), oligo-anovulation, and hyperandrogenism (HA)⁽²⁾. However, the diagnosis of PCOS can be subjective, because counting the ovarian follicular number and measuring the ovarian volume using ovarian ultrasound are technique-dependent and because obtaining standardized measurements is difficult. Serum AMH levels were markedly increased in

women with PCOS and were positively related with small antral follicle number⁽³⁾. Prevalence of the syndrome varies according to diagnostic consensus used, with estimates ranging from 9% according to National Institutes of Health consensus, up to 18% with the Rotterdam consensus⁽⁴⁾.

In females, FSH initiates follicular growth, specifically affecting graffian follicles (GCs). It is thought that each follicle has its own threshold FSH concentration varying from hypo-response to a risk of ovarian hyperstimulation and this concentration has to be exceeded to ensure dominant follicle selection. It is reported that AMH inhibits FSH-stimulated follicle growth in the mouse⁽⁵⁾. and is one of the factors restrains the sensitivity of ovarian follicles for

FSH, thus preventing follicle selection and resulting in follicle arrest at the small antral phase, with failure of dominance⁽⁶⁾.

It is suggested the GCs from polycystic ovaries continue to produce elevated levels of AMH, possibly because of impaired access of FSH to follicles⁽⁷⁾. It is concluded AMH reduces GCs sensitivity to FSH by an effect on aromatase promoter II (PII) activity⁽⁸⁾. In case a decrease of the AMH level, FSH receptor would be stimulated, the block on aromatase is released, and inhibin B production is stimulated with follicle progression. In fact, a low expression of AMH would diminish the threshold level for FSH, allowing these follicles to continue growth and to ovulate in the next estrous cycle⁽⁹⁾. On the other hand, FSH may weaken the inhibiting effect of AMH on follicular growth and induce follicular development by inhibiting AMH's promoter activity through the non-classic Cyclic adenosine monophosphate (cAMP) signal pathway in the ovary⁽¹⁰⁾.

Future research should be performed to clarify the relationship between AMH and FSH and the potential role of AMH on oocyte quality.

In females, LH supports theca cells in the ovaries that provide androgens and hormonal precursors for estradiol production and it is controlled by the pulses of gonadotropin-releasing hormone (GnRH). Although the FSH is at low to normal plasma concentrations, PCOS women often experience an increased frequency of hypothalamic GnRH pulses, so the baseline and stimulated LH concentrations were higher in PCOS resulting in an increased LH/FSH ratio⁽¹¹⁾.

At later stages of follicular differentiation, the dominant follicle becomes less depend on FSH and more responsive to LH. Previous studies have reported that cultured GCs from patients with PCOS increased their AMH production in response to LH, compared with controls⁽¹²⁾. It is suggested the possibility of a direct effect of LH on AMH secretion may reflect the fact that LH contribute to ovarian androgen secretion. And researchers indicated that the high level of LH promote the secreting of androgen and further effect of GCs to produce AMH, so LH may play a synergistic effect on androgen stimulation of GCs to produce AMH⁽¹³⁾.

In PCOS women, patterns of reproductive hormone release are altered, with exhibiting hyperandrogenism. Androgens can augment the FSH responsiveness of GCs in a developmental stage and indirectly protect the follicle from atresia⁽¹⁴⁾. Hyperandrogenism is one of the most important

symptoms of PCOS, affecting at least 60% of women with PCOS⁽¹⁵⁾.

It is indicated the serum AMH is higher in the women of PCOS accompanied with hyperandrogenism. Du *et al.* claimed the increased serum AMH level is the consequence of the androgen-induced excess number of small antral follicles. It is supposed when AMH expression is most pronounced, androgen receptor activity in granulosa cells is most prominent⁽¹⁶⁾.

AIM OF THE STUDY

It is to assess the possible role of AMH as the diagnostic marker for different degrees of PCOS.

PATIENTS AND METHODS

This study will be conducted at El Hussein university Hospital, Faculty of Medicine Al-Azhar University and at Ain Shams General Hospital Cairo Egypt.

Exclusion criteria

Patients with a history of ovarian surgery, an abnormality of thyroid or prolactin hormone levels, a history of hormone therapy in the 3 months before the study, or a FSH level higher than 12 mIU/L were excluded from the study.

Socioeconomic status, medical history, parity, age, and BMI were recorded in a standardized manner. Routine gynecological examination and a basic vaginal ultrasound scan on the day 3–5 of the menstrual cycle were performed.

A total of 150 women aged 18–46 years were included in the study. Patients were consecutively included as they presented in our clinic. 50 patients were diagnosed with sever PCOS, 50 patients were diagnosed with mild PCOS, and 50 women served as controls. Informed written consent was obtained from all women.

Diagnosis of PCOS and definition of the control group:

Diagnosis of PCOS

Diagnosis of PCOS was made according to the **Rotterdam (2004)**.

Signs and Symptoms: The symptomatic presentation of PCOS usually varies with age, young women mainly complaining of reproductive and psychological problems while older women complaining of metabolic symptoms

Endocrine variables: AMH, LH, FSH, LH/FSH ratio (ratio).

Sonographic parameters: Antral follicle count (AFC) and ovarian volume, were assessed between the second and fifth day of menstrual cycle or after artificial bleeding induction in cases of amenorrhoea.

BMI: Was calculated as weight/ (height)².

Definition of control group: All controls presented with regular menstrual cycle, Normal ovarian morphology in the vaginal or pelvic scan and No clinical or serological signs of hyperandrogenism.

Definition of phenotypic subgroups: PCOS patients fulfilling all diagnostic criteria were defined as having severe PCOS group (1), Patients with sonographically polycystic ovaries and menstrual cycle disorders who did not show elevated androgen levels with or without clinical signs of hyperandrogenism were defined as having mild PCOS group (2). And control group (3)

Transvaginal and pelvic scan for diagnosis of PCOS:

All women were scanned by the same experienced physician to avoid inter-observer differences in outcome measurements. Ovarian volume was obtained by measuring the greatest diameter in every plane. We calculated small follicles between 2 and 9 mm diameter in the longitudinal, transverse and anterior– posterior cross-sections of each ovary using the most available magnification factor available for the determination of the AFC.

Patients with any kind of ovarian masses or follicles greater than 10 mm were excluded. For statistical analysis, the ovary showing the maximum AFC value

and maximum ovarian volume per participant was used, respectively.

Biochemical analysis

Automated immunoassay systems were used for the determination of LH, FSH, testosterone.

The study was approved by the Ethics Board of Ain Shams University.

Statistical methods

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 22.0, IBM Corp., Chicago, USA, 2013.

Descriptive statistics were done for quantitative data as minimum& maximum of the range as well as mean±SD (standard deviation) for quantitative normally distributed data.

Inferential analyses were done for quantitative variables using ANOVA test with post hoc Tukey test for more than two independent groups with normally distributed data. While correlations were done using Pearson correlation for numerical normally distributed data. ROC curve was used to evaluate the performance of different tests differentiate between certain groups. The level of significance was taken at P value < 0.050 is significant, otherwise is non-significant.

The study was approved by the Ethics Board of Al-Azhar University.

RESULTS

Table (1): Diagnostic performance of AFC, OV, LH, FSH, ratio and AMH in differentiating mild from control groups

Factors	AUC	SE	P	95% CI	Cut off
AFC	0.980	0.010	<0.001*	0.959–1.000	≥ 9.0
OV	0.767	0.049	<0.001*	0.672–0.863	--
LH	0.698	0.056	<0.001*	0.589–0.808	--
FSH	0.574	0.058	0.202	0.500–0.688	--
Ratio	0.671	0.055	0.003*	0.563–0.779	--
AMH	0.891	0.034	<0.001*	0.825–0.957	≥ 4.10

AUC: Area under curve, SE: Standard error, CI: Confidence interval, *significant

Table (1) and figure (1) show that: Regarding differentiating mild from control groups; **AFC** had significant high diagnostic performance, **AMH** had significant moderate diagnostic performance, **OV, LH& ratio** had significant low diagnostic performance, while **FSH** had no significant diagnostic performance.

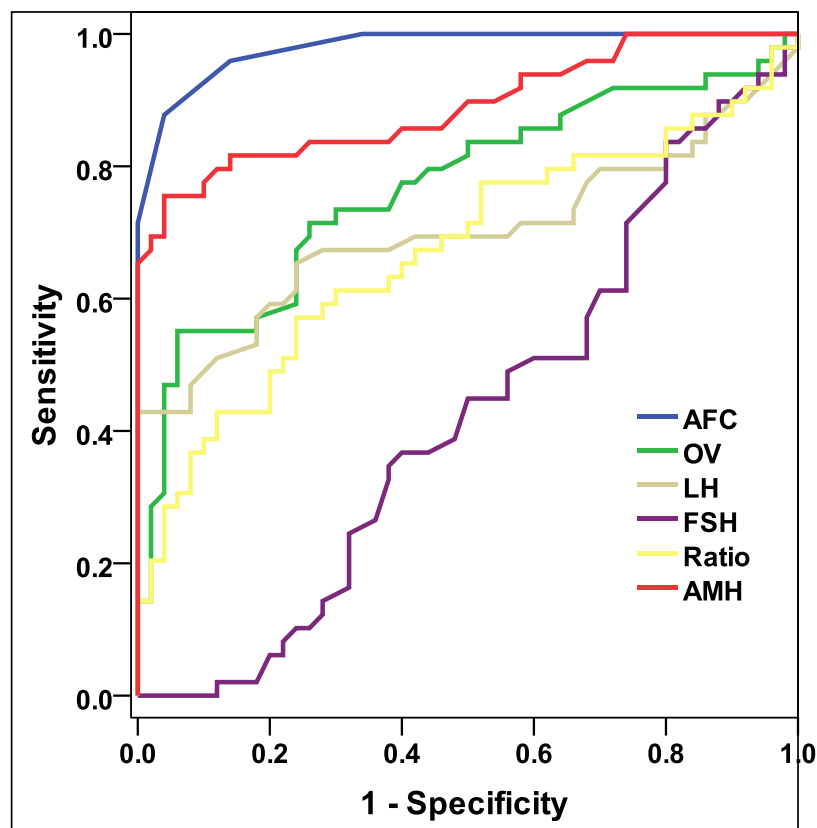


Figure (1): ROC curve for AFC, OV, LH, FSH, ratio and AMH in differentiating mild from control groups

Table (1): Diagnostic performance of AFC, OV, LH, FSH, ratio and AMH in differentiating severe from mild group

Factors	AUC	SE	P	95% CI	Cut off
AFC	0.864	0.036	<0.001*	0.794–0.935	≥ 14.0
OV	0.601	0.057	0.085	0.500–0.712	--
LH	0.593	0.057	0.109	0.500–0.706	--
FSH	0.518	0.059	0.761	0.500–0.633	--
Ratio	0.589	0.057	0.129	0.500–0.701	--
AMH	0.829	0.040	<0.001*	0.750–0.908	≥ 8.50

AUC: Area under curve, SE: Standard error, CI: Confidence interval, *significant

Table (2) and figure (2) show that: Regarding differentiating severe from mild groups; **AFC and AMH** had significant moderate diagnostic performance, **OV, LH, ratio & FSH** had no significant diagnostic performance.

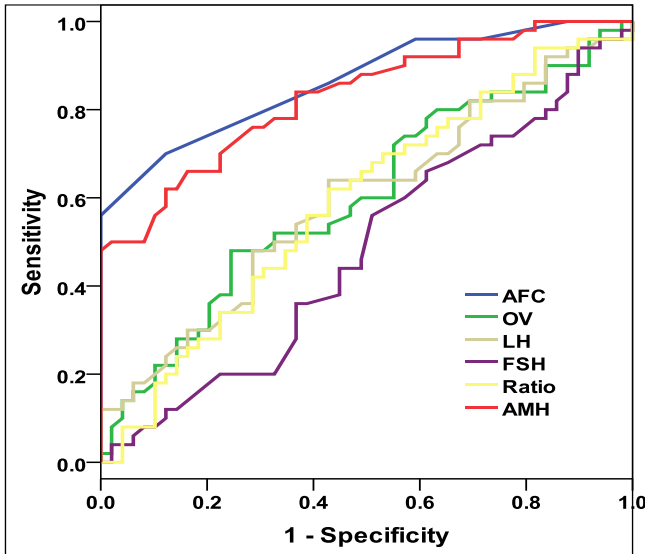


Figure (2): ROC curve for AFC, OV, LH, FSH, ratio and AMH in differentiating severe from mild groups.

Table (3): Diagnostic performance of AFC, OV, LH, FSH, ratio and AMH in differentiating severe from control groups

Factors	AUC	SE	P	95% CI	Cut off
AFC	0.999	0.001	<0.001*	0.500–1.000	≥ 10.0
OV	0.829	0.043	<0.001*	0.746–0.913	--
LH	0.780	0.050	<0.001*	0.683–0.878	--
FSH	0.584	0.057	0.146	0.500–0.697	--
Ratio	0.772	0.048	<0.001*	0.678–0.865	--
AMH	0.993	0.006	<0.001*	0.500–1.000	≥ 4.70

AUC: Area under curve, SE: Standard error, CI: Confidence interval, *significant

Table (3) and figure (3) show that: Regarding differentiating severe from control groups; **AFC** and **AMH** had significant high diagnostic performance, **OV, LH& ratio** had significant low diagnostic performance, while **FSH** had no significant diagnostic performance.

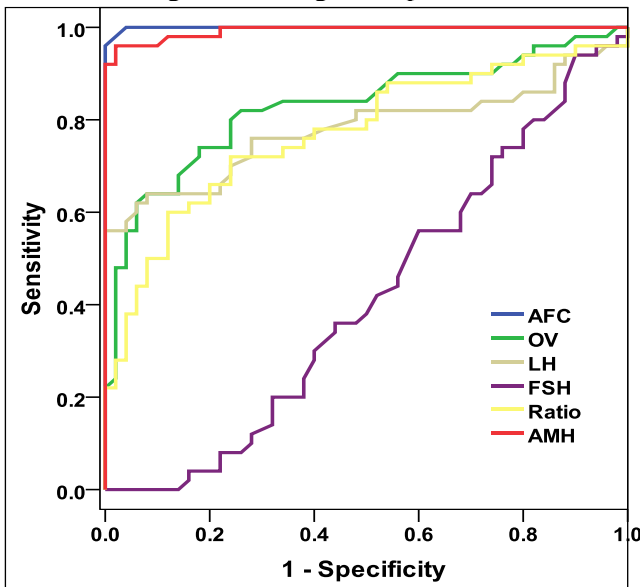


Figure (1): ROC curve for AFC, OV, LH, FSH, ratio and AMH in differentiating severe from control groups

DISCUSSION

The challenge of diagnosing PCOS requires objective and quantitative diagnostic criteria to guide clinicians through the diagnosis and treatment of PCOS. The serum level of AMH as a diagnostic marker for PCOS and showed that the serum AMH levels in patients with PCOS were two to three times the levels in patients without PCOS ($p < 0.001$) (Table 1). So AMH could be noticed as a suitable hormonal marker of the ovarian follicular count and we can assume that serum AMH level is an indirect reflection of ovarian reserve. So serum AMH level could be replaced by AFC and PCOM⁽¹⁷⁾.

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. Increased serum AMH level of > 35 pmol/L (or > 5 ng/mL) has been proposed for the definition of PCOM in the diagnosis of PCOS, as a more sensitive and specific marker than follicle count in ultrasonographic examination⁽¹⁸⁾. With the latest generation of ultrasound equipment, it has been proposed that the threshold of follicle count be increased from 12 (established in 2003 by Rotterdam criteria) to 19 or 25 follicles⁽¹⁹⁾. Lauritsen observed that replacing the criterion for polycystic ovaries above 19 antral follicles or AMH > 35 pmol/L resulted in a PCOS prevalence of 6.3 and 8.5%, respectively, and this was significantly lower in comparison to prevalence of 16.6% according to the standard Rotterdam criteria⁽²⁰⁾. We compared the AMH levels among three groups according to the presence of PCOS (severe PCOS) and PCOM (mild PCOS) and control group. Serum AMH level was the highest in group 3 with severe PCOS and the lowest in group 1 (control). Women with severe PCOS had higher serum AMH levels than did regular cycling women, (control) regardless of the presence of mild PCOS, and women with PCOM (mild PCOS) had higher serum AMH levels than did women without PCOM (control), regardless of the presence of PCOS.

Pigny et al evaluated serum AMH levels in diagnosis of PCOS; they reported a satisfactory specificity of 92% but a low sensitivity of 67% with an AMH cut-off of 8.4 ng/mL (60 μ mol/L) and a mean serum AMH of 11.42 ng/mL (81.6 μ mol/L). 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⁽¹⁷⁾.

Li et al reported that serum AMH levels were elevated in adolescent young adult Chinese patients with PCOS, but the serum AMH measurements

offered relatively poor diagnostic power, with a sensitivity of 61.7% and a specificity of 70% at a cut-off of 8 ng/mL. 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⁽¹⁰⁾.

Hart et al. found the most effective cut-off value of AMH to be 4.2 ng/mL (30 μ mol/L), which is close to our result⁽²¹⁾.

In our study, AMH level was significantly associated with total testosterone, even after adjustment for age, BMI, and the number of menses per year. In multiple regression analysis, the number of small follicles and serum androgen levels were positively correlated with the AMH level in women with polycystic ovaries with and without HA.

CONCLUSION

As group differences in AMH levels between severe and mild PCOS patients compared to controls are stronger than differences in other features involved in the pathogenesis of PCOS, AMH levels seem to be suitable for reflecting PCOS severity at the group level. The value of AMH in discriminating between controls and PCOS patients seems to be equivalent to AFC for severe PCOS cases and slightly inferior to AFC in the detection of mild PCOS cases. However, for the detection of mild PCOS, AMH seems to be the most precise diagnostic tool next to sonographic aspects, while the AFC is superior to the ovarian volume. AMH was also superior to androgens, LH and LH/FSH ratio.

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