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The Adjunct Role of Anti Mullerian Hormone (AMH) in Diagnosing Polycystic Ovary Syndrome

Walid Khaddam[1], Youssef Al Hussein[2], Lojain Alsolaiman[1*]

[1]Department of Biochemistry and Microbiology, Faculty of Pharmacy, Al Baath University.
[2]Department of Obstetrics and Gynecology, Faculty of Human Medicine, Al-Baath University.

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) receives great global attention because it is one of the most common endocrine disorders for women of reproductive age, so this study was directed to prove the role of the Anti-Mullerian Hormone (AMH) in diagnosing this syndrome to start treatment within the earliest possible time.

Methods: This was a prospective case-control study on women attending the Dr. Youssef Al-Hussein Center for Fertility and Infertility Treatment in Tartous, Syria, from July 2022 to October 2022. The study consisted of 93 women diagnosed with PCOS using Rotterdam criteria and 87 controls. Clinical data was collected, including Oligo/Amenorrhea (OA), Hyperandrogenism (HA), examinations including BMI (Body Mass Index), and blood investigations including Thyroid Stimulating Hormone (TSH), Prolactin (PRL), and serum AMH level. Ultrasonography (USG) was done for all women.

Results: AMH was two times higher in women with PCOS than controls, which was statistically significant ($p<0.05$). The maximum diagnostic ability of AMH alone for PCOS was at a cut-off of 4.91 ng/ml, with a sensitivity of 79.1% and a specificity of 81.1%. However, when the polycystic ovarian morphology (PCOM) criterion was replaced by AMH, {(AMH, HA, OA) any two out of three}, it gave the highest sensitivity and specificity (95.3%, 100%), respectively.

Conclusions: AMH levels cannot be used as a single test for diagnosing the syndrome, but when the AMH level was paired with further clinical Rotterdam criteria, it significantly increased the diagnostic power of PCOS and could be suggested as a possible adjunct criterion to diagnose this syndrome.

1. Introduction:

Polycystic Ovarian Syndrome (PCOS) is one of the most important health problems of our times and is receiving considerable attention from gynaecologists worldwide due to its subsequent complications, particularly as it is the most common endocrinopathy in women of reproductive age and the leading cause of ovulation infertility in 80% of patients, with a global prevalence of 8–13%. [1]

The first to describe this disease were two scientists, Michael Leventhal and Irving Stein, in 1935. [2] This disorder includes a variety of clinical symptoms, such as hyperandrogenism represented by hirsutism, acne,

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*Corresponding author & copyright holder: Lojain Alsolaiman Email address: alsolaimanlojain@gmail.com

alopecia, and menstrual irregularities. The real danger conditions, such as type 2 diabetes, metabolic syndrome, and cardiovascular disease, the most important of which is infertility. [3]

Polycystic ovary syndrome is currently diagnosed according to the International Rotterdam Criteria, which are based on the presence of at least two out of three criteria: Oligo/Amenorrhea (OA), clinical or biochemical hyperandrogenism (HA) [hirsutism, acne and excess testosterone] - Polycystic ovarian morphology (PCOM). [4]

Although the Rotterdam criteria are a popular method for PCOS diagnosis, they face many obstacles:

1. Most patients with PCOS are overweight; therefore, it may be difficult to use abdominal ultrasound. Additionally, for virgin teenage girls, the use of vaginal ultrasound is not preferred due to religious or social reasons.
2. The use of oral contraceptives changes the morphology of polycystic ovaries.
3. Assessment Antral Follicle Count (AFC) does not follow a uniform standard as it differs between interobservers.[5]
4. Diagnosing PCOM requires specialists and ultrasonic equipment, and this personalization may play a role in the AFC. Furthermore, certain techniques in this field may result in an artificial increase in PCOM, which may lead to confusion regarding the utilisation of this standard.
5. Various studies have established a suggested threshold value for PCOM due to the advancement of ultrasound devices.[6]

All of these factors can affect the diagnostic ability of PCOM.

It is also difficult to define hyperandrogenism, as the Ferman-Galloway scale is highly subjective.

As a result of these challenges associated with the Rotterdam criteria and given the advantages that anti mullerian hormone (AMH) has, such as the possibility of measuring it at any time, because its levels remain stable throughout the menstrual cycle and also because it is not affected by the use of oral contraceptives; [7-8] it is therefore interesting to use AMH as a new adjunct tool in the diagnosis of PCOS.

The gonadal anti-Müllerian hormone [also known as Mullerian Inhibiting Substance (MIS)] is a 140 kDa glycoprotein and a member of the transforming growth factor-β (TGF-β) superfamily {which includes inhibins, activins, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs)}.[9]

of this syndrome comes from long-term health AMH plays an important role in inhibiting early follicular recruitment and is a hallmark of the ovarian reserve. [10] AMH is expressed in antral follicles with a diameter of (2-9 mm), and its expression is reduced in follicles larger than 9 mm. Many studies have shown that AMH is elevated in patients with PCOS compared to healthy subjects, Which may be due to poor folliculogenesis and inhibition of their maturation, leading to an accumulation of follicles (preantral and small antral), resulting in ovulation defects. In addition, AMH inhibits aromatase expression, resulting in hyperandrogenism. AMH production from granulosa cells is estimated to be 75 times higher in PCOS patients than in healthy women.[11]

Whether AMH can be used alone as a reliable method or as an alternative to PCOM has not been confirmed. Therefore, the aim of this study was to determine the ability of AMH levels to diagnose PCOS.

2. Materials and Methods

The Ethical Committee of Albaath University approved the study protocols, and the ethical approval file has been uploaded as a "**Supplementary File**".

This prospective cross-sectional study was conducted at the laboratory of the Dr. Youssef Al-Hussein Center for Fertility and Infertility Treatment in Tartous, Syria, from July 2022 to October 2022, and informed written consent was obtained from each participanting woman in the study.

PCOS patients: Ninety-three (93) female patients of ageed 21 to 39 years, were diagnosed according to the Rotterdam criteria (two out of three features), and were classified into the following phenotypes: Phenotype **A:** OA-HA-PCOM ; Phenotype **B:** OA-HA ; Phenotype **C:** HA-PCOM and Phenotype **D:** OA-PCOM.

The control group, consisting of eighty-seven (87) women aged 23 to 42 years, consisted of women with no menstrual cycle abnormalities, regular ovulation, normal hormone levels (TSH, prolactin, and AMH), normal ovarian size, and no ovarian cysts observed on ultrasound.

Demographic data (age, weight, blood group, and smoking status) were collected using questionnaires distributed to all the participants.

The clinical history of patients attending to the centre included complaints of menstrual disorders classified as oligomenorrhoea (menstrual cycle length greater

than 35 days or four to nine menstrual cycles per year), secondary amenorrhoea (the absence of menstrual bleeding for more than three to six months in a woman who previously had menstrual bleeding), and hyperandrogenism, manifested by hirsutism (as measured by the Ferriman-Gallwey score), acne, obesity (as a result of insulin resistance and metabolic syndrome), or delayed childbearing and infertility.

PCOM was defined as the presence of more than 12 follicles with a diameter of (2 - 9 mm) in each ovary and/or an increase in ovarian size of more than 10 cm^3 and was diagnosed by transabdominal or transvaginal ultrasound.

Venous blood samples were collected on days 2-4 of the menstrual cycle, and the tubes were centrifuged at 3500 rpm for 15 min. Serum was separated and biochemical assays were performed immediately.

Serum AMH concentration was measured by electrochemiluminescence immunoassay (Elecsys Cobas e411 analysers, Roche Diagnostics GmbH, Mannheim, Germany). The measuring range for serum AMH was (0.03-23 ng/ml).

Serum TSH and PRL levels were measured by immunofluorescence using the I-chroma Boditech/Korea analyser.

All women underwent ultrasound, and the number of antral follicles (days 2-4) was calculated by a specialist using an endovaginal probe (Mindray DC-7 MX29003997 China, 5– 8 mHz). Antral follicles with a diameter between 2-9 mm were measured in both ovaries, and follicles larger than 10 mm were ignored.

2.1. Exclusion criteria were as follows:

Patients with hypothyroidism, hyperprolactinemia, congenital adrenal hyperplasia, surgical removal of the ovaries, radiotherapy, and Cushing's syndrome were excluded from the study.

The study sample was divided into patient and control groups according to the aforementioned inclusion and exclusion criteria.

2.2 Statistical analysis

The Statistical Package for Social Sciences (SPSS) program version 24.0 for Windows was used, and the data are presented as [mean ± standard deviation (SD)]. Independent T test and one-way ANOVA were used for appropriate analyses; multivariate analysis was performed using regression analysis. Receiver operating characteristic (ROC) curve analysis was used to investigate the diagnostic cut-off for AMH. Statistical significance was set at $P < 0.05$.

3. Results

The mean age of the patients and controls was 28.7 and 32.4 years, respectively, with a statistically significant difference between the groups (p-value = 0.002) (Table 1).

The mean serum AMH level in the PCOS group was (7.5 ng/ml) and in the non-PCOS group was (3.5 ng/ml) (p=0.000), demonstrating a statistically significant difference in AMH levels between patients and controls in this study (Table 1).

The mean AFC of the PCOS cases was comparable to that of the control group. The mean AFC in PCOS patients was 19.9 and 9.7 for healthy women, (p=0.000), showing a statistically significant difference between patients and healthy women (Table 1).

Table 1: *A summary of the general characteristics of PCOS patients and controls.*

Variable	**Values [mean ± SD]**			
	Patients group	**Control group**	**P-Value**	
Number of subjects	93	87	-	
Age	28.7 ± 4.45	32.4 ± 5.83	0.002	P<0.05
BMI	25.5 ± 5.26	24.1± 3.94	0.199	---
AFC	19.9 ± 6.77	9.7 ± 5.74	0.000	P<0.05
TSH	4.4 ± 14.35	2.0 ± 1.34	0.318	---
PRL	17.5 ± 11.32	16.7 ± 12.1	0.780	---
AMH	7.5 ± 3.61	3.5 ± 1.95	0.000	P<0.05

The percentage of oligomenorrhoea in PCOS patients was approximately (n = 69) 74%; hyperandrogenism was found in (n = 64) 69% of patients, while the percentage of PCOM was (n = 77) 83%.

Patients with PCOS were classified into the following phenotypes: {(**A:** OA-HA-PCOM), (**B:** OA-HA), (**C:** HA-PCOM), and (**D:** OA-PCOM). The two most common patterns in our study were A and D, with a

ratio of 28 to 93 for each of the two phenotypes (Table 2).

The highest mean AMH concentration was found in type A (9.8 ng/ml), which included all Rotterdam criteria (OA, HA, and PCOM) (Table 2).

Table 2: *AMH levels in the four phenotypes of PCOS patients*

Phenotypes	HA	OA	PCOM	Number	AMH [mean ± SD]
A	+	+	+	28	9.8 ± 3.91
B	+	+	-	16	2.69 ± 0.58
C	+	-	+	21	7.8 ± 3.42
D	-	+	+	28	7.4 ± 2.51

According to the ROC curve analysis in the present study, the maximum diagnostic potential of AMH alone was at a cut-off of 4.91 ng/ml with a specificity of 81.1% and a sensitivity of 79.1%, and the area under the curve was 0.815 (80% CI 0.718 - 0.915); p < 0.000. The positive and negative predictive values were 82.1% and 76.9%, respectively (Figure 1).

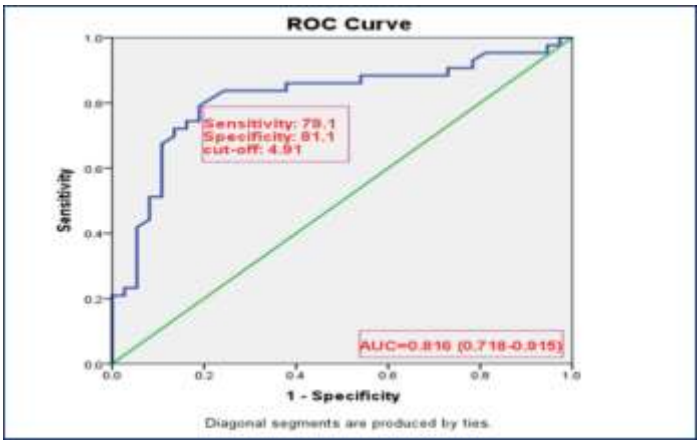

Figure 1: *Receiver operator characteristic curve.*

In the first group of criteria, the combination of AMH (at a cut-off value of 4.91 ng/ml) with the hyperandrogenism criterion, it was found to have a sensitivity of 48.8 % and a specificity of 100% for the diagnosis of PCOS. In the second group, the combination of AMH with the oligo/amenorrhoea (OA) criteria achieved a sensitivity 60,5% and a specificity of 100%. In the third group, the combination of AMH with either hyperandrogenism or oligo/amenorrhoea resulted in a sensitivity 79.1% and a specificity 100% (Table 3).

Table 3: Diagnostic potency of the four proposed criteria with a serum AMH cutoff value of 4.91 ng/mL

Proposed criteria	Sensitivity	Specificity	AUC	P–value
AMH $_{alone}$	79.1%	81.1%	0.816 (0.718-0.915)	0.000
AMH + HA	48.8%	100%	0.744 (0.636-0.853)	0.000
AMH + OV	60.5%	100%	0.802 (0.704-0.901)	0.000
AMH + (OV or HA)	79.1%	100%	0.895 (0.820-0.971)	0.000
Any two out of three (AMH , HA , OV)	95.3%	100%	0.977 (0.940-1.000)	0.000

Patients diagnosed with PCOS according to the Rotterdam criteria were re-evaluated using four proposed diagnostic criteria for PCOS, namely the combination of the serum AMH level (4.91 ng/ml) with OA and HA (Table***4***).

4. Discussion

Common cause of infertility in women worldwide. Therefore, early diagnosis of PCOS is very important to reduce the associated morbidity. Significant differences in the age distribution between PCOS patients (28.7 ± 4.45) and controls (32.4 ± 5.83) have been observed in previous studies, and they may be due to the fact that the severity of symptoms in PCOS patients gradually decreases with age, so that the disease prevalence is mainly seen in younger women.[12] With regard to BMI, there was no statistically significant difference between patients and healthy women in the current study (Table 1), which is consistent with the previous studies by Wiweko *et al.(2014)* and Saxena *et al.(2018).*[8-5] While TSH and PRL levels were similar in PCOS and non-PCOS groups, this finding was consistent with some studies [13-8]**,** and different from others, which may be justified by the exclusion of hypothyroid patients in our study [14] (Table 1).

***Table 4**: Distribution of patients after re-evaluation according to the new proposed criteria*

Diagnosis of polycystic ovary syndrome according to the Rotterdam criteria	Diagnosis of polycystic ovary syndrome according to the new criteria							
	AMH + HA		AMH + OV		AMH+(OV or HA)		Any two out of three (AMH , HA , OV)	
	PCOS	Non PCOS	PCOS	Non PCOS	PCOS	Non PCOS	PCOS	Non PCOS
PCOS (93)	46	47	56	37	73	20	88	5
Non PCOS (87)	0	87	0	87	0	87	0	87

It was observed that the two most common patterns in this study were A and D (Table 2), whereas in the study by Wiweko *et al*. *(2014)*, the most common pattern was D alone .[8] In the study by Singh *et al*. *(2020)*, pattern A ranked first in prevalence .[15] The prevalence of phenotype A in this study may be due to the clear prevalence of the syndrome in the Middle East, while phenotype D may be due to the fact that a large sample of our participants had complaints related to ovulation problems, with the number of patients with ovulation problems reaching 69 out of 93.

In this study, the highest mean AMH concentration (9.8 ng/ml) was found in type A (patients who had an anovulatory disorder, hyperandrogenism, and polycystic ovarian morphology combined) (Table 2), and this is consistent with the study by Wiweko *et al*. *(2014)* [8], who found the highest AMH concentration (11.1 ng/ml) in type A. The same result was found in an Indian study, where type A had the highest concentration of AMH (8.44 ng/ml). From the above, we can conclude that the presence of all clinical symptoms combined is associated with the highest concentration of AMH, and therefore AMH concentration may play a role in determining the severity of the symptoms of this syndrome.[16]-[17]

As AMH quantitatively expresses the number of antral follicles, it is the best laboratory test for the diagnosis of PCOS. This study attempted to confirm the possibility of using AMH as an adjunct diagnostic tool for PCOS and demonstrated that the serum AMH concentrations in PCOS patients were two times higher than the concentrations in the control group (Table 1), Which is similar to study by Sahamy *et al*.'s *(2014)* study, who found that AMH levels were 2-3 times higher in patients compared to controls. [18] Tehrani *et al*. *(2010)* also showed that AMH levels were significantly higher in PCOS patients. [19]

In this study, the maximum diagnostic ability of AMH was found at a cut-off of 4.91ng/ml with a sensitivity and specificity of 79.1% and 81.1%, respectively.

Several studies have reported the use of AMH as a diagnostic tool, but the cut-off values have varied. This may be due to many factors, including ethnicity, geography, sample size, lack of standardisation of laboratory methods for AMH, age, and multiple PCOS phenotypes. [20]

In the study by Woo *et al*. *(2012)*, a cut-off value of 7.82 ng/ml showed a sensitivity of 75.9% and a specificity 86.8% [21]; Hart *et al*. *(2010)* observed a low sensitivity of 53.1% and a low specificity of 69.1% (cut-off 4.2 ng/ml). [22] Lin *et al*. *(2011*) found a sensitivity 76% and a specificity 70% in their study [23]; Pigny *et al*. *(2006)* reported a low sensitivity of 67% but a good specificity of 92% with an AMH cut-off of 8.4 ng/ml. [24] Previous studies have shown low sensitivity when AMH alone is used to diagnose PCOS.

The results of our study also showed low sensitivity, which is consistent with previous studies in which AMH alone was considered an inappropriate tool for the diagnosis of PCOS. Because of the drawbacks associated with the PCOM standard, we investigated the possibility of replacing PCOM with AMH to investigate its auxiliary diagnostic potential for the Rotterdam criteria. Good results were obtained when AMH was combined with other clinical criteria as an alternative to PCOM.

In the fourth group, the presence of any two of the three criteria (HA, OA, and AMH levels) gave results close to those of the Rotterdam criteria, and the system showed 100% specificity and 95.3% sensitivity, which was the optimum combination (Table 3). Therefore, this method is considered a typical diagnostic choice for PCOS. These findings are supported by studies conducted by Eilertsen *et al*. (2012) and Sahmay *et al*. (2014), who concluded that AMH can effectively replace PCOM. [25]-[18]

Patients were re-evaluated using the newly proposed criteria with the AMH cut-off 4.91 ng/ml, which gave satisfactory results and was similar to the Rotterdam criteria for the diagnosis of PCOS (Table 4).

Although AMH alone has been considered a good diagnostic tool in many studies [26], its combination with other criteria gives better results for the diagnosis of PCOS.

5. Conclusion

The results of this study show that AMH can be used reliably as a replacement for PCOM; therefore, it can be described as an improved or modified criterion for the Rotterdam criteria. These results only reflect the cases that came to the center; therefore, there is a need for more similar studies that include different regions of Syria.
We recommend further studies on the relationship between AMH, insulin resistance, and CVD in different PCOS phenotypes. There is emerging evidence that, with improved standardization of assays and established cut-off levels or thresholds based on large-scale validation in populations of different ages and ethnicities, AMH assays will prove to be more accurate than PCOM assays.

List of abbreviations

PCOS: Poly Cystic Ovary Syndrome
BMI: Body Mass Index
USG: Ultrasonography
AFC: Antral Follicle Count
TSH: Thyroid Stimulating Hormone
PRL: Prolactin
AMH: Anti Mullerian Hormone
OA: Oligo/Amenorrhea
HA: Hyperandrogenism
PCOM: Polycystic Ovarian Morphology
TGF-β: Transforming Growth Factor-β
BMPs: Bone Morphogenetic Proteins
GDFs: Growth and Differentiation Factors
ROC: Receiver operating characteristic
AUC: Area under the curve

Declarations:

Ethics approval

The data were adopted from studies' protocols that were performed in line with the principles of the Ministry of Health and the Ministry of Higher Education and Scientific Research in the Syrian Arab Republic.

Ethics approval Included in the "Supplementary File" list.

Consent for publication

A written informed consent was obtained from all individual participants included in the study.

Availability of data and materials

The data that support the findings of this study are available from Dr. Youssef Al-Hussein Center for Fertility and Infertility Treatment, but restrictions apply to the availability of these data due to patients' privacy, and so are not publicly available. Data are, however, available from the authors upon reasonable request and with permission of Dr. Youssef Al-Hussein Center for Fertility and Infertility Treatment.

Competing interests

The authors confirm that they have no competing interests.

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Guarantor

The Guarantor is Lojain Alsolaiman who is the corresponding author of this manuscript. Email: alsolaimanlojain@gmail.com

Authors' contributions

L.A designed this study, performed all the tests, analyzed all the data and wrote the manuscript. W.Kh reviewed the final manuscript. All authors read and approved the final manuscript.

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