

Anti-Müllerian Hormone Level in Patients with Polycystic Ovary Syndrome

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Abstract

Background: Polycystic ovary syndrome (PCOS) is one of the common causes of chronic anovulation and hyperandrogenism in women between the age of 13 and 45 years old. PCOS is characterized by an increased number of follicles at all growing stages particularly in the preantral and small antral follicles, those who primarily produce Anti-Müllerian hormone (AMH). **Objective:** The aim of the study is to measure the AMH levels in serum of the women with PCOS. **Materials and Methods:** The study included 90 women (50 cases with PCOS and 40 control cases healthy women). PCOS patients were diagnosed according to the Rotterdam definition; serum AMH was measured using the ultrasensitive AMH enzyme-linked immunosorbent assay. **Results:** Mean serum of AMH in PCOS patients was 11.52 ng/ml and for the control group was 3.36 ng/ml and was statistically significant with the $P < 0.0001$. **Conclusions:** AMH is elevated in PCOS patients, which can be used in the future as a marker for the diagnosis of PCOS.

Keywords: Anti-müllerian hormone, follicle-stimulating hormone, luteinizing hormone, polycystic ovary syndrome

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the common causes of chronic anovulation and hyperandrogenism in women between the age of 13 and 45 years old, with the prevalence of 5%–10%.^[1] Women with PCOS are at high risk for infertility, preeclampsia, early pregnancy loss, dyslipidemia, hypertension, endometrial cancer, heart disease, and type 2 diabetes mellitus.^[2] The diagnosis of PCOS depends on Rotterdam classification requiring at least 2 out of the 3 following characteristics: (i) cycle disorder, (ii) clinical or biological hyperandrogenism, and (iii) antral follicular excess on ultrasound with ≥ 12 follicles from 2 to 9 mm per ovary and/or ovarian volume ≥ 10 ml.^[3]

Anti-Müllerian hormone (AMH), known also Müllerian inhibiting substance, is a homodimeric glycoprotein linked by disulfide bonds with a molecular weight of 140 kDa.^[4] The AMH is belongs to the transforming growth factor- β superfamily, and the gene encoding AMH is located in the short arm of chromosome number 19.^[5]

AMH action is exerted through two receptors: Type I receptor and Type II receptor which are present on the AMH target organs such as gonads and Müllerian ducts.^[6] AMH has been

known for its role in male sexual differentiation.^[7] In women, AMH expression is restricted to one cell type: the granulosa cells of the ovary. It starts around the 25th week of gestation and continuing until menopause.^[8]

AMH is expressed at all steps of folliculogenesis, which is initiated as soon as primordial follicles are recruited to grow into small preantral follicles and its highest expression is observed in preantral and small antral follicles. AMH expression then decreases with the selection of follicles for dominance and is no longer expressed during the follicle-stimulating hormone (FSH) dependent stages of follicular growth or in atretic follicles.^[9]

AMH has an inhibitory effect on cyclic follicular recruitment *in vivo* by reducing the follicle sensitivity to FSH. *In vitro*, AMH inhibits FSH-induced preantral follicle growth.^[10]

AMH also reduces the number of luteinizing hormone receptors in granulosa cells.^[11] The level of AMH circulating

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in the blood is not affected by the menstrual cycle nor altered during the use of oral contraceptives; this is an important advantage of AMH over FSH.^[12]

PCOS is characterized by an increased number of follicles at all growing stages.^[13] This increase is particularly seen in the preantral and small antral follicles, those which primarily produce AMH.^[14] Thus, elevated serum AMH level, as a reflection of the stock of preantral and small antral follicles, is 2–4 fold higher in women with PCOS than in healthy women and is found in all PCOS populations.^[15]

This increase in serum AMH was first thought to be only due to the higher number of preantral and small antral follicles. However, production of AMH by granulosa cells was found *in vitro* to be 75-fold higher in anovulatory PCOS and 20-fold higher in normo-ovulatory PCOS than in normal ovaries. This suggests increased serum AMH levels in PCOS would also reflect an intrinsic dysregulation of the granulosa cells.^[16]

Several studies have suggested that AMH serum levels may be a marker for PCOS. As the diagnostic criteria for ultrasound findings are the presence of more than 12 follicles with a diameter of 2–9 mm or when the ovarian volume is more than 10 cm³, it may correlate with the level of serum AMH.^[3] This is the first study of AMH in PCOS patients in our region.

The aim of the present study was to assess levels of AMH in PCOS and healthy controls.

MATERIALS AND METHODS

Study design and sampling

The current case–control study was performed at Azadi Teaching Hospital/gynecological outpatient clinic in Duhok city/Kurdistan Region/Iraq. The study included 90 women (50 cases with PCOS and 40 control cases healthy women) from their companion without PCOS. This study started from the August 2017 to March 2018. Their age was between 15 and 40 years old.

Inclusion and exclusion criteria

Inclusion criteria for the current study were as follows: a diagnosis of PCOS patients done by gynecologist according to the Rotterdam classification (2003) requiring at least 2 out of the 3 following characteristics: (i) cycle disorder, (ii) clinical or biological hyperandrogenism, (iii) antral follicular excess on ultrasound with ≥ 12 follicles from 2 mm to 9 mm per ovary and/or ovarian volume ≥ 10 ml. Exclusion criteria from the liver, renal, thyroid, and adrenal diseases done by self-report.

Diagnostic and measurement criteria

Levels of AMH were measured in the serum of the participants after collection by gel tube and then centrifugated, the serum is separated and stored in the refrigerator in a -20°C degrees and frozed, then measured using the ultrasensitive AMH enzyme-linked immunosorbent assay. Kit of AMH provided from diagnostic systems (Ansh Labs) from America. The reference range of (0.9–9.5 ng/ml) is used.^[17]

Statistical methods

The descriptive purposes of the study were determined in frequency distribution whether mean and standard deviation or frequency and percentage. The difference of AMH between cases and controls was examined in independent *t*-test. The null hypothesis was rejected in $P < 0.05$. The statistical calculation was performed by Statistical Package for Social Sciences version 24:00 (SPSS; IBM; USA).

Ethical considerations

This study was approved by the Kurdistan Board for Medical Specialties/Directorate of Training Affairs/Scientific Research Units. The committee responsible for ethical approval, written informed consent was obtained from each participant.

RESULTS

In this study, the cases (patients) and controls were comparable (similar) in age, 26.88 years versus 27.57 years, respectively, ($P = 0.620$) and marital status ($P = 0.924$). However, the body mass index (BMI) of cases (patients) was significantly higher (27.17) compared to BMI of controls (24.18), $P = 0.001$. In addition, the AMH patients in the case group were significantly abnormal in contrast with the control which was normal ($P < 0.0001$) [Table 1].

The study showed that the mean AMH had a significantly higher level in cases (patients) compared to controls, 11.52 versus 3.36, and statistically significant with $P < 0.0001$ [Table 2].

Table 1: Difference of general characteristics between cases and controls

General characteristic	Study groups		P (two-sided)
	Cases (n=50)	Controls (n=40)	
Age (year), mean $\pm$ SD	26.88 $\pm$ 6.76	27.57 $\pm$ 6.45	0.620*
BMI, mean $\pm$ SD	27.17 $\pm$ 5.24	24.18 $\pm$ 3.21	0.001*
Marital status, frequency (%)			
Single	28 (56.0)	22 (55.0)	0.924**
Married	22 (44.0)	18 (45.0)	
AMH categories, frequency (%)			
Normal (0.9–9.5 ng/mL)	20 (40.0)	2 (5.0)	<0.0001***
Abnormal AMH	30 (60.0)	38 (95)	

*Independent *t*-test and **Chi-squared test, and ***Fishers' exact tests were performed for statistical analysis. AMH: Anti-müllerian hormone, BMI: Body mass index, SD: Standard deviation

Table 2: Difference of anti-müllerian hormone between cases and controls

AMH	Study groups (mean $\pm$ SD)		P (two-sided)*
	Cases (n=50)	Controls (n=40)	
AMH (ng/mL)	11.52 $\pm$ 5.42	3.36 $\pm$ 2.23	<0.0001

*Independent *t*-test was performed for statistical analysis.

AMH: Anti-müllerian hormone, SD: Standard deviation

The mean AMH was comparable and statistically not significant between the different BMI, in cases with normal AMH which were (9.40, 6.11, and 6.63) in underweight, normal, and overweight, respectively, and in cases with abnormal AMH were (14.10, 14.77, 14.89) in underweight, normal, and overweight, respectively, with $P = 0.521$. Furthermore, in controls with normal AMH were (3.22, 0.13, 0.0) and in abnormal AMH were (0.00, 3.54, 3.00) in underweight, normal, and overweight, respectively, with $P = 0.744$ [Table 3].

The mean AMH was comparable and statistically not significant between singles and married in cases and controls, in cases of normal AMH were (6.78) and (6.14), respectively, and with abnormal AMH were (15.54) and (14.13), respectively, with $P = 0.295$, and in controls of normal AMH were (3.43) and (3.14), respectively, and in abnormal AMH were 0.0 and 0.0, respectively, and with the $P = 0.719$ [Table 4].

DISCUSSION

PCOS is associated with an increase prevalence of AMH. In the present study, mean AMH shows high significant differences ($P < 0.0001$) between PCOS patients and control groups.

Pigny *et al.* found that the specificity and sensitivity of serum AMH measurement reached 92% and 67%, respectively.^[18] In the Piltonen *et al.* study, serum AMH levels were 2–3 fold higher in PCOS women than in healthy women.^[19] Lin *et al.* obtained a cut-off AMH level of 7.3 ng/mL, giving 76% specificity and 70% sensitivity to predict PCOS.^[20] Many other published studies have confirmed elevated concentrations of AMH in the blood of women with PCOS.^[21–23]

Anti-Müllerian hormone production gradually declines as follicles grow, once follicles reach a size at which they are dominant, it has largely disappeared. Its removal from these

larger follicles appears to be an important requirement for dominant follicle selection and progression to ovulation as AMH has an inhibitory role in the ovary, reducing both primordial follicle initiation and follicle sensitivity to FSH by inhibition of aromatase. It is for this reason that AMH is a focus of interest in PCOS. Serum levels are doubled, and granulosa cell production is greatly increased.^[22]

The results of the present study revealed that there were no significant differences in age between women with PCOS and non-PCOS with the $P = 0.620$. This finding is similar to that reported by previous studies in Iraq and Erbil/Kurdistan Region/Iraq.^[24–26]

In this study, there was no significant difference in AMH between PCOS patients and non-PCOS healthy women regarding marital states with the $P = 0.924$.

In our study, a significant increase in mean of BMI was found in women with PCOS when compared with healthy women without PCOS with the $P = 0.001$. This finding is consistent with previous study^[26] that found a greater BMI in women with PCOS than in women without PCOS, because PCOS is also associated with metabolic aberrations.

The incidence of metabolic syndrome is 2–3 fold higher among women with PCOS compared to healthy women of similar age and BMI, while 20% of women with PCOS, aged <20 years have already manifested the metabolic syndrome.^[27] Although there is no prognosis regarding the outcome, especially for women with PCOS, the risk of fatal myocardial infarction is double among postmenopausal hyperandrogenemic women with a history of severe oligomenorrhoea, who are actually expected to be PCOS patients, compared to women with regular menstrual cycle.^[28] Women with PCOS have a 2–6 fold greater number

Table 3: Anti-müllerian hormone among the patients with different body mass index in cases and controls

Study group	AMH categories	BMI categories, frequency (%), mean $\pm$ SD			P (two-sided)*
		Underweight	Normal	Overweight/obese	
Cases	Normal AMH	1 (50.0), 9.40	8 (50.0), 6.11 $\pm$ 1.28	11 (34.4), 6.63 $\pm$ 1.98	0.521
	Abnormal AMH	1 (50.0), 14.10	8 (50.0), 14.77 $\pm$ 2.89	21 (65.6), 14.89 $\pm$ 4.98	
Controls	Normal AMH	2 (100), 3.22 $\pm$ 1.30	2 (8.7), 0.13 $\pm$ 0.0	0 (0.0)	0.744
	Abnormal AMH	0 (0.0)	21 (91.3), 3.54 $\pm$ 1.76	15 (100), 3.00 $\pm$ 1.60	

*Fishers' exact tests were performed for statistical analyses. The numbers are in frequency (%). AMH: Anti-müllerian hormone, SD: Standard deviation, BMI: Body mass index

Table 4: Anti-müllerian hormone levels of cases and controls between single and married subjects

Study group	AMH categories	Marital status, frequency (%), mean $\pm$ SD		P (two-sided)*
		Single	Married	
Cases	Normal AMH	13 (46.4), 6.78 $\pm$ 1.69	7 (31.8), 6.14 $\pm$ 2.00	0.295*
	Abnormal AMH	15 (53.6), 15.54 $\pm$ 3.32	15 (68.2), 14.13 $\pm$ 5.26	
Controls	Normal AMH	22 (100), 3.43 $\pm$ 1.78	18 (100), 3.14 $\pm$ 1.52	0.719**
	Abnormal AMH	0 (0.0)	0 (0.0)	

*Chi-squared and **Fishers' exact tests were performed for statistical analyses. The numbers are in frequency (%). AMH: Anti-müllerian hormone, SD: Standard deviation

of follicles (primary, secondary, and antral) in their ovaries, possibly due to the hyperandrogenism.

Obesity or overweight have negative impact on the consequences of PCOS.^[29] In contrast, other previous study^[30] did not report any differences in BMI value between women with PCOS and without PCOS. Obesity is one of the clinical characteristics of the PCOS; however, not all obese women have PCOS and not all PCOS women are obese.^[27]

Lim *et al.* reported that obesity prevalence in PCOS was lower in Asian women than Caucasian women. However, clinical evaluation and management of obesity are still an important in PCOS women and should be assessed in each patient.^[31]

Some studies have evaluated the relationship between weight loss and serum AMH levels in overweight women with PCOS. Moran *et al.* showed better menstrual improvement after weight loss in women with lower baseline serum AMH.^[32]

Serum AMH could, therefore, be used as a potential predictive factor of cycle normalization after weight loss. Nybacka *et al.* described a significant decrease in serum AMH levels after diet in obese women with PCOS.^[33] Nybacka A *et al.* demonstrated long-term weight loss (20 weeks) resulted in improvements in reproductive function but did not change serum AMH levels and various effects on AMH level were obtained with metformin usage in women with PCOS.^[34]

Pigny *et al.* found that BMI did not influence circulating AMH concentrations in women with PCOS.^[35] Nardo *et al.* also found no relationship between AMH and BMI, which was explained by the differences in study populations and clinical settings, mainly the preselection of patients based on BMI cut-offs, which was the case in their study.^[36]

The use of AMH may also be useful in the therapeutic approach of PCOS patients. Indeed overweight women with PCOS who respond to weight loss with menstrual improvements have significantly reduced pre-weight-loss AMH levels, indicating that baseline AMH may provide a potential clinical predictor of menstrual improvements with weight loss in PCOS.^[32]

CONCLUSIONS

Serum AMH was elevated in PCOS which can be used in the future as a marker for the diagnosis of PCOS.

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Conflicts of interest

There are no conflicts of interest.

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