Anti-Mullerian hormone, a marker for metformin therapy efficacy in polycystic ovarian syndrome: a pilot study on an Egyptian population

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Background
Polycystic ovarian syndrome (PCOS) is the most common endocrinopathy in adult women, and is emerging as a common cause of menstrual disturbance in the adolescent population. Insulin resistance, which is considered one of its underlying causes, has increased markedly in the past decade, placing more adolescent girls at risk for PCOS and its complications. Anti-Mullerian hormone (AMH) is secreted by the granulose cells of ovarian follicles and correlated with the count of small antral follicles and it is expressed throughout folliculogenesis.
Objective
This study aimed to evaluate AMH in Egyptian women with PCOS and to determine whether it might serve as a prognostic marker for treatment efficacy with metformin.
Patients and methods
This study included 30 women with PCOS (group 1) and 30 healthy women without PCOS (group 2). AMH was measured in both groups, and before and after treatment with metformin (2550 mg) for 3 months in group 1.
Results
AMH levels were higher in PCO groups before (3.54±0.58 ng/ml) and after treatment (2.79±0.39 ng/ml) than the control group (2.14±0.49 ng/ml), with *P* value less than 0.01. In the PCO group, it was higher before (3.54± 0.58 ng/ml) than after treatment (2.79±0.39 ng/ml), with *P* value less than 0.01.
Conclusion
AMH is higher in PCO patients and its levels decrease significantly with the insulin sensitizer metformin, and it can be used as a marker for treatment efficacy with metformin.

Keywords:
anti-Mullerian hormone, metformin, polycystic ovary

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Introduction

The prevalence of polycystic ovaries (PCOs) increases throughout puberty and may reach as high as 26% by 15 years of age. Polycystic ovarian syndrome (PCOS) is the most commonly diagnosed ovarian cause of hirsutism and menstrual disturbance and controversy exists about whether the basic defect is central (hypothalamic or pituitary) or ovarian [1].

Insulin resistance, which is considered one of its underlying causes, has increased markedly in the past decade, placing more adolescent girls at risk for PCOS and its complications [2].

Insulin-sensitizing agents (biguanides and thiazolidinediones), metformin is the most commonly used, are among the lines of treatment for PCOS [3]. Metformin suppresses hepatic gluconeogenesis, improves the peripheral resistance to insulin, increases the consumption of glucose in skeletal muscles, and decreases the intestinal glucose absorption. Metformin also enhances insulin action at cellular levels by enhancing the disposal of glucose in adipose and muscular cells and by increasing ligation to the insulin receptors [4].

Metformin may also play a beneficial role in serum androgens by increasing the hepatic production of sex hormone-binding globulin (SHBG), thus decreasing the circulating free testosterone, and adrenal and ovarian androgen production [5].

Anti-Mullerian hormone (AMH) belongs to the transforming growth factor-β (TGF-β) family. It is encoded by the *AMH* gene. It plays a role in fetal sex

differentiation; in males, AMH is produced by sertoli cells, where it induces degeneration of the Mullerian ducts, thus suppressing the development of the uterus and other Mullerian structures, whereas the absence of AMH in female embryo allows for the development of the upper vagina, uterus, cervix, and oviducts, an action that is mediated through the AMH type II receptors [6].

In females, AMH is secreted by the granulosa cells of ovarian follicles [7]. The AMH has no cyclic variation and it has a strong correlation with the antral follicle count and the ovarian follicle pool; therefore, it is considered a marker of the quantitative amount of ovarian reserve, the size of the ovarian follicles pool, and also ovarian dysfunction [8]. The serum levels of AMH are increased in women with PCOS and may reach 2–3 folds higher than normal values; AMH can be used as a marker with high specificity and sensitivity for PCOS [9].

Aim

This study aimed to evaluate AMH in women with PCOS and to determine whether it could serve as a prognostic marker for treatment efficacy with metformin.

Patients and methods

Participants

Sixty Egyptian women who were visitors at the outpatient clinic of Ain Shams University hospital participated in this study. They were classified as follows:

(1) Group 1: 30 women with PCO disease.
(2) Group 2: 30 healthy women matched for age as a control group.

Exclusion criteria

Individuals who fulfilled any of the following criteria were excluded from our study: (a) individuals with major illnesses such as cancer, intermittent hemodialysis, and chronic kidney disease, and pregnant women; (b) individuals with autoimmune diseases; (c) infertile women because of any other cause of infertility; and (d) individuals with endocrinal diseases such as thyroid dysfunction and hyperprolactinemia. All these women were excluded on the basis of assessment of their medical history.

Methods

Both patients and controls were subjected to the following: (a) detailed assessment of history, with a special focus on age, parity, menstrual cycles, induction of ovulation, and history of medical disease or thyroid disorders; (b) full general examination, with a special focus on weight, BMI, blood pressure, acne, and hirsutism; (c) diagnosis of PCOS (according to Rotterdam criteria if any two out of three were present), in the absence of other causes that might cause them; (i) oligoovulation and/or anovulation (menstrual disorders, oligomenorrhea, amenorrhea, and infertility); (ii) excess androgen activity (hirsutism, acne, and obesity); (iii) PCOs (by ultrasound); and (d) laboratory tests: measurements of AMH at the beginning of the study and after treatment with metformin for 3 months in group 1. Metformin (2550 mg daily) was administered at three divided doses (850 mg in each dose) to all PCO patients and for 3 months. Ethical approval was obtained from Ain Shams University, Faculty of Medicine, Research Ethics Committee, FWA00017858.

Measurement of anti-Mullerian hormone

The human AMH ELISA Kit (Sun Red Company, Shanghai, China) was used in the test procedure. Sample collection was performed.

Principle of the test

This AMH enzyme-linked immunosorbent assay uses a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit is precoated with a multiclonal antibody specific for standards; samples are then added to the microtiter plate wells and AMH, if present, will bind to the antibody-precoated wells.

To quantitatively determine the amount of AMH present in the sample, a standardized preparation of horseradish peroxidase-conjugated polyclonal antibody, specific for AMH, is added to each well to sandwich the AMH immobilized on the plate. The microtiter plate is subjected to incubation, and then the wells are washed thoroughly to remove all unbound components. Next, A and B substrate solution is added to each well. The enzyme horseradish peroxidase and substrate are allowed to react over a short incubation period. Only those wells that contain AMH and enzyme-conjugated antibody will show a change in color. The enzyme–substrate reaction is terminated by the addition of a sulfuric acid solution and the color change is measured spectrophotometrically at a wave length of 450 nm.

Statistical methodology

Data were collected and statistically analyzed using the statistical program for the social sciences, version 20 (SPSS; SPSS Inc., Chicago, Illinois, USA).

Results

(1) Group 1: included 30 women with PCO, mean age of 24.2±4.9 years. Their BMI was 28.7±6.2 kg/m^2 before treatment and 27.7±6.0 kg/m^2 after treatment. Their AMH was 3.54±0.58 ng/ml before treatment and 2.79±0.39 ng/ml after treatment.
(2) Group 2: included 30 healthy women. Their mean age was 26.2±3.3 years. Their BMI was 26.6±3.8 kg/m^2 and their AMH was 2.14±0.49 ng/ml.
(3) There was no difference between the two groups in age, weight, and BMI ($P>0.05$) (Tables 1 and 2). In terms of the menstrual cycle, there was a significant difference between the two groups ($P<0.01$). In the PCO group, 3.3% had regular cycles, 80.0% had oligomenorrhea, and 16.7% had amenorrhea, whereas all the women in the control group had regular cycles (100%). Among the PCO patients, 66.7% were infertile and nullipara. However, all the controls (100%) were fertile. A total of 83.3% of the PCO patients had induction of ovulation whereas this was found in none of the controls ($P<0.01$).

On comparing the signs and symptoms, PCO patients (83.3%) had acne, 86.7% had hirsuitism, 13.3% had diabetes, and 13.3% had hypertension, whereas none of the women in the control group had acne, hirsuitism, diabetes, or hypertension. There was a significant difference between the two groups ($P<0.01$) in terms of the presence of acne and hirsuitism, but no statistically significant difference in the presence of diabetes and hypertension.

On comparing the family history (FH) in the two groups, in the PCO group, 36.7% had FH of diabetes, 16.7% had FH of hypertension, 6.7% had FH of obesity, and 13.3% had FH of PCO. In the control group, 6.7% had FH of diabetes, 10% had FH of hypertension, and none of the women had FH of obesity or PCO. There was a difference between the two groups in FH of diabetes and hypertension ($P<0.01$).

In terms of the different parameters in the PCO group before and after treatment, there was a significant difference ($P<0.01$) in weight, BMI, and AMH levels before and after treatment with metformin (Table 3).

The difference in AMH levels between controls (2.14±0.49 ng/dl) and PCO patients before treatment (3.54±0.58 ng/dl) and between controls and PCO patients after treatment (2.79±0.39 ng/dl) and in PCO patients before and after treatment was significant ($P<0.01$) (Table 4).

Table 1 Comparison between polycystic ovary patients and controls in terms of weight, BMI, and anti-Mullerian hormone before metformin treatment using the Student *t*-test

Parameters	Control (n=30) (mean±SD)	PCO patients before treatment (n=30) (mean±SD)	t	P
Weight (kg)	73.4±10.6	78.4±18.5	–1.280	0.207
BMI (kg/m^2)	26.6±3.8	28.7±6.3	–1.563	0.123
AMH (ng/ml)	2.14±0.49	3.54±0.58	–8.916	0.000**

AMH, anti-Mullerian hormone; PCO, polycystic ovary. **$P<0.01$, highly significant.

Table 2 Comparison between polycystic ovary patients and controls in weight, BMI, and anti-Mullerian hormone after metformin treatment using the Student *t*-test

	Control (n=30) (mean±SD)	PCO patients after treatment (n=30) (mean±SD)	t	P
Weight (kg)	73.4±10.6	75.6±17.8	–0.584	0.561
BMI (kg/m^2)	26.6±3.8	27.7±6.0	0.868	0.389
AMH (ng/ml)	2.14±0.49	2.79±0.39	–5.195	0.000**

AMH, anti-Mullerian hormone; PCO, polycystic ovary. **$P<0.01$, highly significant.

Table 3 Comparison between weight, BMI, and anti-Mullerian hormone in the polycystic ovary group before and after metformin treatment using the Student *t*-test

	PCO patients		t	P
	Before treatment (mean+SD)	After treatment (mean+SD)		
Weight (kg)	78.4±18.5	75.6±17.8	5.594	0.000**
BMI (kg/m^2)	28.7±6.2	27.7±6.0	5.570	0.000**
AMH (ng/ml)	3.54±0.58	2.79±0.39	8.199	0.000**

AMH, anti-Mullerian hormone; PCO, polycystic ovary. **$P<0.01$, highly statistically significant.

Table 4 Comparison between the control group and the polycystic ovary group in anti-Mullerian hormone 'before and after treatment' and in the polycystic ovary group 'before and after treatment' using the Student *t*-test

AMH (ng/ml)	Mean±SD	*t*	*P* value
Controls (*n*=30)	2.14±0.49	–8.916	0.000**
PCO patients 'before treatment' (*n*=30)	3.54±0.58		
Controls (*n*=30)	2.14±0.49	–5.195	0.000**
PCO patients 'after treatment' (*n*=30)	2.79±0.39		
PCO patients 'before treatment' (*n*=30)	3.54±0.58	8.199	0.000**
PCO patients 'after treatment' (*n*=30)	2.79±0.39		

AMH, anti-Mullerian hormone; PCO, polycystic ovary. **$P<0.01$, highly statistically significant.

Table 5 Correlation between anti-Mullerian hormone levels and characteristics of the polycystic ovary group before treatment using Pearson's correlation test

Parameters	AMH in PCO before treatment	
	Pearson's correlation significance (two-tailed)	*P* value
Age (years)	0.265	0.158
Parity	0.023	0.909
Weight (kg)	0.378	0.040*
Height (cm)	0.122	0.519
BMI (kg/m^2)	0.371	0.043*

AMH, anti-Mullerian hormone; PCO, polycystic ovary. *$P<0.05$, significant.

There was a positive correlation between AMH with weight and BMI ($P<0.05$), but no correlation with age, parity, and height in the PCO group before treatment ($P>0.05$) (Table 5).

Discussion

In the present work, we studied the difference in the AMH levels in Egyptian women with PCOS in comparison with controls and the effect of metformin therapy 2550 mg daily dose for 3 months on the levels.

The PCO cases had higher weight and BMI than the controls ($P<0.05$). This result might support the presence of a strong link between PCO and metabolic syndrome manifestations including obesity, insulin resistance, type 2 diabetes mellitus, dyslipidemia, hypertension, and nonalcoholic fatty liver disease [10,11].

The control group had regular menstrual cycles whereas only 3.3% of the women in the PCO group had regular cycles; 80% had oligomenorrhea and 16.7% had amenorrhea, with *P* value less than 0.001, between the two groups. This might be the result of high AMH in the PCOS group in comparison with the controls. We found that the AMH levels in PCOS was 3.54±0.58 ng/ml before treatment whereas it was 2.14±0.49 ng/ml in the controls ($P=0.000$). It has been reported that AMH physiologically decreases the expression of both the follicle-stimulating hormone (FSH) receptor and ovarian aromatase. This mechanism protects the small follicles from premature aromatase expression. However, when AMH increases and/or lasts longer than it should in larger follicles, this could result in 'follicular arrest' as this results in a defect in the selection of the dominant follicle [7].

This is in agreement with another study that reported that AMH levels had a significant positive correlation with cycle length ($P<0.01$). Cycle length can be considered a reflection to the degree of anovulation [12,13]. Zhu *et al.* [13] found that AMH was associated significantly with menstrual cycle length and each SD increase in AMH was associated with a 2.8 folds increased risk for longer menstrual cycles. These findings are consistent with evidence indicating a role for the ovary, in particular AMH, in the regulation of the menstrual cycle. Per each day increase in menstrual cycle length, serum AMH was reported to increase by 14·0% in a Danish population [14].

Many lines of evidence argue for AMH as the ovarian mediator for menstrual cycle length. The human menstrual cycle has follicular and luteal phases. Cycle length variation is determined mainly by the follicular phase, the luteal phase being much less variable [15]. Low estradiol levels in the early follicular phase are known to be associated with longer menstrual cycles [16]. AMH can reduce estradiol production by inhibiting FSH-stimulated aromatase mRNA expression [17]. Its suppressive effect on FSH-stimulated estradiol production from antral follicles could be one mechanism by which AMH may lengthen the follicular phase, whereas low AMH and rapidly increasing estradiol levels are associated with early emergence of dominant follicles [14] and shorter menstrual cycles. Genetic polymorphisms that reduce AMH protein function are associated with increases in estradiol levels and a shorter follicular phase [18].

The present study showed that 66.7% of the women in the PCO group were infertile whereas all the women in the control group were fertile ($P<0.001$). PCO is the

most common endocrine cause of infertility because of anovulation and elevated levels of AMH as it is hypothesized that the high AMH concentrations present in women with PCOS play an integral role in causing anovulation because of its inhibitory influence on the actions of FSH, which normally promotes follicular development from the small antral to the ovulatory stage [17,19]. These results are consistent with a study that reported that fertility increases when AMH levels decrease in the PCO group [20].

In the present study, the PCO women had symptoms and signs of hyperandrogenism (acne and hirsutism) than the controls ($P<0.001$). This can be explained by the strong link between biochemical hyperandrogenemia and PCO [21]. These results support the results of the study by Rosenfield *et al.* [22], who reported an independent correlation between AMH with PCO and ovarian hyperandrogenism.

The PCO group had a FH of diabetes than the controls ($P<0.001$). Some studies suggested that genetic plays an important role in the pathogenesis of PCOS. The high prevalence of women with PCOS and the wide range of phenotypes can be explained by the interaction of key genes with environmental factors. Some evidences have shown that there are associations between cytochrome P450 17-hydroxylase/17, 20-desmolase (CYP17), and PCOS [23].

Another study reported that the prevalence of PCO among mothers and sisters of women with PCO was 24 and 32%, respectively [21]. Nevertheless, the prevalence of a parental history of diabetes and hypertension was higher in PCOS patients than in non PCOS women [24].

In the present study, we reported that AMH was higher in PCO patients than in the controls ($P<0.001$). Our results support those reported by Pigny *et al.* [25], who detected higher serum AMH levels in PCOS patients than in controls and were significantly related to the follicle number in the two groups. Caglar *et al.* [26] also found that the AMH values were higher in PCOS cases compared with the controls.

In addition, we found a positive correlation between AMH and weight and BMI in PCO patients before treatment ($P<0.05$), and this can be explained by the fact that obesity is a common finding in women with PCOS and between 40 and 80% of women with this condition are reported to be overweight, obese, or centrally obese [27]. Also, this might be because of lower levels of adiponectin in women with PCOS than normal women without PCOS (adiponectin enhances insulin sensitivity and fatty acid oxidation, thus reducing glucose and lipid concentrations) [28]. Saleh *et al.* [29] also reported that there was a positive correlation between serum levels of AMH and the values of BMI.

In the present study, AMH levels decreased after treatment with metformin therapy (850 mg tablets three times daily for 3 months) ($P<0.01$). The possible reason for this reduction remains controversial. The beneficial role of metformin might be because of an increase in the hepatic production of SHBG, thus lowering the circulating free testosterone, decreasing the adrenal androgen production, decreasing androgen production in the ovary, normalizing luteinizing hormone, and slightly increasing FSH levels and decreasing the insulin concentrations. Moreover, metformin has been shown to induce regular menstrual cycles, increase ovulation, ameliorate hirsutism, and produce slight weight loss [30].In addition, metformin markedly improves peripheral resistance to insulin and restores ovarian morphology, suppresses hepatic gluconeogenesis, increases glucose consumption in skeletal muscles, and decreases the intestinal glucose absorption. It also enhances insulin action at cell levels by enhancing the disposal of glucose in adipose and muscular cells and by increasing the ligation to the insulin receptors [4,30]. Other studies have reported similar results, but at lower doses and for longer durations [29–32].

However, our findings are not consistent with the result reported by Nascimento *et al.* [33], who found that AMH levels were higher in an untreated PCOS group than the control group ($P<0.0001$), but these AMH levels did not change after treatment with metformin 1500 mg/day for 8 weeks despite an improvement in the metabolic parameters. Perhaps the dose and the duration of metformin therapy are factors associated with the reduction of AMH levels [33].

Weight and BMI decreased in PCO patients after metformin therapy ($P<0.001$). This reduction might be because of the action of metformin, which increases insulin sensitivity and peripheral glucose uptake, whereas it decreases hepatic gluconeogenesis, insulin-induced suppression of fatty acid oxidation, and absorption of glucose from the gastrointestinal

tract [34]. Our results support those of Sova *et al.* [35], who found that weight and BMI decrease after metformin therapy (1000 mg twice daily) in obese PCO patients.

Finally, multiple linear regression analysis of our data showed that weight, height, BMI, and hyperandrogenic state manifested as acne and hirsuitism; FH irregular cycles and FH PCO are independent predictors of AMH levels after metformin treatment ($P<0.05$).

Our study provides evidence that patients with PCO have higher levels of AMH, which might play a pivotal role in the irregular ovulation and infertility in these patients. Metformin, through its effect on insulin resistance and hepatic production of SHBG, might improve the anovulation and aid the management of this condition. In addition, AMH could serve as a prognostic marker for the efficacy of metformin therapy.

Conclusion

Increased levels of AMH are found in PCOS patients, and the use of metformin in the treatment of PCO disease patients is beneficial. AMH can be used as a marker for treatment efficacy with metformin.

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Conflicts of interest

There are no conflicts of interest.

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