



## Research Article

# The Relationship between Serum Levels of Anti-Mullerian Hormone and Body Mass Index in Adolescents with Polycystic Ovary Syndrome

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## ABSTRACT

**Introduction:** Polycystic ovary syndrome is widely prevalent among adolescents and early diagnosis is crucial. While accurate diagnosis is not straightforward, anti-Mullerian hormone was found to be a reliable marker among young patients with polycystic ovary syndrome. However, its serum level was found to be affected by many other variables. This study assessed the relation between serum levels of anti-Mullerian hormone and body mass index in adolescents with polycystic ovary syndrome.

**Methods:** This was a cross sectional analytical study, conducted at the Gynecology and Obstetrics outpatient clinics at Suez Canal University hospitals in Ismailia. It included 100 adolescents with PCOS who fulfilled the revised Rotterdam diagnostic criteria for PCOS, attending secondary stage or higher levels of education in Ismailia, Egypt. The recruited patients were divided into two groups. Group one reported to have BMI  $\geq 30$  and group two included those who have BMI  $< 30$ . Complete history taking, clinical examination (BMI) and biochemical markers including assessment of serum LH and AMH level, and ultrasound assessment to detect ovarian volume, antral follicular count, and presence of ovarian cysts.

**Results:** AMH levels were not affected by BMI in adolescent patients with PCOS ( $r$  0.19,  $p$  value 0.185). There were significant correlations between serum AMH and LH level, & between AMH and ovarian morphology among the studied population. The AMH was higher among those with menstrual irregularity and those with hyper-androgenism. Accordingly, AMH level is not affected by patients' weight.

**Conclusion:** The AMH was correlated to clinical and biochemical findings of PCOS.

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## Introduction

Polycystic ovary syndrome (PCOS) is considered a major cause of anovulation and hyper-androgenism among females [1]. It is established that PCOS trigger multiple systems affection on the long term such as cardiovascular incidents, type 2 diabetes mellitus, metabolic syndrome, in addition to infertility [2, 3]. Most commonly affected population is female patients at adolescence and childbearing period, with an estimated prevalence of 6 to 10 percent [4]. The Rotterdam consensus workshop, which was held in 2003, recommended that at least two of the following three features are for PCOS to be diagnosed:

i. Oligomenorrhea or amenorrhea.

- ii. Evidence of clinical or laboratory hyper-androgenism.
- iii. Polycystic ovaries (as defined on ultrasonography as 12 or more follicles in at least one ovary measuring 2-9 mm in diameter or a total ovarian volume of  $>10\text{m}^3$ ) [5].

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein merely produced by the granulosa cells of the three types of follicles: primary, pre antral and small antral. In the following phases of follicle's development, AMH level experiences a steady reduction. It may not be detected in larger follicles of more than 8 mm [6]. This hormone has a significant importance in infertile patients because it has a predictive value for the evaluation of ovarian reserve and predicting response to assisted reproductive technologies [7]. Nonetheless, serum AMH levels

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were found to be modified by some conditions. Obesity is one of the factors that could affect serum AMH levels. This aspect was initially evaluated in women in the advanced spectrum of the reproductive age [8].

Further studies, which included a wider spectrum of age categories, reported conflicting results, either confirming the negative relationship between AMH and body mass index (BMI) or reporting no relationship at all [9-15]. Moreover, the severity of excess adiposity in adolescents, which can negatively influence or have no effect on the AMH production, is incompletely clarified. Recently, there is a wide acceptance that nutritional status affects follicular development through specifically perceived energy mechanisms [16]. Nowadays, obesity affects a large number of people especially the young. However, the data regarding the relationship between adiposity and serum AMH level in those obese adolescents are scarce. Considering the important predictive role of AMH in ovarian reserve, the possible contribution of BMI to its productivity in younger patients with PCOS needs to be analyzed.

## Patients and Methods

Receiving the ethical and institutional approval, 100 adolescents diagnosed as polycystic ovary syndrome were enrolled in this cross sectional study which was conducted among students of secondary schools and colleges in Ismailia, Egypt, after fulfilling the following inclusion criteria: Age spectrum ranging from 16-21 years, BMI  $\geq$  30 for group one patients and  $<$  30 for group two, menarche at least 2 years before enrollment. All PCOS patients fulfilled 2003 revised Rotterdam diagnostic criteria based on the association of at least two of the three following criteria: (a) Oligomenorrhea defined as cycles at intervals  $>$  45 days or amenorrhea defined as absent cycles more than 3 months. (b) Clinical Hyperandrogenism (the presence of hirsutism (modified Ferriman and Gallwey score  $>$  8), acne, or androgenic alopecia.) or hyperandrogenemia (total testosterone  $>$  0.7 ng/ml). (c) Ultrasound criterion of polycystic ovary syndrome, either; 1) Presence of 12 or more follicles in each ovary measuring 2-9 mm in diameter, 2) And/or increased ovarian volume ( $>$ 10mL), 3) And/or an ovarian area more than 5.5 cm<sup>2</sup> unilaterally or bilaterally. We excluded the following cases: pregnant females, patients using an oral contraceptive pill or metformin, chronic medical conditions, users of glucocorticoid or other hormonal therapy, thyroid abnormalities, Cushing syndrome and pituitary disorders. Patients were divided into two groups; group one included obese patients with BMI  $\geq$  30 and group two patients with BMI  $<$  30. All included patients were subjected to clinical examination and investigations which included: general examination (weight, height, BMI), assessment of serum AMH and LH level, and Ultrasound assessment to detect: ovarian volume, antral follicular count and presence of ovarian cysts. The required sample size was calculated using a-error of 0.05 [17].

## I Sample Collection and Storage

Two mL of human serum were obtained from every participant using plain vacutainers. Blood was coagulated and centrifuged to eliminate fibrin. Serum was separated and stored at  $-20^{\circ}\text{C}$  till ready for use. Frozen-stored samples were thawed and were mixed using a vortex.

## II Assessment of Serum Anti-Müllerian Hormone Level by Vidas® Immunoanalyzer

VIDAS® is a compact automated immunoassay system based on the Enzyme Linked Fluorescent Assay (ELFA) principles. The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection. The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips. All the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

The sample was transferred into the wells containing anti-Müllerian antibody labeled with alkaline phosphatase (conjugate). The sample/conjugate mixture is cycled in and out of the SPR® several times. This operation enables the anti-Müllerian hormone to bind with the antibodies coated on the interior of the SPR® and with the conjugate to form a sandwich. Unbound components are eliminated during the washing steps. During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR®. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferon), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration present in the sample. At the end of the assay, the results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

## III Test Procedure

- Test strips were removed from the refrigerator immediately before use.
- One "AMH" strip and one "AMH" SPR® were used for each tested sample, control (C1) or calibrator (S1).
- The calibrator "S1" and the control "C1" were tested before testing the samples.
- Before pipetting, samples, calibrators, controls and diluent were ensured to be free of bubbles.
- The "AMH" SPRs and "AMH" strips were inserted into the instrument, and the color labels with the assay code on the SPRs and the Reagent Strips were ensured to be matched.
- Hundred microliters (200  $\mu\text{L}$ ) of each sample, calibrator, and control, were infused into the sample well on the test strip.
- All the assay steps were performed automatically, and the assay was completed within  $\sim$ 35 minutes.

## IV Results and Interpretation

Once the assay was completed after collecting the identified sample, results were analyzed automatically by the computer. Fluorescence was measured twice in the Reagent Strip's reading cuvette for each sample tested. The first reading was a background reading of the substrate cuvette before the SPR was introduced into the substrate. The second reading was taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (Relative Fluorescence Value) was calculated by subtracting the background reading from the final result. The results were automatically calculated using calibration curves stored by the instrument and were expressed in ng/mL.

## Results

Our patients were matched regarding their age with a range from 16-21 years. They were all students and virgins. There was a significant difference between them in BMI (p value 0.005), amenorrhea as well as clinical evidence of hyperandrogenism (Table 1). There was a significant difference between both groups in serum levels of LH only with a p value of 0.005 (Table 2).

**Table 1:** Basic demographic and clinical data of the studied population.

		Group one (50)	Group two (50)	P value
<b>Age</b>		18.16± 1.89	18.12± 1.67	0.933
<b>BMI</b>		32.81± 1.87	23.18± 2.48	0.005*
<b>Menstrual irregularity</b>	<b>Oligomenorrhea</b>	25 (50.0%)	17 (34.0%)	0.105
	<b>Amenorrhea</b>	25 (50%)	0 (0%)	0.005*
<b>Clinical hyperandrogenism</b>		28 (56.0%)	17 (34.0%)	0.027*

\*significant

**Table 2:** Mean level of AMH and LH.

	Group one	Group two	P value
<b>AMH</b>	11.06 ± 2.37	11.36 ± 2.47	0.97
<b>LH</b>	10.72 ± 1.48	5.12 ± 1.68	0.005*

\*significant

Ultrasonography diagnostic criteria were similar in both groups (Table 3). There was no correlation between AMH levels and BMI in the studied population however; it showed a significant negative correlation with patients' age (corr. coeff. -0.293 and p value 0.003) (Table 4). Serum AMH levels were higher in patients with amenorrhea (p value 0.0001) and those with clinical hyperandrogenism with a mean level of 13.4 ± 2.4 and a p value 0.002.

**Table 3:** Ultrasound parameters among both groups:

	Group one	Group two	P value
<b>Follicle number</b>	14.18± 2.93	14.38± 3.05	0.745
<b>Ovarian volume (mm<sup>3</sup>)</b>	12.68± 2.34	14.18± 1.95	0.102

**Table 4:** Correlation between AMH and other parameters of the studied population:

Correlations		Age	BMI	Follicular number	Ovarian volume	AMH	LH
<b>LH (mIU/ml)</b>	<i>R</i>	-0.71	0.195	0.033	0.057	0.142	-----
	<i>P-value</i>	0.623	0.175	0.822	0.694	0.001*	-----
<b>AMH (ng/ml)</b>	<i>R</i>	-0.586	0.19	0.496	0.245	-----	0.348
	<i>P-value</i>	0.0001*	0.185	0.0001*	0.0001*	-----	0.001*

\*significant

## Discussion

The study revealed that there was no relation between body mass index and serum levels of AMH. There was significant negative correlation between AMH levels and patients' age. Albu reported that BMI was not significantly correlated with AMH in a cohort of infertile patients which was similar to our results. But when the association between AMH serum

level and BMI was analyzed according to age group, AMH and BMI were positively correlated in young patients (≤ 30 years old and 30-35 years old,  $r = 0.139$ ,  $p = 0.021$  and  $r = 0.077$ ,  $p = 0.027$ , respectively) [18]. However, Bernardi et al, reported that BMI at the age of 18 ( $\beta = -0.016$ ; 95% CI -0.024, -0.008), which is considered the heaviest reported lifetime weight ( $\beta = -0.002$ ; 95% CI -0.003, -0.001) was inversely associated with AMH. This difference may be related to different target population as they included premenopausal women of African-American origin [19]. Our study contradicted others who found negative correlation between the two parameters [8, 10, 19, 20]. Our study reported significant negative correlations between AMH levels and patients' age which was similar to what was reported by others [21].

Leonte et al. reported increased levels of AMH in patients with hyperandrogenism as well as ours [22]. The explanation of the higher level of AMH among PCOS with hyper-androgenism than non-hyperandrogenic is that with hyper-androgenism there is excess follicular arrest with a large mass of 2-5 mm follicular pool, and these are the main population of follicular cohort secreting the AMH leading to marked increase in its level. AMH levels correlated strongly with LH level which contradicted what was reported by previous studies.

This contradiction might be explained by the selection criteria in this research, as all the included patients were anovulatory, which may entail more disturbed ovarian physiology and higher LH level which was strongly related to the high AMH level arising from the profoundly arrested small follicles [22, 23]. There was moderate positive correlation between AMH level and AFC and ovarian volume which was also as reported by others [24, 25]. Since AMH is secreted from these follicles, AMH level increases proportionally to their number. The more AFC, the more AMH level, which exactly happens in PCOS [26]. Given the fact that AMH level is positively correlated to AFC, hence it is predictable that AMH is correlated to ovarian volume.

In our study, the recruited patients were unmarried virgin with ultrasound evaluation carried out abdominally. Whether this would influence the accuracy of the ultra-sonographic criteria or not needs to be evaluated. Also, the possibility to use serum biomarkers in such patients instead of ultrasound needs to be evaluated.

## Strengths and Limitations of the Study

We included 100 adolescent patients aged 16-21 divided into two groups according to their BMI which empowers the results. Larger sample would increase the strength of the results. Also, evaluation of patients with morbid obesity was not properly evaluated in our study.

## Conclusion

AMH was not correlated with BMI but significantly correlated with patients' age, ovarian volume and follicular count.

## Informed Consent

Informed consent was obtained from all participants before enrollment in the study.

## Funding

Self-funded research.

## Conflicts of Interest

None.

## Ethical Approval

All procedures performed in in the study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

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