

Correlation between Anti-Mullerian Hormone Levels and Antral Follicle Numbers in Polycystic Ovary Syndrome

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Abstract

Introduction: Polycystic Ovary Syndrome (PCOS) is characterized by clinical and biochemical hyperandrogenism, polycystic ovarian morphology, altered gonadotropin secretion, insulin resistance and /or hyperinsulinemia associated with obesity.

Objective: The objective of this study was to compare the correlations between levels of FSH, LH, estradiol, Anti-Mullerian Hormone (AMH), and antrafollicle numbers in women with polycystic ovary syndrome.

Methods: PCOS was diagnosed when at least two out of the three parameters were present, according to the Rotterdam criteria. Patients were grouped according to the presence of oligomenorrhea and amenorrhea. Levels of AMH, FSH, LH and Estradiol were determined and a transvaginal ultrasound was performed to determine ovarian volume and the number of antral follicles.

Results: Pearson's correlation revealed a significant correlation (0.283, $p=0.01$) between AMH and antrafollicle number. It was appetent that the higher the number of antral follicles the concentrations of AMH is also higher. A linear correlation showed that the AMH concentration and the number of antral follicles correlated positively ($r=0.303$, $p=0.002$). While the levels of FSH correlated negatively with the number of antral follicles ($r=-0.182$, $p=0.05$). The ovarian volume also correlated positively with the number of follicles ($r=0.708$, $p=0.000$).

Conclusions: A significant correlation of AMH with the number of antral follicles was revealed.

Keywords: polycystic ovary syndrome, anti-Müllerian hormone, ovarian reserve.

reserve. Furthermore, women with higher serum levels of **Anti-Mullerian Hormone (AMH)** have longer menstrual cycles compared with those with lower levels. In females, AMH is expressed in granulosa cells of growing follicles up to the antral stage, suggesting an important role in early ovarian folliculogenesis. AMH is able to inhibit the initiation of primordial follicle growth and may also decrease the sensitivity of antral follicles to follicle-stimulating hormone (FSH). In humans, however, the association between androgens and AMH remains uncertain, and its exact function in follicular recruitment and long-term effects is not well understood. Serum AMH levels could be used also to identify the girls at risk for PCOS in early adulthood, and allow early prevention by life style counselling. The aim of this study was to compare the correlations between hormone levels of FSH, LH, estradiol, AMH, and antrafollicle numbers of women with polycystic ovary syndrome.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive-aged women with a prevalence of 6%–18%¹, which is characterized by clinical² and biochemical hyperandrogenism, polycystic ovarian morphology, altered gonadotropin secretion, insulin resistance and/or hyperinsulinemia associated with obesity.³⁻⁴

Several authors have described altered levels of gonadotropins, prolactin and sex steroids in PCOS⁵. Menstrual disturbances commonly observed in PCOS include oligomenorrhea and amenorrhea. About 70%–90% of women with oligomenorrhea have PCOS while 30%–40% of women with amenorrhea could have PCOS⁶. More than 63% of women presenting with symptoms of androgen excess have PCOS and 47% of them have hormonal alterations.⁷⁻⁸

Women with PCOS often seek care for menstrual disturbances, clinical manifestations of hyperandrogenism, and infertility. PCOS is the most common cause of anovulatory infertility.⁹ Women with PCOS are likely to have not only a higher antral follicle count (AFC) but also a greater ovarian volume.¹⁰

AFC is essential to correlate biochemical and clinical markers to prognosticate infertility and ovarian

METHODS

This was a descriptive and analytical study. The selection of patients was randomly done by the Gynecology Service by two specialists. All patients underwent a complete medical history including reproductive background. In the participating Gynecology Service, a medical professional approached potential participants to ascertain interest in the study and obtain informed consent. Assurance was given that consent or refusal to participate would not affect the quality of care or eligibility for any future clinical studies and that all information provided would be confidential to the study team. Ethical approval was provided by the Institutional Ethics Committee of The University of Guanajuato with the

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A total of 50 women were included, between 25-30 years of age, who were diagnosed with PCO according to the Rotterdam criteria.¹¹ The PCOS was defined with at least two out of the three criteria were present. The diagnosis was carried out by two experienced Gynecologists. The patients were separated in to two different groups; patients with oligomenorrhea were placed in group 1, while group 2 was set up by the patients with amenorrhea. In this study, amenorrhea is described as absence of at least 3 to 6 consecutive menstrual cycles or four or fewer menstrual period per year and oligomenorrhea is described as menstrual cycles fewer than 8 cycles per year, or the duration of the cycle exceeds 35 days.

The determinations of LH, FSH and estrogen levels were taken in the 1-3 days of the menstrual cycle in patients with oligomenorrhea. The determinations levels of LH, FSH and estrogen were taken any day of the menstrual cycle in patients with amenorrhea. The determinations of levels of AMH were taken any day of the menstrual cycle in all women, during the second study visit. Patients who had a history of previous ovarian surgery and those who had been taking oral contraceptives were excluded from the present study.

Determination of hormone levels

After at least 12 hours of fasting 10ml of peripheral venous blood, on any day of the menstrual cycle, was drawn for determining levels of AMH by an ultra sensitive ELISA (AMHGenII, Beckman Coulter Inc., Webster, TX, USA) with a sensitivity of 0.08ng/mL. To determine levels of LH, FSH and estrogen, 10 ml of peripheral venous blood were taken in the 1-3 days of the menstrual cycle of each patient and analyzed by means of radio immunoassay.

Transvaginal ultrasonography (USG)

Each patient had a USG performed by the same observer, a reproductive

biologist with a 5 MHz transvaginal probe by Medison Sono Ace 6000C (Medison, Seoul, Korea). The measurements were performed in real time using the highest possible magnification to observe the ovaries. Transvaginal ultrasound was performed in all participants in the early follicular phase between day 2 and day 3 of the menstrual cycle.

Axes measured were length, thickness and width to calculate ovarian volume (OV).¹² Ultrasonographic image was considered characteristic of PCOS in the presence of 12 or more follicles 2-9 mm in diameter located in the periphery of the ovaries by scanning each ovary from the inner to the outer margins in a longitudinal cross section, according to the Rotterdam criteria. The total ovarian volume (OV) was defined as the sum of the right and the left ovarian volumes, and the total antral follicle number was defined as the sum of the right and the left antral follicle count (AFC).

Statistical analysis

We used the statistical package SPSS version 21.0 for the statistical analysis. Descriptive statistics were used to represent the demographic characteristics of women. We performed Pearson's correlation

between the number of antral follicles number and AMH levels, and a linear correlation between LH, FSH, estradiol, AMH, OV and antral follicle number. In all cases the p value accepted as significant was <0.05.

RESULTS

The demographic and clinical characteristics of oligomenorrhea and amenorrhea patients with PCOS are given in Table 1. No significant differences in age, body mass index and education. The patients showed differences only in the presence of menstruation as expected.

When the women in the current study were classified according to their menstrual cycle into oligomenorrhea and amenorrhea (Table 2) it was found that FSH and LH concentrations were significantly higher in amenorrhea patients ($p=0.024$ and $p=0.028$, respectively).

Analysis of Pearson's revealed a significant correlation (0.283, $p = 0.01$) between AMH and the number of antral follicles, which showed that the higher the number of antral follicles more the concentrations of AMH.

AMH correlated positively with the number of antral follicles ($r = 0.303$, $p = 0.002$). FSH negatively correlated

Table 1: Demographic and Clinical Characteristic of the Patients

Variable	Oligomenorrhea n=25	Amenorrhea n=25	p
Age(years)	24.2±5.2	25.7±5.0	0.190
BMI(kg/m ²)	26.4±2.62	19.8±1.70	0.180
Schooling (years)	11 ±3.5	10±3.0	0.440

Data are expressed as mean±standard deviation.* $p<0.05$.

Table 2: Endocrine parameters among investigated groups

Hormone	Oligomenorrhea n=25	Amenorrhea n=25	p
FSH U/L	9.1±4.5	11.8 ±4.1	0.024
LH U/L	17.3±12.6	29.4±22.4	0.028
Estradiol ng/ml	92±22	85±11	NS
AMH ng/ml	8.8±2.6	8.5±2.2	NS

Data are expressed as mean±standard deviation.* $p<0.05$.

Table 3: Ultrasonographic volumen parameters

Variable	Oligomenorrhea n=25	Amenorrhea n=25	p
Number of antrafollicles	32 (25-46)	36 (28-57)	0.001
OvarianVolume	5.0 (2.3-9.2)	6.7 (3.9-12.4)	0.001

Data are expressed as median(range)*p<0.05

with the number of antral follicles in both groups ($r = -0.182$, $p = 0.05$).

The serum AMH level was not significantly related to the ovarian volume on ultrasonography.

Ovarian volume positively correlated with the number of follicles ($r = 0.708$, $p = 0.000$) (Table 3).

DISCUSSION

In this study, we evaluated the relationship between level of FSH, LH, estradiol, AMH, and antral follicle numbers and ovarian volume in women with polycystic ovary syndrome. The results showed a significant correlation between serum AMH level and the number of antral follicles, which showed that the higher the number of antral follicles more the concentrations of AMH.

Some authors have reported that the highly significant relationship between the serum AMH level and AFC.¹³⁻¹⁴ AMH is regarded as the most useful marker of the status of ovarian reserve.¹⁵

Swellam et al. Other authors suggest that, serum AMH levels might be introduced as a marker to be utilized clinically in the differential diagnosis of hyperandrogenemic patient¹⁶. Our data confirm these findings, which showed that higher the number of antral follicles the more the concentration of AMH.¹⁶

Patients who were diagnosed with PCOS- Amenorrhea showed elevated levels of FSH and AMH, ovarian volume and number of antral follicles. In addition it has been reported that AMH levels between hyperandrogenic and non-hyperandrogenic PCOS patients are different.¹⁷ In the current study elevated FSH levels were reported in oligomenorrhea patient with PCOS.

Increased LH and normal to low FSH such as young PCOS women with hyperandrogenemia, serum AMH levels are increased,¹⁸ this was seen in our study.

In contrast, we found not significant correlation between the serum AMH level and the ovarian volume, and this result is in disagreement with that of a previous study showing that the AMH levels positively correlate with the ovarian volume.¹⁹ One possible explanation is that the ovarian stromal volume is not significantly affected by the serum AMH level.

Hormone levels of FSH showed significant differences in the two groups. Stimulatory effect of FSH on AMH secretion in oligo/anovulatory PCOS women is known. Cateau-Jonard and Dewailly, suggest that the defective selection of a dominant follicle in anovulatory patients, results in a local inhibition of FSH action.²⁰

Current tests to determine the ovarian response, which include AMH are designed to predict how a woman can respond to controlled ovarian stimulation, so it is considered that assessing functional ovarian reserve through AMH is not enough. FSH levels increase with age, hindering the estimation of ovarian reserve and decrease possible fertility. The number of antral follicles, an indirect measure of ovarian reserve, seen through a transvaginal ultrasound is now reliable and allows us to have a more accurate estimation even when it comes to starting a treatment protocol for infertility. Antral follicle count with transvaginal sonography is a non-invasive and easy to perform method that provides predictive information about ovarian responsiveness before stimulation.²¹ Further research is needed to determine the relationship of AMH and PCOS. AMH may be a useful initial diagnostic test for PCOS

subject to validation in prospective population.²² The study was restricted to women who sought treatment for infertility. Moreover, we believe that AMH can be used as a marker in conjunction with ultrasound values to assess ovarian reserve in the future.

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AUTHOR CONTRIBUTIONS

Silva-Vera M conceived and designed the study, performed the experiments and data analysis, and drafted the manuscript. Beltran-Campos V carried out the hormonal analysis. All authors have read and approved the final manuscript. ■

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