

ASSOCIATION OF SERUM AMH WITH LABORATORY AND PHENOTYPE IN WOMEN WITH POLYCYSTIC OVARY SYNDROME: A RETROSPECTIVE STUDY

POLİKİSTİK OVER SENDROMLU KADINLARDA SERUM AMH İLE LABORATUVAR VE FENOTİP İLİŞKİSİ: RETROSPEKTİF ÇALIŞMA

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ABSTRACT

Objective: The aim of this study was to compare the endocrine and phenotypic characteristics of women with polycystic ovary syndrome (PCOS) with serum Anti-Mullerian Hormone (AMH) levels of 4-10 ng/mL and >10 ng/mL, and to investigate the importance of high AMH levels in the diagnosis of PCOS.

Materials and Methods: In this retrospective cohort study, the laboratory and demographic characteristics of women with serum AMH ≥4 ng/mL whose follow-up was initiated at the infertility outpatient clinic of a tertiary health care institution were investigated. Fasting levels of Homeostasis Model Assessment (HOMA), Luteinizan hormon (LH), Follkül stimulan hormon (FSH), estradiol, androstenedione, total testosterone, prolactin, 17- hydroxyprogesterone (170HP), dehydroepiandrosterone sulphate (DHEAS), and AMH were recorded from hospital records. Age, gravidity, BMI, infertility status, hirsutism, menstrual cycle and ultrasonographic ovarian morphology were recorded. Patients were divided into two groups, as those with serum AMH values of 4-10 ng/mL and those with serum AMH values of >10 ng/mL. Women with AMH >10 ng/mL were defined as the "high AMH group" and those with AMH >14 ng/mL were defined as the "very high AMH group." Women on hormone-containing drugs, metformin, and/or chronic medication, as well as women with endocrine organ tumors and/or who have had ovarian surgery were excluded.

Results: The patients were between 21 and 38 years of age. Fifty-four women with AMH values of 4-10 ng/mL, 12 women with 10-14 ng/mL and 16 women with >14 ng/mL were included in the study. Forty-four patients were primary infertile, and 28 patients had clinical hirsutism. Women with high AMH values had more primary infertility and hirsutism. Thirty-four patients had menstrual irregularities, and there was no statistically significant difference between the two groups in terms of menstrual irregularities. Among laboratory values, total testosterone, androstenedione, and the LH/FSH ratio were significantly associated with high AMH. Primary infertility and hirsutism were more common in women with high AMH. PCOM was seen in all the women with AMH >10 ng/mL. There was no difference between the two groups in terms of HOMA and BMI criteria. Other endocrine values were not associated with AMH levels. Conclusion: There is a positive correlation between androgens and AMH in women with high AMH values, with the possibility of high AMH being an additional marker for the diagnosis of PCOS. We believe that women with hirsutism and high amounts of androgens and especially women with PCOM may have high AMH, and infertility treatments should be organized by taking into account the related drug resistance. Keywords: Anti-mullerien hormone, hirsutism, PCOS, androgens

ÖZ

Amaç: Bu çalışmanın amacı serum Anti Müllerien Hormon (AMH) değeri 4-10 ng/ml ve >10 ng/ml olan polikistik over sendromlu kadınlardaki endokrin ve fenotipik özellikleri karşılaştırmak ve polikistik over sendrom (PCOS) tanısında yüksek AMH seviyesinin önemini araştırmaktır.

Gereç ve Yöntem: Bu retrospektif cohort çalışmada tersiyer sağlık kuruluşunda infertilite polikliniğinde takipleri başlatılan, serum AMH değeri ≥4 ng/ml kadınların laboratuar, demografik özellikleri araştırıldı. Hastane kayıtlarından kadınların foliküler fazda açlık Homeostasis Model Assessment (HOMA), Luteinizan hormon (LH), Folikül stimulan hormon (FSH), östradiol, androstenedion, total testosteron, prolaktin, 17 hidroksiprogesteron (17OHP), dehidroepiandrosteron sülfat (DHEAS) ve AMH düzey sonuçları kaydedildi. Hastaların yaşı, gravidası, BMI, infertilite durumu, hirsutizm, adet düzeni ve ultrasonografik over morfolojisi kaydedildi. Hastalar serum AMH değeri 4-10 ng/ml ve >10 ng/ml olanlar olarak 2 gruba ayrıldı. AMH'ı 10 ng/ml'dan yüksek olan kadınlar " yüksek AMH grubu", AMH > 14 ng/ml olanlar da "çok yüksek AMH grubu" olarak adlandırıldı. Hormon içeren ilaç kullanan kadınlar, metformin kullanan kadınlar, kronik bir ilaç kullanan kadınlar, endokrin organ tümörü tanısı olanlar ve over cerrahisi geçirmiş kadınlar çalışmaya alınmadı.

Bulgular: Hastalar 21- 38 yaş aralığındaydı. AMH değeri 4-10 ng/ml olan 54, 10-14 ng/ml arası 12 ve >14 ng/ml 16 kadın çalışmaya alınmıştı. 44 hasta primer infertildi; 28 hastada klinik hirsutizm şikayeti vardı. Yüksek AMH değeri olan kadınlarda daha çok primer infertilite ve hirsutizm vardı. 34 hastada adet düzensizliği vardı ve iki grup arasında adet düzensizliği açısından fark yoktu. Laboratuar değerlerinden total testosteron, androstenedion, LH/FSH oranı ile yüksek AMH arasında anlamlı ilişkili bulundu. Yüksek AMH olan kadınlarda daha çok primer infertilite ve hirsutizm vardı. Tüm >10 ng/ml AMH değeri olan kadınlarda PCOM görülmüştü. Adet düzensizliği açısından iki grup benzerdi. 2 grup arasında HOMA ve BMI kriterleri açısından fark yoktu. Diğer endokrin değerler AMH düzeyi ile ilişkili değildi.

Sonuç: Yüksek AMH değeri olan kadınlarda androjenler ile AMH arasında pozitif korelasyon vardır, AMH yüksekliği PCOS tanısı konulmasına ek bir marker olabilir. Hirsutizm ve yüksek androjen olan kadınlarda ve özellikle PCOM olan kadınlarda yüksek AMH olabileceği ve infertilite tedavilerinde buna bağlı ilaç dirençlerinin dikkate alınarak tedavinin düzenlenmesi gerektiğini düşünüyoruz.

Anahtar Kelimeler: Antimüllerien hormon, hirsutizm, PCOS, androjenler

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INTRODUCTION

Apart from the infertile patient population, the laboratory and clinical effects of AMH in women have received little attention. AMH is a biomarker indicating ovarian reserve (1). There are few studies describing which value of AMH leads to which results. Studies mostly focus on polycystic ovary syndrome (PCOS), responses to assisted reproductive technology (ART) treatments and the relationship between AMH levels and menopause prediction.

AMH is a glycoprotein produced in primary follicles and antral follicles prior to FSH-dependent follicle selection. It functions as an autocrine and paracrine regulator of follicular maturation. Since the size of the residual follicular pool depends on the number of small antral follicles and decreases over time, the serum AMH level in women reaches its highest value at the age of 25, plateaus for a while, and starts to decline. This decline accelerates in menopause and becomes undetectable shortly after menopause (2).

Ovarian folliculogenesis is mediated by the joint interactions of FSH, AMH, estradiol and androgens. AMH inhibits follicle recruitment and development and promotes follicle atresia. In fact, AMH is an inhibitory factor that prevents folliculogenesis from reaching a point of being difficult to impossible to control. This inhibition may explain the anovulation that accompanies the increased number of antral follicles in PCOS. In women with PCOS, AMH increases ambient androgen and insulin resistance by inhibiting FSH-induced aromatase. Therefore, weight loss becomes difficult in these women and the response to ovulation induction decreases. Patients' clinical course recovered with the lowering of AMH. This allows us to see that AMH has an active role in PCOS, which is an example of exaggerated folliculogenesis (3). Nevertheless, a number of studies show that weight loss does not decrease AMH in obese women with or without PCOS (4).

The two opposite poles in the clinical reflection of folliculogenesis are polycystic ovary syndrome and menopause. The normal range for AMH is not clear; however, serum AMH levels are high in women with PCOS and unmeasurable in menopause.

In women with PCOS, both the number of pre-antral and the number of antral follicles increase and serum AMH is high because of the increased production per follicle (due to increased granulosa cells) (5). Different results can be found in the literature related to AMH-secreting cell sizes (4, 6, 8 and 9 mm) (6). In the literature, abnormalities secondary to increased AMH are variable. We still therefore cannot predict which hormones are affected in women, the development of clinical abnormalities independent of hormone levels, and the phenotype changes of women based on the AMH level. Conversely, we do not know what changes occur in problems such as menstrual irregularity and hirsutism that should, but cannot, be measured in the laboratory and whether AMH changes can explain this abnormality despite normal hormone levels. The main aim of this study was to investigate the association of AMH with PCOS phenotypes and laboratory tests in addition to discussing the role of AMH in the diagnosis of PCOS.

MATERIALS and METHODS

The study was initiated after obtaining detailed verbal and written consent from all participants following the approval of the ethics committee. Non-interventional clinical trials were approved by the ethics committee (Date: 10.11.2022, No: 950). This study was conducted in full accordance with the guidelines of the Declaration of Helsinki. The data of women who applied to the infertility clinic of a tertiary health institution between January 2019 and May 2023 were reviewed from hospital records and files by obtaining the verbal and written consent from the patients.

Age, menstrual cycle, hirsutism (Ferriman-Gallwey score >8 points), primary or secondary infertility status, BMI, and the presence of polycystic morphology in the ovaries were recorded in 81 women aged 20 to 40 years who applied at the infertility outpatient clinic and did not receive any treatment. Homeostasis Model Assessment (HOMA), (fasting glucose x fasting insulin ÷405), Luteinizan hormone (LH), Follicle stimulating hormone (FSH), prolactin, AMH, androgens (androstenedione, 17- hydroxyprogesterone (170HP), total testosterone, dehydroepiandrosterone sulphate (DHEAS) measured in the morning blood on the second, third, and fourth day of menstruation were recorded.

Serum AMH levels were measured using the Elica technique (Roche E411, USA).

Every patient underwent morning transvaginal ultrasonography (TVUSG) on the second, third and fourth days of menstruation. Polycystic ovarian morphology (PCOM) was defined as \geq 12 follicles in either ovary, measuring 2-9 mm in diameter and/ or increased ovarian volume for each ovary >10 mL on the ultrasound scan (7).

Women who were taking hormone-containing drugs for any reason, women with known ovarian mass and disease, and women with a history of adnexal surgery were excluded. Women with congenital adrenal hyperplasia, thyroid dysfunction and diabetes mellitus were also excluded.

Statistical analysis

Mean, Standard Deviation and Median IQR values were given in the descriptive statistics for the continuous data, and the number and percentage values were given in the discrete data. The Shapiro-Wilk test was used to examine the conformity of continuous data to the normal distribution.

An independent samples t test was used to compare patient ages in the AMH groups, and the Mann Whitney U test was used to compare the laboratory values.

The relationships between the AMH values and ages and laboratory values were analyzed using Spearman's correlation

	(n= 81)
Age Mean±SD (Min-Max)	28.89±3.88; (21-38)
BMI n (%)	
18-25	39 (48.1%)
25-30	16 (19.8%)
≥ 30	26 (32.1%)
Menstruation n (%) (n=78)	
Irregular	34 (42.0%)
Regular	47 (58.0%)
Infertility n (%)	
Primary	44 (54.3%)
Secondary	37 (45.7%)
PCOM	
No	13 (16%)
Yes	68 (84%)
Hirsutism (n=78)	
No	53 (65.4%)
Yes	28 (34.6%)
HOMA index	
<2.5	44 (54.3%)
≥2.5	37 (45.7%)

Table 1: Patient characteristics

BMI: Body mass index, PCOM: Polycystic ovarian morphology, HOMA: Homeostasis Model Assessment

coefficient.

Chi-Square and Fisher's Exact test were used for comparisons of nominal variables between the AMH groups (in cross-tabulations).

The IBM SPSS version 20 (Chicago, IL, USA) program was used in the evaluations, and p<0.05 was accepted as the limit of statistical significance.

In the study including 54 patients with AMH values ≤10 ng/mL and 27 patients with AMH values >10 ng/mL, and comparing the presence of hirsutism as the primary outcome, the power of the test was found to be power=0.88 (88%) with Type I error=0.05.

In the study examining the relationship between AMH values

Table 2: Laboratory findings of the patients

		Mean±SD	Median (IQR)
Estradiol	81	46.83±17.55	43 (34-55)
LH/FSH	81	1.39±0.67	1.26 (0.96-1.80)
Androstenedione	81	2.47±1.33	2.19 (1.35-3.50)
Testosterone	81	0.50±0.27	0.41 (0.32-0.64)
170HP	81	1.40±1.52	0.85 (0.63-1.50)
DHEAS	81	248.53±104.90	229 (161.30-317.0)
AMH	81	10.11±8.48	7.52 (4.97-11.88)
Prolactin	81	19.17±8.59	18 (6.5-48)

IQR:Inter quantile range, LH: Luteinizan hormone, FSH: Follicle stimulating hormone, DHEAS: dehydroepiandrosterone sulphate, AMH: Anti-Mullerian Hormone, 170HP: 17 hydroxyprogesterone

Table 3: AMH distribution (ng/mL)

AMH n (%)		
54 (66.7%)		
27 (33.3%)		

and LH/FSH values as the primary outcome in 81 patients included in the study, the power of the test with Type I error=0.05, power=0.99 (99%).

The calculation was performed using the 'GPower 3.1.9.2' program.

RESULTS

A total of 81 infertility patients with AMH levels of 4 ng/mL and above were included in the study. The mean age of the patients was 28.89±3.88 years (minimum 21 and maximum 38 years). Patients with serum AMH>10 ng/mL were younger than patients with AMH≤10 ng/mL (p<0.01) (Table 1).

AMH values were found to be >10 ng/mL in 33.3% of the patients (Table 3).

Approximately half of the patients (48%) had a normal body mass index (BMI 18-25 kg/m²), and there was no significant difference in the BMI levels between the two groups (p>0.05) (Table 1).

Primary infertility was found in 54.3% of the patients and secondary infertility in 45.7%. Primary infertility was found more frequently in patients with AMH >10 ng/mL (p<0.05) (Table 1).

Menses were regular in the majority of the patients (58%), and

Table 4: Comparison of the characteristics of patients with an AMH value ≤10 ng/mL and patients with an AMH value >10 ng/mL **Table 5:** Comparison of laboratory values of patients with $AMH \le 10 \text{ ng/mL}$ and patients with AMH > 10 ng/mL

	AMH ≤10		AMH >10		p value
Age (year) Mean±SD	29.80)±3.92	27.07	7±3.16	0.002ª
BMI n (%)					
18-25	29	53.7	10	37.0	
25-30	11	20.4	5	18.5	0.224°
≥ 30	14	25.9	12	44.4	
Menstruation n (%)					
Irregular	19	35.2	15	55.6	0.0800
Regular	35	64.8	12	44.4	0.080
Infertility n (%)					
Primary	24	44.4	20	74.1	
Secondary	30	55.6	7	25.9	0.012
PCOM n (%)					
No	13	24.1	0	0	
Yes	41	75.9	27	100	0.004 [°]
Hirsutism n (%)					
No	42	77.8	11	40.7	
Yes	12	22.2	16	59.3	0.001°
HOMA n (%)					
<2.5	28	51.9	16	59.3	
≥2.5	26	48.1	11	40.7	0.528°

AMH: Anti müller hormone, a: Independent samples t test, c: Chi-square Test/Fisher's Exact test, BMI: Body mass index, PCOM: Polycystic ovarian morphology, HOMA: Homeostasis Model Assessment

there was no statistically significant difference between the two groups in terms of menstrual irregularity (p>0.05). Polycystic ovarian morphology was seen in 84% and all the patients with an AMH value >10 ng/mL had PCOM (p<0.01) (Table 1).

Approximately one-third of the patients (34.6%) had hirsutism, which was significantly more common in patients with AMH values >10 ng/mL (p<0.01) (Table 1).

It was found that 45.7% of the patients had HOMA values $^{3}2.5$ (Table 1). However, there was no statistically significant difference between the two groups in terms of HOMA elevation (p>0.05) (Table 1).

Median (IQR)	AMH ≤10	AMH >10	p value
Estradiol	40 (34-53.2)	44.3 (35-59)	0.300 ^b
LH/FSH	0.52 (0.81-1.57)	1.54 (1.24-2.20)	< 0.001 ^b
Androstenedione	2.0 (1.19-3.09)	2.98 (1.88-3.86)	0.020 ^b
Testosterone	0.36 (0.29-0.52)	0.68 (0.46-0.86)	< 0.001 ^b
170HP	0.88 (0.67-1.43)	0.78 (0.45-1.83)	0.227 ^b
DHEAS	251.5 (158.7-317.0)	216.0 (162.6-323.0)	0.648 ^b
Prolactin	18.5 (11.8-24.0)	18.0 (12.2-26.0)	0.722 ^b

IQR: Inter quantile range, AMH: Anti müller hormone, b: Mann Whitney U test, DHEAS: dehydroepiandrosterone sulphate, 17OHP: 17hydroxyprogesterone

 Table 6: Correlations between AMH, age and laboratory parameters

	АМН		
	r*	р	
Age (year)	-0.453	<0.001	
Estradiol	0.128	0.255	
LH/FSH	0.471	<0.001	
Androstenedione	0.140	0.212	
Testosterone	0.458	<0.001	
17OHProgesterone	-0.156	0.166	
DHEAS	-0.090	0.422	
Prolactin	0.068	0.548	

AMH: Anti müller hormone, *Spearman's correlation coefficient, LH: Luteinizan hormone, FSH: Follicle stimulating hormone, 17OHP: 17hydroxyprogesterone, DHEAS: dehydroepiandrosterone sulphate

LH/FSH, androstenedione and testosterone values were higher in patients with AMH>10 ng/mL (p<0.001) (Table 2).

There was a negative correlation between the ages of the patients and AMH values (r=-0.453 p<0.001) (Table 6).

Positive correlations were found between LH/FSH values, testosterone values and AMH (r=0.471 and r=0.458, respectively, p<0.001) (Table 6).

No difference could be seen between the E2, 17OHP, DHEAS, and PRL values of the patients with AMH \leq 10 ng/mL and pati-

ents with AMH >10 ng/mL (p>0.05) (Table 5).

DISCUSSION

According to the results of our study, we found androgenic effects correlated with AMH, therefore making AMH possibly one of the diagnostic markers of PCOS.

Unlike the publications showing that AMH does not change intra- and inter-cyclically, the lowest level of AMH is measured in the luteal phase immediately after ovulation (8). Considering these differences, we included women in the study in whom AMH was measured in the follicular phase. In this study, a serum AMH level >4 ng/mL was accepted as the optimal cut-off for the diagnosis of PCOS (9).

In a study conducted on IVF patients, relationships were found between AMH>5.7 ng/mL and hyperandrogenism, PCOM, menstrual irregularity and high LH/FSH. Accordingly, the LH/ FSH ratio may increase in women with high AMH because in PCOS, FSH is secreted normally, while LH is secreted at normal or increased levels. However, it would not be correct to diagnose PCOS only by looking at this ratio. In the same study, a correlation between menstrual irregularity and the LH/FSH ratio was also reported (10). We also found a higher LH/FSH ratio in the high AMH group (>10 ng/mL).

In our study, no correlation was found between AMH and insulin resistance (HOMA) in accordance with a number of studies in the literature (11).

In a study conducted with a small group of cases in the literature, it was reported that women with high AMH levels were thinner, had higher androgen levels and suffered more from amenorrhea. According to the results of our study, testosterone is positively correlated with AMH levels (12). Although we did not find a correlation between androstenedione and AMH, we did find a correlation between androstenedione levels of <10 ng/mL and >10 ng/mL (see Tables 5 and 6). These two androgens are expected to cause clinical hyperandrogenism and changes in body fat distribution. In a study conducted on a small number of patients, the garnered data reported that there was no correlation between BMI and LH/FSH (13). Although the BMI of the patients was similar in our study, we were not able to compare the waist/hip ratio with fat distribution, so we cannot comment on the fat distribution.

It is natural that high AMH and accompanying androgen levels make some changes in the phenotype. Although obesity is common in women with PCOS, women with very high AMH have been shown to be leaner. In a study conducted by dividing 134 patients into three groups according to AMH value, Tal et al. reported that PCOS was present in a large proportion of women at AMH >10 ng/mL. These women were thinner with AMH levels and PCOS severity being correlated (14). Among the women in our study, being overweight was more common in those with high AMH (about half of the patients). However, in those with AMH ≤10 ng/mL, half of the patients were of nor-

mal weight. As a result, we did not find a relationship between elevated AMH levels and BMI.

A positive correlation between serum AMH levels and androgens has been previously reported. It has been reported that hyperandrogenism is an intrinsic defect of thecal cells in women with PCOS and is positively associated with testosterone levels and ovarian volume (15). In our study, a relationship was found between hirsutism and AMH, but we observed that the frequency of menstrual cycle abnormalities did not increase with high AMH (Table 4). This is directly related to serum AMH levels in patients with oligo-/amenorrhea and increased AFC (PCOM) (16). Although menstrual irregularity is common in women with PCOS, menstrual irregularity in women in our two groups was seen in half of the patients with AMH>10 ng/mL.

In our study, PCOM was observed in all of our patients with AMH>10 ng/mL. We also detected 2/3 PCOM in women with an AMH of 4-10 ng/mL. Patients with accepted PCOS also have PCOM or increased ovarian volume. In our study, we compared the clinical changes associated with AMH by comparing women with high AMH levels and two groups with far higher AMH (such as 63 ng/mL and 34 ng/mL).

In this study, we grouped not according to the presence of PCOS but according to the AMH level. We accepted a limit of 4 ng/mL, which is believed to mark the onset of PCOS pathology, and thus included all women with normal and abnormal conditions. We believe that our classification of clinical and laboratory features with AMH in a mixed group similar to that in the general population is the strength of our study and will provide more information than working with a selected group with PCOS. However, the most important limitation is that it was not performed with a higher number of age groups and patients. The absence of a control group was a limitation, but it is very difficult to find similarities in AMH <4 ng/mL in and of itself and between different age groups. There will already be a known and incomparable difference between women with high AMH and those without.

It should not be forgotten that the phenotypic appearance in PCOS does not change only with measurable values. The age, activity status and diet of the woman are also having effects. In this study, data are limited due to the fact that, with the exception of age, we do not have any information about these factors. Moreover, there are a small number of patients and they are not age-matched.

As a result, we believe that high AMH can be used in the diagnosis of PCOS in accordance with ovarian appearance on ultrasonography and clinical and/or laboratory hyperandrogenism.

Ethics Committee Approval: This study was approved by Istanbul Medipol University Non-Interventional Clinical Research Ethics Committee (Date: 10.11.2022, No: 950).

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