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ORIGINAL

Relationship between serum anti-Mullerian hormone and clinical parameters in polycystic ovary syndrome

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Abstract. Polycystic ovary syndrome (PCOS) is an ovulatory disorder that affects 6-10% of women of reproductive age. Serum AMH level may be an additional factor, or surrogate of PCOM, in the diagnostic criteria of PCOS. We evaluated the correlations between the serum AMH level and various endocrine and metabolic features in PCOS using the latest fully automated assay. Serum AMH level was compared between 114 PCOS patient (PCOS group) and 95 normal menstrual cycle women (Control group). Correlations between serum AMH level and various endocrine and metabolic factors were analysed in PCOS group. The serum AMH level was significantly higher in the PCOS group (8.35±8.19 ng/mL) than in the Control group (4.99±3.23 ng/mL). The serum AMH level was independently affected by age and the presence of PCOS on multiple regression analysis. Ovarian volume per ovary (OPVO) showed the strongest positive correlation (r=0.62) with the serum AMH level among related factors. On receiver operating characteristic (ROC) curve analysis, the cut-off value of AMH for the diagnosis of PCOS was 7.33 ng/mL, but this value did not have high efficacy (sensitivity 44.7%, specificity 76.8%). A cut-off value of 10 ng/mL had a high specificity of 92.6%, although the sensitivity was low (24.6%). The serum AMH level was elevated and reflected ovarian size in PCOS patients. The serum AMH level could be a surrogate for ultrasound findings of the ovaries in PCOS and might be useful for estimating ovarian findings without transvaginal ultrasound in the diagnosis of PCOS.

Key words: Anti-Mullerian hormone (AMH), Polycystic ovary syndrome (PCOS), Polycystic ovarian morphology (PCOM), Ovarian volume per ovary (OVPO)

POLYCYSTIC OVARY SYNDROME (PCOS) is a common ovulatory disorder that affects 6-10% of women of reproductive age [1-3]. PCOS is diagnosed according to the Rotterdam criteria defined by the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM). The Rotterdam criteria include the following clinical and biochemical features and require the presence of at least two of three features: 1. oligo/or anovulation; 2. hyperandrogenism or hyperandrogenemia; 3. polycystic ovarian morphology (PCOM) [4].

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In recent years, researchers have been evaluating whether an elevated serum AMH level may be an additional factor, or surrogate of PCOM, in the diagnostic criteria of PCOS. AMH is a member of the transforming growth factor-β (TGF-β) family, which is released from granulosa cells of primary, preantral, and small antral follicles [5]. The serum AMH level in PCOS patients was 2 or 3-fold higher than that of normal women, and it was highly related to the antral follicle count (AFC) in PCOS patients [6-9]. Furthermore, serum AMH seems to have more diagnostic potential than PCOM. Dewailly *et al*. reported that the specificity and sensitivity of serum AMH were higher than of PCOM in the diagnosis of PCOS when the cut-off value of the serum AMH level was 35 pmol/L (5 ng/mL) [6]. Other researchers also reported, that serum AMH level has a good predictor when the cut-off value of the serum AMH level was more than 4.7 ng/mL or 33

pmol/L for the diagnosis of PCOS based on Rotterdam criteria and NIH classification [10, 11].

The threshold of PCOM in the Rotterdam criteria is defined as antral follicles with a diameter of 2-9 mm and a count of 12 or more in one ovary, or an ovarian volume per ovary (OVPO) of 10 cm^3 or more. Dewailly *et al*. reported that the diagnostic potential of PCOM was higher in PCOS when the antral follicle count (AFC) was 19 or more and ovarian volume was more than 7 mL [6]. Lujan *et al*. reported that an AFC of one ovary of 26 or more and ovarian volume of more than 10 cm^3 showed more sensitivity and specificity than the original cut-off of the Rotterdam criteria in the diagnosis of PCOS [12]. Detection of PCOM has been affected by the evolution of ultrasound equipment and inter-observer variability. In fact, the finding of PCOM in the normal population has increased due to newer ultrasound equipment, which complicates the diagnosis of PCOS [13, 14]. Therefore, more objective and quantitative diagnostic measures are needed.

The Elecsys® AMH assay (Roche Diagnostics GmbH, Mannheim, Germany) is one of the latest fully automated electrochemiluminescence immunoassays for measuring the serum AMH level, and it has a lower threshold for the AMH level and high sensitivity and specificity compared with several types of manual (Gen II (Beckman Coulter Inc., CA, USA); ELISAs-EIA AMH/MIS (Immunotech, Beckman Coulter Inc., CA, USA) and Ultrasensitive AL-105i (Anshlabs, Webster, TX, USA)) or automatic immunoassays-Access Dxi (Beckman Coulter Inc., CA, USA) [15, 16]. The objective of this study was to evaluate whether AMH is an independent factor related to PCOS pathophysiology among phenotypic, anthropometric, and reproductive endocrine and metabolic changes and to determine the serum AMH level using the latest fully automated assay.

Materials and Methods

Subjects

A total of 114 PCOS patients (PCOS group) and 95 women with normal cycles (Control group) participated in this study. Mean age and distribution age of enrolled participants were 30±6.4, 18-48 (mean±SD, range) in PCOS group and 28.2±7.9, 20-46 in Control group. The lean participants (BMI <18.5) were absent in Control group, because these population showed higher rate of irregular menstrual cycle. The menstrual cycle of the Control group was 25-38 days in length with biphasic basal body temperatures. PCOS was diagnosed using the Rotterdam criteria [4]. This study was approved by the institutional ethics Committee (No.2138), and was conducted in accordance with the ethical standards of the Committee. Written informed consent was obtained from all participants.

Clinical findings

Weight, height, hip, waist, waist to hip ratio, and blood pressure were measured on physical examination. Body fat percentage was measured by Bioelectrical Impedance Analysis (InnerScan TANITA Corporation, Tokyo, Japan), and the body mass index (BMI) was determined using the following formula: weight in kilograms divided by height in meters squared (kg/m^2). Likewise, hyperandrogenism was determined by the presence of hirsutism or acne.

Blood sampling

Blood samples were collected on day 6-8 of the menstrual cycle (mid-follicular phase) in the Control group. Sampling in the PCOS group was not timed to the menstrual cycle, because all patients were oligomenorrheic or amenorrheic. However, absence of developing follicles (≥10 mm) was checked by transvaginal ultrasonography on the day of sampling. The blood samples were used to determine serum concentrations of AMH, LH, FSH, E_2, T, free T, AN, DHEA-S, fasting glucose, and fasting insulin.

Hormone assays

The serum AMH concentration was determined using a fully automated electrochemiluminescence immunoassay (Elecsys®AMH, Roche Diagnostics GmbH). The analytical sensitivity of AMH assay was 0.03 ng/mL, and the intermediate precision coefficient variation (CV) of AMH was 2.9-4.4% [17]. Inter- and intra-assay CV of this assay were also reported as 3.7 and 2.1%, respectively [15]. The serum LH and FSH concentrations were measured using immunoradiometric assays ARCHITECT LH and FSH kit (ARCHITECT; Abbott Japan Inc., Tokyo, Japan). Serum total T concentration was determined using an electrochemiluminescence immunoassay kit (ECLusys TESTO II, Roche Diagnostics, K.K., Tokyo, Japan). Serum free T concentration was measured

using Coat-a-Count Free Testosterone™ (Diagnostic Products Corporation, Los Angeles, CA, USA). The concentration of serum DHEA-S was assayed by a radioimmunoassay kit (Diagnostic Products Corporation). Serum E_2 was determined by electrochemiluminescence immunoassay kit (Elecsys® Estradiol II, Roche Diagnostics K.K.). Serum fasting glucose and insulin concentrations were measured using the glucose oxidase method on an Automated Glucose analyzer GA04 (A&T, Kanagawa, Japan) and an enzyme immunoassay on an AIA200 (TOSOH Co., Tokyo, Japan), respectively. Insulin resistance was estimated with HOMA-IR, which was calculated by the following formula: fasting serum insulin (μU/mL) × fasting serum glucose (mg/dL) / 405 [18].

Transvaginal ultrasound

The ovaries were evaluated using transvaginal ultrasonography with a 7.5-MHz transducer in the PCOS group on the day of blood sampling (Sonovista FX, Siemens Healthcare K.K. Tokyo, Japan). OVPO was determined by the following formula: 0.5 × length × width × thickness [19]. The ultrasound measurements were done by one examiner for all patients under the highest possible magnification.

Statistical analysis

Differences in values were analyzed using Student's *t*-test, Welch's *t*-test, and Mann-Whitney's U test after considering the variance and distribution. Correlations between variables were calculated by Spearman's rank order analysis and Pearson's correlation test. Correlations between the various endocrine and metabolic factors and the serum AMH level of the PCOS group were examined using multiple regression analysis. The diagnostic potential of serum AMH was analyzed by receiver operating characteristic (ROC) curve analysis. All data are presented as means±SD values. Significance was defined as $p<0.05$.

Results

The clinical findings showed significant differences between the Control and PCOS groups (Table 1); age, BMI, waist, W/H, and body fat percentage were significantly greater in the PCOS group than in the Control group. The serum AMH level was significantly higher in the PCOS group than in the Control group (Fig. 1).

Table 1 Clinical findings of the subjects

	Control	PCOS
Age (y)	28.2 ± 7.9	30 ± 6.4 **
BMI (kg/m^2)	21.6 ± 2.8	23.9 ± 6.3 *
Waist (cm)	70.5 ± 6.6	78.9 ± 15.2 **
W/H	0.75 ± 0.05	0.83 ± 0.08 **
Body fat (%)	28.0 ± 5.7	32.3 ± 10.6 *

Data are presented as mean±SD. * $p<0.05$; ** $p<0.001$ *vs*. Control

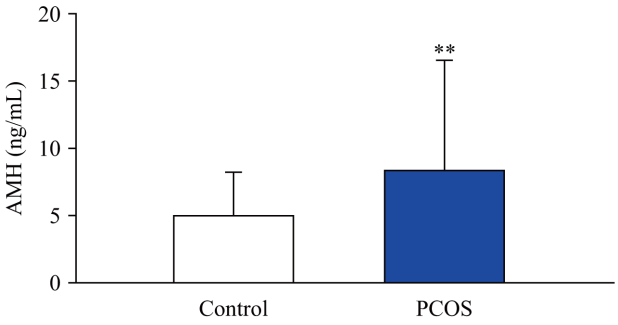

Fig. 1 Serum AMH level of the PCOS group
Data are presented as means±SD. ** $p<0.01$ *vs*. Control.

In the Control group, a strong negative correlation was observed between the serum AMH level and age (r= -0.543; $p<0.01$), whereas this correlation was not observed in the PCOS group (r= -0.066; $p<0.469$). In the PCOS group, a negative correlation was observed between the serum AMH level and BMI (r= -0.21; $p<0.05$), whereas this correlation was not observed in the Control group (r= -0.066; $p<0.469$) (Fig. 2). Serum AMH level of PCOS group was significantly higher than that of Control group at BMI 18.5-25. Serum AMH level was significantly higher in BMI<18.5 group than other BMI groups within PCOS patients, Control group did not contain BMI<18.5 (Table 2). The serum AMH level was independently affected by the presence of PCOS (Partial correlation coefficient (Partial Corr)= 0.320; $p<0.01$), BMI (Partial Corr= -0.201; $p<0.01$) and age (Partial Corr= -0.193; $p<0.01$) on multiple regression analysis (Table 3). The cut-off value of AMH for the diagnosis of PCOS was 7.33 ng/mL from the ROC curve analysis, but this value did not have high efficacy (sensitivity 44.7%, specificity 76.8%). A cut-off value of 10 ng/mL had high specificity (92.6%), but sensitivity was low (24.6%) (Fig. 3, Table 4).

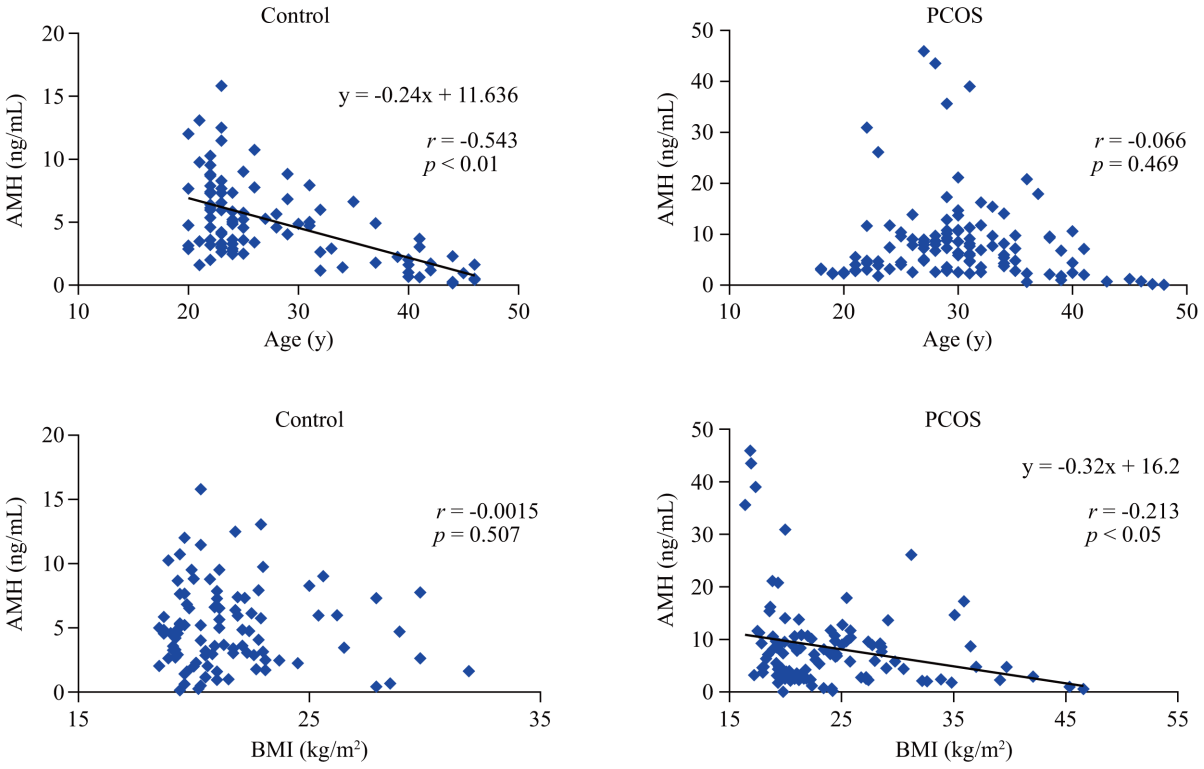

Fig. 2 Relationship the serum AMH level with age and BMI in the Control and PCOS groups

The serum AMH level and age show a strongly negative correlation in the Control group, but not in the PCOS group. In the PCOS group, a negative correlation was observed between the serum AMH level and BMI, but not in the Control group.

Table 2 Serum AMH level according to BMI

BMI	Control group (n)	PCOS group (n)
< 18.5	–	17.4 ± 16.7 (13) ##
18.5-25	5.0 ± 3.3 (83)	7.2 ± 5.6 (62) *
≥ 25	4.8 ± 3.0 (12)	7.4 ± 5.7 (39)

Data are presented as mean±SD ng/mL. * $p<0.05$ *vs*. Control, ## $p<0.01$ *vs*. BMI 18.5-25, ≥ 25. BMI, Body mass index; (n), number of subject. There were no subject who had lower BMI than 18.5 in Control group.

Table 3 The multiple regression analysis of groups, BMI and age on serum AMH

Constant term	Explanatory variable	Regression coefficient	Partial correlation coefficient	*p* value
X_1	Groups (PCOS and Control)	4.31	0.320	3.19E-06
X_2	BMI	-0.257	-0.201	0.00402
X_3	Age	-0.172	-0.193	0.00572

$y = 4.31X_1 - 0.257X_2 - 0.172X_3 + 15.4$

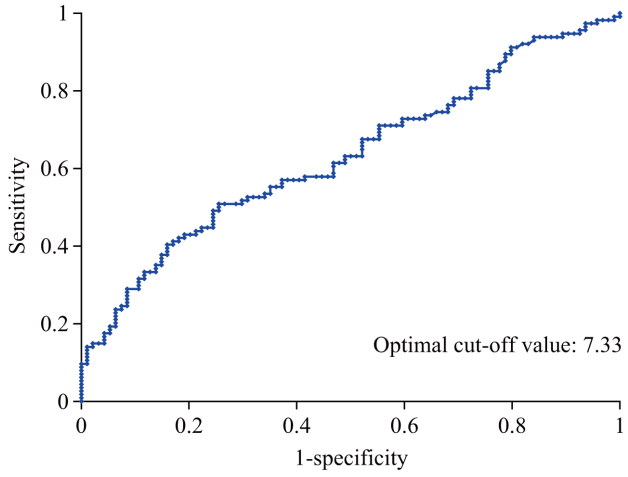

Fig. 3 ROC curve of AMH in the diagnosis of PCOS

Table 4 Sensitivity and specificity of each a cut-off value

Cut-off value of AMH	Sensitivity	Specificity
2	92.1	16.8
3	75.4	31.6
4	68.4	44.2
5	57.9	55.8
6	52.6	68.4
7	48.2	74.7
7.33	44.7	76.8
8	40.4	84.2
9	32.5	88.4
10	24.6	92.6
11	18.4	94.7
12	14.9	95.8
13	14.0	97.9
14	12.3	98.9
15	10.5	98.9
16	9.6	100.0

The serum AMH level and OPVO showed the strongest positive correlation (r=0.62, p<0.01) among related factors, although serum LH, LH/FSH ratio, T, free T, AN, LDL cholesterol, acne, height and weight also had significant positive correlations with the serum AMH level. However, AMH was not correlated with adrenal androgen (DHEA-S) and metabolic factors (IRI and HOMA-IR) in the PCOS group (Fig. 4, Table 5).

The serum AMH level was independently affected by OVPO (r=0.425; p<0.01) on multiple regression analysis, whereas serum LH, AN, free T, TG, acne, and BMI had no independent effects on the serum AMH level on multiple regression analysis (Table 6).

Discussion

In this study, the serum AMH level was significantly higher in the PCOS group than in the Control group, and OVPO was the only independent factor that affected the serum AMH level among various phenotypic, anthropometric, reproductive endocrinological, and metabolic features. This is the first study to prove the independent relationship using the multiple regression analysis among various factors that were involved in PCOS pathophysiology. In previous decades, researchers reported higher ovarian volume and 2-3 fold higher serum AMH levels in PCOS patients than in normal ovulatory women [6, 7, 20-22]. A positive correlation between serum AMH and OVPO is a relatively new finding in PCOS patients [23]. However, there has been no other report of such an association. The results of the present study confirm previous studies and add a new finding about the independent significance of ovarian size for the serum AMH level in PCOS.

AMH, a main member of the TGF family, is secreted from granulosa cells of developing follicles, including primary, preantral, and small antral follicles. AMH has a critical role in the regulation of folliculogenesis, inhibiting the initial requirement of the primordial pool and the responsiveness of the follicle to FSH [5, 24, 25]. The inhibiting effect of AMH on the responsiveness of follicles to FSH is mediated by AMHRII, which inhibits FSH-induced adenylyl cyclase activation and aromatase expression [26]. Reduction of aromatase expression from granulosa cells increases intra-ovarian androgens such as T and AN [27]. Androgens stimulate the follicle requirement in the preantral and antral stages of follicular development [28] and, therefore, increase ovarian volume and the AFC. Accordingly, the interaction of AMH and several androgens affects ovarian size and the number of antral follicles. A strong positive correlation between the serum AMH level and OVPO in the present study could be explained by the interaction of AMH and androgens in the folliculogenesis described above. Reduction of aromatase activity also decreases estrogen synthesis. A reduction of the serum estrogen level increases LH secretion mediated by negative feedback. Moreover, Cimino *et al*. demonstrated that AMH directly increased LH pulsatility and secretion, mediated by AMHRII receptors on the surface of GnRH neurons [29]. The positive correlation between AMH and LH could be explained by these mechanisms.

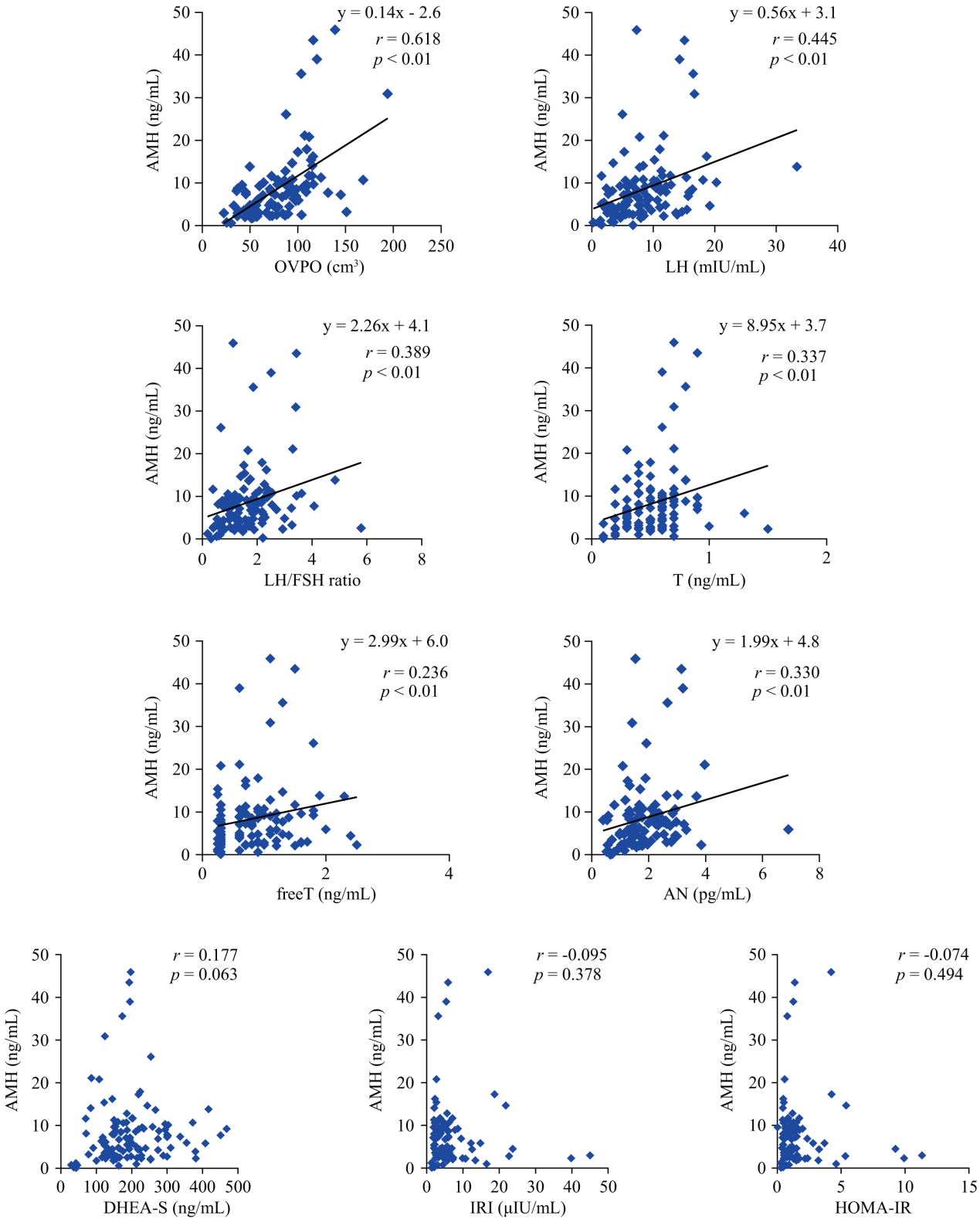

Fig. 4 Relationship between the serum AMH level and other factors in the PCOS group

The serum AMH level shows strong positive correlations with OVPO, serum LH, LH/FSH ratio, and ovarian androgens (T, free T and AN), but no correlations with adrenal androgen (DHEA-S), IRI, and HOMA-IR.

Table 5 Correlation of AMH and other factors in the PCOS group

Significant correlation		
	Correlation coefficient (*r*)	*p* value
OVPO	0.62	1.13E-09
Right ovary volume	0.60	2.81E-09
LH	0.44	3.47E-06
Left ovary volume	0.42	3.56E-05
Acne	0.39	5.28E-04
LH/FSH ratio	0.39	5.28E-05
T	0.34	0.000492
AN	0.33	0.000960
TG	-0.30	0.00194
Height	0.25	0.00935
freeT	0.24	0.0164
BMI	-0.21	0.0262
LDL-C	-0.20	0.0358
Body weight	-0.20	0.0368
No significant correlation		
	Correlation coefficient (*r*)	*p* value
DHEA-S	0.18	0.0625
Hairiness	-0.17	0.134
Hip	-0.16	0.0922
Waist	-0.16	0.103
HDL-C	0.15	0.111
W/H	-0.14	0.143
T-CHO	0.13	0.161
Body fat percentage	-0.13	0.179
HbA1c (NGSP)	-0.12	0.210
DHEA	0.11	0.260
IRI	-0.095	0.378
Pulse	0.092	0.343
E2	-0.074	0.434
HOMA-IR	-0.074	0.494
Systolic blood pressure	-0.074	0.438
Menstrual disorder	0.073	0.512
Age	-0.066	0.469
E1/E2 ratio	0.066	0.501
Diastolic blood pressure	-0.057	0.553
E1	-0.025	0.793
FSH	0.025	0.796
FBS	0.017	0.883

Table 6 The multiple regression analysis between serum AMH and other factors in the PCOS group

Constant term	Explanatory variable	Regression coefficient	Partial correlation coefficient	*p* value
X_1	Acne	4.491	0.235	0.094
X_2	OVPO	0.134	0.425	0.0017
X_3	LH	-0.0264	-0.0108	0.940
X_4	AN	0.255	0.0276	0.846
X_5	TG	-0.0125	-0.0595	0.675
X_6	Height	-0.0563	-0.0405	0.776
X_7	freeT	1.76	0.0764	0.590
X_8	BMI	-0.303	-0.159	0.261

$y = 4.49X_1 + 0.134X_2 - 0.0264X_3 + 0.255X_4 - 0.0125X_5 - 0.0563X_6 + 1.76X_7 - 0.303X_8 + 12.3$

Several researchers reported that the serum AMH level and AFC decreased with aging in normo-ovulatory women in a longitudinal study, and that the serum AMH level was positively correlated with age and AFC in a cross-sectional study [30-33]. On the other hand, the serum AMH level and AFC were not correlated with age in PCOS patients, and the serum AMH and AFC were higher in PCOS patients than in normal women throughout the reproductive period [8, 32, 34]. In the present study, the serum AMH level had a strong inverse correlation with age in the Control group, whereas this correlation was not seen in the PCOS group. The serum AMH level would decrease with aging in each PCOS subject. It seems that a cross-sectional study does not show a correlation between AMH and age, indicating that there is heterogeneity of severity or other factors that affect the AMH level in PCOS. Given these results, AMH, rather than aging, could be a special marker of pathophysiology in individual PCOS patients.

In the present study, lean PCOS patients (BMI <18.5) had significantly higher serum AMH level compared with normal BMI and overweight/obese PCOS patients. The lean participants (BMI<18.5) were absent in Control group, because these population showed higher rate of irregular menstrual cycle. However, previous reports have already shown that serum AMH does not have relationship with BMI in reproductive age normal women including lean (BMI<18.5) women [35, 36]. Therefore, lean PCOS patients would have higher serum AMH than lean normal women, not only than PCOS patients with BMI >18.5. BMI was not independent factor to affect serum AMH level in multiple regression analysis among factors of PCOS pathogenesis and OVPO was the only independent factor to affect serum AMH. From these findings, lean PCOS patients had higher serum AMH level related to larger ovary and not to their slim body. Overweight/obese PCOS patients have insulin resistance, which stimulates androgen synthesis in ovary and induces PCOS phenotype [37]. On the other hand, lean PCOS patients, who do not have insulin resistance, have larger ovary, which synthesizes excess androgen in itself and induces PCOS phenotype. Present data highlights special character of lean PCOS among heterogeneous syndrome.

As for age, Control group had negative relationship with AMH and Control group showed significantly lower age than PCOS group. This bias would bring lower AMH in PCOS group. As for BMI, PCOS group had negative relationship with AMH and PCOS group would significantly higher BMI than Control group. This bias would bring lower AMH in PCOS group, because BMI showed negative relationship with serum AMH in PCOS group. On the contrary to these bias, our result showed higher AMH in PCOS group, indicating PCOS group really had higher AMH irrespective of the bias of age and BMI.

The present results also indicated that AMH was closely related to various key features of PCOS, such as anovulation, hyperandrogenism, and elevated serum LH, indicating that AMH has a critical role in the pathogenesis of PCOS. Therefore, assessment of serum AMH levels might be valuable in the diagnosis of PCOS. There have been several reports that assessed serum AMH levels in the diagnosis of PCOS. Lin *et al*. reported that the cut-off value of AMH was 7.3 ng/mL, with sensitivity of 76% and specificity of 70% [38]. Woo *et al*. determined that the threshold of the serum AMH cut-off value was 7.84 ng/mL, with sensitivity of 75.9% and specificity of 86.8% for the diagnosis of PCOS on ROC curve analysis [39]. In the present study, the Elecsys® AMH assay, which is one of the latest fully automated electrochemiluminescence immunoassays, was used to measure the serum AMH level. The advantages of this assay are short assay time, high sensitivity, and high specificity, with a broad linear range better than other assays, and the lower limit of detection of AMH is the lowest [16, 17, 40]. ROC curve analysis was used to investigate the diagnostic potential of the serum AMH level for PCOS, and the cut-off value was 7.33 ng/mL, with sensitivity of 44.7% and specificity of 76.8%. However, this value did not have high efficacy. A cut-off value of 10 ng/mL had high specificity (92.6%). This cut-off value is more effective as a surrogate for PCOM in the diagnostic criteria, because the AMH level was correlated with OVPO. The inconsistent sensitivity and specificity of the suggested cut-off value of AMH in the present and other studies is likely the result of differences in the subjects and assay methods.

The serum AMH level related to outcome of ovulation induction treatments in the literature. PCOS patients who had elevated serum AMH level showed poor response to ovulation induction and needed higher doses of hMG and longer duration of treatment when serum AMH level was higher than 4.7 ng/mL [41]. Mahran *et al*. reported, that the potential of ovulation

and pregnancy rates in clomiphene citrate cycle were lower, when serum AMH level was higher than 3.4 ng/mL, and these PCOS patients needed higher doses of clomiphene citrate in PCOS patients [42]. Moreover, PCOS patient who had serum AMH level more than 7.7 ng/mL showed lower ovulation recovery rate after laparoscopic ovarian diathermy (LOD) treatment than those who had serum AMH level lower than 7.7 ng/mL [43]. From these findings, serum AMH level would be useful predictor to adjust the doses of ovulation induction in PCOS patients.

In conclusion, the serum AMH level was elevated and reflected ovarian size in PCOS patients. The serum AMH level could be a surrogate for the ultrasound findings of PCOS. AMH measurements might be useful to estimate ovarian findings without transvaginal ultrasound in the diagnosis of PCOS.

Disclosure

Funding and conflict of interest

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