ORIGINAL ARTICLE

Comparative Evaluation of Diagnostic Efficacy of Serum Anti-Müllerian Hormone and Ultrasound in Polycystic Ovarian Syndrome

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ABSTRACT

Aim: To study the diagnostic efficacy of serum anti-Müllerian hormone (AMH) in polycystic ovarian syndrome (PCOS) and its comparison with ultrasonography (USG).

Study design: Prospective observational study.

Materials and methods: In the study group, a total of 80 women in the age group of 20 to 35 years with diagnosis of PCOS by Rotterdam's criteria were recruited at B.R.D. Medical College, Gorakhpur, India, during the period from January 2015 to December 2016. Women without PCOS, having regular cycles which were selected randomly comprised the control group (n = 80). Serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, androstenedione, estradiol, fasting insulin, and AMH were measured on days 2 to 3 of natural cycle or progesterone-induced withdrawal bleeding together with transvaginal sonography (TVS) for detection of the number of small follicles (<10 mm) and measurement of ovarian volume (OV). The AMH levels above 5 ng/mL were taken as diagnostic cut-off for diagnosing PCOS.

Results: Anti-Müllerian hormone was positively correlated to LH, estradiol, testosterone, number of follicles (<10 mm), and OV; and negatively correlated to FSH. No correlation was found between AMH and age, body mass index (BMI), waist–hip ratio (WHR), and fasting insulin. The AMH-based diagnosis of PCOS showed sensitivity of 91.25% and a specificity of 95%.

Conclusion: Serum AMH level is a promising marker for diagnosis of PCOS, especially in cases where reliable USG data are not available or when there are no typical clinical and laboratory findings.

Keywords: Anti-Müllerian hormone, Body mass index, Ovarian volume, Polycystic ovarian syndrome, Waist–hip ratio.

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INTRODUCTION

Polycystic ovary syndrome is the most common endocrine disorder in reproductive-aged women with a prevalence of 6 to 18%,[1] which is characterized by clinical[2] and biochemical hyperandrogenism, polycystic ovarian morphology, altered gonadotropic secretion, insulin resistance, and/or hyperinsulinemia associated with obesity.[3,4] Women with PCOS are likely to have not only a higher antral follicle count (AFC) but also a greater OV.[5] The AFC is essential to correlate biochemical and clinical markers to prognosticate infertility and ovarian reserve. In females, AMH is expressed in granulosa cells of growing follicles up to the antral stage, suggesting an important role in early ovarian folliculogenesis. The AMH is able to inhibit the initiation of primordial follicle growth and may also decrease the sensitivity of antral follicles to FSH. In humans, however, the association between androgens and AMH remains uncertain, and its exact function in follicular recruitment and long-term effects are not well understood. Serum AMH levels could be used to identify the girls at risk for PCOS in early adulthood, and allow early prevention by lifestyle counseling.

The aim of this study was to evaluate the diagnostic efficacy of serum AMH in PCOS and its comparison with USG.

MATERIALS AND METHODS

The present prospective observational study was carried out among female patients attending the Gynecological Outpatient Department indoor section of B.R.D. Medical College, Gorakhpur, India (tertiary academic hospital of North Eastern India) between January 2015 and December 2016. An informed consent was obtained from all the participants. The protocol was approved by the institutional ethical committee. Two hundred fifty female

patients aged 20 to 35 years, who visited the outpatient department and indoor section with complaints of menstrual irregularities and/or hirsutism and/or infertility were subjected to initial screening that included detailed history, physical examination (especially height, weight, BMI, and WHR), routine clinical investigations, and hormone immunoassays.

The inclusion criteria were age between 18 and 35 years, both ovaries present, no previous ovarian operation, adequate visualization of ovaries on transvaginal USG (transabdominal in unmarried), no current hormone therapy and no preexisting medical illness. Women with other causes of endocrinological abnormalities, such as hyperprolactinemia, thyroid dysfunction, Cushing syndrome, congenital adrenal hyperplasia, menarche less than 2 years, pregnancy, and lactation were excluded from the study. Women on drugs known to cause abnormal bleeding, e.g., hormonal contraceptive and drugs known to produce hirsutism and galactorrhea (e.g., corticosteroids, androgens, cyclosporine, minoxidil, phenytoin, diazoxide, histamine receptor blocker, methyldopa, etc.) were also excluded.

After initial screening, patients were further subjected to baseline hormone study on days 2 to 3 of natural cycle or progestin-induced withdrawal bleeding (in amenorrhea) together with TVS (or transabdominal sonography in unmarried) for the detection of number of small follicles (<10 mm) and measurement of OV.

Compliance with Ethical Standards

Ethical Approval

All procedures performed in the study involving human participants 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.

Informed Consent

Informed consent was obtained from all the participants included in the study.

Determination of Hormone Levels

After at least 12 hours of fasting, 6 mL of peripheral venous blood was drawn on days 2 to 3 of menstrual cycle and serum AMH was measured using an ultrasensitive generation 2 enzyme-linked immunosorbent assay (AMH–EIA, Beckman Coulter). Results were expressed in nanogram per milliliter. AMH levels above 5 ng/mL were taken as arbitrary cut-off for diagnosing PCOS.

To determine levels of LH, FSH, testosterone, androstenedione, estradiol, thyroid-stimulating hormone, and prolactin, 10 mL of peripheral venous blood was taken on days 2 to 3 of menstrual cycle of each patient and analyzed by means of radioimmunoassay.

Transvaginal Sonography

The TVS was performed by a single observer on the same day that the blood sample was obtained. Measurements were performed in real time using the highest possible magnification to observe ovaries. Transabdominal sonography was performed in unmarried females. Axes measured were length, thickness, and width to calculate OV. Ultrasonographic image was considered characteristic of PCOS in the presence of enlarged ovaries (>10 mL in volume) with 12 or more follicles 2 to 9 mm in diameter located in the periphery of ovaries by scanning each ovary from inner to outer margins in the longitudinal cross-section, according to Rotterdam's criteria. The total OV was defined as the sum of the right and left OVs and the total antral follicle number was defined as the sum of right and the left AFC.

So, finally, in 80 women, diagnosis of PCOS was established based on Rotterdam's 2003 consensus which is the finding of two out of the three following criteria: (1) oligo- and/or anovulation; (2) hyperandrogenemia defined as hirsutism (Ferriman–Gallwey score >8), or minor signs, such as acne, seborrhea, and (3) polycystic ovaries by USG.

Women with PCOS (n = 80) comprised the study group and women without PCOS having regular cycles which were selected randomly comprised the control group (n = 80). Women in the control group were apparently healthy females, not on any hormonal medications, no known infertility and endocrinologic or dermatologic problems.

These women in the study as well as control groups were observed to evaluate the role of serum AMH as a diagnostic marker in comparison with polycystic ovarian morphology by USG.

Statistical Analysis

We used the statistical package Statistical Package for the Social Sciences version 17 for the statistical analysis. Data were analyzed by using chi-square test. A p-value <0.05 was considered statistically significant. Correlation analysis was performed using Pearson's test. The diagnostic value of serum AMH level was evaluated by receiver operating characteristic (ROC) curve analysis.

RESULTS

The sociodemographic characteristics of PCOS and control group are given in Table 1. Most of the patients in the study group (47.5%) as well as in the control group

Table 1: Sociodemographic characteristics of PCOS and non-PCOS women

Parameters		PCOS group	Percent	Control group	Percent	p-value
Age (in years)	20–25	38	47.5	26	32.5	>0.05
	26–30	23	28.75	22	27.5	
	31–35	19	23.75	32	40	
Residence	Urban	57	71.25	55	68.75	>0.05
	Rural	23	28.75	25	31.25	
Education	Literate	46	57.5	48	60	>0.05
	Illiterate	34	28.75	32	40	
BMI (kg/m^2)	Underweight (<18.5)	5	6.25	3	3.75	<0.05
	Normal body weight (18.5–24.9)	27	33.75	48	60	
	Overweight (25–29.9)	31	38.75	23	28.78	
	Obese (≥30)	17	21.25	6	7.5	
WHR	<0.8	31	38.75	47	58.75	<0.05
	>0.8	49	21.25	33	41.25	
Marital status	Married	68	85	72	90	>0.05
	Unmarried	12	15	8	10	
Religion	Hindu	63	78.75	66	82.5	>0.05
	Muslim	15	18.75	13	16.25	
	Christian	2	2.5	1	1.25	
	Others	0	0	0	0	

Table 2: Clinical presentation of PCOS and control group

Clinical features		PCOS group		Control group		p-value
		No.	%	No.	%	
Types of infertility	Primary	40	68.96	5	6.25	>0.05
	Secondary	18	31.03	2	2.5	
Menstrual cycle	Regular	11	13.75	80	100	
	Oligomenorrhea	53	66.25	0	0	
	Secondary amenorrhea	16	7.5	0	0	
	Primary amenorrhea	2	2.5	0	0	
	Menorrhagia	8	10	0	0	
Features of hyperandrogenism	Hirsutism (Ferriman–Gallwey score >7)	28	35	5	6.25	>0.05
	Acne	30	37.5	10	12.5	
	Alopecia	2	2.5	1	1.25	
	Acanthosis nigricans	1	1.25	0	0	

belonged to 20 to 25 years of age; 71.25% PCOS patients were from urban area, while 28.75% patients were from rural area. The less percentage of rural patients may be due to difference in catchment area of hospital and lack of awareness. In the present study, 60% patients had BMI >25 and 40% patients had BMI <25. Most of the PCOS patients belonged to Hindu religion (78.75%), while 18.75% were Muslims.

In all, 72.5% (n = 58) of PCOS women were concerned about infertility; out of them 68.96% women had primary infertility and 31.03% had secondary infertility (Table 2); 66.25% of the PCOS cases presented with oligomenorrhea, 2% presented with primary amenorrhea, 6 and 8% had secondary amenorrhea and menorrhagia respectively. Features of hyperandrogenemia, such as hirsutism, acne, and alopecia were present in 76% (n = 61) of PCOS women.

In the present study, level of serum AMH was found in 91.25% cases to be > 5 ng/mL while only 8.75% has serum AMH less than the cut-off (Graph 1). Table 3 presents the correlation between AMH with clinical and hormone parameters in the PCOS and control group. In the PCOS group, AMH was positively correlated to LH, testosterone, androstenedione, estradiol, number of follicles (<10 mm), and OV; and negatively correlated to FSH. No correlation was found between AMH and age, BMI, WHR, and fasting insulin. Also, there was no significant correlations between AMH and all the parameters in the control group.

On USG, 72.5% (n = 58) of PCOS women had more than 12 cysts (<10 mm) as compared with only 6.25% in the control group and their difference was statistically significant; 75% (n = 60) of PCOS women had enlarged ovaries measuring more than 10 cm^3 as compared with
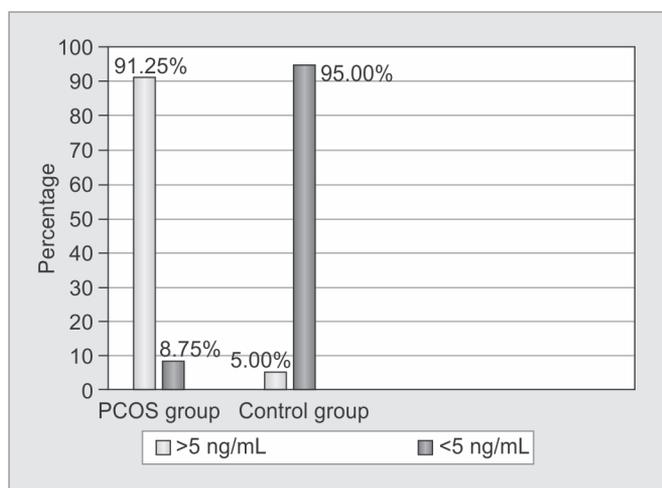
Graph 1: Distribution of cases and controls according to serum AMH levels
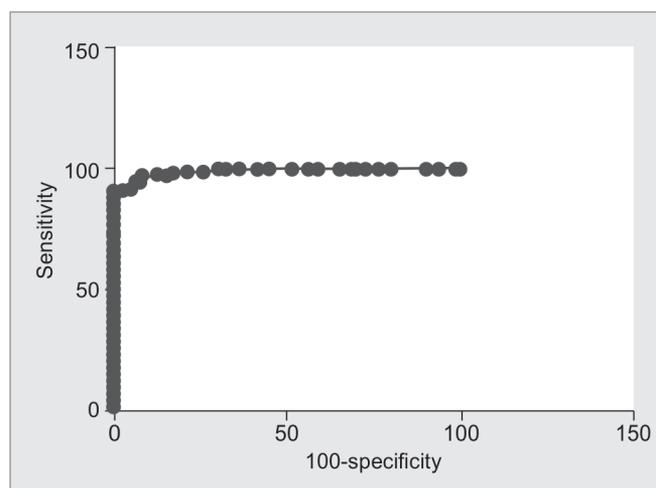
Graph 2: Receiver operating characteristic curve for evaluating the diagnostic role of AMH in PCOS. AUC = 0.990. Cut-off value of AMH = 5 ng/mL (specificity 95% and sensitivity 91.25%) for PCOS diagnosis

Table 3: Correlation of serum AMH and studied variables

	PCOS group			Control group		
Parameters	Mean ± SD	Pearson's correlation coefficient (r)	p-value	Mean ± SD	Pearson's correlation coefficient (r)	p-value
Age (years)	26 ± 4.5	0.06846	0.54 NS	28 ± 4.66	0.1051	0.3533 NS
BMI (kg/m^2)	25.65 ± 5.22	−0.1826	0.15 NS	23.73 ± 4.37	0.0813	0.4703 NS
WHR	0.84 ± 0.085	−0.1431	0.20 NS	0.80 ± 0.085	−0.0101	0.9284 NS
FSH	5.336 ± 0.558	−0.8819	<0.0001	4.37 ± 0.68	−0.0617	0.5844 NS
LH	12.23 ± 1.05	0.9256	<0.0001	5.95 ± 1.06	−0.0054	0.9616 NS
Testosterone	0.873 ± 0.239	0.7193	<0.0001	0.68 ± 0.17	−0.0339	0.7635 NS
Androstenedione	2.47 ± 0.228	0.7355	<0.0001	2.07 ± 0.58	0.0311	0.7827 NS
Estradiol	31.128 ± 1.382	0.7588	<0.0001	23.66 ± 1.13	−0.0835	0.4585 NS
Fasting insulin	5.89 ± 2.99	0.0529	0.64 NS	4.50 ± 0.86	0.0739	0.5118 NS
OV	22.607 ± 10.858	0.8409	<0.0001	9.23 ± 4.5	−0.5007	0.6571 NS
No. of follicles	17.125 ± 7.08	0.8125	<0.0001	8.42 ± 2.97	−0.0051	0.9639 NS

SD: Standard deviation; NS: Not significant

Table 4: Ultrasonography parameters of PCOS and control group

USG parameters	No. of patients	Percent	No. of controls	Percent	p-value
OV (mm^3)					
OV >10	60	75	9	11.25	<0.0001
OV ≤10	20	25	71	88.75	
Significant cyst in ovary					
Cyst in ovary ≥ 12	58	72.5	5	6.25	<0.0001
Cyst in ovary < 12	22	27.5	75	93.75	

Table 5: Comparison of serum AMH with PCOM in diagnosing PCOS

Comparative parameter	Serum AMH (%)	PCOM in USG (%)
Sensitivity	91.25	75
Specificity	95	88.75
PPV	94.8	86.95
NPV	91.57	78.02

PCOM: Polycystic ovarian morphology; PPV: Positive predictive value; NPV: Negative predictive value

11.25% in the control group and their difference was statistically significant (Table 4). Graph 2 shows the ROC curve analysis of serum AMH concentration for discriminating PCOS. The area under ROC curve yielded a satisfactory result of 0.990 (95% confidence interval, 0.980–1.000; p-value <0.0001). The best compromise between specificity (95%) and sensitivity (91.25%) for PCOS diagnosis was obtained with a cut-off value of 5 ng/mL. Table 5 evaluates the role of serum AMH in diagnosing PCOS as compared with USG.

DISCUSSION

Polycystic ovary syndrome is a complex and heterogeneous disorder, affecting mostly women in reproductive age group. It is characterized by chronic anovulation, hyperandrogenemia, altered LH:FSH ratio (>2/3:1) and polycystic ovaries; excess androgen levels lead to menstrual disturbances like oligomenorrhea and amenorrhea, development of ovarian cysts, hirsutism, and other related disorders.

In the present study, the age of most of the PCOS women and non-PCOS women ranged from 20 to 25 years with a mean of 26 ± 4.5 years in the PCOS group as compared with 21.83± years in the non-PCOS group. These findings were similar to Haider et al[6] and Ramprasad et al.[7] Mean BMI was found to be 25.65 ± 5.22 and 27.73 ± kg/m^2 in the study group and control group respectively, which is in corroboration with the studies done by Nazir et al[8] who reported 86.5% patients with BMI>25 kg/m^2. Lim et al,[9] in a systematic review and meta-analysis, concluded that women with PCOS had a greater risk of overweight, obesity, and central obesity. The mean WHR was 0.84 ± 0.085 in PCOS women while 0.81 ± 0.085 in the control group, which is in accordance with findings of Panda et al.[10]

As seen from Table 2, about 72.5% PCOS patients were having infertility problem, including primary infertility 50% and secondary infertility 22.5%. This is consistent with the study conducted by Nazir et al.[8] The present study found oligomenorrhea in 66.25% cases which is quite high in frequency, while the regular menses were seen in 13.75% of cases, amenorrhea in 10% (both primary and secondary), and menorrhagia in 10% of PCOS women. This is similar to the study done by Yousouf et al.[11] In our study, 76.25% of patients presented with features of hyperandrogenism, 35% of patients complained of hirsutism according to Ferriman–Gallwey score, 37.5% complained of acne, 2.5 and 1.25% complained of alopecia and acanthosis nigricans respectively. This is in accordance with the study by Vignesh and Mohan[12] who found that 70% of patients presented with signs of hyperandrogenism and maximum patients mainly of hirsutism and others with acne and alopecia.

In 91.25% of cases, serum AMH was found to be higher than 5 ng/mL which is twofold higher than the serum AMH levels in normal women aged between 20 and 35 years (Graph 1). This is close to the study by Leonte et al[13] who found serum concentration of AMH almost threefold higher in patients with PCOS. The current study revealed no significant correlations between AMH and age, BMI, and WHR (Table 3). This is in agreement with Sadiq and Jarah[14] and Pingy et al.[15] The AMH correlated positively with LH (r = 0.9256, p < 0.0001), testosterone (r = 0.7193, p < 0.0001), androstenedione (r = 0.7355, p < 0.0001), and serum estradiol (r = 0.7588, p < 0.0001). The FSH was negatively correlated to serum AMH (r = –0.8819, p < 0.0001). These finding are in accordance with the study done by Veena et al[16] and Begawy et al.[17]

Pearson's analysis revealed a significant correlation (r = 0.8125, p = <0.0001) between AMH and the number of antral follicles ,which showed that the higher the number of antral follicles, more the concentration of AMH. Serum AMH level was significantly related to OV on USG. This is consistent with the study done by You et al.[18] From Table 3, it is observed that the mean LH level in PCOS cases was 12.23 ± 1.05 and FSH was 5.336 ± 0.558. This corresponds to the study done by Pingy et al[15] in which mean levels of LH and FSH were found to be 8 and 5.6 IU/L respectively.

Increased OV was identified in 75% (n = 80) cases of PCOS. It is similar to the study done by Hann et al[19] who studied the USG appearance of 28 patients of PCOS and found that 71% of the cases had bilaterally significantly enlarged ovaries(>10 mm^3). The presence of 12 or more follicles between 2 and 9 mm diameter was seen in 72.5% PCOS women, which was close to the study done by Esmaeilzadeh et al[20] who found it in 89% of cases.

The area under the curve (AUC) of the serum AMH assay in PCOS patients reached a value of 0.990 (95% confidence interval 0.980–1.000). The sensitivity and specificity of AMH in diagnosing PCOS were 91.25 and 95%, while that of USG were 75 and 88.75% respectively. This is comparable to the studies done by Pingy et al.[15] In their work, the serum AMH showed a good specificity of 92%, but a relatively poor sensitivity of 67%.

CONCLUSION

The present study suggests that the serum AMH level is a valuable diagnostic marker for PCOS. Challenges in obtaining good USG imaging of ovarian morphology, substantial interobserver variation, and ever-evolving USG technology make serum AMH a plausible standard criterion in PCOS diagnosis. Future studies should be done, in large cohorts, so that universally accepted methods for serum AMH measurements and international standards could be established.

ACKNOWLEDGMENTS

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