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Correlation of Anti-Müllerian hormone with clinical, hormonal and ultrasonographic parameters in PCOS and normo-ovulatory women: an experience of single tertiary care center

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ABSTRACT

Objectives: to compare the serum AMH levels in PCOS & normo-ovulatory women and investigate correlation between clinical, hormonal, ultrasonographic parameters and AMH levels in both groups.

Methods: a comparative cross-sectional study was performed in sixty-four women (32-PCOS based on Rotterdam consensus; 32 normo-ovulatory-matched controls investigated for male, tubal or unexplained infertility). Serum LH, FSH, androstenedione, fasting-insulin and AMH were measured in early phase (day 3–4) of natural menstrual cycle or progestin-induced withdrawal bleeding (in PCOS). Transvaginal USG performed for calculation of small sized ovarian follicles (<10 mm).

Results: AMH levels were significantly higher in patients of PCOS group as compared to control group (ROC curve; Cut off >3.6; Sensitivity–78.12% & Specificity-87.50%). AMH was positively correlated with LH, LH/FSH, androstenedione and number of small follicles in PCOS group while in control group with number of small follicles only. Multiple regression analysis demonstrated that number of small follicles has only determinant for AMH level (r =0.509; P < 0.005).

Conclusions: number of small sized ovarian follicles has strong association with AMH level in PCOS patients, possibly lead to increased androgen levels. So AMH could be helpful in planning and management for ovulation induction therapy in PCOS patients.

Keywords: PCOS (polycystic ovarian syndrome); AMH (antimullerian hormone); hyperandrogenism.

SOMMARIO

Obiettivi: per confrontare i livelli sierici di AMH nelle donne con PCOS e normo-ovulatorie e studiare la correlazione tra parametri clinici, ormonali, ultrasonografici e livelli di AMH in entrambi i gruppi.

Metodi: uno studio comparativo trasversale è stato condotto in sessantaquattro donne (32-PCOS basate sul consenso di Rotterdam; 32 controlli di confronto normo-ovulatorio studiati per l'infertilità maschile, tubarica o inspiegabile). Siero LH, FSH, androstenedione, insulina a digiuno e AMH sono stati misurati nella fase iniziale (giorno 3-4) del ciclo mestruale naturale o dell'emorragia da sospensione indotta da progestinico (in PCOS). USG transvaginale eseguito per il calcolo dei follicoli ovarici di piccole dimensioni (<10 mm).

Risultati: i livelli di AMH erano significativamente più alti nei pazienti con gruppo PCOS rispetto al gruppo di controllo (curva ROC, Cut off> 3.6, Sensibilità-78,12% e Specificità-87,50%). L'AMH era correlata positivamente con LH, LH / FSH, androstenedione e numero di piccoli follicoli nel gruppo PCOS, mentre nel gruppo di controllo solo con il numero di piccoli follicoli. L'analisi di regressione multipla ha dimostrato che il numero di piccoli follicoli ha solo determinante per il livello di AMH (r = 0,509; P <0,005).

Conclusioni: il numero di follicoli ovarici di piccole dimensioni ha una forte associazione con il livello di AMH nei pazienti con PCOS, probabilmente portare ad un aumento dei livelli di androgeni. Quindi AMH potrebbe essere utile nella pianificazione e gestione della terapia di induzione dell'ovulazione nei pazienti con PCOS.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common disorder in the reproductive age group females. It is characterized by anovulation and commonly showed as oligomenorrhea or amenorrhea, elevated levels of androgens and LH hormone and polycystic ovaries on ultrasound[1].

The distinguishing feature of PCOS is failure of follicular maturation, despite initial recruitment, resulting in anovulation followed by accumulation of preantral and small antral follicles. They lead to increased production of AMH hormone[2,3].

AMH is a dimeric glycoprotein, the member of transforming growth factor-β superfamily. In male, produced by the fetal Sertoli cells and stimulated regression of Müllerian ducts and regulates male sex differentiation. In females, AMH is produced after birth by granulosa cells from preantral and small antral follicles and produces inhibitory effect on the primordial follicle recruitment as well as on responsiveness of growing follicles to FSH[4,5].

PCOS is one of the challenging issues, which causes infertility. There are few existing parameters and criteria used in diagnosis of PCOS. Rotterdam consensus (2003) diagnostic criteria based on three parameters, are oligo-and/or anovulation (OA), hyperandrogenism (either clinical or biochemical) (HA), and the ultrasound feature of polycystic ovaries[6]. The diagnosis is made in presence of at least two criteria, after excluding diseases associated with excessive androgen production.

We have planned the present study with following aims and objectives:

1. To compare the serum AMH levels in PCOS vs. normo-ovulatory group

2. To explore the correlation between clinical, hormonal, ultrasonographic parameters with AMH levels in both the groups.

MATERIALS AND METHODS

This comparative cross-sectional study was conducted at Rabindra Nath Tagore Medical College and attached hospital, pannadhay mahila chikitsalaya, Udaipur, Rajasthan, INDIA between September 2013 to March 2015. A total 64 women of reproductive age group are recruited for study after taking informed consent. The inclusion criteria were: age between 21-35 year, both ovary present, no previous history of ovarian surgery, adequate visualization of ovary by transvaginal ultrasound and no regular & continuous hormonal therapy since six months. The prevalence of PCOS in Mewar region of Rajasthan state India was quite low so sample size was relatively lower. There was no dropout in present study.

In PCOS group, 32 women were diagnosed as PCOS based on Rotterdam consensus. All enrolled patients have at least two out of three following criteria's:

1. oligomenorrhea oramenorrhea (<6 menstrual periods/year);

2. hyperandrogenism, identified either by hirsutism (modified Ferriman–Gallwey score >6), or minor signs such as acne or seborrhea, and/or testosterone >3 nmol/l and/or androstenedione >12 nmol/l; and

3. on ultrasound minimum 9 small sized follicles (<10 mm) per ovary, and/or increased ovarian volume of at least 10 cm3.

Controls were 32 normoovulatory-matched investigated for male, tubal or unexplained infertility. They had regular menstrual cycle (25-35 days), no endocrine abnormalities (normal prolactin, basal FSH and estradiol, and no hyperandrogenism), and normal ultrasonic ovarian morphology. The control women were matched with PCOS women for mean age (±5 years) and mean body mass index, BMI (±3kg/m^2).

Blood sampling for hormone measurement and biochemical parameter assays was performed in the first 3–4 days of the natural menstrual cycle (control group) or progesterone-induced vaginal bleeding in PCOS group. The serum levels of AMH were determined by enzyme –linked immunosorbent assay (ELISA) test kit. Serum hormones, such as FSH, LH, androstenedione and insulin levels were determined by the chemiluminescence detection system. Transvaginal USG was also performed for calculating the number of small sized (<10 mm) ovarian follicles. Approval of the institutional ethical committee was obtained for this retrospective study. However this study was not registered on Clinicaltrials.gov or similar repositories.

STATISTICAL ANALYSIS

Continuous data was presented as mean + SD in both groups. Differences in the various parameters (age, BMI, serum LH, FSH, LH-FSH ratio, AMH, fasting insulin and USG value) in both the groups were analyzed by using independent t-test. The Cut-off value with sensitivity and specificity of serum AMH levels as a predictor of the diagnosis of PCOS was analyzed using a ROC procedure. The relationship between AMH levels and various parameter in PCOS and normo-ovulatory phenotypes were assessed by

Pearson correlation coefficient . The Multivariate regression analyses used to study the association of these variables with AMH in PCOS patients. Data analyses were performed with SPSS version 11.0 (Chicago, Illinois) and P<0.05 considered as the significant.

RESULTS

Table 1: presents the clinical, hormonal and ultrasonographic parameters of the PCOS and control groups (mean ±SD). The mean LH, LH/FSH, androstenedione, AMH and number of small follicles (<10 mm) were significantly higher in the PCOS group as compared to control group. The mean age, BMI, FSH and fasting insulin were not significantly different between the two groups.

We used the ROC curve to investigate the diagnostic potential of AMH level. The AUC of AMH level was 0.907 (95 % CI 0.808 to 0.965) and optimal AMH cut-off level was 3.6 ng/ml, with 78.1 % sensitivity and 87.5 % specificity (**Figure 1**).

Table 2: presents the correlation of AMH

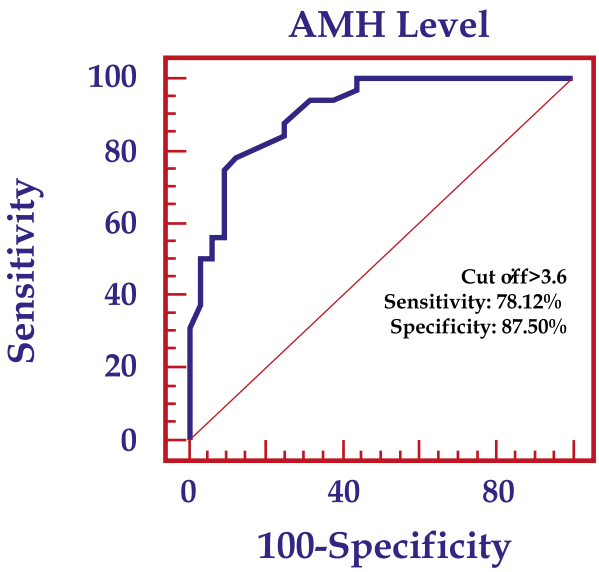

Figure 1.
ROC curve of AMH level in the present study group
AMH Cut off value of PCOS diagnosis: >3.6. Area under the ROC curve (AUC): 0.907. 95% Confidence Interval: 0.808 to 0.965. Sensitivity: 78.12%; Specificity: 87.50%; Significance level P (Area=0.5): < 0.0001.

Table 1.
Clinical, hormonal and ultra sonographic parameters of the PCOS and control groups. Results are expressed in mean ± SD.

	PCOS	Non PCOS	P	Significant
Age (Years)	28.72 ± 3.5	27.1 ± 3.4	0.911	NS
BMI (Kg/m²)	25.8±3.5	26.1± 3.0	0.407	NS
LH (IU/ml)	7.9± 1.7	4.1 ± 1.2	0.05	S
FSH (IU/ml)	4.6 ± 0.8	4.9 ± 1.0	0.286	NS
LH/FSH	1.9 ± 0.4	0.9 ± 0.2	0.001	S
AMH	4.9±1.3	2.7±1.1	< 0.0001	S
Androstenedione (nm/l)	8.6± 2.4	3.5± 1.1	< 0.001	S
Fasting Insulin	7.1± 1.4	5.2 ± 1.0	0.096	NS
No of follicles; Size <10mm	17.8 ±3.5	7.3 ± 1.9	0.001	S

NS = non-significant and S = significant.

with clinical, hormonal and ultrasonographic parameters in the whole group (n = 64). Significant positive correlations were found between AMH, and LH, LH/FSH, androstenedione, fasting insulin and number of small sized ovarian follicles (size <10 mm); while no significant correlation was found between AMH and age, BMI and serum FSH levels.

Table 3: presents the correlation between AMH and clinical, hormonal and ultrasonographic parameters in the PCOS and control groups separately. In PCOS group, significant positive correlations were found between AMH, and LH, LH/FSH, androstenedione and number of small follicles (size <10 mm); while no correlation was found between AMH and age, BMI, fasting insulin and serum FSH levels. In control group, only a significant positive correlation was found between AMH and number of small follicles (size <10 mm); while no correlation was found between AMH and rest of parameters.

Table 4: in the PCOS group, multiple regression analysis was performed with AMH as the dependent variable, and LH, LH/FSH, androstenedione and number of small sized follicles (<10 mm) as independent variables. The number of small sized follicles was the only determinant for AMH level (r =0.509; P < 0.005) as compared to the other parameters.

DISCUSSION

The pathophysiology of PCOS is exactly not known so it is determined as heterogeneity of disease[7]. However; undoubtedly the high level of androgen plays an important role and is responsible for symptomatology of PCOS. The possible mechanism for hyperandrogenism is high level of AMH by inhibiting the peripheral aromatization[8]. Serum AMH levels in the PCOS group was significantly higher than the control group (4.9±1.3 vs. 2.7±1.1; P < 0.0001).

These similar findings are also noted in other related studies. In present study, we found that serum AMH level could be a valuable diagnostic marker for the PCOS patients. The ROC, AUC of AMH level was 0.907 (95 % CI 0.808 to 0.965) and optimal cut off was >3.6 (Sensitivity – 78.12% Specificity- 87.50%). The possible cut off value of AMH with sensitivity and specificity was also compared with previous conducted studies for diagnosis of PCOS[7,9-11]. We found present study is well correlated with most of studies except Li L et al[12].

The cause of higher level of serum AMH was the increased number of small sized follicles (<10mm). Similarly, in our study the number of small sized ovarian follicles is closely correlated with serum AMH level in whole as well as isolated control and PCOS group. So AMH is considered as a good marker for ovarian reserve[2, 13]. In the

Table 2.
Correlation between AMH and clinical, hormonal and ultrasonographic parameters in the whole group of patients (n = 64).

	r	P	Significance
Age (Years)	0.1084	0.3940	NS
BMI (Kg/m²)	-0.07863	0.5368	NS
LH (IU/ml)	0.6244	<0.0001	S
FSH (IU/ml)	-0.1594	0.2083	NS
LH/FSH	0.7051	<0.0001	S
Androstenedione (nm/l)	0.7249	<0.0001	S
Fasting Insulin	0.3708	0.0026	S
No of follicles; Size <10mm	0.7755	<0.0001	S

NS = non-significant and S = significant.

Table 3.
Correlation between AMH and clinical, hormonal and ultra sonographic parameters in the PCOS and control groups separately.

	PCOS (n=32)			Control (n=32)		
	r	P	Significance	r	P	Significance
Age (Years)	-0.0146	0.9367	NS	-0.1259	0.4923	NS
BMI (Kg/m^2)	-0.2432	0.1798	NS	0.1721	0.3462	NS
LH (IU/ml)	0.3096	0.0447	S	-0.0198	0.9143	NS
FSH (IU/ml)	-0.1620	0.3758	NS	0.0616	0.7376	NS
LH/FSH	0.5077	0.0030	S	-0.0057	0.9749	NS
Androstenedione (nm/l)	0.5023	0.0034	S	0.2019	0.2678	NS
Fasting Insulin	-0.1053	0.5662	NS	-0.1445	0.4301	NS
No of follicles; Size <10mm	0.5089	0.0029	S	0.5349	0.0016	S

NS = non-significant and S = significant.

Table 4.
Summary of correlation (significant) between AMH and different parameters.

	Age	BMI	LH	FSH	LH/FSH	Androstenedione (nm/l)	Fasting Insulin	No of follicles (Size <10mm)
Whole Group	N	N	Y	N	Y	Y	Y	Y
PCOS group	N	N	Y	N	Y	Y	N	Y
Normo-Ovulatory woman	N	N	N	N	N	N	N	Y

N-no significant co-relation and Y-significant co-relation.

present study, significant positive correlations were found between AMH and androstenedione in the PCOS and whole group similar to previous studies (**Table 2, 3**).

AMH does not vary throughout the menstrual cycle because it is not under the influence of gonadotrophic hormones thus, it better reflects the follicular pool and its maturation, sensitivity of FSH action on ovarian follicle[3,14-16]. So AMH is considered as a better marker for ovarian reserve.

Present study shows correlation of AMH with LH and LH/FSH (**Table 2, 3**) in whole as well as PCOS group are comparable with the results of previous studies[3,17,18]. However, Pigny et al.[2] demonstrated no significant relationship of AMH with LH and LH/FSH in PCOS and whole group.

Present study also revealed no significant correlations between AMH and age, BMI, or fasting insulin (**Table 3**) as shown by Begawy et al.[18] However, Nardo et al.[19] demonstrated significant negative correlation between AMH and age & insulin; and Chen et al.[20] found that AMH had a significant inverse relation with both BMI and insulin resistance.

In the present study, significant positive correlations were found between AMH and androstenedione in the PCOS and whole group (**Table 2, 3**) similar to previous studies[11,12,18,20,21]. However, Nardo et al.[19] indicated that AMH is related in same manner with androgens in PCOS and non-PCOS women. FSH induced aromatase activity of human granulosa cells is inhibited by

AMH. This mechanism contributes to increase in androgen levels[22,23].

Multiple regression analysis demonstrated that number of small sized follicle was the only determinant for AMH level in the PCOS group ($r = 0.509$; $P < 0.005$) as demonstrated by Pigny et al.[2] In contrast Begawy et al.[18] revealed only androgens were significantly related to AMH level but not with the number of small sized follicles while Eldar-Geva et al.[17] showed both the number of small sized follicles and androgens were significantly related to AMH levels.

The present study is also untouched with following limitations:

1. Our study was conducted in the limited geographical area and on relatively small sized sample. To extrapolate our results in the whole population, this study should be conducted in the large geographical area and on big sized sample.

2. Serum AMH level measurement is slightly expensive so lower class patients cannot afford it.

Finally we concluded that the serum AMH level is an important parameter in the diagnosis of the PCOS. And by inhibiting peripheral aromatization it increases the androstenedione level, which is responsible for symptomatology of PCOS. In present study the number of small sized ovarian follicles has strong association with AMH level in PCOS patients, possibly leading to increased androgen levels. So we hypothesize that in PCOS group increased AMH level is due to follicular arrest so AMH could be helpful in planning and managing ovulation induction therapy in PCOS patients.

CONFLICTS OF INTEREST IN THE WORK

Nil

FINANCIAL INTEREST DISCLOSURE

Nil

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