Academic Journal of Clinicians
Published by Academic Journals Publishing Group
ISSN: XXXX-XXXX

Serum Anti-Müllerian Hormone in Women with Polycystic Ovarian Syndrome

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Original Article

ABSTRACT

***Background**: Early diagnosis of polycystic ovarian syndrome (PCOS) is essential in treatment of infertility and prevention of cancer. The serum anti-Müllerian hormone (AMH) was found to be correlated with folliculogenesis in ovaries.* ***Objective**: To assess the relationship between serum AMH level and PCOS in comparison to women with normal ovulation and to identify the appropriate cutoff value of serum AMH in prediction of PCOS.* ***Patients & Methods:** This study was a cross sectional study conducted in Gynecology Outpatient Department of Azadi Teaching hospital through the period between 1st of September, 2016 to the 30th of August, 2018 on sample of 90 women with PCOS and 90 healthy control women in reproductive age period. Diagnosis of PCOS was based on two diagnostic criteria by the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine Rotterdam consensus.*

***Results**: The obesity and oligomenorrhea were significantly related with PCOS women ($p<0.001$). Follicular stimulating hormone and testosterone hormone were significantly higher among PCOS women. Mean serum AMH was 6.07 ng/ml for PCOS and 2.9 ng/ml for controls with a highly significant difference between both groups ($p<0.001$). The appropriate cutoff value of AMH in diagnosis of PCOS with an acceptable validity was 5.05 ng/ml.* ***Conclusions:** The serum Anti-Müllerian hormone is good marker for early diagnosis of polycystic ovarian syndrome.*

Keywords: Polycystic ovarian syndrome, Anti-Müllerian Hormone

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1.INTRODUCTION

The polycystic ovary syndrome (PCOS) is the most common endocrine disorder of women in reproductive age as (6.6-8%) of women in this age group affected globally. The hyperandrogenism and ovulatory dysfunction are main characteristics of PCOS and it is the common cause of anovulatory infertility (1). The definite etiology of PCOS is still unknown, however, many risk factors are related to PCOS development like insulin resistance, distorted gonadotrophin levels and obesity in addition to genetic and environmental effect (2). Many authors suggested that ovarian hyperthecosis and high androgen release are the predominant endocrine abnormalities of PCOS and its pathogenesis is the result of interaction between genetic and environmental factors (3, 4). In Iraq, the PCOS prevalence detected by pelvic ultrasound examination in reproductive age women was in range of (14.3-17%) (5, 6) and it represented 29% of infertility cause among Iraqi infertile women attending fertility centers at 2015 (7).

The diagnosis of PCOS according to Rotterdam criteria (2003) after exclusion of related disorders is depending on identification of two of three characteristics; oligo-or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries observed by ultrasonography (Twelve follicles or more, diameter of two to nine mm and/or ovarian volume as ten cm3 or more) (8). These diagnostic criteria relay on the antral follicle count (AFC) detected by ultrasonography (9). Nowadays and due to technological advancement of ultrasound equipment and its higher accuracy, there is an improvement in validity findings of ultrasonography in AFC detection. In spite of that, assessment of polycystic ovarian morphology (PCOM) is characterized by high variability with obstacles in accounting AFC trans-abdominally in unmarried or obese women (10). So, there was an urgent need for objective parameters and the serum Anti-Müllerian hormone (AMH) test could be the best choice to solve this problem (11). Variation in luteinizing hormone (LH) production is regarded as common feature of women with PCOS that lead to variation in LH/FSH ratio (12).

The AMH is dimeric glycoprotein considered as an interesting regulator of folliculogenesis in the ovaries (10). Granulosa cells of ovarian follicles is responsible of AMH secretion that is increased to about three fold among women with PCOS in comparison to women with normal ovulation. Elevated serum levels of AMH in PCOS are parallel to increase in number of AFC (13, 14). The AMH contributes to physiology of ovaries by its role in follicular growth inhibition (15). Some authors suggested that AMH interfere with action and concentration of follicular stimulating

hormone (16). High AMH levels among women with PCOS are related to hyperandrogenism (17), insulin resistance (18) and obesity (19). It was shown that metformin therapy lead to prominent reduction in AMH level in PCOS (20). Although these positive findings, there was inability in definition of an appropriate standard thresholds of AMH (3, 21).

Prevalence of PCOS among reproductive age women within general population was found to be ranged from 4-12% (22); in Iraq, it was (14.3%) among adult females (23), while more than one forth of infertile women in Iraq was diagnosed with PCOS (7). High rates of infertility related increased prevalence of PCOS among reproductive age women in Iraq in last decades, scarcity of screening techniques of PCOS and in order to standardize an optimal cutoff values for AMH in diagnosis of PCOS, for all of these reasons, this study was carried out to assess the relationship between serum AMH level and PCOS in comparison to women with normal ovulation and to identify the appropriate cutoff value of serum AMH in prediction of PCOS.

2. PATIENTS and METHODS

The present study was a cross sectional study conducted in Gynecology Outpatient Department of Azadi Teaching hospital through the period between 1st of September, 2016 to the 30th of August, 2018. Women presented clinically with symptoms of PCOS were the study population. Inclusion criteria were reproductive age period (18-42 years), oligomenorrhea, hirsutism, abnormal investigations findings (FSH, LH, LH/FSH and testosterone) and ultrasound finding of PCOS. Patients on metformin therapy, hormonal therapy, other endocrine disease and refuse to participate were excluded from the study. A convenient sample of 90 women with PCOS was selected after eligibility to inclusion and exclusion criteria. Another convenient sample of 90 healthy women in reproductive age period was selected from relatives of patients attending to the Consultancy clinic. An approval for study was taken from the Ethical Committee of Kirkuk Health Directorate and an oral informed consent was taken from each study participantt before enrolled in the study in addition to the researchers' responsibility for maintenance of PCOS patients' management accordingly. The data were collected by the researcher using a prepared questionnaire. The selected patients were known cases of PCOS diagnosed by the researcher or other Gynecologist according to diagnostic criteria by the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine Rotterdam consensus (ESHRE/ASRM) like an-or oligoovulation, clinical or biochemical features of hyperandrogenism and ultrasound morphology of PCOS (10). The PCOS women were visiting the researcher for treatment and follow

up. The collected characteristics of patients were age, body mass index (BMI) and menstrual status. The studied hormones of study participants were luteinizing hromone (LH), follicular stimulating hormone (FSH), prolactin, testosterone and serum Anti-Müllerian hormone (AMH). All women participated in this study were assessed during early follicular phase (1st week of menstrual cycle). The weight and height were measured by the researcher in the outpatient department using standardized scale and the BMI was measured using the equation BMI=Wt/ (Ht in meter) 2. The BMI of women was categorized into normal (<25 Kg/m2), overweight (25-29.9 Kg/m2) and obese (≥30 Kg/m2). A sample of 10 ml venous blood was drawn from study participant after night fasting for hormonal assay and after centrifuge, they were sent to private laboratory in Kirkuk. The normal ranges of studied hormones of LH, FSH, prolactin and testosterone were (1.4-15.4 mIU/ml), (1.24-7.8 mIU/ml), (2-17 ng/ml) and (15-70 ng/ml), respectively. The statistical analysis was conducted by using Statistical Package for Social Sciences (SPSS) version 22. Multiple contingency tables were performed. Chi-square test was used to compare between categorical variable and independent sample t-test was used to compare between two means. Receiver Operating Curve (ROC) was used to predict the appropriate cutoff value of AMH. The level of significance was ≤0.05.

3. RESULTS

Ninety women with PCOS and 90 healthy control women were included in this study. No significant difference in age was observed between PCOS women and controls (p=0.3). There was a highly significant difference in BMI between PCOS women and controls (p<0.001), obesity was present in 43.3% of PCOS women, while obesity represented 10% of controls. (**Table 1**) . A highly significant association was observed between oligomenorrhea and PCOS women (p<0.001), (**Table** 2). Studying hormonal profile of both study groups revealed no significant differences between PCOS women and controls regarding luteinizing hormone (p=0.7), prolactin hormone (p=0.2) and LH/FSH ratio (p=0.2). The follicular stimulating hormone was significantly higher among women with PCOS (p<0.001). Similarly, testosterone hormone level was significantly higher among women with PCOS (p<0.001), (**Table 3**). The mean Anti-Müllerian hormone (AMH) level of PCOS women was (6.07 ng/ml) and mean AMH of controls was (2.9 ng/ml). There was a highly significant increase in AMH level among women with PCOS (p<0.001), (**Figure 1**). The ROC curve analysis showed that an appropriate cutoff value of AMH in prediction of PCOS with high sensitivity of 80% and specificity of 61.1% was more than 3.3 ng/ml, while with increasing AMH

level, the sensitivity was decreased and specificity was increased until cutoff value of 7 ng/ml of 42.2% sensitivity and 100% specificity. The appropriate cutoff value of AMH in prediction of PCOS was 5.05 ng/ml with sensitivity of 64.4% and specificity of 83.3%. (**Table 5 and Figure 2**).

Table 1. Distribution of age and body mass index according to PCOS women and controls.

Variable		PCOS(n=90) No. (%)	Control(n=90) No. (%)	P. value
Age (year)	<20 years	15 (16.7)	9 (10.0)	0.300* NS
	20-29 years	29 (32.2)	38 (42.2)	
	30-39 years	38 (42.2)	38 (42.2)	
	≥40 years	8 (8.9)	5 (5.6)	
BMI	Normal	13 (14.5)	42 (46.7)	<0.001* S
	Overweight	38 (42.2)	39 (43.3)	
	Obese	39 (43.3)	9 (10.0)	

** Chi-square test, S=Significant, NS=Not significant.*

Table 2. Distribution of menstrual cycle according to PCOS women and controls.

Menstrual cycle	PCOS(n=90) No. (%)	Control(n=90) No. (%)	P. value
Normal	15 (16.7)	86 (95.6)	**<0.001**
Oligomemorrhea	75 (83.3)	4 (4.4)	

Table 3. Distribution of hormones according to PCOS women and controls.

Variable		PCOS(n=90) No. (%)	Control(n=90) No. (%)	P
LH	Normal	21 (23.3)	19 (21.1)	0.700
	High	69 (76.7)	71 (78.9)	
FSH	Normal	12 (13.3)	50 (55.6)	**<0.001**
	High	78 (86.7)	40 (44.4)	
LH/FSH ratio	Mean± SD	0.82±0.4	0.75±0.34	0.200
Prolactin	Normal	30 (33.3)	37 (41.1)	0.200
	High	60 (66.7)	53 (58.9)	
Testosterone	Normal	18 (20.0)	79 (87.8)	**<0.001**
	High	72 (80.0)	11 (12.2)	

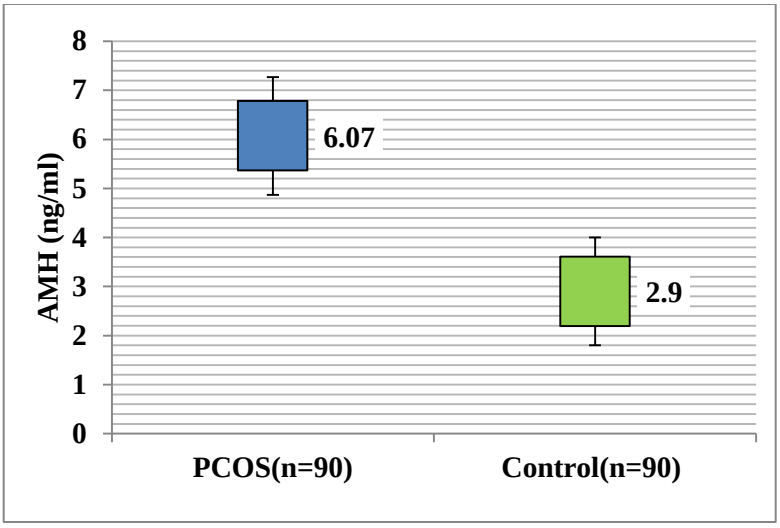

Figure 1. Comparison of mean AMH of PCOS cases and controls (Difference was significant P. value < 0.001)

Table 5. Results of ROC curve analysis for the validity of AMH in prediction of PCOS at different cutoff points .

AMH values	Sensitivity	Specificity
3.3 ng/ml	80%	61.1%
4.05 ng/ml	76.7%	68.9%
5.05 ng/ml	**64.4%**	**83.3%**
6.03 ng/ml	55.6%	96.7%
7 ng/ml	42.2%	100%

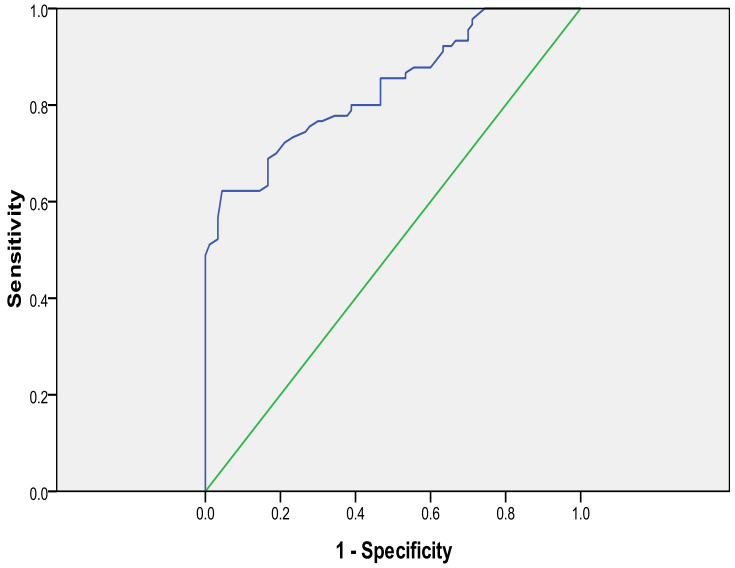

Figure 2. Diagram of ROC curve analysis for AMH prediction of PCOS (area under curve=0.83).

DISCUSSION

The early detection and diagnosis of PCOS is essential in managing infertility among reproductive age women and in prevention of seious endometrial malignancies. The serum AMH has hopeful results in improving the screening and diagnosis of PCOS in collaboration with ultrasonography (24). The current study showed a highly significant increase in AMH level among women with PCOS in comparison to controls ($p<0.001$). This finding is consistent with results of Al-Moayad et al (25) study in Iraq which found that PCOS women had a significantly higher AMH mean in comparison to normal ovulatory women. Similarly, Pellatt et al (26) study in United Kingdom reported higher AMH level among reproductive age women with PCOS in comparison to normal ovulatory women and an inhibitory role for ovulation in PCOS women. It was proved that serum AMH are affected directly by small ovarian follicles and the AMH is responsible in decreasing the sensitivity of these follicles to follicular stimulating hormone (27). Recent study in Egypt (28) found that serum AMH was strongly related to clinical, biochemical and sonography findings of PCOS. Many researchers documented that serum AMH level is not only predictor for diagnosis of PCOS, but also it is helpful in detection of good response to different PCOS treatment types like clomiphene citrate, gonadotrophins and laparoscopic drilling of ovaries (29, 30).

The present study showed that serum AMH level of 5.05 ng/ml was an appropriate cutoff value in prediction of PCOS with an acceptable validity results (sensitivity 64.4% and specificity 83.3%) and the specificity was increased with increase of AMH cutoff value to 7 ng/ml to reach 100%, although low sensitivity. This finding is similar to results of Matsuzaki et al (31) study in Japan which reported an appropriate serum AMH cutoff value in prediction of PCOS as 7.3 ng/ml and also stated that increase specificity of diagnosis increased with increase AMH level. In Iraq, Obeid et al iq study in Iraq documented that best cutoff value of serum AMH in prediction of PCOS was 7.9 ng/ml (sensitivity 82.1%, specificity 100%). Different literatures like Sahamay et al (21) stydu in Turkey, Zadehmodarres et al (32) study in Iran and Mahran et al (33) study in UK assessing the appropriate AMH cutoff value in detection of PCOS and some of them reported lower values of AMH in range of 3-3.4 ng/ml. These discrepancies between different studies in confirmation of an appropriate cutoff value for AMH in diagnosis of PCOS represented an obstacle in application of AMH. However, Stracquadanio et al (34) study in Italy reported that predictive serum AMH levels for PCOS diagnosis were ranging between 3-7 ng/ml and AMH level of 5 ng/ml was the best cutoff value.

In the current study, the obesity was significantly predominant for PCOS women ($p<0.001$). This finding coincides with results of many previous literatures such as Lim et al (35) study in Australia and Schulte et al (36) study in USA. The oligomenorrhea was the significant menstrual disturbance of PCOS women in our study ($p<0.001$). Recently, Harris et al (37) suggested that menstrual disturbances specifically the oligomenorrhea which accompany the PCOS increased risk of cancer that might be attributed to ovulation hypothesis which documented that recurrent damage and repair to the ovarian surface increases ovarian cancer risk Ref. The FSH and testosterone levels were significantly higher among studied PCOS women in comparison to controls. These findings are in agreement with results of AL-Deresawi et al (38) study in Iraq which revealed an increase of FSH and testosterone hormone in PCOS women and these hormones especially the androgen hormones are related directly to BMI of women. Higher FSH levels in current study might be due to irregular hormonal levels of some studied women that related to recent treatment with hormonal replacement therapy. The LH/FSH ratio in current study is not significantly different between PCOS women and controls. Recent literature by Saucido et al reported that LH/FSH ratio was not diagnostic for PCOS although, it is a valuable test (39).

CONCLUSIONS

Serum Anti-Müllerian hormone is good marker for early diagnosis of polycystic ovarian syndrome. The appropriate AMH level in diagnosis of PCOS was 5.05 ng/ml that is accompanied by 64.4% sensitivity and 83.3% specificity. Encouraging gynecologist to adopt the serum AMH testing in combination with clinical features or ultrasonography to confirm the diagnosis of PCOS.

Ethical Clearance: The study protocol was approved by the scientific committee of the Iraqi Ministry of health, Kirkuk Health directorate. Verbal informed consent was taken from each participant women and the data were collected in accordance to the World Medical Association (WMA) declaration of Helsinki , 2013, for the ethical issues or researches including human.

Conflict of interest: The authors declared none

Funding: Self-Funded by the authors

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Acknowledgement

We would like to thank all our colleagues ; medical and Paramedical staff of Azadi Teaching hospital for their efforts and help in this work. Special thanks to all participant women in this study for their cooperation and time, we hope they be in good health and life.