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Threshold value of Anti Mullerian Hormone (AMH) and its correlation with other related hormones in Polycystic Ovarian Disease

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Abstract

Background: Polycystic ovarian disease (PCOD) is the commonest endocrine disorder affecting women at reproductive age. As a complex multisystem disorder, its background can be confusing to understand. The key feature is an increased production of AMH by antral follicles.

Aim: The aim of the present study was to assess the threshold value of serumAMH in PCOD and its correlation with other related hormones such as FSH, LH and Prolactin.

Materials and Methods: Serum AMH, FSH, LH and Prolactin in all subject (PCOD and Non PCOD groups) were estimated using Cobas-e-411analyser. The results analyzed statistically by Using Z-test (p value <0.05 considered as significant), Correlation test and ROC analysis.

Result: Serum AMH and LH shown significant increase level in PCOD groups compared to Non PCOD groups (p value=0.00, 0.001 respectively). Serum FSH and Prolactin have shown no much significant variation between the two groups (p value=0.75, 0.8 respectively). Threshold values of serum AMH and LH was observed as 8.47 ng/ml and 15.57 mIU/ml respectively. Additionally, serum AMH and LH has shown the strong positive correlation in PCOD group; no other parameters shown any correlation in both groups.

Conclusion: Serum AMH level estimation can be considered as a better choice for diagnosis of PCOD especially in the situation wherever ultrasonography examination is not convenient to conduct.

Keywords: Polycystic ovarian disease; Anti Mullerian hormone; Follicle stimulating hormone; Luteinizing hormone

1. Introduction

PCOD is a hormonal disorder common among women of reproductive age. Women with PCOD may have infrequent or prolonged menstrual period or excess male hormone (androgen) levels. The ovaries may develop numerous small collections of fluid (follicles) and fails to regularly release eggs. There is a marked association with insulin resistance, dyslipidaemia, obesity, gestational diabetes and heart disease. In addition, it is an established cause of endometrial hyperplasia, and is linked to endometrial cancers. The short- and long-term consequences of PCOD represent an increased burden on health resources. Polycystic ovarian syndrome is otherwise known as Stein-Leventhal (1935) syndrome [1].

Around 20% of women have the characteristic appearance of polycystic ovaries on ultrasound scans [1] and 7-8% has the additional clinical and biochemical features of PCOD itself [2]. Obesity increases the proportion of women with

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polycystic ovaries who develop the syndrome, the current epidemic of obesity is likely to make PCOD even more common [3].

Polycystic ovaries found on ultrasound scanning will often have nonclinical effect, but PCOD is the most common diagnosis made in women presenting with amenorrhea, oligo menorrhea or heavy, irregular and prolonged periods. It is the commonest cause of hirsutism and of infertility due to anovulation.

Anti-mullerian hormone (AMH), also known as mullerian inhibiting hormone (MIH), is a glycoprotein hormone structurally related to inhibin and activin from the transforming growth factor beta super family, whose key role are in growth differentiation and folliculogenesis. It is a hormone secreted by cells in developing egg sacs (follicles). AMH was discovered by Alfred Jost, French endocrinologist [1]. Serum AMH level can be used as a good indicator of ovarian reserve.

The level of AMH can reflect the number of ovarian antrum follicles, ovarian reserve and ovarian function [4]. Women with PCOD have an increased number of follicles in the pre -antral and antral stage, and therefore it is observed that their AMH serum concentrations are higher than their counterpart [5]. In women, serum AMH concentration are low during the pubertal years, and appear to be elevated in pre pubertal daughters of woman with PCOD syndrome. Furthermore, AMH levels are positively correlated with individual features of PCOD including FSH, LH and prolactin [6]. The role of AMH in the pathogenesis of PCOD is uncertain [7].

AMH (in male foetal development) prevents the mullerian duct from developing into the uterus and other mullerian structures, resulting in normal development of male reproductive tract. In the absence of AMH the mullerian duct and structure develop into the female reproductive tract. AMH serum concentration is elevated in males under 2-year-old then progressively decreases until puberty. AMH (In female) is also expressed in the follicles of female reproductive age and inhibit the transition of follicles from primordial to primary stages. Follicular AMH production begins during the primary stage, peaks in the preantral and small antral stage, and then decreases to undetectable concentration as follicles grow larger. By contrast, AMH concentration is low in female children until puberty [8]. The aim of this study was to evaluate the role and significance of AMH and other related hormones in PCOD. Additionally, threshold value of serum AMH and LH in PCOD and correlation of AMH with other hormones also studied.

2. Material and methods

2.1. Subject

This retrospective study was conducted in the gynaecological department of Lisie hospital, Cochin. PCOD was diagnosed according to the Rotterdam criteria 2003. The Rotterdam criteria are the most widely-used tool for diagnosing PCOD.This include:

- Oligo/anovulation (OA)
- Hyperandrogenism
- Presence of polycystic ovary /increased ovarian volume

According to these criteria, if the subject is diagnosed with any two of the above features will be considered as suffering PCOD

Total 105 women (55 individual with PCOD and 50 individuals without PCOD), aged between 18-40 years were included in the study. Women who diagnosed with other etiologies such as Cushing syndrome, Down syndrome, ovarian tumour etc or receive hormones or drug therapy for major medical disease were excluded from the study.

2.2. Sample

The blood samples (5 ml) were obtained from median cubital vein through standard phlebotomy procedures during the days 2-5 of the menstrual cycle. The sample was collected in a serum separator tube and allowed to clot for 30 min before centrifugation at 1000 x g for 15 min. All samples were processed within 2 hrs of collection.

2.3. Diagnosis

2.3.1. Blood test

Serum AMH, FSH, LH and Prolactin level were estimated by Sandwich ELISA method using Cobas e 411 analyser.

2.3.2. Ultrasonography

Ultrasound was performed for the entire participant on the same day as blood sample collection. A trans-vaginal or trans-rectal ultrasound examination was performed. Measurements were obtained in real time at the early follicular phase for determination of ovarian morphology. Each ovary was scanned in both longitudinal and transverse cross section from the inner to the outer margin to count the total number of follicles. The volume of each ovary and all follicles with a diameter of 2-9mm was counted in each ovary. Ovarian volume was calculated using the formula, 6x (D1xD2xD3)

2.4. Statistical analysis

The significance of serum AMH, FSH, LH and Prolactin were statistically analysed between PCOD and NON PCOD individuals by using Z test.

2.4.1. Z test

Here Z test is used because the sample size is more than 30 and this test also enables to compare two scores that are from different normal distribution. Compared the Z score with critical value and then look for significance and p value ≤ 0.05 was considered as the significant difference.

2.4.2. Correlation Study

Correlation studies of serum AMH with FSH, LH and Prolactin separately have done in PCOD as well as in NON PCOD groups by using Pearson correlation coefficient test. The concentration between the parameters interpreted as positive, negative and no correlation based on calculated 'r" value and graph obtained.

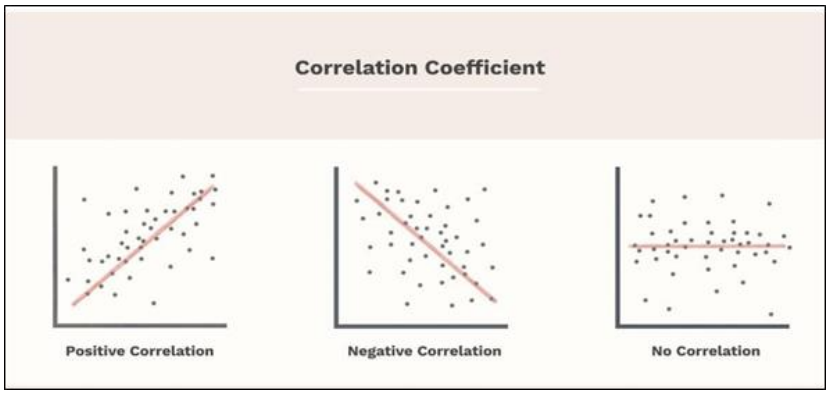

Figure 1 Correlation Coefficient

Correlation	Interpretation
-1.00—0.76	Strong negative correlation
-0.51 to 0.75	Good negative correlation
-0.50 to -0.26	Pair negative correlation
-0.25 to 0.01	Poor negative correlation
0	No correlation
0.01 to 0.25	Poor positive correlation
0.26 to 0.50	Fair positive correlation
0.51 to 0.75	Good positive correlation
0.76 to 1.00	Strong positive correlation

2.4.3. ROC (Receiver Operator Curve)

ROC was used to find out the diagnostic effective of AMH and LH in PCOD and cut-off values were calculated from the curve. The value of sensitivity was plotted on Y axis and false positive values on X axis. The coordinate point with highest sensitivity and less false positive value were taken as the cut-off value.

3. Results

3.1. Z test

The serum AMH FSH, LH, Prolactin values were statistically analysed with Z test used to find out the significance of variation between the groups. Serum AMH and LH showed significant variation but serum FSH and Prolactin didn't show the significant variation between the groups (Table-1).

Table 1 Significance of Serum AMH, FSH, LH and Prolactin in PCOD and Non PCOD- Z Test

Hormones	PCOD (N=55) Mean+/- SD	NON PCOD(N=50) Mean+/- SD	Z test- P value
AMH (ng/ml)	7.63+/-3.97	2.01+/- 1.50	0 *
FSH (mIU/ml)	6.70+/-10.39	6.09 +/-3.97	0.75
LH (mIU/ml)	14.70 +/-10.69	7.80+/-8.18	0.001*
Prolactin (ng/ml)	12.88+/-5.95	12.56 +/-7.87	0.8

* Significant variation

3.2. Pearson correlation coefficient test

In PCOD Groups, Pearson correlation coefficient study showed the strong correlation of serum AMH and LH levels and poor correlation of serum AMH with other parameters such as FSH and Prolactin. (Table-2). In Non-PCOD Groups, correlation study showed the fair/poor correlation between all parameters (Table-3).

Table 2 Correlation of serum AMH with FSH, LH and Prolactin in PCOD

Hormones	r value	Result
AMH and FSH	-0.03	Poor negative correlation
AMH and Prolactin	-0.22	Poor negative correlation
AMH and LH	0.79	Strong positive correlation

Table 3 Correlation of serum AMH with FSH, LH and Prolactin in Non-PCOD

Hormones	r value	Result
AMH and FSH	0.14	Fair positive correlation
AMH and Prolactin	0.29	Poor positive correlation
AMH and LH	0.13	Poor positive correlation

3.3. Receiver Operating Curve Analysis

ROC analysis used to determine the cut off value of serum AMH and LH levels by using. Details were shown in table 4 and figures 2 and 3 below.

Table 4 ROC Analysis of Serum AMH and LH

Parameters	Sensitivity (Y Axis)	False Positive (X Axis)	Cut Off Value
Serum AMH	0.509	0.02	8.47 ng/ml
Serum LH	0.431818	0.0357	15.57 mIU/ml

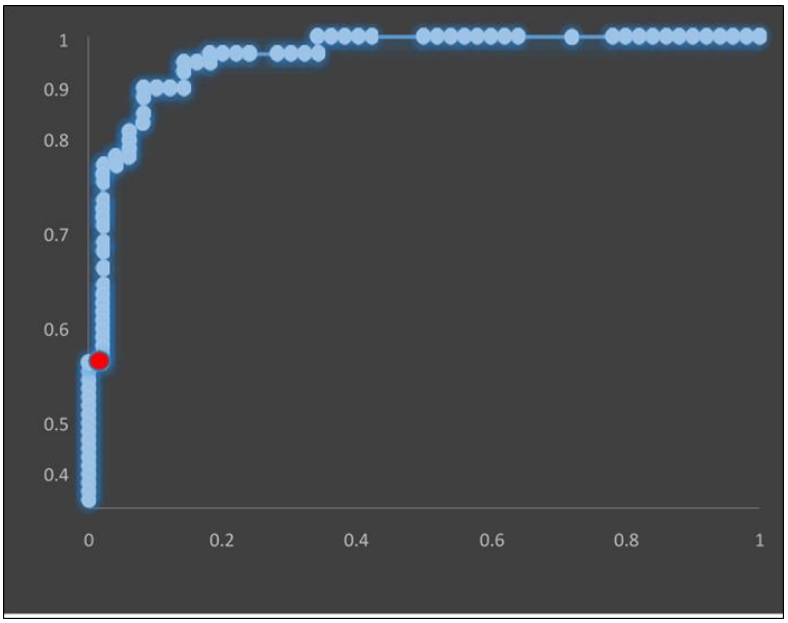

Figure 2 ROCof AMH

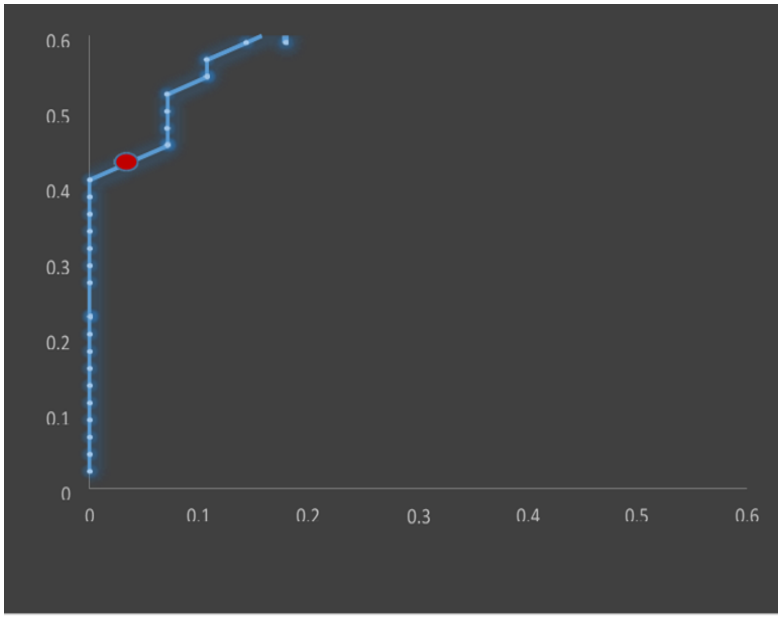

Figure 3 ROC of LH

4. Discussion

In this study, women with PCOD showed a significant higher AMH level compared to non PCOD groups. *Wiweko et al* also reported that AMH level are higher in PCOD [9]. This may be due to increased synthesis and secretion of AMH by polycystic ovary [10]. *Pellat et al* reported that AMH production increases approximately 75 times higher in each polycystic ovarian granulosa cells and found increased mRNA expression of AMH in ovarian granulosa cells [11].

The serum LH level was also significantly higher in PCOD patient when compared with Non PCOD patient in this study. This is because when granulosa cells are cultured with LH, the expression of AMH is up regulated in anovulatory polycystic ovary, which is not observed in normal ovaries [12].

However, as per our results, serum FSH level has not shown any significant variation when compared with non PCOD [13]. This is probably because the AMH level in patient with PCOD is not only related to increase in follicle pool but also increase in the production per follicle [14]. Also, LH was found elevated because GnRH secretion in patient with PCOD is relatively fast. This may lead to increased LH level, which in turn leads to poor egg development and inability to ovulate, and this finally leads to no significant variation in FSH level.

The serum prolactin has shown no significant variation in our study. In a similar study, *Chao -yan Yue et al* found that serum AMH and LH level were significantly elevated in PCOD than control group but FSH and prolactin have showed no significant variation between two groups [15].

In the current study, threshold value of serum AMH and LH was observed as 8.58 ng/ml and 15.57 mIU/ml as per the ROC analysis. The studies conducted by *Chao -yan Yue et al* [15] also suggested that the threshold for serum AMH level is 8.16 ng/ml; very closely related to our findings. Moreover, serum AMH and LH has shown the strong positive correlation in PCOD group; no other parameters shown any strong correlation in both groups.

Most researchers agree that serum AMH should be considered as a marker for increased ovarian reserve. Impaired folliculogenesis may cause excess accumulation of pre antral and small antral follicle, which may ultimately cause the increased serum AMH level associated with PCOD [16]. However, it must be recognized that polycystic ovarian morphology in normal women does not commonly predisposes them to develop PCOD [17].

5. Conclusion

Our results have shown that the serum level of AMH and LH was found significantly elevated PCOD patients, and no significant variation have shown in serum level of FSH and prolactin when compared to Non-PCOD groups.

From the ROC, the cut-off value of serum AMH and LH derived as 8.47 ng/ml and15.57 mIU/ml respectively. Additionally, serum AMH and LH has shown the strong positive correlation in PCOD group; no other parameters shown any strong correlation in both groups.

Serum AMH level can reflect the number of antral follicle and its estimation is easier and less expensive. In addition, measurement of serum AMH levels may also be used as an indicator of PCOD patients' response to therapeutic approaches. Hence Serum AMH level estimation can be considered as a better choice for diagnosis of PCOD especially in the situation wherever ultrasonography examination is not convenient to conduct.

Compliance with ethical standards

Acknowledgments

We are thankful to all who have helped to complete the research.

Future aspects

Since this study was conducted with comparatively a smaller number of samples, we are planning to conduct the similar study with larger sample size along with other parameters in order to find out whether serum AMH and LH can be used as a Prognostic and diagnostic indicator of PCOD.

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding publication of this article.

Statement of ethical approval

The study was approved by institutional research committee.

Statement of informed consent

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

References

[1] Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. Nature reviews endocrinology. 2011 Apr; 7(4):219-31.

[2] Lakhani K, Seifalian AM, Atiomo WU, Hardiman P. Polycystic ovaries. The British Journal of Radiology. 2002 Jan; 75(889):9-16.

[3] Duncan WC. A guide to understanding polycystic ovary syndrome (PCOS). Journal of Family planning and reproductive health care. 2014 Jul 1; 40(3):217-25.

[4] Findlay JK, Hutt KJ, Hickey M, Anderson RA. What is the "ovarian reserve"? Fertility and Sterility. 2015 Mar 1; 103(3):628-30.

[5] Kruszewska J, Laudy-Wiaderny H, Kunicki M. Review of Novel Potential Insulin Resistance Biomarkers in PCOS Patients—The Debate Is Still Open. International Journal of Environmental Research and Public Health. 2022 Jan; 19(4):2099.

[6] Crisosto N, de Guevara AL, Echiburú B, Maliqueo M, Cavada G, Codner E, Paez F, Sir-Petermann T. Higher luteinizing hormone levels associated with antimüllerian hormone in postmenarchal daughters of women with polycystic ovary syndrome. Fertility and sterility. 2019 Feb 1; 111(2):381-8.

[7] Catteau-Jonard S, Dewailly D. Pathophysiology of polycystic ovary syndrome: the role of hyperandrogenism. Polycystic ovary syndrome. 2013; 40:22-7.

[8] Visser JA, McLuskey A, Verhoef-Post M, Kramer P, Grootegoed JA, Themmen AP. Effect of prenatal exposure to diethylstilbestrol on Mullerian duct development in fetal male mice. Endocrinology. 1998 Oct 1; 139(10):4244-51.

[9] Wiweko B, Maidarti M, Priangga MD, Shafira N, Fernando D, Sumapraja K, Natadisastra M, Hestiantoro A. Anti-mullerian hormone as a diagnostic and prognostic tool for PCOS patients. Journal of assisted reproduction and genetics. 2014 Oct 31(10):1311-6.

[10] Pasquali R, Stener-Victorin E, Yildiz BO, Duleba AJ, Hoeger K, Mason H, Homburg R, Hickey T, Franks S, Tapanainen JS, Balen A. PCOS Forum: research in polycystic ovary syndrome today and tomorrow. Clinical endocrinology. 2011 Apr; 74(4):424-33.

[11] Pellatt L, Rice S, Dilaver N, Heshri A, Galea R, Brincat M, Brown K, Simpson ER, Mason HD. Anti-Müllerian hormone reduces follicle sensitivity to follicle-stimulating hormone in human granulosa cells. Fertility and sterility. 2011 Nov 1; 96(5):1246-51.

[12] Pierre A, Peigné M, Grynberg M, Arouche N, Taieb J, Hesters L, Gonzalès J, Picard JY, Dewailly D, Fanchin R, Catteau-Jonard S. Loss of LH-induced down-regulation of anti-Müllerian hormone receptor expression may contribute to anovulation in women with polycystic ovary syndrome. Human reproduction. 2013 Mar 1; 28(3):762-9.

[13] Pigny P, Merlen E, Robert Y, Cortet-Rudelli C, Decanter C, Jonard S, Dewailly D. Elevated serum level of anti-mullerian hormone in patients with polycystic ovary syndrome: relationship to the ovarian follicle excess and to the follicular arrest. The Journal of Clinical Endocrinology and Metabolism. 2003 Dec 1; 88(12):5957-62.

[14] Desforges-Bullet V, Gallo C, Lefebvre C, Pigny P, Dewailly D, Catteau-Jonard S. Increased anti-Müllerian hormone and decreased FSH levels in follicular fluid obtained in women with polycystic ovaries at the time of follicle puncture for in vitro fertilization. Fertility and sterility. 2010 Jun 1; 94(1):198-204.

[15] Chang TY, Chueh KH. Relationship between elderly depression and health status in male veterans. Journal of nursing research. 2011 Dec 1; 19(4):298-304.

[16] Ong ML. The effects and mechanisms of paeoniflorin on murine ovarian cells for the treatment of polycystic ovarian syndrome (Doctoral dissertation).

[17] Broekmans FJ, Fauser BC. Diagnostic criteria for polycystic ovarian syndrome. Endocrine. 2006 Aug; 30(1):3-11.

[18] Casadei L, Madrigale A, Puca F, Manicuti C, Emidi E, Piccione E, Dewailly D. The role of serum anti-Müllerian hormone (AMH) in the hormonal diagnosis of polycystic ovary syndrome. Gynecological Endocrinology. 2013 Jun 1; 29(6):545-50.

[19] Tal R, Tal O, Seifer BJ, Seifer DB. Antimüllerian hormone as predictor of implantation and clinical pregnancy after assisted conception: a systematic review and meta-analysis. Fertility and sterility. 2015 Jan 1; 103(1):119-30.

[20] Saleem F, Rizvi SW. New therapeutic approaches in obesity and metabolic syndrome associated with polycystic ovary syndrome. Cureus. 2017 Nov 13;9(11).