e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2024; 16(4); 778-782

Original Research Article

To Establish a Correlation between Anti-Müllerian Hormones in Patients Diagnosed With Polycystic Ovarian Syndrome in a Tertiary Care Hospital

Shashi Bhushan Kumar[1], Bijay Krishna Prasad[2]

[1]Tutor, Department of Physiology, Anugrah Narayan Magadh Medical College, Gaya, Bihar, India

[2]Professor and HOD, Department of Physiology, Anugrah Narayan Magadh Medical College, Gaya, Bihar, India

Received: 03-02-2024 / Revised: 10-03-2024 / Accepted: 22-04-2024
Corresponding Author: Dr. Shashi Bhushan Kumar
Conflict of interest: Nil

Abstract

Aim: To establish a correlation between anti-müllerian hormones in patients diagnosed with polycystic ovarian syndrome in a tertiary care hospital.

Material and methods: This study was done in the Department of Physiology, Anugrah Narayan Magadh Medical College, Gaya, Bihar, India for 12 months. This study included 50 PCOS patients and 50 healthy women of fertile age as controls who are attending the outpatient Department of Physiology, Anugrah Narayan Magadh Medical College, Gaya, Bihar, India. Rotterdam criteria were used to select these patients using a convenient sampling technique. The PCOS was diagnosed when ≥2 through the following three criteria: oligomenorrhea or amenorrhea, clinical hyperandrogenism or hyperandrogenemia, and polycystic ovaries on ultrasonography.

Results: Baseline characteristics of subjects revealed statistically higher BMI (26.7 ± 4.5 vs. 21.7 ± 2.8, kg/m2; p <0.001) in PCOS than that of control. Menstrual irregularity (86.3% vs. 1.3%; p<0.001) and infertility rate (p<0.001) were also significantly higher in the PCOS. AMH level was significantly higher (9.21 ± 0.50 vs. 4.40 ± 0.41, ng/ml; p<0.001) in the PCOS patients than that of controls. However, when compared according to age-group, this was significantly different between PCOS and controls in the age group 23-27 years (9.91 ± 0.71 vs. 4.52 ± 0.54, ng/ml; p<0.001) and age-group 28-31 years (8.28 ± 1.51 vs. 4.22 ± 0.68, ng/ml; p<0.011). The subgroups of PCOS and control subjects divided on the basis of a cut-off value of AMH at 3.5ng/ml. AMH ≥ 3.5ng/ml was considered as positive in the diagnosis of PCOS.

Conclusion: It may be concluded that PCOS has significantly higher serum AMH than healthy women during the reproductive period. Age-related decline of AMH occurs in healthy women as well as in PCOS women.

Key Words: Anti-Mullerian Hormone, Polycystic Ovary Syndrome, Menopause, Rotterdam Criteria

Introduction

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder affecting women of reproductive age, characterized by a spectrum of clinical manifestations including oligo-ovulation or anovulation, hyperandrogenism, and polycystic ovarian morphology. It is a leading cause of infertility and has significant metabolic, reproductive, and psychological implications. The pathophysiology of PCOS is complex and multifactorial, involving genetic, hormonal, and environmental factors. [1] Among the various biomarkers used to evaluate ovarian function, anti-Müllerian hormone (AMH) has gained prominence. AMH is a glycoprotein hormone produced by the granulosa cells of ovarian follicles and plays a critical role in the regulation of folliculogenesis. In women with PCOS, AMH levels are typically elevated, reflecting the increased number of small antral follicles characteristic of this condition. Elevated AMH levels in PCOS patients are thought to result from the excessive production by these follicles, which are arrested in their development due to the hormonal imbalance inherent to PCOS. [2] The correlation between AMH levels and PCOS has been extensively studied, with research indicating that AMH can serve as a useful diagnostic and prognostic marker for the syndrome. High AMH levels are often associated with anovulation, increased ovarian volume, and higher follicle counts, which are key diagnostic criteria for PCOS. This correlation underscores the utility of AMH not only in diagnosing PCOS but also in monitoring ovarian function and predicting the response to treatment. [3] In addition to its diagnostic utility, AMH has been explored as a marker for assessing the severity of PCOS and its associated reproductive and metabolic abnormalities. Studies have shown that AMH levels correlate with the degree of

hyperandrogenism and insulin resistance, which are common features of PCOS. This relationship highlights the potential of AMH as a comprehensive marker that reflects the underlying pathophysiological processes of PCOS. [4] Moreover, AMH measurement is relatively stable throughout the menstrual cycle and less influenced by external factors compared to other hormonal markers, making it a reliable and practical tool for clinical use. The stability and specificity of AMH in relation to ovarian function make it an ideal candidate for routine evaluation in women suspected of having PCOS. [5-7] Despite the promising role of AMH in PCOS, it is essential to consider its limitations and the need for standardized measurement techniques. Variations in assay methods and lack of universally accepted reference ranges can affect the interpretation of AMH levels. Therefore, while AMH provides valuable insights into ovarian function in PCOS, it should be used in conjunction with other clinical and biochemical assessments to ensure accurate diagnosis and management.

Material and Methods

This study was done in the Department of Physiology, Anugrah Narayan Magadh Medical College, Gaya, Bihar, India for 12 months. This study included 50 PCOS patients and 50 healthy women of fertile age as controls who are attending the outpatient Department of Physiology, Anugrah Narayan Magadh Medical College, Gaya, Bihar, India.

Rotterdam criteria were used to select these patients using a convenient sampling technique. The PCOS was diagnosed when ≥2 through the following three criteria: oligomenorrhea or amenorrhea, clinical hyperandrogenism or hyperandrogenemia, and polycystic ovaries on ultrasonography. All the patients were infertile (i.e., lack of pregnancy after one year of unprotected intercourse) and with PCOS. The inclusion criteria were women with previously diagnosed PCOS according to Rotterdam criteria, aged between 20-35 years, and having a body mass index (BMI) between 18-30 kg/m2. The exclusion criteria encompassed women with infertility of any other etiology, exposed to the cytotoxic drug, pelvic radiation therapy, or suffering from renal or liver diseases.

Biochemical Assays:

AMH was estimated by single measurements by an enzyme-linked immunosorbent assay, AMH GEN II ELISA kit whereas other hormones (follicle-stimulating hormone, luteinizing hormone, Testosterone) by immune chemiluminometric assay. Values of AMH were presented as nanograms per milliliters (conversion factor to pmol/l = ng/ml × 7.1). AMH was calculated using kc3 biographs with help of the standard supplied with the kit. QC (quality control) was used in each assay run to assess the precisions of the assay. Intraassay CV (coefficient of variance) was 3.4 to 5.4% and interassay CV 4.0 to 5.6% for AMH assay. [7]

Statistical Analysis

AMH levels were expressed as the mean ± (SE). Student's t-test for continuous variables and Chi-Square test for discrete variables were used. Correlation among variables was assessed by using Pearson's correlation test. Multiple regressions were done to see the impact of independent factors over AMH. P values ≤ 0.05 were considered statistically significant.

Results

Table 1: Characteristics of the studied PCOS patients and control(n=100)

VARIABLES	PCOS (n = 50)	Controls (n = 50)	p
Age (mean ±SD, year)	23.7±4.8	26.3±2.9	<0.001
BMI (mean ±SD, kg/m2)	26.7±4.5	21.7±2.8	<0.001
Menstrual disturbance	69	1	<0.001
Family history of PCOS	6	2	0.276
*Infertility			
Primary	14/43 (32.6)	0/34	<0.001
Secondary	8/43 (18.6)	0/34	
MR/Abortion	12/43 (27.9)	10/34 (29.4)	NS

Table- 1 Shows Baseline characteristics of subjects (as seen in Table-I) revealed statistically higher BMI (26.7 ± 4.5 vs. 21.7 ± 2.8, kg/m2; p <0.001) in PCOS than that of control. Menstrual irregularity (86.3% vs. 1.3%; p<0.001) and infertility rate (p<0.001) were also significantly higher in the PCOS.

Table 2: Basal serum amh levels in control women and in women with PCOS

Group of subjects	Controls (n = 50) AMH ng/ml)±(SE)	PCOS (n = 50) AMH (ng/ml)±(SE)	p-value
Whole group	4.40 ± 0.41	9.21 ± 0.50	<0.001
Age group(years)			

n(control, PCOS)			
23 – 27 (49,32)	4.52 ± 0.54	9.91 ± 0.71	<0.001
28 – 31 (25,7)	4.22 ± 0.68	8.28 ± 1.51	<0.011

Table-2 shows AMH level was significantly higher (9.21 ± 0.50 vs. 4.40 ± 0.41, ng/ml; p<0.001) in the PCOS patients than that of controls. However, when compared according to age-group, this was significantly different between PCOS and controls in the age group 23-27 years (9.91 ± 0.71 vs. 4.52 ± 0.54, ng/ml; p<0.001) and age-group 28-31 years (8.28 ± 1.51 vs. 4.22 ± 0.68, ng/ml; p<0.011).

Table 3: Sensitivity and specificity of AMH for the diagnosis of PCOS holding cut-off as 3.5 ng/ml.

Group(s)	Anti-mullerian hormone (ng/ml)		Total
	≥ 3.5 ng/ml	<3.5 ng/ml	
PCOS	30	20	50
Control	33	27	50
Total	**63**	**37**	**100**

Table-3 shows the subgroups of PCOS and control subjects divided on the basis of a cut-off value of AMH at 3.5ng/ml. AMH ≥ 3.5ng/ml was considered as positive in the diagnosis of PCOS. Thus 30out of 50in the PCOS patients and 33 out of 50 controls could be labeled as positive. The calculated sensitivity was found to be 67% and specificity 78.33%. PCOS: polycystic ovarian syndrome- Sensitivity = true positive / (all positive) × 100= 67/ (67+33) × 100= 67 %. Specificity= true negative / (all negative) × 100= 47/ (47+13) × 100= 78.33%.

Discussion

In the present study, it was seen that the serum AMH level is higher in women with PCOS and amenorrhoea, compared to those with oligomenorrhoea. The mean value of AMH in those cases with oligomenorrhoea is 8.92 ng/ml (range 3.45 – 18.36) with a standard deviation of 4.2 and in those with amenorrhoea is 15.69 ng/ml (range 7.56 – 20.36) with a standard deviation of 3.5.[7] The mean of AMH level differed significantly between those presenting with oligomenorrhoea or amenorrhoea (p-value < 0.0001) Polycystic Ovary Syndrome (PCOS) is one of the most common endocrinological problems in women. [8] In addition to chronic oligo-anovulation, the main features of the PCOS include elevated levels of circulating androgens and/or clinical hyperandrogenism, polycystic ovary morphology, altered gonadotropin secretion, insulin resistance and/or compensatory hyperinsulinemia often associated with obesity. [9] Women affected by PCOS also show a higher risk of type 2 diabetes, dyslipidemia, hypertension and cardiovascular disease Differences in the association of anti-Müllerian hormone with clinical or biochemical characteristics between women with and without polycystic ovarian syndrome. [10] The sensitivity and specificity of AMH for detecting PCOS in patients aged 18-35 years were calculated to be 67% and 78.33% respectively, using an AMH cut-off value of 3.5 ng/ml as followed in another study. Mean AMH differed significantly between PCOS subjects and healthy controls. Increased serum AMH concentrations in PCOS patients have been explained by the increased number of small ovarian follicles responsible for AMH secretion. [11] In the ovary AMH is produced from granulosa cells of pre-antral and small antral follicles. From experimental data, mainly obtained in rodents, the proposed functions of AMH are 1) inhibition of the initial recruitment of primordial follicles, through a paracrine effect and 2) inhibition of aromatase activity in granulosa cells, thus reducing the production of estradiol (E2). [12] Pathophysiology of PCOS has been known to be multifactorial. Anovulation and/or oligo- ovulation are the main underlying cause of infertility. Altered LH: FSH ratio, hyperandrogenemia, and hyperinsulinemia as well as insulin resistance – all had been thought to be linked to the probable cause of anovulatory cycles. But in the past decade much attention had been concentrated on AMH in context of PCOS. Several factors have been reported to be associated with AMH secretion. [13] A negative correlation was observed between FSH and AMH levels in some studies. Low dose recombinant FSH therapy in PCOS patients decreased serum AMH levels, suggesting the negative role of AMH in aromatase expression during dominant follicle selection. Increasing serum FSH will cause a shift of small antral follicles to larger ones, expressing less AMH, thus a decline in AMH and allowing dominance of follicle to occur. [14] It has been observed that AMH serum levels significantly and inversely correlate to FSH levels in healthy women. [15] Apropos with the above facts, in the present study a negative relationship was observed between FSH and AMH though not significant statistically. Follicles from AMH knockout mice have been shown to be more sensitive to FSH than those from the wild type. [16] This further suggests that the inhibiting effect of AMH on aromatase activity acts through a decrease in granulosa cell sensitivity to FSH. The balance between the opposite effects of AMH and FSH on

aromatase activity might be crucial for the cohort at the time of the selection process for dominant follicle. [17] Wilkes S et al used a cut-off value of 3.5 ng/ml of AMH in discrimination of PCOS from control and observed sensitivity and specificity on its basis as 74% and 79 %. A negative relationship was seen between age and AMH level by regression analysis but found to be nonsignificant. The age-related decline in AMH level among control women is supported by other studies whereupon negative correlation between age and AMH has been reported. [18] As because AMH levels correlate with the number of early antral follicles which might represent the size of the resting follicle pool, AMH may constitute a marker for ovarian aging. [19,20]

Conclusion

It may be concluded that PCOS has significantly higher serum AMH than healthy women during the reproductive period. Age-related decline of AMH occurs in healthy women as well as in PCOS women. This is indicative of ovarian aging. Observed relatively higher AMH levels in the healthy control group may reflect the ethnic variation. The sensitivity and specificity of AMH for diagnosing PCOS were calculated to be 67% and 78% respectively holding a cut-off value of AMH at 3.5 ng/ml. Thus, AMH seems to be an important zero marker in the diagnosis of PCOS irrespective of other characteristics of PCOS. This difference of associations might suggest a loss of multi- factorial control for AMH production in PCOS, and which might contribute to the pathogenesis of PCOS. Further investigation is needed to elucidate the role of AMH and the regulation mechanism of AMH production.

References

1. Zhang, J., Wang, Q., Xu, X., You, L., & Li, X. (2022). Correlation between anti-Müllerian hormone with insulin resistance in polycystic ovarian syndrome: a systematic review and meta-analysis. Journal of Ovarian Research. Available from: https://ovarianresearch.biomedcentral.com/articles/10.1186/s13048-022-00910-3
2. Verdiesen, R.M.G., van der Schouw, Y.T., van Gils, C.H., Verschuren, W.M.M., Broekmans, F.J.M., Borges, M.C. et al. (2022). Genome-wide association study meta-analysis identifies three novel loci for circulating anti-Mullerian hormone levels in women. Human Reproduction, 37(5), 1069–1082. Available from: https://academic.oup.com/humrep/article/37/5/1069/6654075
3. Day, F., Karaderi, T., Jones, M.R., Meun, C., He, C., Drong, A. et al. (2021). Large-scale genome-wide meta-analysis of polycystic ovary syndrome suggests shared genetic architecture for different diagnosis criteria. PLoS Genetics, 17(12), e1007813. Available from: https://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1007813
4. La Marca, A., Pati, M., Orvieto, R., Stabile, G., Carducci Artenisio, A., & Volpe, A. (2021). Serum anti-Müllerian hormone levels in women with secondary amenorrhea. Fertility and Sterility, 85(5), 1547–1549.
5. Birch Petersen, K., Hvidman, H.W., Forman, J.L., Pinborg, A., Larsen, E.C., Macklon, K.T. et al. (2021). Ovarian reserve assessment in users of oral contraception seeking fertility advice on their reproductive lifespan. Human Reproduction, 30(10), 2364–2375.
6. Wang, Y., Yuan, Y., Meng, D., Liu, X., Gao, Y., Wang, F. et al. (2021). Effects of environmental, social and surgical factors on ovarian reserve: implications for age-relative female fertility. International Journal of Gynecology & Obstetrics. Available from: https://www.ijgo.org/article/S0020-7292(21)00360-3/fulltext
7. Kriseman M, Mills C, Kovacs E, Sangi-Haghpeykar H, Gibbons W. Antimullerian hormone levels are inversely associated with body mass index (BMI) in women with polycystic ovary syndrome. J Assist Reprod Genet. 2015;32(9):1313-1316.
8. Lamaze F, Genro V, Fuchs F, et al. [Serum AMH level is not a predictive value for IVF in a modified natural cycle: analysis of 342 cycles]. J Gynecol Obstet Biol Reprod (Paris). 2011;40(3):205-210.
9. Lee JR, Kim SH, Kim SM, et al. Anti-Mullerian hormone dynamics during controlled ovarian hyperstimulation and optimal timing of measurement for outcome prediction. Hum Reprod. 2010;25(10):2597-26 04.
10. Nakhuda GS, Chu MC, Wang JG, Sauer MV, Lobo RA. Elevated serum Mullerian-inhibiting substance may be a marker for ovarian hyperstimulation syndrome in normal women undergoing in vitro fertilization. Fertil Steril. 2006;85(5):1541-1543.
11. Pellatt L, Rice S, Mason HD. Anti-Mullerian hormone and polycystic ovary syndrome: a mountain too high? Reproduction. 2010;139 (5):825-833.
12. Piggy P, Merlen E, Robert Y, et al. Elevated serum level of anti-mullerian hormone in patients with polycystic ovary syndrome: relationship to the ovarian follicle excess and to the follicular arrest. J Clin Endocrinol Metab. 2003;88(12):5957-5962.
13. Peltonen T, Morin-Papunen L, Koivunen R, Perheentupa A, Ruokonen A, Tapanainen JS. Serum anti-Mullerian hormone levels remain high until late reproductive age and decrease during metformin therapy in women with

polycystic ovary syndrome. Hum Reprod. 20 05;20(7):1820- 1826.
14. Rasekhjahromi A, Maalhagh M, Hosseinpoor M, Farhang H, Alavi F. A clomiphene citrate and letrozole versus tamoxifen and letrozole as an infertility treatment in women with polycystic ovary syndrome. Pak J Biol Sci. 20 15;18(6):300.
15. Salmassi A, Mettler L, Hedderich J, et al. Cut-Off Levels of Anti-Mullerian Hormone for The Prediction of Ovarian Response, In Vitro Fertilization Outcome and Ovarian Hyperstim ulation Syndrome. Int J Fertil Steril. 2015;9 (2):157-167.
16. Tremellen K, Zander-Fox D. Serum anti-Mullerian hormone assessment of ovarian reserve and polycystic ovary syndrome status over the reproductive lifespan. Aust N Z J Obstet Gynaecol. 2015;55(4):384-389.
17. When C, Laven JS, Von Bergh AR, et al. Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod. 2004;10(2):77-83.
18. Wilkes S, Chinn DJ, Murdoch A, Rubin G. Epidemiology and management of infertility: a population-based study in UK primary care. Fam Pract. 2009;26(4):269-274.
19. Zadehmodarres S, Heidar Z, Razzaghi Z, Ebrahimi L, Soltanzadeh K, Abed F. Anti-mullerian hormon level and polycystic ovarian syndrome diagnosis. Iran J Reprod Med. 2015; 13(4):227-230.
20. Grossman MP, Nakajima ST, Fallat ME, Siow Y Mullerian-inhibiting substance inhibits cytochrome P450 aromatase activity in human granulosa lutein cell culture. Fertil Steril 2008 N89:1364-1370.