ORIGINAL ARTICLE

ANTI-MÜLLERIAN HORMONE: A BETTER BIO MARKER FOR ASSESSMENT OF ANOVULATION IN POLYCYSTIC OVARIAN SYNDROME COMPARED TO CONVENTIONAL BIOMARKERS

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Background: Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder affecting women of childbearing age. The objective of this study was to determine the diagnostic accuracy of Anti-Müllerian Hormone (AMH) in predicting anovulatory PCOS, keeping day 21 progesterone level as gold standard. **Methods:** This cross-sectional validation study was conducted on 289 women aged 15–30 years with PCOS, from 11 Aug 2021 to 10 Feb 2022, in Pathology Department, Bahawal Victoria Hospital, Bahawalpur. AMH levels were measured and anovulatory events documented. AMH levels were compared with progesterone levels on the 21st day of the menstrual cycle. **Results:** In the cohort of individuals demonstrating a positive presence of AMH, a total of 149 were accurately identified as true positives, while 6 were mistakenly classified as false positives. Within the group of patients exhibiting a negative status for AMH, 8 were incorrectly categorized as false negatives, whereas 126 were correctly identified as true negatives. When evaluating the diagnostic efficacy of AMH as a predictor of anovulatory PCOS, with the 21st day progesterone measurement, the following metrics were ascertained: a commendable sensitivity of 94.90%, a robust specificity of 95.45%, a notable positive predictive value of 96.13%, a substantial negative predictive value of 94.03%, and an impressive overall diagnostic accuracy of 95.16%. **Conclusion:** The diagnostic accuracy of Anti-Müllerian hormone in predicting anovulatory PCOS is very high.

Keywords: Anti-Müllerian Hormone, AMH, Polycystic ovarian syndrome, PCOS, Sensitivity

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INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder affecting women of childbearing age, with varying prevalence among different ethnic groups and age ranges. British women aged 20–25 years have a 33% prevalence[1], while Finnish women under 36 have 21.6%[2]. South Asian, particularly Pakistani women, have a striking 52% prevalence, surpassing white populations in the UK, which range from 20% to 25%.[3]

The Rotterdam criteria states that diagnosis, requiring at least two of these: anovulation or oligo-, hyperandrogenism, and polycystic ovaries.[4] PCOS can be categorized into four types based on historical records and physical examinations: Complete PCOS, polycystic ovaries plus anovulation/oligo-, polycystic ovaries plus hyperandrogenism, and anovulation/oligo- plus hyperandrogenism.[5] Clinical manifestations include hyperandrogenism, insulin resistance, and sarcopenic obesity. Insulin resistance and hyperinsulinemia contribute to hyperandrogenism, increasing the risk of gestational diabetes, pregnancy-induced hypertension, preterm birth, and complications during pregnancy.[6]

PCOS often leads to elevated serum Anti-Müllerian Hormone (AMH) levels due to multiple active antral follicles. The use of AMH as a diagnostic indicator remains controversial, with some suggesting an AMH level >3.8–5 ng/mL. Combining the Rotterdam criteria and AMH levels can aid in early and accurate diagnosis.[7]

Approximately 60% of PCOS women have elevated AMH values, associated with reduced pregnancy success rates during controlled intrauterine insemination cycles.[8] The presence of two out of three clinical attributes (AMH, Hyperandrogenism, and Oligo-anovulation) demonstrates strong sensitivity (96%) and specificity (100%) in diagnosing PCOS based on the Rotterdam criteria.[9]

The use of AMH levels as a diagnostic marker for early detection of PCOS, particularly among unmarried women of reproductive age group in Pakistan has garnered significant attention as compared to transvaginal ultrasonogram. This study aims to elucidate the patterns and practicality of serum AMH levels as a supplementary diagnostic tool within the context of the Rotterdam criteria for women diagnosed with PCOS.

MATERIAL AND METHODS

This was a cross-sectional validation study done at the Department of Pathology, Bahawal Victoria Hospital, Bahawalpur from 11 Aug 2021 to 10 Feb 2022. It involved a cohort of 289 female individuals aged 15–30 years, with a diagnosis of PCOS. The sample size was determined as 289 through OpenEpi online software, with a 95% confidence interval, a margin of error at 6.3%, a prevalence rate of anovulatory PCOS at 25%,

and sensitivity and specificity values of elevated AMH levels for predicting anovulatory PCOS at 92% and 97% respectively, using a non-probability, consecutive sampling method. Individuals with Cushing's disease, chronic renal failure, hypothyroidism, congenital adrenal hyperplasia, or tumours were excluded from the study.

After approval from Institutional Ethical Review Committee, a total of 289 cases meeting the inclusion criteria were selected, with informed consent. Serum AMH levels were estimated, and a record was maintained regarding the presence or absence of ovulation. The values of AMH levels were subjected to a comparative analysis alongside the results of serum progesterone levels estimated on the 21st day of the menstrual cycle. Data including weight, age, obesity, height, body mass index (BMI), anovulation as indicated by serum AMH levels, and the presence or absence of anovulation determined by serum 21st day progesterone levels (absent/present) were recorded using a specially designed proforma.

The data were analysed using SPSS-25. Mean and standard deviation were computed for variables such as age, duration of PCOS, height, weight, BMI, serum AMH level, and serum 21st day progesterone levels. Parameters such as obesity and the presence or absence of anovulation, both based on serum AMH and serum 21st day progesterone levels, were given as percentages and frequencies. Specificity, sensitivity, negative predictive value, positive predictive value, and the diagnostic accuracy of elevated Anti-Müllerian hormone in predicting anovulation in PCOS were calculated using 2×2 contingency table. Stratification was conducted for variables such as age, BMI, and obesity, with diagnostic accuracy subsequently calculated after stratification.

RESULTS

The age range of the patients spanned from 15 to 30 years, with a mean age of 25.52±2.55 years. The majority (200, 69.2%) of the patients were 15 to 25 years of age. The average height among the participants was 154.33±11.23 Cm, and the mean weight was 71.24±8.98 Kg. The BMI was calculated as 28.99±3.47 Kg/m². The average duration of PCOS was 8.93±4.32 months. Mean concentration of Anti-Müllerian Hormone was 41.55 pmol/L, and the mean levels of 21st day progesterone measured 8.25 ng/mL.

Among patients who 's serum AMH was more than cut-off (4.9 ng/ml), a total of 149 individuals were correctly identified as true positive, while 6 were inaccurately classified as false positive. In contrast, within the group of patients who tested negative for AMH, 8 were erroneously categorized as false negative, while 126 were accurately identified as true negative.

Evaluation of the diagnostic capability of Anti-Müllerian Hormone produced a sensitivity of 94.90%, specificity of 95.45%, positive predictive value of 96.13%, negative predictive value of 94.03%, and an overall diagnostic accuracy of 95.16%.

Table-1: Stratification of diagnostic accuracy

Variables	AMH Level*	21st day progesterone level		*p*
		Positive	Negative	
Anti-Müllerian hormone diagnostic accuracy in predicting anovulatory PCOS, keeping 21st day progesterone as gold standard of ovulation	>cut-off	149 (TP)	6 (FP)	0.0001
	<cut-off	8 (FN)	126 (TN)	
Diagnostic accuracy in the context of the age group 15–25 years (n=200)	>cut-off	106 (TP)	3 (FP)	0.001
	<cut-off	6 (FN)	85 (TN)	
Diagnostic accuracy in the context of the age group 26–30 years (n=89)	>cut-off	43 (TP)	3 (FP)	0.001
	<cut-off	2 (FN)	41 (TN)	
Diagnostic accuracy in the context of the BMI ≤30 Kg/m² (n=186)	>cut-off	103 (TP)	6 (FP)	0.001
	<cut-off	3 (FN)	74 (TN)	
Diagnostic accuracy with respect to BMI ≤30 Kg/m² (n=103)	>cut-off	46 (TP)	0 (FP)	0.001
	<cut-off	5 (FN)	52 (TN)	
Diagnostic accuracy in the context of the non-obese (n=186)	>cut-off	103 (TP)	6 (FP)	0.001
	<cut-off	3 (FN)	74 (TN)	
Diagnostic accuracy with respect to obese (n=103)	>cut-off	46 (TP)	0 (FP)	0.001
	<cut-off	5 (FN)	52 (TN)	

*cut-off value for AMH=4.9 ng/ml

Table-2: Anti-Müllerian Hormone diagnostic accuracy in predicting anovulatory PCOS, keeping 21st day progesterone as gold standard

Variable	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy
Anti-Müllerian Hormone diagnostic accuracy in predicting anovulatory PCOS	94.90%	95.45%	96.13%	94.03%	95.16%
Diagnostic accuracy in age group 15–25 years (n=200)	94.64%	96.59%	97.25%	93.41%	95.50%
Diagnostic accuracy in age group 26–30 years (n=89)	95.56%	93.18%	93.48%	95.35%	94.38%
Diagnostic accuracy in BMI ≤30 Kg/m² (n=186)	97.17%	92.50%	94.50%	96.10%	95.16%
Diagnostic accuracy with respect to BMI ≤30 Kg/m² (n=103)	90.20%	100.0%	100.0%	91.23%	95.15%
Diagnostic accuracy in non-obese (n=186)	97.17%	92.50%	94.50%	96.10%	95.16%
Diagnostic accuracy in obese (n=103)	90.20%	100.0%	100.0%	91.23%	95.15%

DISCUSSION

This study was undertaken to assess the diagnostic precision of Anti-Müllerian Hormone in predicting anovulatory PCOS, with the 21st day progesterone measurement serving as the reference standard. The study revealed that AMH exhibited a sensitivity of 94.90%, specificity of 95.45%, positive predictive value of 96.13%, negative predictive value of 94.03%, and an overall diagnostic accuracy of 95.16% in predicting anovulatory PCOS. In another study[10], prevalence of anovulatory PCOS was established as 25%, and the sensitivity and specificity of elevated AMH levels in forecasting anovulatory PCOS were 92% and 97% respectively. Pigny *et al*[11] reported that serum AMH measurement achieved a specificity of 92% and sensitivity of 67%. Lin *et al*[12] identified a cut-off AMH level of 7.3 ng/mL, which conferred a specificity of 76% and sensitivity of 70% for predicting PCOS. The strong correlation between AMH and Antral Follicle Count (AFC) has prompted some researchers to compare their performance in the diagnosis of PCOS.[12] Butt *et al*[13] showed. AMH levels tend to increase with weight, menstrual abnormalities, and hirsutism. LH was the only reproductive hormone that increased with the elevation of serum AMH levels among PCOS women.[13]

Nonetheless, the findings within the literature exhibit a lack of uniformity. A portion of this variation arises from the absence of a clearly defined study population.[14] Notably, some researchers have adhered to the PCOS definition established during the Rotterdam Conference of 2003, which specifies the presence of 12 follicles measuring 2–9 mm in diameter per ovary as the criterion for diagnosing polycystic ovary morphology (PCOM).[15] It is crucial to acknowledge that this specific cut-off is heavily reliant on the quality of ultrasound equipment and the skill of the operator, as observed by Dewailly *et al*[8]. Consequently, with the introduction of more advanced ultrasound technologies and equipment in recent times, this threshold has undergone modifications and can now range from 19 to 25 follicle per ovary. This threshold is likely to continue evolving with the ongoing development of ultrasound technologies.[16] Significant issues pertain to the criteria for including or excluding specific populations from the normative reference group, contributing to the observed heterogeneity in the results.[17] Lastly, technical concerns related to serum Anti-Müllerian Hormone assays further contribute to the variability in the literature. Consequently, to date, it remains unfeasible to establish a universally accepted and unanimous diagnostic threshold for serum AMH in the prediction of PCOS.[18] However, we have found that a threshold of 35 pmol/L or 4.9 (ng/mL) using the enzyme immunoassay AMH-EIA exhibits a commendable specificity and sensitivity of 97% and 92% respectively when compared to Antral Follicle Count (AFC) in predicting PCOs. This outcome was achieved after excluding women with asymptomatic Polycystic Ovaries (PCO) from the control group through the application of cluster analysis.[19]

Pigny *et al*[11] have undertaken a comparison of the five different serum Anti-Müllerian Hormone assays, as described previously, for the purpose of diagnosing PCOS. Their recommendations include a higher cut-off of 5.6 ng/mL or 40 pmol/L when employing manual ELISA assays. This particular threshold is considered biologically indicative of Polycystic Ovary Morphology (PCOM) and corresponds to the 95th percentile of individuals classified as 'pure' controls. In addition, they have suggested a threshold of 4.2 ng/mL (30 pmol/L) for the automatic assays.[11]

If these findings are subsequently validated with the application of new automated serum AMH assays or an ultrasensitive assay, it is conceivable that a heightened serum AMH level could emerge as a dependable and precise marker for PCOM. This could potentially supplant Antral Follicle Count (AFC), which is also a subject of significant debate in the scientific literature.[20] The level of serum Anti-Müllerian Hormone is also associated with the intensity of symptoms in Polycystic Ovary Syndrome, and it tends to be higher when hyperandrogenism or oligo-anovulation is present.[21] Through a principal component analysis, it has been demonstrated that a markedly elevated serum AMH level can serve as an indicator of hyperandrogenism and could help harmonize the diverse PCOS classifications currently in use.[22]

In the case of adolescents and young women diagnosed with PCOS, assessing the ovaries via ultrasonography can sometimes be a challenging task. In such scenarios, serum AMH assays emerge as a viable alternative, a recommendation put forth by the American Association of Clinical Endocrinologists.

CONCLUSION

Anti-Müllerian Hormone exhibits a notably high level of diagnostic accuracy in the prediction of anovulatory Polycystic Ovary Syndrome. It is recommended that Anti-Müllerian Hormone be employed as the primary diagnostic test for anticipating anovulation in PCOS and ultimately contributing to a reduction in the morbidity experienced by these specific patients.

REFERENCES

1. Michelmore KF, Balen AH, Dunger DB, Vessey MP. Polycystic ovaries and associated clinical and biochemical features in young women. Clin Endocrinol 1999;51(6):779–86.
2. Koivunen R, Laatikainen T, Tomas C, Huhtaniemi I, Tapanainen J, Marti-kainen H. The prevalence of polycystic ovaries in healthy women. Acta Obstet Gynecol Scand 1999;78(2):137–41.

3. Akram M, Roohi N. Endocrine correlates of polycystic ovary syndrome in Pakistani women. J Coll Physicians Surg Pak 2015;25(1):22–6.
4. Stein IF, Leventhal ML. Amenorrhea is associated with bilateral polycys- tic ovaries. Am J Obstet Gynecol 1935;29(2):181–91.
5. Sobti S, Dewan R, Ranga S. Metabolic syndrome and insulin resistance in PCOS phenotypes. Int J Reprod Contracept Obstet Gynecol 2017;6(11):5067–73.
6. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes 1989;38(9):1165–74.
7. Di Paola R, Garzon S, Giuliani S, Laganà AS, Noventa M, Parissone F, *et al*. Are we choosing the correct FSH starting dose during controlled ovarian stimulation for intrauterine insemination cycles? Potential application of a nomogram based on woman's age and markers of ovarian reserve. Arch Gynecol Obstet 2018;298(5):1029–35.
8. Dewailly D, Gronier H, Poncelet E, Robin G, Leroy M, Pigny P, *et al*. Diagnosis of polycystic ovary syndrome (PCOS): revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the definition of polycystic ovaries. Hum Reprod 2011;26(11):3123–9.
9. Weerakiet S, Lertvikool S, Tingthanatikul Y, Wansumrith S, Leelaphiwat S, Jultanmas R. Ovarian reserve in women with polycystic ovary syndrome who underwent laparoscopic ovarian drilling. Gynecol Endocrinol 2007;23(8):455–60.
10. Begawy AF, El-Mazny AN, Abou-Salem NA, El-Taweel NE. Anti-Müllerian hormone in polycystic ovary syndrome and normo-ovulatory women: correlation with clinical, hormonal and ultrasonographic parameters. Middle East Fertil Soc J 2010;15(4):253–8.
11. Pigny P, Jonard S, Robert Y, Dewailly D. Serum anti-Müllerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. J Clin Endocrinol Metab 2006;91:941–5.
12. Lin YH, Chiu WC, Wu CH, Tzeng CR, Sen Hsu C, Hsu MI. Anti-Müllerian hormone and polycystic ovary syndrome. Fertil Steril 2011;96(1):230–5.
13. Butt MS, Saleem J, Aiman S. Zakar R, Sadique I, Fischer F. Serum anti-Müllerian hormone as a predictor of polycystic ovarian syndrome among women of reproductive age. BMC Women's Health 2022;22:199.
14. Eilertsen TB, Vanky E, Carlsen SM. Anti-Müllerian hormone in the diagnosis of polycystic ovary syndrome: can morphologic description be replaced? Hum Reprod 2012;27(8):2494–502.
15. Iliodromiti S, Kelsey TW, Anderson RA, Nelson SM. Can anti-Müllerian hormone predict the diagnosis of polycystic ovary syndrome? A systematic review and meta-analysis of extracted data. J Clin Endocrinol Metab 2013;98(8):3332–40.
16. Tenajas R, Miraut D, Illana CI, Alonso-Gonzalez R, Arias-Valcayo F, Herraiz JL. Recent advances in artificial intelligence-assisted ultrasound scanning. Appl Sci 2023;13(6):3693.
17. Dewailly D, Alebic MS, Duhamel A, Stojanovic N. Using cluster analysis to identify a homogeneous subpopulation of women with polycystic ovarian morphology in a population of non-hyperandrogenic women with regular menstrual cycles. Hum Reprod 2014;29(11):2536–43.
18. Dewailly D, Lujan ME, Carmina E, Cedars MI, Laven J, Norman RJ, *et al*. Definition and significance of polycystic ovarian morphology: a task force report from the Androgen Excess and Polycystic Ovary Syndrome Society. Hum Reprod Update 2014;20(3):334–52.
19. Asada Y, Tsuiki M, Sonohara M, Fukunaga N, Hattori Y, Inoue D, *et al*. Performance of anti-Müllerian hormone (AMH) levels measured by Beckman Coulter Access AMH assay to predict oocyte yield following controlled ovarian stimulation for *in vitro* fertilization. Reprod Med Biol 2019;18(3):273–7.
20. Tadros T, Tarasconi B, Nassar J, Benhaim JL, Taieb J, Fanchin R. New automated antimüllerian hormone assays are more reliable than the manual assay in patients with reduced antral follicle count. Fertil Steril 2016;106(7):1800–6.
21. Sivanandy MS, Ha SK. The role of serum Anti-Müllerian hormone measurement in the diagnosis of polycystic ovary syndrome. Diagnostics (Basel) 2023;13(5):907.
22. Tal R, Seifer CM, Khanimov M. Seifer DB, Tal O. High serum Antimüllerian hormone levels are associated with lower live birth rates in women with polycystic ovarian syndrome undergoing assisted reproductive technology. Reprod Biol Endocrinol 2020;18(1):20.

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ABS: Drafting and Acquisitions and Analysis

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