Original Article

The Significance of Anti-Mullerian Hormone in the Identification of Polycystic Ovarian Syndrome among Pakistani Women

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Abstract

Objective: To assess the precision of serum Anti-Müllerian hormone (AMH) levels in identifying cases of polycystic ovary syndrome (PCOS), and to ascertain its potential as an alternative for the evaluation of PCOM (polycystic ovarian morphology) as outlined in the Rotterdam norms.
Methodology: The research was conducted at Pak General Hospital Peshawar from January 2023 to June 2023, The ethical approval was taken from the dept. of medical research at Pak General hospital Peshawar. Utilizing multiple parameters for diagnosis, serum AMH levels were determined through the use of an ELISA (Gen-II) assay. Additionally, the maximum follicle counts in a single section as well as ovarian volume were obtained through Transvaginal Sonography, executed by a single observer. The unit of measurement was ng/mL, which equates to 7.14 pmol/L
Results: Results from the study indicated significantly elevated levels of anti-Mullerian hormone (AMH) in individuals affected by polycystic ovary syndrome (PCOS) as compared to the control group.
Conclusion: Although AMH alone wasn't deemed a fully dependable investigative sign for PCOS, the outcomes underscored the potential benefit of integrating AMH levels as an added parameter within the prevailing criteria of Rotterdam. Such inclusion could potentially enhance the precision of PCOS identification. Therefore, AMH levels hold promise as a valuable supplementary indicator for diagnosing PCOS.
Keywords: Polycystic Ovary Syndrome (PCOS), Hormonal Imbalance, Ovulatory Dysfunction, Insulin Resistance, Androgen Excess

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Introduction

Polycystic ovary syndrome (PCOS) is a multifaceted condition characterized by a range of clinical manifestations. These manifestations exhibit varying degrees of severity and impact both metabolic and propagative endocrine roles. The symptoms of PCOS display diversity, spanning from mild to severe presentations. The roots of PCOS are known to be complex, involving a blend of genetic and environmental factors, reflecting its polygenic and multifactorial nature. This intricacy complicates the identification of a singular root cause for PCOS. Nevertheless, research indicates that abnormal hormone levels, insulin resistance, and persistent low-level inflammation might contribute. The precise mechanisms driving the pathophysiology of PCOS remain under investigation and constitute an ongoing area of research.

In accordance with the Rotterdam criteria established in 2003, the diagnosis of polycystic ovary syndrome (PCOS) typically requires the presence of at least two out of the following three criteria. 1) Oligo and Lack of Ovulation, 2) Excessive Androgen Levels and Ovarian Polycystic Characteristics

Polycystic Ovary Syndrome (PCOS) is a prevalent condition found in young women of reproductive age, affecting approximately 20-30% of this population. Women afflicted with PCOS often experience central obesity, insulin resistance, and dyslipidemia factors that heighten their vulnerability to emerging DM and CVDs.[1,2]

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Figuring out if an individual have healthy ovaries often involves blood tests and ultrasounds. There's a hormone called AMH that's a more solid sign than other blood markers.[3] Spanning from 24 to 50 years old, AMH levels gradually decline with advancing age.[4] AMH quantities are closely connected to the count of antral follicles, making them a more reliable indicator of ovarian reserves when compared to FSH and estradiol levels. The link between AMH and the root causes of PCOS has sparked conversations about the possibility of using it as a more sensitive and specific indicative symbol. It could possibly replace or enhance ultrasonography in identifying polycystic ovaries. In women of reproductive age without health issues, both the ovaries and adrenal cortex play significant roles in steroid biosynthesis pathways, producing similar levels of testosterone and androstenedione. Even though each organ produces more androstenedione than testosterone on its own, about half of the circulating testosterone actually comes from the peripheral conversion of androstenedione.[5-9] Inside the ovary, androgens are generated in the internal layer theca of ovarian follicles, while within the adrenal cortex, the synthesis takes place in the zona fasciculate.[10]

Methodology

The study was conducted at Pak General Hospital Peshawar from January 2023 to June 2023, The ethical approval was taken from the dept. of medical research at Pak General hospital Peshawar. We enrolled women aged 18 to 40, who had no prior history of any diseases. The group without PCOS included women from the general population with a good health record, and who had experience in giving birth to at least one child. The participants had about 5ml of blood drawn on the third day of their periods or during a progesterone-induced cycle. These blood samples were then sent to a clinical laboratory for the analysis of different hormones, including LH, TSH, FSH, AMH, E2, Ft3, Ft4, PRL and total testosterone.

The study's inclusion criteria encompassed women with regular menstrual cycles lasting between 25 to 35 days and lasting 3 to 8 days, who had refrained from hormonal medications for a minimum of three months and had not undergone any surgeries related to the reproductive system.

Exclusion criteria included post-menopausal women, those with ovarian tumors, thyroid snags, Cushing syndrome, autoimmune conditions or congenital adrenal hyperplasia,

This study considered various factors such as Body Mass Index, the presence of Hirsutism, the use of Ultrasonography, hormone assays, including Anti-Mullerian hormone, Testosterone, Oestradiol, Follicle Stimulating Hormone, Luteinizing Hormone, and Prolactin.

Results

When examining an association between AMH levels and different aspects in individuals with PCOS, we discovered a significant negative correlation between AMH levels and LH as well as FSH levels (with a p-value less than 0.002 for both). Moreover, there was a positive correlation between AMH levels and T3 levels (with a p-value of 0.025). Additionally, a positive correlation was observed between AMH levels and T3 levels (p = 0.025). No significant correlations were found between AMH levels and factors such as age, BMI, FBS, T4, TSH, testosterone, prolactin, AFC, ovary volume, and estrogens.

Table I: Association between Hormone and various study aspects in the study cohort

Characteristics	Anti-Müllerian hormone	
	R-value	P-value
Age	-0.020	0.660
BMI	-0.004	0.846
FBS	-0.018	0.717
FSH	-0.290	<0.002*
LH	-0.387	<0.002*
TSH	0.050	0.441
T3	0.166	0.025*
T4	-0.012	0.662
Prolactin	0.064	0.323
Testosterone	-0.006	0.884
Estrogens	0.050	0.390
Volume right ovary	0.048	0.464
Volume left ovary	0.124	0.050
AFC right ovary	-0.014	0.786
AFC left ovary	0.090	0.211
*Significant value		

Table II presents the optimal threshold for AMH levels, which was determined to be 3.98 units, showcasing a sensitivity of 92% and a specificity of 92.82%. This threshold was statistically significant.

Table II: Receiver Operating Characteristic (ROC) curve depicting AMH levels (in ng/ml) among individuals with PCOS.

Area under curve	Cut-off	P value	Sensitivity	Specificity	95% confidence interval	
					Upper bound	Lower bound
0.884	3.98	0.000*	92.0%	92.82%	0.992	0.976

The study's findings, coupled with the data in the ROC table, support the suitability of an AMH level cut-off of 3.98 units for distinguishing between individuals with PCOS and control subjects, given its heightened sensitivity and specificity.

Regarding the logistic regression analysis involving various factors and their association with PCOS. The data in Table III indicates that the serum anti-mullerian hormone levels have the greatest impact on the occurrence of PCOS. Age and FSH levels did not show significant odds ratios, and therefore, they cannot be considered linked to the development of PCOS.

Table III: Logistic regression analysis of different variables associated with PCOS.

Independent Variables	Odd ratio with 95% confidence interval	P value
Age (years)	0.988 (range 0.904-1.562)	0.672
FSH (mIU/ml)	1.024 (range 0.810-1.121)	0.886
LH (mIU/ml)	1.198 (range 1.076-1.368)	0.002*
AMH levels (ng/ml)	478.22 (range 238-780)	<0.001*
Testosterone (ng/ml)	1.008 (range 1.022-1.154)	<0.001*

*****Significant value*

Elevated serum levels of LH, testosterone, and AMH emerged as statistically significant predictors of PCOS, with particularly high odds associated with AMH levels in developing the condition.

The average BMI within the PCOS group measured 29.44±4.34kg/m², while it was 23.98 ± 3.44 kg/m² in the control group. The average BMI exhibited a notable increase in the PCOS group in comparison to the control group (p<0.001). The results are displayed in figure 1.

In the PCOS cohort, out of 80 individuals exhibited acne/hirsutism, while in the comparison group, 68 individuals displayed acne/hirsutism; however, there was no notable correlation between the two groups (p=0.912). The results are outlined in figure 2.

The PCOS group exhibited a mean T3 level of 1.642±0.224, while the control group showed a mean T3 level of 2.111±0.446. Regarding T4 levels, the PCOS group had a mean of 1.164±0.240, whereas the control group had a mean of 1.282±1.662. The mean TSH level for the PCOS group was 2.272±0.628, while for the control group, it was 2.622±0.228. Notably, the PCOS group exhibited significantly lower mean levels of T3, T4, and TSH compared to the control group (p<0.002, p=0.010, and p=0.042 respectively). The results are displayed in figure 3.

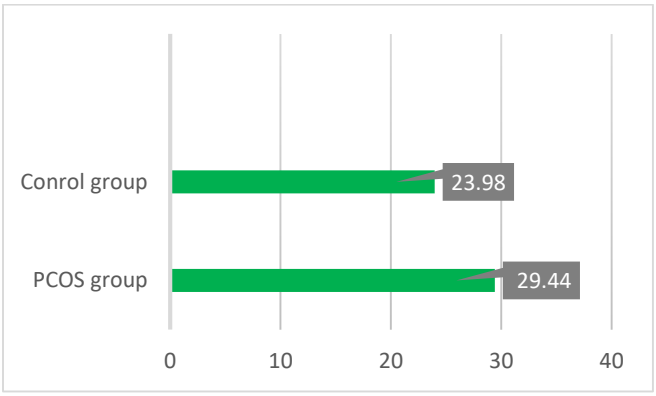

Figure 1. The average BMI (kg/m²) in the PCOS group vs control (p<0.001).

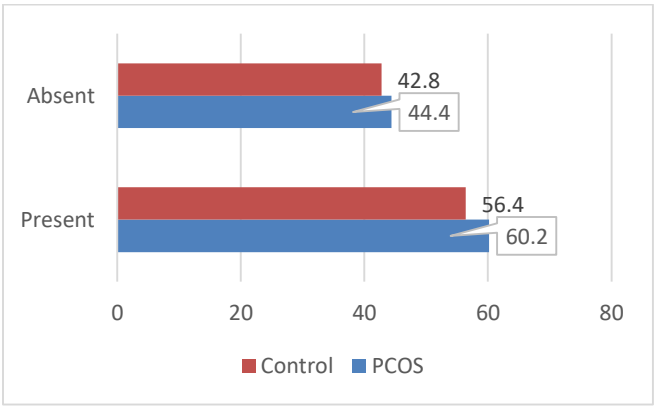

Figure 2. Comparative analysis of presence of hirsutism/acne in the study groups (p=0.862)

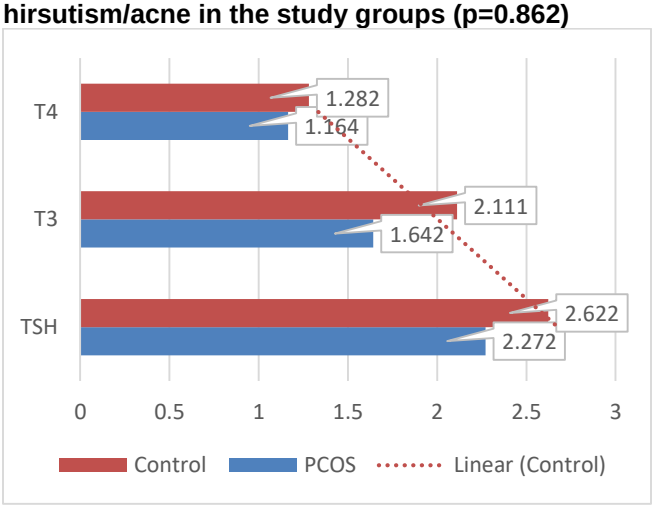

Figure 3. Comparative analysis of mean TSH, T3 and T4 levels in the study groups.

Discussion

PCOS encompasses a range of indications linked to an inequality in hormones, varying in intensity from slight to severe.[5] This syndrome has implications for reproduction, endocrine function, and metabolism. Among women of reproductive age, PCOS stands as a primary contributor to female subfertility and holds the

status of being the most prevalent endocrine disorder. Despite decades of dedicated research, the precise origins and development of this intricate disorder remain veiled.

Utilizing ultrasound for PCOS diagnosis presents challenges; interpreting ultrasound outcomes can require subjective judgments and is susceptible to differences in interpretation among observers.[11] Additionally, the main demographic affected includes teenagers and women of reproductive age, who might not be amenable to transvaginal ultrasound assessments due to factors such as virginity or obesity.

Further complicating diagnosis is the existence of various phenotypes within PCOS.[12] Technological advancements in imaging have artificially inflated the detection of polycystic ovarian morphology, leading to uncertainty about its suitability as a diagnostic criterion. Establishing a diagnosis for PCOS necessitates clear and quantifiable criteria to aid clinicians in identifying and treating individuals grappling with this intricate endocrine disorder. Anti-Müllerian hormone (AMH) emerges as a promising marker in this regard, as its levels remain consistent throughout the menstrual cycle and remain unaffected by vacillations in other reproductive hormones.[13]

Even though AMH serum levels are utilized as a prognostic indicator for ovarian response in IVF, conflicting findings exist regarding its predictive efficacy for folliculogenesis during clomiphene citrate-induced ovulation.[5] The measurement of AMH levels enables further exploration of PCOS and its clinical inferences. The current investigation seeks to evaluate the investigative capability of serum AMH in diagnosing PCOS. Additionally, this study seeks to explore whether serum AMH can serve as a replacement for PCOM in the Rotterdam Criteria and to scrutinize the association between Anti-Müllerian hormone and hyper-androgenemia.

In this study, there was no statistically significant disparity in mean age between PCOS cases and the control group. This observation lines up with the research conducted by Singh et al.[14] where they found no significant age difference between the PCOS and control groups. Similar results were found by Ahmed, Saxena, and Sahmay.[15-17] However, Wiweko, Johnstone, and Hsu[18-20] reported different outcomes, noting that patients with PCOS were generally young than those without syndrome. This led them to the conclusion that the percentage of women with PCO decreased with age. Additionally, in this study, there was no notable difference in the age of menarche between the PCOS and control groups.

Regarding BMI, the PCOS group displayed significantly higher values compared to the control group. PCOS typically affects females in their reproductive years but can manifest in both women in their earlier and later stages of life. Further investigation is warranted to ascertain whether age plays a pivotal role in PCOS development or impacts the diagnostic accuracy of serum AMH.

Significantly, this study observed a considerable increase in AMH levels among individuals in the PCOS group. The median AMH levels were 6.142 ± 2.446 ng/ml in the PCOS group, almost twice as high as the control group's 2.602 ± 0.422 ng/ml ($p<0.001$). A separate investigation revealed that women meeting all three Rotterdam criteria and having an AMH level of at least 11 ng/ml had an 80% chance of being diagnosed with PCOS. Another study indicated that 97% of women with AMH levels exceeding 10 ng/ml received a PCOS diagnosis.[8]

In this current study, a specific threshold demonstrated the most effective diagnostic capability for AMH, showing sensitivity and specificity rates of 90% and 90.6%, respectively. Dewailly et al.[9] reported a similar AMH cut-off value of 4.90 ng/ml, associated with higher sensitivity and specificity rates of 92% and 97%. As a result, they concluded that AMH provides a more accurate prediction compared to counting follicles per ovary. This is because AMH not only signifies antral follicle count but also mirrors the extent of hyperandrogenism.

Conclusion

The existing Rotterdam criteria, known as PCOM, serve the purpose of identifying PCOS, a multifaceted and prevalent antenatal complaint. Yet, the assessment of PCOM is noticeably subjective and shows restricted reliability. In spite of the inherent weaknesses in its sensitivity and specificity, and the absence of a single definitive AMH cutoff for diagnosis, the utilization of AMH shows promise as an additional diagnostic approach for PCOS alongside the current Rotterdam criteria. This potential is particularly evident when considering the replacement of PCOM with AMH. AMH stands out as a biological, objective, and quantifiable marker, unaffected by menstrual cycle timing or oral contraceptive pill use, thereby offering distinct advantages as a diagnostic tool. Consequently, further investigations are recommended

in the future to substantiate the role of AMH as a reliable diagnostic tool for PCOS.

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