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Original Research Article

Determination of PCOS by Assessing Anti-Mullerian Hormone

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Abstract:

Introduction: Polycystic Ovarian Syndrome **(**PCOS) affects 20.2% of pubertal women and presents differently, making diagnosis difficult. Many employ the Rotterdam criteria, which emphasise PCO morphology, hyperandrogenism, and oligo or amenorrhea. PCOS diagnosis by AMH, which measures ovarian follicle count, is promising. GnRH neurons are affected by AMH, causing PCOS. As high as 3.8–5 ng/mL, AMH levels in PCOS women may be diagnostic. AMH and Rotterdam criteria may improve PCOS diagnosis, especially in culturally diverse communities.

Aim and Objectives: The objective is to assess the association of anti-Müllerian hormone (AMH) levels as a diagnostic marker for polycystic ovarian syndrome (PCOS) and to determine its cut-off value for suspecting PCOS.

Method: Roche Diagnostics compared PCOS-positive patients to controls in a retrospective, trial conducted from May 2022 to April 2023. According to Rotterdam, the study assessed PCOM thresholds. In addition, ovulatory failure and ovarian volume were considered in 25-45-year-old women, excluding specific disorders. Testosterone and sex hormone-binding globulin levels were measured in control subjects by drawing blood samples taken on days 2-4 of the menstrual cycle.

Result: This study was conducted among70 women with polycystic ovary syndrome (PCOS) and seventy healthy controls were used to study the effects of age, race, and reproductive indicators. The study plotted cumulative distribution plot for Anti-Müllerian Hormone (AMH) cutoff values to diagnose Polycystic Ovary Morphology (PCOM AMH). It displays specificity and sensitivity percentages against PCOM AMH levels ranging from 0 to 23 ng/mL. The graph aids in determining an optimal AMH cutoff point that balances sensitivity and specificity for PCOM diagnosis. Specific cutoff values are associated with corresponding sensitivity and specificity percentages, facilitating diagnostic test interpretation.

Conclusion: This study has concluded that there is strong association betweem higher AMH level and PCOS and the cut-off value that was selected by the author (based on ROC) was 3.2 ng/mL

Keywords: PCOS, Morphology, Hyperandrogenism, Oligo Or Amenorrhea, Anti-Mullerian Hormone.

Introduction

Up to 20.2% of women of pubertal age suffer from anovulatory infertility, with PCOS being a major contributing factor. A variety of symptoms and signs, including increased androgen, irregular ovulation, metabolic abnormalities, and polycystic ovarian (PCO) morphology are indicative of PCOS; however, not a single trait is necessary for the diagnosis. Therefore, the intricacy and variability of PCOS's phenotypic presentation might make diagnosis difficult [1].

The most popular criteria for diagnosing PCOS is the Rotterdam criteria, which calls for the existence of two or more of the following characteristics: PCO morphology on ultrasound, biochemical or clinical hyperandrogenism, and oligo/amenorrhea. International guidelines have modified the PCOS diagnostic criteria in more recent times [2]. Identifying the morphology of polycystic ovaries (PCO) using ultrasonography is crucial for diagnosing polycystic ovarian syndrome (PCOS). This involves observing the typical peripheral pattern of follicular dispersion surrounding a central stroma in the ovary. Nevertheless, follicular number per ovary (FNPO) is a more widely used metric to determine PCO morphology because of the subject's perception. The Rotterdam criteria, which formerly characterised PCO morphology as having several at least 12 FNPO, have been amended to include at least 20 FNPO in light of advancements in ultrasound resolution [3]

A defining feature of PCOS is hypersecretion of LH, in response to GnRH and basally. It is identifiable as the main anomaly in typical PCOS that results in androgen excess. Anti-Müllerian

hormone (AMH) is produced by small ovarian follicles of preantral and antral, and it is a fundamental primordial factor for PCOS diagnostic indicators and folliculogenesis. Because it accounts for the tiny antral and preantral ovarian follicles, the serum AMH was more sensitive than the antral follicle count. Research has demonstrated a robust correlation between antral follicle reserves and hyperandrogenism in the body, indicating that AMH is a potent diagnostic tool for PCOS [3,4].

Ovarian antral follicles stimulate the production of transforming growth factor-β, which includes AMH. The proposed diagnostic tool for diagnosing PCOS is AMH, as it is closely associated with the total quantity of follicles of antrum within both ovaries. While the examination of ovarian morphology and antral follicle count (AFC) is most accurate in the follicular phase, anti-Müllerian hormone (AMH) offers the benefit of being non-invasive and relatively consistent during the course of the menstrual cycle. However, AMH has not been incorporated into the diagnostic criteria for PCOS due to the inconsistencies in amounts measured using outdated and less reliable tests, as well as the lack of a globally accepted standard [5].

A novel notion has emerged, indicating that AMH may serve as a significant endocrine signal in the development and progression of PCOS, rather than solely functioning as an indicator of follicular count of. A certain group of neurons that produce gonadotropin-releasing hormone (GnRH) have receptors for anti-Müllerian hormone (AMH). When AMH is given, it increases the activity of these GnRH neurons. Pituitary gonadotropes are responsive to changes in the frequency and intensity of GnRH pulses. Therefore, an increase in pulsatility of GnRH is associated with the generation of LH-predominant gonadotropins, which is a characteristic feature of PCOS that is unique but not universally present. AMH levels are greater in PCOS-afflicted women than in matched controls. As people age, their AMH levels decrease along with some PCOS clinical aspects getting better [6,7].

Due to elevated AMH synthesis per follicle and concurrent stimulation of many antral follicles, PCOS women are known to have elevated serum AMH levels. There is insufficient data to determine the AMH cut-off value as a PCOS diagnosis tool. According to certain research, the Rotterdam criteria and levels of AMH should be utilised in tandem for an immediate and precise diagnosis of PCOS. AMH values of more than 3.8–5 ng/mL have also been proposed as a diagnostic factor. A high blood AMH value and inadequate pregnancy outcomes during controlled intrauterine insemination cycles were reported by about 60.3% of PCOS women [8]. Numerous populations have explored the usage of AMH level in determining the presence of PCOS in the female menstrual age; however, there is a lack of information about the association between AMH level and PCOS in Pakistani communities. Furthermore, due to the religious and social stigmas associated with transvaginalultrasonograms, unmarried women and adolescent girls in Pakistan are hesitant to choose this approach for PCOM identification. For these women, it will be crucial to employ serum AMH levels as a biochemical diagnostic for early PCOS detection by detecting ovarian dysfunction [9].

Method

Research Design: This retrospective, nonintervention, single-center, case-control study was conducted from May 2022 to April 2023. The study authors collected the patients' data and analysed it. The data including the age, BMI, AMH level This study compared the threshold for PCOM in PCOS-positive women compared to PCOS-negative controls, which was developed. The Rotterdam criteria were used to identify PCOS cases in women aged 25-45 with ovulatory failure and ovarian volume >10 mL in at least one ovary. To conduct this study, the patients needed to undergo several diagnostic tests to exclude other diseases like Congenital Adrenal Hyperplasia, Cushing disease, androgen-secreting tumours, and oral contraceptives. Women aged 25-45 with regular menstrual cycles, no severe uterine or ovarian abnormalities visualized by TransvaginalSonography or TVS, no previous in-vitro fertilisation cycles, and an AFC of 20 per ovary. In order to conduct this study, it was included PCOS, and obese patients were not considerd as they can show high level of AMH which will be false positive. Even if it had serum human chorionic gonadotrophin, major ovarian abnormalities, endocrine or metabolic abnormalities, or were currently treated for cancer. When possible, blood samples were taken on days 2–4 of the menstrual cycle. Experimental investigations examined testosterone and sex hormone-binding globulin levels in a subset of control samples.

Inclusion and Exclusion Criteria

Inclusion Criteria

1. Women aged 25-45 years presented in gynae-cology OPD with PCOS.
2. Diagnosis of polycystic ovary syndrome (PCOS) was done according to Rotterdam cri-teria.
3. Healthy controls were also taken who had no PCOS.

Exclusion Criteria

1. Patients with Congenital Adrenal Hyperplasia, obesity (BMI more than 24.99) and suspected insulin resistance.

2. Patients with history of infertility treatment.

Statistical Analysis

Patients characteristics and biomarker levels were reported using descriptive statistics. The authors used SPSS 27 for statistical analysis and plotting ROC. The sensitivity and specificity of case-control status was estimated, with 95% CIs. Statistical significance was determined using a 1-sided binomial test (significance level, ¼ 0.05) and specificity and sensitivity requirements of >75% and >70%, respectively. ANOVA was employed in determining p-value between AMH level of Case and Control group. The level of significance was considered to be P<0.05.

Result

Table 1 describes the cohort of 70 cases and 70 controls. The median age of patients is 29 years (IQR: 27–32), significantly younger than the controls' 36 years (IQR: 32–39). Cases are more common in 25–29-year-olds (57.14%) than in controls (50.00%). The median BMI for patients and controls is 28.1 kg/m2 (IQR: 23.7–33.1). However, reproductive markers differ, with patients having a higher median anti-Müllerian hormone (AMH) level (6.32 ng/mL, IQR: 4.24–9.30) than controls (1.58 ng/mL, IQR: 0.760–2.56). Also, patients have a median of 42.5 follicles, compared to 12.0 in controls. The percentage of cases with an AFC of 12 or more is 90%, while all controls had 12 or less. The findings show that age, race, and reproductive biomarkers distinguish cases from controls.

Table 1: Basline characteristics of the patients in this study

Characteristic	Case (n=70)	Control (n=70)
Median age, y (IQR	29 (27–32)	36 (32–39)
Age group, y—no. (%)		
25–29	40 (57.14%)	35 (50.00%)
30–34	20 (28.57%)	25 (35.71%)
35–39	7 (10.00%)	8 (11.42%)
40–45	3 (4.28%)	2 (2.85%)
Median BMI, kg/m2 (IQR)	24.85±0.85	22.65±0.58
Median number of follicles—no. (IQR)	42.5 (30.0–60.0)	12.0 (8.0–16.0)
Antral Follicular Count (AFC) > 12	63 (90%)	0

Cumulative distribution map for polycystic ovary morphology anti-Müllerian hormone (PCOM AMH) cutoff in Figure 1. The X-axis shows PCOM AMH cutoff values in ng/mL from 0 to 23, while the y-axis shows specificity and sensitivity percentages. A PCOM AMH cutoffof 0 ng/mL is highly specific in identifying individuals without polycystic ovary morphology, as the specificity curve starts at 100%. Specificity drops to 99% at a PCOM AMH cutoffof 9 ng/mL and stays high afterwards. The sensitivity curve starts at 0% and reaches 100% at 0 ng/mL PCOM AMH. Sensitivity drops to 0% at a PCOM AMH cutoffof 23 ng/mL. The cumulative distribution map's specificity and sensitivity curves overlap to show their trade-off at different PCOM AMH cutoff values. The graph helps determine an AMH cutoff point that balances sensitivity and specificity for diagnosing polycystic ovary morphology. At specific points on the curve, the derived cutoff values show the PCOM AMH values associated with specific sensitivity and specificity percentages, aiding diagnostic test interpretation and application.

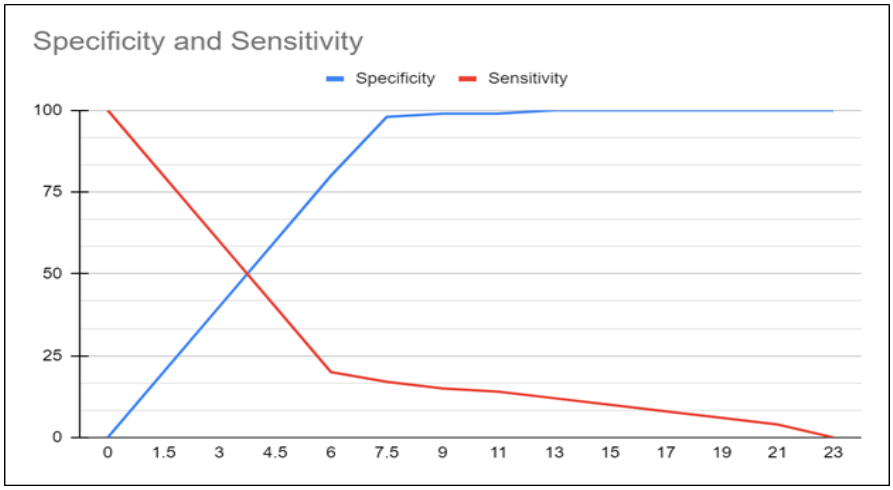

Figure 1: A cumulative distribution plot for AMH cutoff to diagnose Polycystic Ovarian Morphology

The average Anti-Müllerian Hormone (AMH) levels in the case group, diagnosed with Polycystic Ovary Syndrome (PCOS), and the control group are 6.838 ng/mL and 1.651 ng/mL, respectively. The p-value of 0.325 from ANOVA analysis suggests no statistically significant difference in AMH levels between the two groups.

Additionally, the range of AMH levels varies between the case and control groups. In the case group, the range is from 4.24 ng/mL to 9.30 ng/mL, while in the control group, it ranges from 0.760 ng/mL to 2.56 ng/mL. This indicates higher variability and potentially higher AMH levels in the PCOS group compared to the control group. However, the statistical significance of this difference is not explicitly provided.

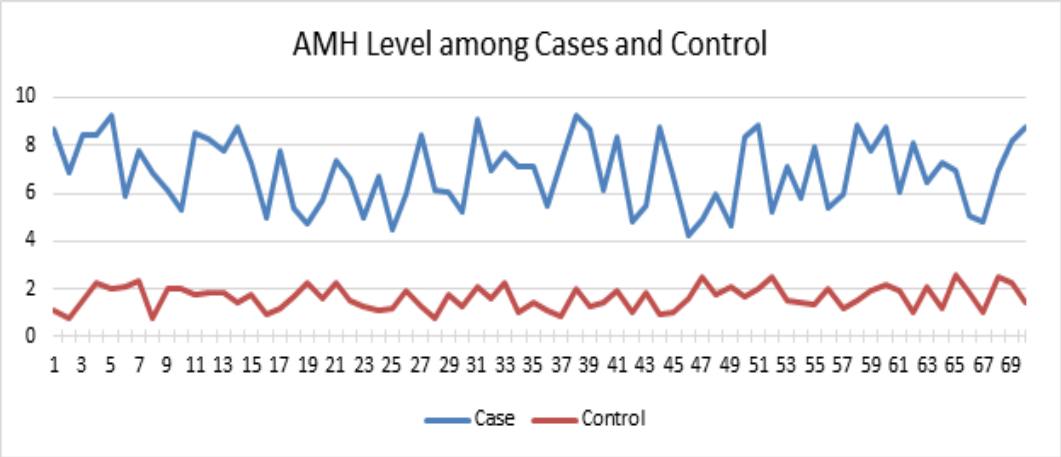

Figure 2: Variation of AMH among the subjects of Case and Control

Figure 3 shows diagnostic test Receiver-Operator Characteristic (ROC) curves with specificity percentages on the x-axis and cutoff values on the y-axis. At a threshold of 0, the test is very specific for identifying those without the ailment. The specificity curve starts at 100%. Based on the ROC coordinates, the authors have selected 3.2 ng/mL as the cut-off for AMH level. More than this level, PCOS can be suspected. The curve's cutoff values reflect test performance assessment points. These numbers illustrate sensitivity and specificity trade-offs at different evaluation thresholds. The ROC curve shows the test's ability to distinguish positive and negative instances at various cutoff points. Diagnostic accuracy, sensitivity, and specificity are higher when the curve closely follows the top-left corner of the graph. The diagnostic context's ideal sensitivity-specificity balance determines the ROC curve cutoff point. Figure 3 can help clinicians and researchers choose a cutoff value based on the test's diagnostic requirements. The ROC curve shows the test's performance and helps assess its ability to identify cases from non-cases.

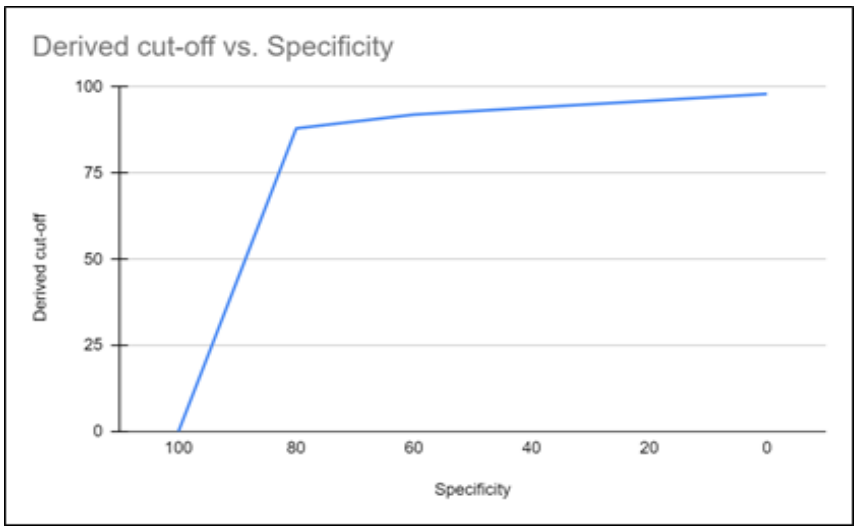

Figure 3: ROC of the AMH level

Discussion

The current biochemical assays for polycystic ovarian syndrome (PCOS) exhibit low specificity and sensitivity. PCOS is often characterised by elevated levels of AMH in women, making it a potentially valuable inclusion in the diagnostic criteria. A comprehensive literature study was conducted to identify the precise accuracy of Anti-Müllerian Hormone in predicting Polycystic Ovary Syndrome and to identify the most effective diagnostic threshold. AMH could serve as a valuable initial diagnostic test for PCOS, pending confirmation in future population cohorts [10].

A study used sophisticated automated Elecsys AMH assay technology to determine the standard range of serum anti-Mullerian hormone. The aim was to determine the indicators of early depletion of ovarian reserve, increased susceptibility to a strong reaction during stimulation of in vitro fertilisation (IVF), and potential for diagnosing polycystic ovary syndrome. AMH in serum is a highly responsive indicator of the diminishing ovarian reserve associated with ageing. A blood Anti-Müllerian Hormone level of more than 36 picomoles per litre (pmol/L), or above 75% for age, strongly indicates a diagnosis of Polycystic Ovary Syndrome. A blood anti-Müllerian hormone level less than the 10th percentile for age indicates a faster decline in the reserve of ovaries, whereas an AMH level above 20.2 pmol L−1 indicates a higher likelihood of developing ovarian hyperstimulation syndrome (OHSS) after IVF treatment [11].

A study was undertaken to analyse the disparity in levels of AMH in the different phenotypic groups of PCOS and assess the significance of AMH in predicting the severity of PCOS. Levels of AMH were highest in cases when all three primary diagnostic criteria were present. Levels of AMH exhibit the strongest correlation with Polycystic Ovary Morphology (PCOM). Furthermore, oligo-anovulation is a contributing factor to elevated AMH levels. The influence of the hyperandrogenism criterion on AMH levels was found to be minimal. AMH levels appear to play a diagnostic function in assessing the extent of PCOS [12].

Polycystic ovarian syndrome is a prevalent endocrine condition that mostly impacts women during their reproductive years. The cause of Polycystic ovarian syndrome is not fully understood, however, one of the potential factors that has been identified is anti-Müllerian hormone. At present, there is no apparent association between levels of AMH and the prevalence of PCOS among women from Saudi Arabia. The research aimed to determine the threshold levels of AMH and its association with clinical features of PCOS to improve the precision of PCOS diagnosis. Increased levels of anti-Müllerian hormone (AMH) have been found to be positively correlated with the initiation of menstruation, the existence of polycystic ovarian morphology (PCOM), and irregular or interrupted menstrual cycles (oligo/amenorrhea). The anti-Müllerian hormone (AMH) seen in serum has the potential to be a dependable diagnostic marker for ovarian dysfunction in individuals with polycystic ovary syndrome, especially in cases where evaluating the morphology of the ovaries has been difficult [13].

The determination of PCOS is a complicated process because of the varied and unclear causes of its signs and symptoms. The aim of the research was to investigate the serum concentrations of anti-Mullerian hormone and the luteinizing hormone to follicle-stimulating hormone (FSH) ratio in Sudanese women with polycystic ovarian syndrome (PCOS). The study also aimed to evaluate the diagnostic accuracy of these markers for detecting PCOS. The values of Serum AMH and LH/FSH ratio were higher in patients when compared to those in the control group. Nevertheless, the AMH level exhibits superior discriminatory ability and strong diagnostic potential for identifying PCOS in Sudanese women [14].

A previous meta-analysis examining the predictive capability of AMH for PCOS found that AMH by itself could be a good first diagnostic tool for PCOS. The study's objective was to conduct a recent meta-analysis and assess the diagnostic effectiveness of anti-Müllerian hormone (AMH) as a proxy for polycystic ovary morphology (PCOM) based on the Rotterdam criteria. Replacing the abbreviation PCOM with AMH in the Rotterdam criteria improves the accuracy of diagnosing polycystic ovary syndrome [15].

Conclusion

This study has concluded that there is strong association betweem higher AMH level and PCOS and the cut-off value that was selected by the author (based on ROC) was 3.2 ng/mL. Our study examined how well anti-Müllerian hormone (AMH) levels can be used to find the condition. In a retrospective, multicenter Roche Diagnostics investigation from May 2022 to April 2023, we compared PCOS-positive patients to controls using Rotterdam PCOM standards. We found substantial age, racial, and reproductive indicator differences between PCOS-positive patients and controls. Women with PCOS had greater AMH and follicle counts than controls. The cumulative distribution map of the PCOM AMH cutoff showed specificity-sensitivity trade-offs, suggesting AMH could be a PCOS diagnosis marker. Specificity and sensitivity curves with cutoff values showed how diagnostic accuracy and cutoff selection affect case-control

group identification. Receiver-operating characteristic (ROC) curves allow doctors to select ideal diagnosis thresholds by demonstrating the test's capacity to discriminate positive and negative cases using different cutoff points. This research contributes to the expanding body of evidence supporting AMH levels in PCOS diagnosis, especially in culturally diverse communities where standard approaches may be less effective. Clinicians can improve PCOS diagnosis and patient care by using AMH with Rotterdam criteria. Further study is needed to confirm our findings and improve PCOS detection methods.

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