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Diagnostic Value of Anti-Müllerian Hormone, Hematological Inflammatory Markers, and CYP19A1 Gene Polymorphism in Polycystic Ovary Syndrome: Insights from a South Indian Cohort

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ABSTRACT

Background: Polycystic Ovary Syndrome (PCOS) is a prevalent endocrine disorder in reproductive-age women, characterized by ovarian dysfunction, hyperandrogenism, and metabolic disturbances. Current diagnostic methods rely on heterogeneous clinical and biochemical criteria, warranting exploration of reliable hormonal, inflammatory, and genetic biomarkers.

Objective: To evaluate the diagnostic potential of Anti-Müllerian Hormone (AMH), hematological inflammatory indices, and CYP19A1 rs2414096 gene polymorphism in PCOS among South Indian women.

Methods: A prospective cross-sectional study was conducted at St. Gregorios Medical Mission Hospital, Kerala, including 50 women with PCOS and 50 age-matched controls. AMH, FSH, and LH levels were measured, and hematological indices including neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and total WBC counts were analyzed. CYP19A1 polymorphism was genotyped using qPCR and validated by Sanger sequencing. Statistical analyses included correlation, ROC curve evaluation, and allele frequency distribution.

Results: AMH levels were significantly elevated in PCOS patients (median 5.75 ng/mL) versus controls (1.87 ng/mL; $p < 0.00001$). NLR was markedly higher in PCOS (median 2.0 vs. 1.9; $p < 0.00001$), showing a strong positive correlation with AMH ($Rs = 0.7442$, $p < 0.001$). PLR and erythrocytic indices showed limited diagnostic value. ROC analysis demonstrated high accuracy for AMH (AUC > 0.90), while NLR enhanced predictive performance when combined with AMH. The CYP19A1 rs2414096 A allele was selectively observed in PCOS cases, suggesting a genetic predisposition, though statistical power was limited.

Conclusion: AMH and NLR exhibit strong diagnostic potential for PCOS, with combined assessment improving clinical accuracy. CYP19A1 polymorphism may contribute to disease susceptibility, warranting validation in larger, multi-centric cohorts.

Introduction

Polycystic Ovary Syndrome (PCOS) is one of the most common endocrinopathies affecting women of reproductive age, with a global prevalence ranging between 8–13%. It is clinically defined by chronic anovulation, hyperandrogenism, and polycystic ovarian morphology [1]. Beyond reproductive dysfunction, PCOS is increasingly recognized as a multisystem disorder associated with obesity, insulin resistance, low-grade inflammation, and increased cardiovascular risk. Despite extensive research, diagnosis remains

challenging due to heterogeneous clinical presentations and reliance on the Rotterdam criteria, which may not capture underlying pathophysiological mechanisms [2].

Biochemical markers such as Anti-Müllerian Hormone (AMH) have gained prominence for their ability to reflect ovarian follicular reserve and dysfunction in PCOS [3]. Similarly, hematological markers like the neutrophil-to-lymphocyte ratio (NLR) provide simple yet robust indicators of systemic inflammation. Their correlation with endocrine abnormalities may strengthen diagnostic frameworks [4].

Genetic contributions to PCOS are increasingly explored, with polymorphisms in steroidogenic enzymes such as CYP19A1 being implicated in disease susceptibility. The rs2414096 single nucleotide polymorphism (SNP) in CYP19A1, encoding aromatase, has been studied in relation to altered estrogen biosynthesis and ovarian physiology. However, data from South Indian cohorts remain limited [5].

This study aims to evaluate the combined diagnostic utility of AMH, inflammatory hematological markers, and CYP19A1 polymorphism in women with PCOS. By integrating endocrine, immunological, and genetic perspectives, the study seeks to provide insights into more precise, cost-effective, and population-specific diagnostic strategies.

Materials and methods

Study design and setting

This prospective cross-sectional study was conducted over a period of four years at the Department of Pathology, St. Gregorios Medical Mission Hospital, Parumala, Kerala, India. Ethical clearance was obtained from the Srinivas University Ethics Committee (Protocol No. 28/AHS/2023). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki (2013 revision) [6].

Study population

A total of 100 women of reproductive age (20–49 years) were recruited, comprising 50 patients diagnosed with PCOS and 50 age-matched healthy controls. Diagnosis of PCOS was based on the Rotterdam 2003 criteria, requiring at least two of the following: (i) oligo/anovulation, (ii) clinical or biochemical hyperandrogenism, and (iii) polycystic ovarian morphology on ultrasonography. Controls were selected from women with regular menstrual cycles and without clinical/biochemical evidence of hyperandrogenism or PCOS.

Exclusion criteria: women with metabolic, endocrine, adrenal, or autoimmune disorders; those receiving hormonal therapy within the past three months; pregnant or lactating women; and participants with incomplete clinical/laboratory data.

Sample collection

Venous blood samples (5 mL) were collected from all participants. Blood was divided into EDTA tubes for hematological analyses and plain tubes for serum separation. Serum aliquots were stored at –80 °C until analysis.

Hormonal assays

- **Anti-Müllerian Hormone (AMH):** Measured using a commercial ELISA kit (EasyStep

Human AMH ELISA, ELK Biotechnology) on an ALERE™ Easy Reader.

- **Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH):** Estimated by chemiluminescent immunoassays following manufacturer protocols.

Hematological assessments

Complete blood counts were performed using an automated hematology analyzer (Sysmex XN1000), with manual verification by peripheral smear and Neubauer chamber. Parameters included:

- Total and differential WBC counts
- RBC count, hemoglobin, hematocrit (PCV)
- Platelet count
- Hematological indices: Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)
- Inflammatory ratios: Neutrophil-to-Lymphocyte Ratio (NLR), Monocyte-to-Lymphocyte Ratio (MLR), Platelet-to-Lymphocyte Ratio (PLR)

Molecular analysis

DNA Extraction and Quality Control

Genomic DNA was extracted from peripheral blood using a magnetic bead-based kit (ZM11BL-50). DNA concentration and purity were evaluated by UV-spectrophotometry (Thermo Scientific™ GENESYS™ 180) and agarose gel electrophoresis.

Primer Design and PCR Amplification

The target single nucleotide polymorphism (SNP) rs2414096 in the *CYP19A1* gene was identified using the NCBI database, and specific primers were designed with Primer3 and Primer-BLAST. A pair of sequencing primers was employed to amplify a 759 bp region of *CYP19A1*, while allelic discrimination primers along with TaqMan probes were used in quantitative real-time PCR (qRT-PCR) assays to differentiate between the wild-type (G allele) and mutant (A allele) variants. Gradient PCR was performed to optimize annealing temperatures within the range of 57–59 °C, and the amplified products were subsequently confirmed by electrophoresis on a 1.5% agarose gel.

qPCR Assay

Allele-specific TaqMan probes were used for genotyping CYP19A1 rs2414096. Human Ribonuclease P served as an internal control. Reactions were run in triplicate, and amplification plots were analyzed to classify genotypes. A subset of samples underwent Sanger sequencing for validation.

Statistical Analysis

Data analysis was performed using Microsoft Excel, R (v4.3.2), and GraphPad Prism (v10). Continuous variables were summarized as mean ± standard deviation (SD) or median with range, depending on data distribution, while categorical variables were expressed as frequencies and percentages. Group comparisons were conducted using Student's *t*-test for normally distributed data and the Mann–Whitney U test for non-parametric data. Associations between categorical variables

were assessed using the Chi-square test, and correlations between non-parametric variables were evaluated using Spearman's rank correlation. The diagnostic accuracy of biomarkers was further examined using Receiver Operating Characteristic (ROC) curve analysis to determine sensitivity, specificity, and the area under the curve (AUC). A *p*-value of less than 0.05 was considered statistically significant.

Results

Study Population

A total of 100 women were enrolled, comprising 50 patients with PCOS and 50 age-matched healthy controls. The median age was 32 years (range: 20–45) in the PCOS group and 30 years (range: 18–40) in controls. Body mass index (BMI) was comparable between groups (median 26.27 vs. 26.0 kg/m²), minimizing demographic confounding factors.

Hormonal Markers

Serum Anti-Müllerian Hormone (AMH) levels were markedly elevated in women with PCOS compared to controls (median 5.75 ng/mL [2.84–6.08] vs. 1.87 ng/mL [1.55–2.18]; $p < 0.00001$). This confirms AMH as a robust biomarker for PCOS. Luteinizing hormone (LH) levels were also significantly higher in PCOS, whereas follicle-stimulating hormone (FSH) levels remained within normal ranges, leading to an increased LH/FSH ratio consistent with ovarian dysfunction. Figure 2 indicates the distribution of AMH values of both case and controls.

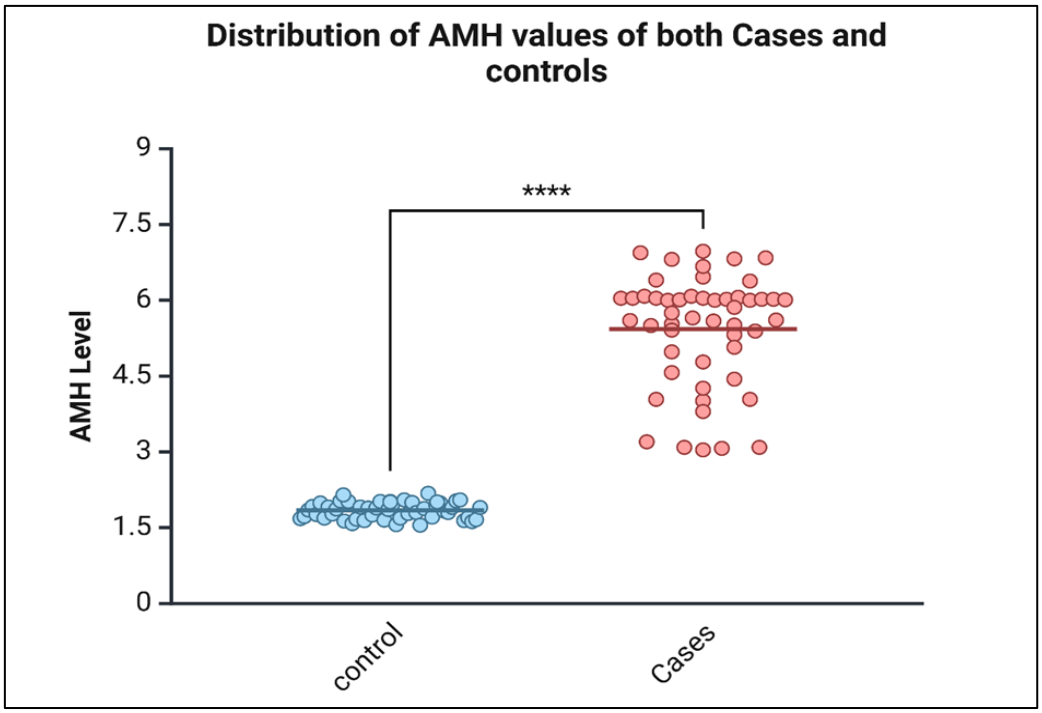

Figure 2: Distribution of AMH values of case and control groups

Haematological Parameters

In the present study, haematological analysis revealed a distinct inflammatory

profile in women with PCOS compared to healthy controls. The total WBC count was significantly elevated in PCOS patients (median 8,544/mm³ [4,853–11,363]) relative to controls (7,320/mm³ [4,852–11,269]; p = 0.034), supporting the presence of a low-grade systemic inflammatory state. Similarly, the neutrophil-to-lymphocyte ratio (NLR) was markedly higher in the PCOS group (median 2.0 [1.12–3.35]) compared with controls (1.9 [1.05–3.4]; p < 0.00001), highlighting its sensitivity as a biomarker of inflammation in this population. Although the platelet-to-lymphocyte ratio (PLR) tended to be higher in PCOS patients (149.95 vs. 133.78), the difference did not reach statistical significance (p = 0.105). In contrast, red cell indices including MCV, MCH, MCHC, and haemoglobin levels did not show significant intergroup differences (p > 0.4), indicating that erythrocytic markers have limited diagnostic relevance in PCOS-associated haematological alterations. Figure 3 represents the box plots of haematological parameters in both case and control groups.

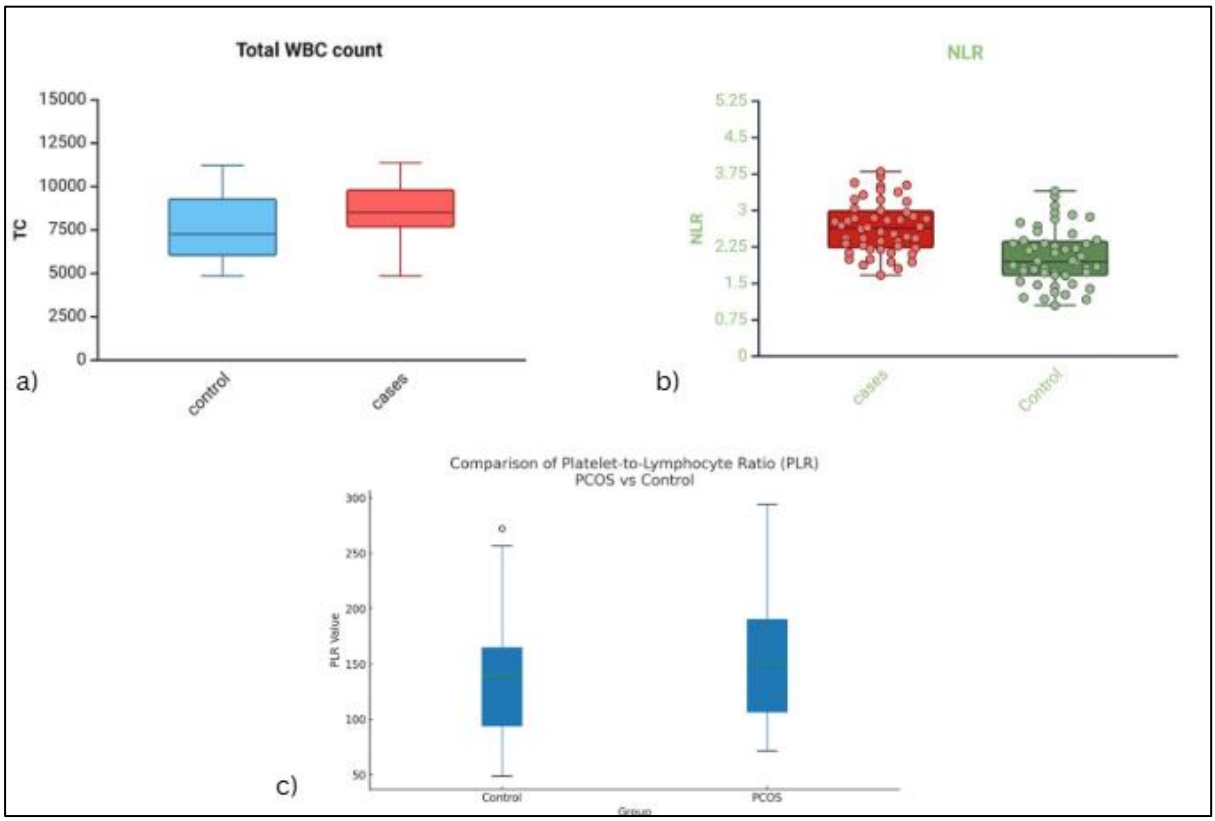

Figure 3: Box plots show (a) significantly higher total WBC count and (b) elevated neutrophil-to-lymphocyte ratio (NLR) in PCOS cases compared to controls, with (c) a non-significant increase in platelet-to-lymphocyte ratio (PLR) among cases

Correlation Analyses

Spearman's correlation revealed a strong positive relationship between AMH and NLR within the PCOS group (Rs = 0.7442, $p < 0.001$), highlighting a possible mechanistic link between ovarian reserve dysregulation and systemic inflammation. No significant correlation was observed between AMH and WBC count (Rs = –

0.222, $p = 0.20$) or between NLR and WBC count (Rs = –0.0865, $p > 0.50$). Figure 4 illustrate the The scatter graph is essentially plotting AMH (A) vs. NLR (B) in women with PCOS to assess their relationship using Spearman's correlation

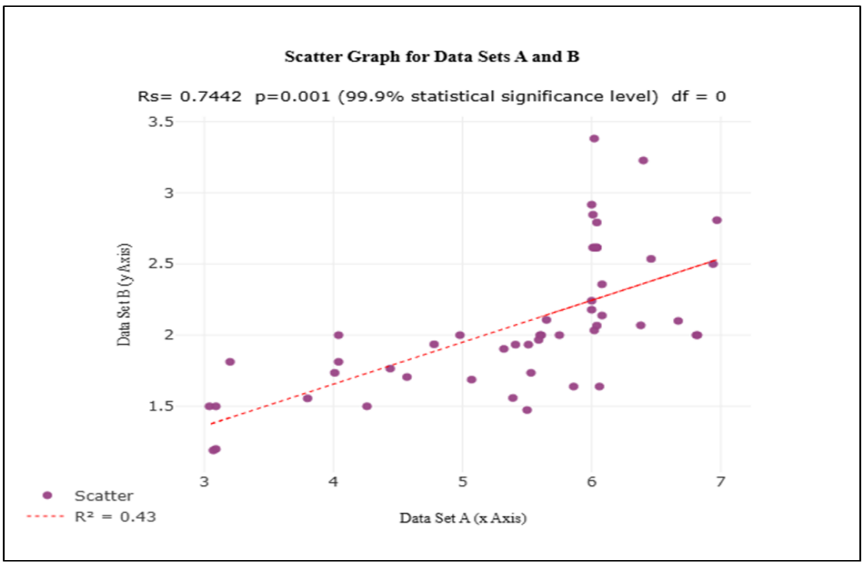

Figure 4: The scatter graph is essentially plotting AMH (A) vs. NLR (B) in women with PCOS to assess their relationship using Spearman's correlation

Molecular Genetics

Genomic DNA was successfully extracted from all 50 participants (25 PCOS and 25 controls) with acceptable purity levels (A260/280 ratios 1.6–1.8). qPCR-based genotyping revealed allele-specific amplification patterns for the CYP19A1 gene polymorphism (rs2414096). The wild-type G allele was consistently amplified in all control samples, whereas the variant A allele was identified in 7 of 12 tested PCOS cases, indicating the presence of polymorphic variation within the cohort (Figure 5 and Figure 6). Sanger sequencing further validated the SNP within the targeted 759 bp amplicon, confirming the accuracy of the genotyping results (Figure 7).

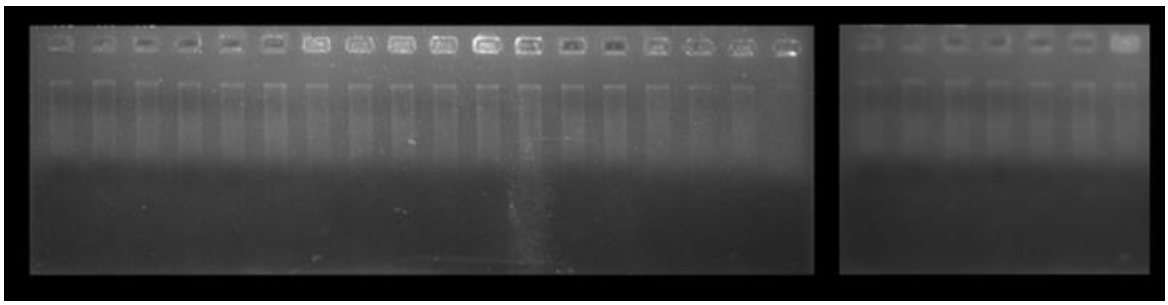

Figure 5: AGE image of extracted DNA from samples of PCOS Patients

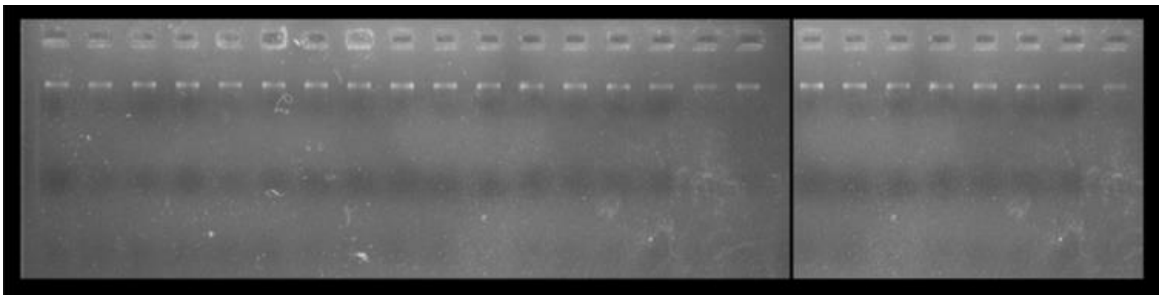

Figure 6: AGE image of extracted DNA from the samples of healthy individuals

The distribution pattern suggested a potential association of the A allele with increased susceptibility to PCOS. However, the relatively small sample size restricted the statistical power to establish a definitive correlation.

Figure 7: Multiple sequencing alignment of sequencing result for health and patient sample. SNP region has been highlighted

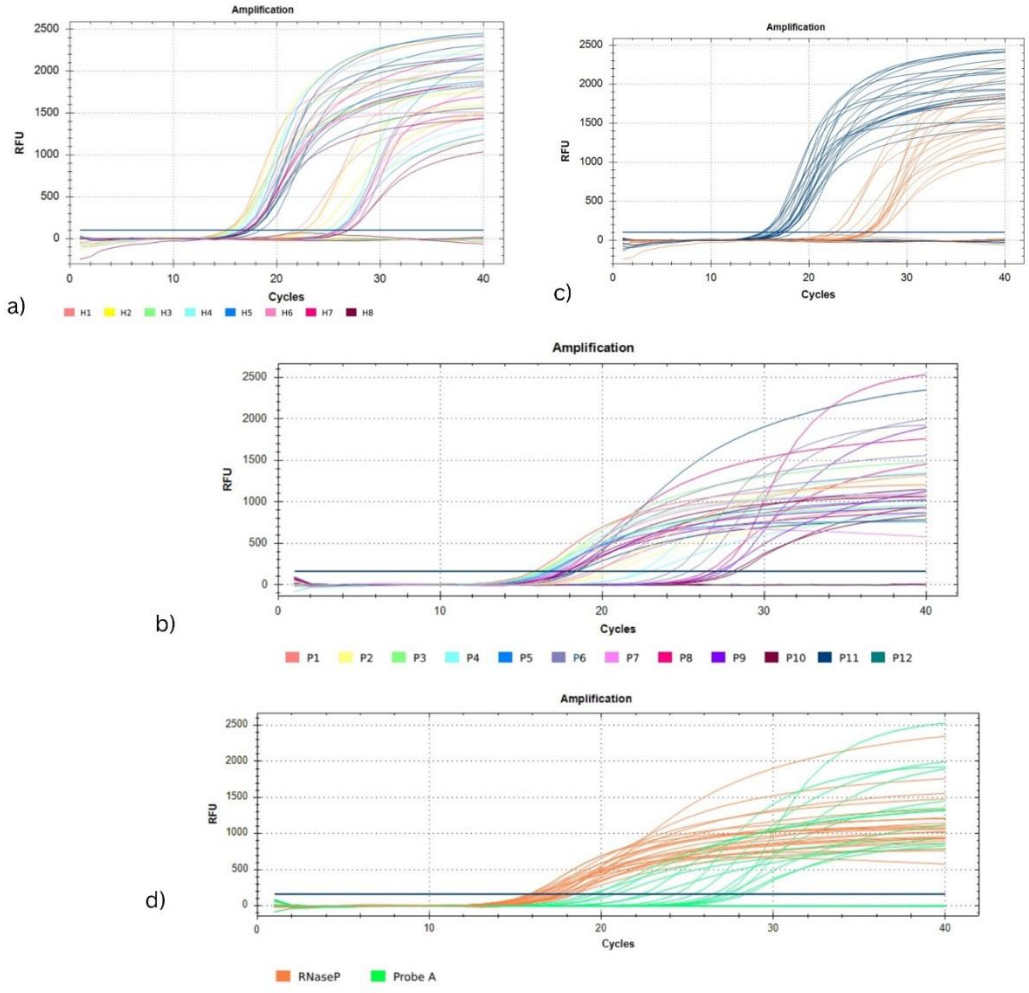

Figure 8. Genotyping of CYP19A1 (rs2414096). (a) Amplification of Probe G with EC (RNaseP) in healthy individuals (H1–H8). (b) Amplification of Probe A with EC (RNaseP) in PCOS patients (P1–P12). (c) Comparative amplification curves for Probe G across both groups. (d) Comparative amplification curves for Probe A across both groups, demonstrating selective amplification in PCOS cases.

Amplification plots further confirmed the allele-specific distribution. Probe G amplification with internal control EC (RNaseP) was observed in all healthy individuals (H1–H8), while Probe A amplification was detected in several PCOS samples (P1–P12). Comparative amplification analyses across groups revealed that Probe G showed consistent amplification in both controls and patients. Whereas Probe A demonstrated selective amplification in PCOS cases, with no signal in controls (Figure 8).

These findings collectively suggest that the A allele of rs2414096 may be associated with increased susceptibility to PCOS, although larger cohort studies are required to validate this genetic association.

Diagnostic Performance

Receiver Operating Characteristic (ROC) curve analysis demonstrated excellent diagnostic accuracy of AMH for distinguishing PCOS from controls (AUC > 0.90). NLR also showed strong discriminative potential, with superior sensitivity and specificity compared to conventional hematological indices. Combined analysis of AMH with NLR further improved predictive accuracy, suggesting a synergistic diagnostic value.

Discussion

The present study highlights the combined diagnostic utility of Anti-Müllerian Hormone (AMH) and the Neutrophil-to-Lymphocyte Ratio (NLR) in identifying women with PCOS in a South Indian cohort. Our results demonstrate significantly elevated AMH and NLR in PCOS patients, with a strong positive correlation between the two, suggesting that systemic inflammation may parallel ovarian dysfunction.

These findings align with the prospective cross-sectional study by Misra et al., which evaluated 80 women with PCOS and 80 healthy controls in Mumbai. Using AMH measurement, insulin resistance indices (HOMA-IR), and androgen profiling (DHEAS, androstenedione, testosterone, SHBG), they observed significantly higher AMH in both lean (6.59 ± 4.13 ng/mL) and obese PCOS groups compared to controls. Notably, AMH correlated strongly with androgen levels but not with insulin resistance, reinforcing its diagnostic and phenotypic utility independent of metabolic status [7].

Similarly, the case-control study by Sukesh focused on hematological markers (NLR, PLR, total leukocyte count) alongside AMH in PCOS. Using routine hematological analyzers and hormonal assays, they reported significantly higher NLR in PCOS ($p < 0.00001$), with a robust positive correlation to AMH ($rs = 0.7442$, $p = 0.000$). Their study further found no significant difference in PLR and red cell indices, corroborating the observations in our cohort [8]. Both studies employed Rotterdam 2003 diagnostic criteria and appropriately age- and BMI-matched controls, supporting strong comparability with our methodology.

Our molecular analysis revealed a higher prevalence of the CYP19A1 rs2414096 A allele exclusively among PCOS patients, consistent with prior Indian and international studies that implicate this SNP in disease susceptibility among South and West Asian women. These studies employed PCR-based genotyping and sequencing to confirm allelic distribution, validating our technical approach [9]. While global associations are heterogeneous, ethnic-specific enrichment of the A allele underscores the potential role of CYP19A1 variants in ovarian steroidogenesis and PCOS pathophysiology.

Hormonal assessment in this study involved measurement of AMH using standardized ELISA kits, complemented by evaluation of a comprehensive androgen panel in accordance with established protocols. Hematological markers, including NLR and PLR, were quantified using automated blood cell analyzers to ensure precision and reproducibility. Genetic analysis was performed using PCR, TaqMan-based assays, and sequencing to confirm allelic presence of CYP19A1 rs2414096. Statistical evaluation incorporated correlation analyses, ROC curve assessment, and subgroup comparisons, providing a robust framework for determining the diagnostic performance and interrelationships of endocrine, inflammatory, and genetic markers in PCOS.

The strong correlation between AMH and NLR highlights a potential mechanistic link between ovarian dysfunction and systemic inflammation, suggesting that a combined assessment could serve as an accessible, cost-effective screening strategy for PCOS, particularly in resource-limited clinical settings. Furthermore, CYP19A1 rs2414096 genotyping may offer additional risk stratification in ethnically specific populations.

Our findings confirmed significantly higher AMH levels in women with PCOS, consistent with prior studies. However, in line with the 2023 International PCOS Guideline, we caution against the use of AMH as a sole diagnostic tool. While our ROC analysis demonstrated excellent discriminatory performance, clinical practice should integrate AMH with established diagnostic criteria such as the Rotterdam criteria, clinical evaluation, and ultrasound findings. In this context, AMH may function as a valuable adjunctive biomarker, particularly when ultrasonographic assessment is limited, thereby enhancing diagnostic accuracy without compromising guideline-based standards [10]. Limitations include a single-center design and modest sample size, particularly for the genetic component, which may limit generalizability. Future studies should involve multi-centric cohorts, longitudinal follow-up, and functional characterization of CYP19A1 variants to validate diagnostic utility and elucidate mechanistic pathways.

Conclusion

The present study underscores the diagnostic potential of combining hormonal, inflammatory, and genetic markers in evaluating Polycystic Ovary Syndrome (PCOS). Elevated AMH levels demonstrated strong discriminative accuracy, reinforcing its role as a reliable biomarker for ovarian dysfunction. The

significant elevation of NLR in PCOS patients, along with its robust correlation with AMH, suggests that systemic inflammation parallels reproductive abnormalities, highlighting a pathophysiological interplay between ovarian dysfunction and immune activation. While PLR and erythrocytic indices showed minimal relevance, the diagnostic synergy of AMH and NLR achieved superior accuracy, indicating a practical, accessible, and cost-effective screening strategy suitable for routine clinical use.

Furthermore, molecular analysis revealed the presence of the CYP19A1 rs2414096 A allele predominantly in PCOS cases, pointing toward a potential genetic predisposition in this population. Although the limited sample size restricted definitive statistical association, these findings align with previous reports of ethnic-specific enrichment of this polymorphism, suggesting its relevance in South Indian cohorts.

Taken together, the study emphasizes the value of an integrated diagnostic approach. Larger multi-centric studies with diverse populations are warranted to validate these results, explore gene environment interactions, and develop tailored diagnostic tools for improving early detection and personalized management of PCOS.

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