

Assessment of Hematological Parameters and the Role of Anti Müllerian Hormone in Women with Polycystic Ovarian Syndrome: A Case Control Study

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Keywords Anti-Müllerian Hormone (AMH), Inflammatory Biomarkers, Polycystic Ovary Syndrome (PCOS),	Abstract Background: Polycystic Ovary Syndrome (PCOS) is a multifactorial endocrine disorder among women, often associated with chronic low-grade inflammation. This study evaluates the diagnostic relevance of inflammatory markers, including Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), and Total White Blood Cell (WBC) count, along with other hematological parameters such as Hb, RBC count, PCV, MCV, MCH, MCHC and hormonal marker Anti-Müllerian Hormone (AMH). Methods: A case-control study was conducted with PCOS patients and healthy controls. Significant difference in the haematological and inflammatory markers was analysed using the Kruskal-Wallis test, and the correlation of all significant markers was evaluated by Spearman's correlation. Results: AMH levels showed a significant elevation in the PCOS group with the P value < 0.00001; NLR also showed a highly significant difference between PCOS and control groups ($p < 0.00001$), with a strong positive correlation ($r_s = 0.7442$, $P = 0$) with AMH levels in PCOS patients. While Total WBC Count exhibited moderate significance ($p = 0.03436$), it highlighted a potential link to inflammation. PLR demonstrated a trend of elevation in PCOS cases but lacked statistical significance ($P = 0.10522$). Hemoglobin levels, Packed Cell Volume(PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) did not show any significant differences between the groups. Conclusion: NLR and AMH emerged as reliable markers for PCOS, with NLR strongly correlating with AMH and highlighting the role of systemic inflammation in PCOS pathophysiology. AMH showed strong significance as a diagnostic marker ($p < 0.00001$). However, Total WBC Count and NLR hold promise as cost-effective markers for assessing PCOS-associated inflammation. These findings emphasize the potential of haematological parameters in the diagnostic strategies of PCOS to improve patient outcomes.
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Introduction

Polycystic ovarian syndrome (PCOS) is a prevalent endocrine and metabolic disorder affecting approximately 6–20% of women of reproductive age. The symptoms of PCOS often begin to appear during puberty [1]. Hyperandrogenism, chronic anovulation, polycystic ovaries, and metabolic abnormalities are typical characteristics of PCOS [2]. Hormonal imbalances contribute to the development of pathological symptoms in women with this condition, especially the Anti-Müllerian Hormone (AMH), which plays a key role in regulating various stages of folliculogenesis and also exhibits neuroendocrine effects [3]. Additionally, the significance of serum AMH as an accurate indicator of ovarian reserve and its utility in predicting PCOS and primary ovarian failure is widely recognized. Furthermore, substantial evidence highlights the role of AMH in the development and progression of PCOS [4].

Systemic inflammation plays a role in the progression of various long-term illnesses like PCOS. Haematological parameters derived from complete blood count have recently been recognized as markers of systemic inflammation, primarily due to their low cost and practical accessibility [5,6]. Neutrophils are crucial immune defense cells that originate from the bone marrow, along with other immune cells, they enhance the release of cytokines during the early stages of inflammation, while cytokines, in turn, amplify inflammation by promoting the production of additional neutrophils. There have been studies conducted on

the neutrophil-lymphocyte ratio (NLR) showing their association with inflammatory conditions, which makes them potential markers of inflammation [7,8].

The majority of the studies indicate that women with PCOS have a higher NLR value, indicative of low-grade chronic inflammation. Thus, NLR can be used as an indicator in assessing inflammation and potentially predicting the progression of disease in PCOS [9,10].

In the last couple of years, several studies were conducted to reveal the association of NLR with PCOS and with other metabolic and hormonal parameters. However, till date, all of them lacked consistency due to limited case-control studies. Hence, the main objective of this study was to understand the association of AMH, an established marker of diagnosis for PCOS, in relation to NLR. We assessed the absolute neutrophil count, absolute lymphocyte count, and NLR in relation to the levels of AMH in women with PCOS to see if there is any significant correlation between these hematological measures and AMH levels.

Materials and Methods

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and was approved by the Srinivas University Ethics Committee (SUEC) (Protocol No: 28/AHS/2023). All participants provided written informed consent after receiving a detailed explanation of the study objectives and procedures.

A total of 100 participants were enrolled, comprising 50 age-matched healthy controls (women aged 18–40) and 50 women diagnosed with PCOS, all of whom were not receiving any medication at the time of recruitment. PCOS diagnosis was based on the Rotterdam criteria. Demographic data for all participants were collected and analysed.

Blood samples were collected from both control and PCOS groups and serum was separated for ELISA. Serum Anti-Müllerian Hormone (AMH) was measured using the "EasyStep Human AMH ELISA Kit" (ELK Biotechnology) with an ELISA reader (ALERE™ Easy Reader). Complete blood counts (CBC), including red blood cell (RBC) count, hemoglobin concentration, packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leukocyte count, and differential leukocyte count, were performed for both groups. CBCs were initially assessed using an automated hematology analyzer (Sysmex XN1000) and subsequently verified manually with an Improved Neubauer counting chamber. Peripheral blood smears were prepared to analyze differential leukocyte counts. Absolute neutrophil count (ANC) and absolute lymphocyte count (ALC) were calculated, and the neutrophil-to-lymphocyte ratio (NLR) was determined. All statistical analyses were conducted using Jamovi software (version 9).

Results

Table1: Demographic characteristics of the groups

S.no	Group characteristics	Control (N=50)	PCOS (N=50)
1.	Age in years (Median with range)	30 (18-40)	32 (18-40)
3.	BMI (Body Mass Index) *	26.0 (19.49-37.58)	26.27 (15.78 -40.89)

*The body mass index (BMI) was calculated based on height and weight measurements, with a median BMI of 26 for the control group and 26.27 for the PCOS group.

Table 2: All Biomarkers with Median, Range, and P-Values from Kruskal-Wallis Test.

STUDY PARAMETER S	CONTROL Group (Median & Range)	PCOS Group (Median & Range)	Kruskal Wallis (P-value)
AMH (ng/mL)	1.87 (1.55 -2.18)	5.75 (2.84-6.08)	<.00001
TC (Total Count) (cells/mm ³)	7320 (4852-11269)	8544 (4853– 11363)	.03436

NLR (%)	1.9 (1.05 -3.4)	2.0 (1.12-3.35)	<.00001
PLR (%)	133.78 (48.89-272.06)	149.95 (71.62-294.37)	.10522
PCV (%)	37.1 (22.33-44.8)	35.5 (12.6-44.72)	.24821
MCV (μm^3)	86 (68–99)	86 (68-119)	0.82529
MCH (pg)	28.3 (22.2-51.4)	32.2 (24.7-43.8)	0.86936
MCHC (g/dL)	31.1 (20.9- 57)	28.2 (20.44-52.03)	0.82312
Hb (g/dL)	12.8 (10.55-16.42)	8.98 (7.83-16.39)	1.4905

The homogeneity of the sample population was maintained in the study. The median values were calculated for demographic parameters, including age and BMI. The median age for the healthy control group was 30, while the median age for the PCOS group was 32. The age range for both groups was maintained between 18 and 45 years. Participants below 18 years were excluded as they might not fully understand the consent process or provide accurate data, while those above 45 years were excluded to avoid potential confounding factors such as other hormonal issues or health risks (Table 1).

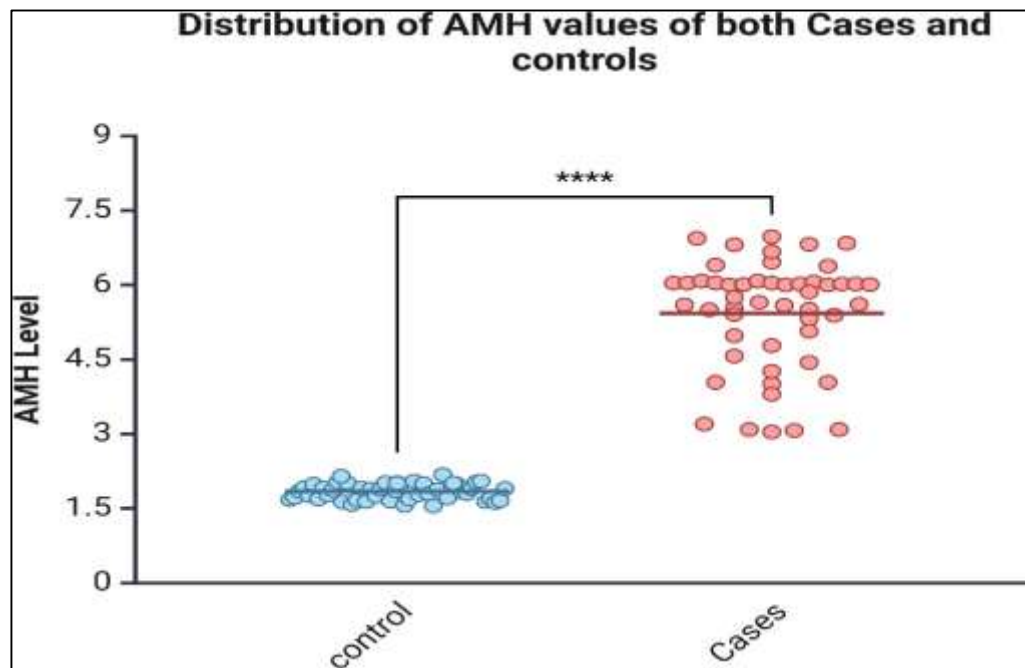
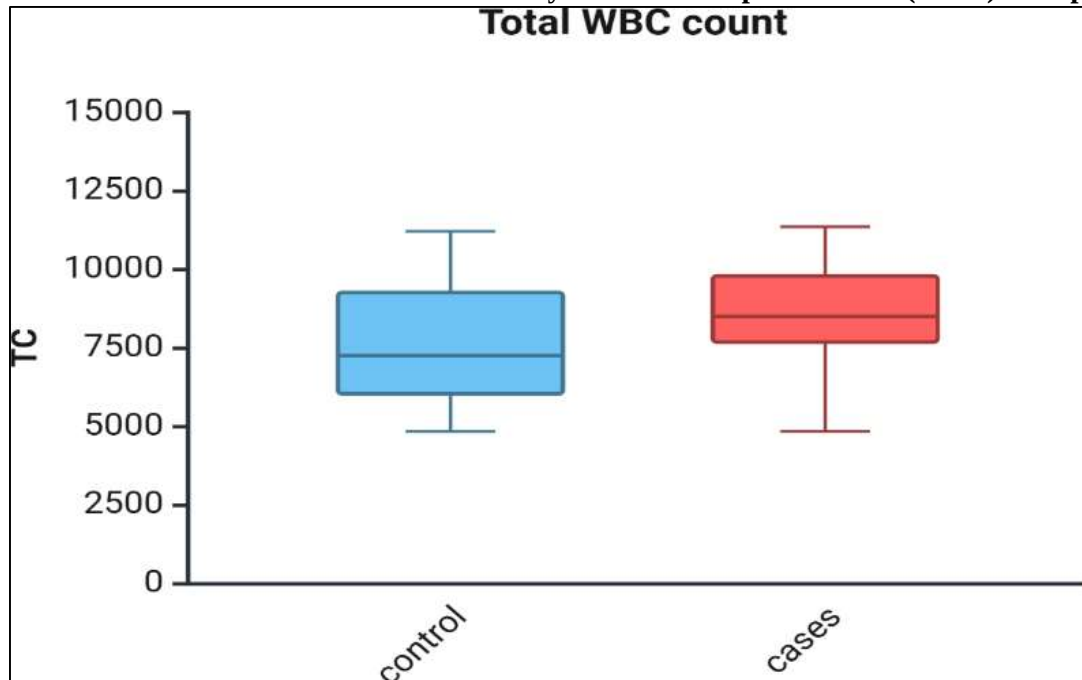


Chart 1. Distribution of AMH value of both cases and Control groups

Anti-Müllerian Hormone (AMH) levels showed a significant difference between the control group and the PCOS group ($p < 0.0001$) as determined by the Kruskal-Wallis's test. The median AMH level for the PCOS group was 5.75 (2.84-6.08) and in control group the median was 1.87 within the range of 1.55 to 2.18, these results indicating that the AMH is an excellent diagnostic marker for PCOS.

Chart 2 Total WBC count Between Healthy Control Group and PCOS (Cases) Group



The Kruskal-Wallis test (Table2) was performed for all the markers, and the Total WBC Count yielded a p-value of 0.03436. This indicates a statistically significant difference in the Total WBC Count between the PCOS and control groups when compared with the standard significance level of 0.05. While this result is significant, it is not considered as stronger. However, this finding suggests a potential link between the inflammatory processes associated with PCOS. The Total WBC Count could serve as a useful diagnostic marker for assessing the level of inflammation or understanding the pathophysiology of PCOS and it could be helpful to give personalized treatment strategies to the patient.

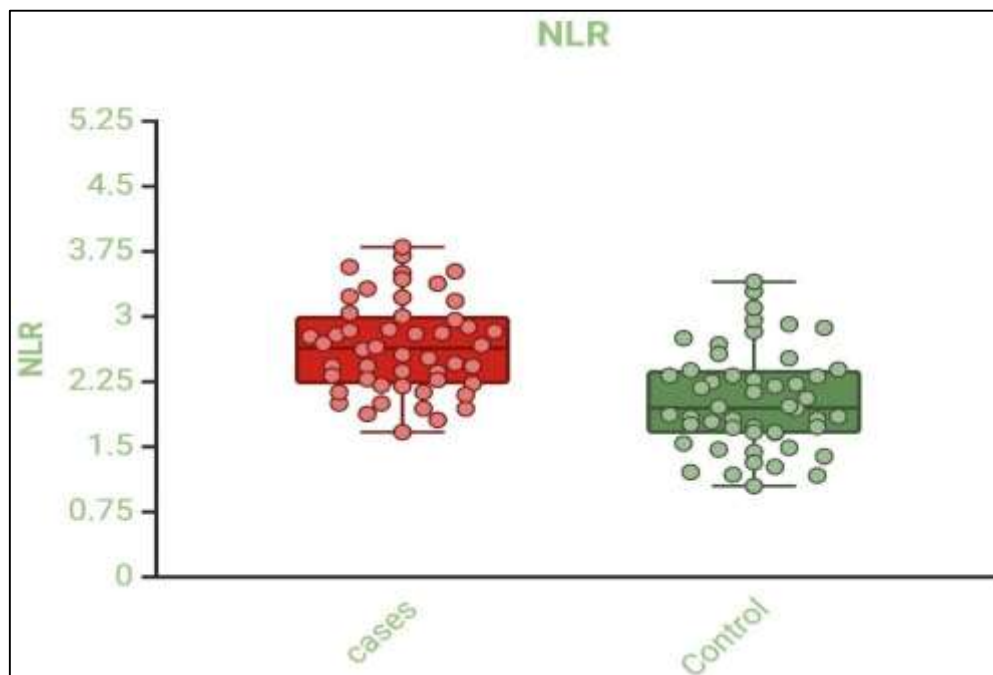


Chart 3 NLR values of both Healthy control and PCOS Group

The NLR ratio were significant ($p < .00001$) and this indicating a highly significant difference between the PCOS case and the control group. This extremely low p-value suggests a very strong statistical association, implying that NLR is markedly different between the two groups. Such a result highlights the potential of

NLR as a reliable marker for distinguishing individuals with PCOS from healthy controls. The high significance underscores its relevance in understanding the inflammatory mechanisms linked to the condition of PCOS and its potential role as a diagnostic or prognostic marker in clinical practices. The median NLR was identified as 1.9, with a range of 1.05 to 3.4, in the control group, while in the PCOS group, the median was 2.0, with a range of 1.12 to 3.35.

The Platelet-Lymphocyte Ratio (PLR) was analyzed in both the PCOS and control groups. Although the individual values were slightly elevated in the PCOS group, the difference was not statistically significant, with a p-value of 0.10522 obtained by performing Kruskal-Wallis test, This suggests that PLR may not be a strong marker for distinguishing PCOS from healthy controls in this study. The median and range values for the control group were 133.78(48.89–272.06), while the PCOS group had a median of 149.95(71.62–294.37), these results proved that there is a trend toward higher PLR values in the PCOS group, the lack of statistical significance limits its utility as a reliable indicator compared to NLR. Rather than a diagnostic marker, PLR can be used as an indication marker along with NLR for the better understanding of the disease. The P-value for the Packed Cell Volume (PCV) is .24821, which indicates, statistically there is not significant difference between the PCOS and control groups when comparing with standard significance level of 0.05. This result suggests that PCV values were not showing a significant difference between the two groups, implying that changes in PCV could be associated with PCOS. However, since the P- value is not closer to 0.01.

The other markers, such as MCV, MCH, and MCHC, did not show any significant difference between the case and control groups, as the p-value obtained for all these parameters was 0.8. The p-value obtained for hemoglobin was 0.4, indicating no significant difference between the control and PCOS groups. The range of hemoglobin levels in the control group was 10.55 to 16.42, with a median of 12.8, while in the PCOS group, the range was 7.83 to 16.39, with a median of 8.98. the lack of statistical significance suggests that hemoglobin levels are not strongly affected by the presence of PCOS in this study. However, based on the median and range, it is important to note that hemoglobin levels can vary based on the individual's health status, disease characteristics, symptoms like excessive bleeding and potential underlying factors, meaning that the value of hemoglobin might be depend with the pathophysiology of PCOS in this context.

Table 3 Spearman's correlation test

S No:	Parameter	RS value	P-value (2-tailed)
1.	AMH and NLR	RS=0.7442	p= 0.001
2.	AMH and TC	RS=-0.222	0.20
3.	TC and NLR	RS=-0.0865	>0.50

The Spearman's correlation test was performed for all three significant markers of NLR, Total WBC count and AMH. The Spearman's correlation test between AMH and total WBC count yielded a correlation coefficient 'Rs' of -0.222, with ap-value of 0.20. This indicates a weak negative correlation between AMH and total WBC count. The p-value of 0.20 is greater than the commonly used significance threshold of 0.05, suggesting that the relationship between AMH and total WBC count is not statistically significant. Therefore, there is no strong evidence to support a meaningful association between AMH levels and WBC count in this study, The correlation of NLR and WBC count obtained RS= -0.0865and P value>0.50, both indicate that there is not a significant correlation between WBC count and NLR statistically.

The Spearman's correlation between NLR and AMH of PCOS group yielded a correlation coefficient 'Rs' of 0.7442, with a p-value of 0(2-tailed). This indicates a strong positive correlation between NLR and AMH levels. The p-value of 0 is highly significant, suggesting that the relationship between NLR and AMH is statistically robust and the correlation is positive, All the higher NLR values were associated with all the higher AMH levels, supporting the potential link between systemic inflammation as indicated by the higher values of NLR and ovarian function as indicated by the higher AMH values in PCOS patients (Table 3).

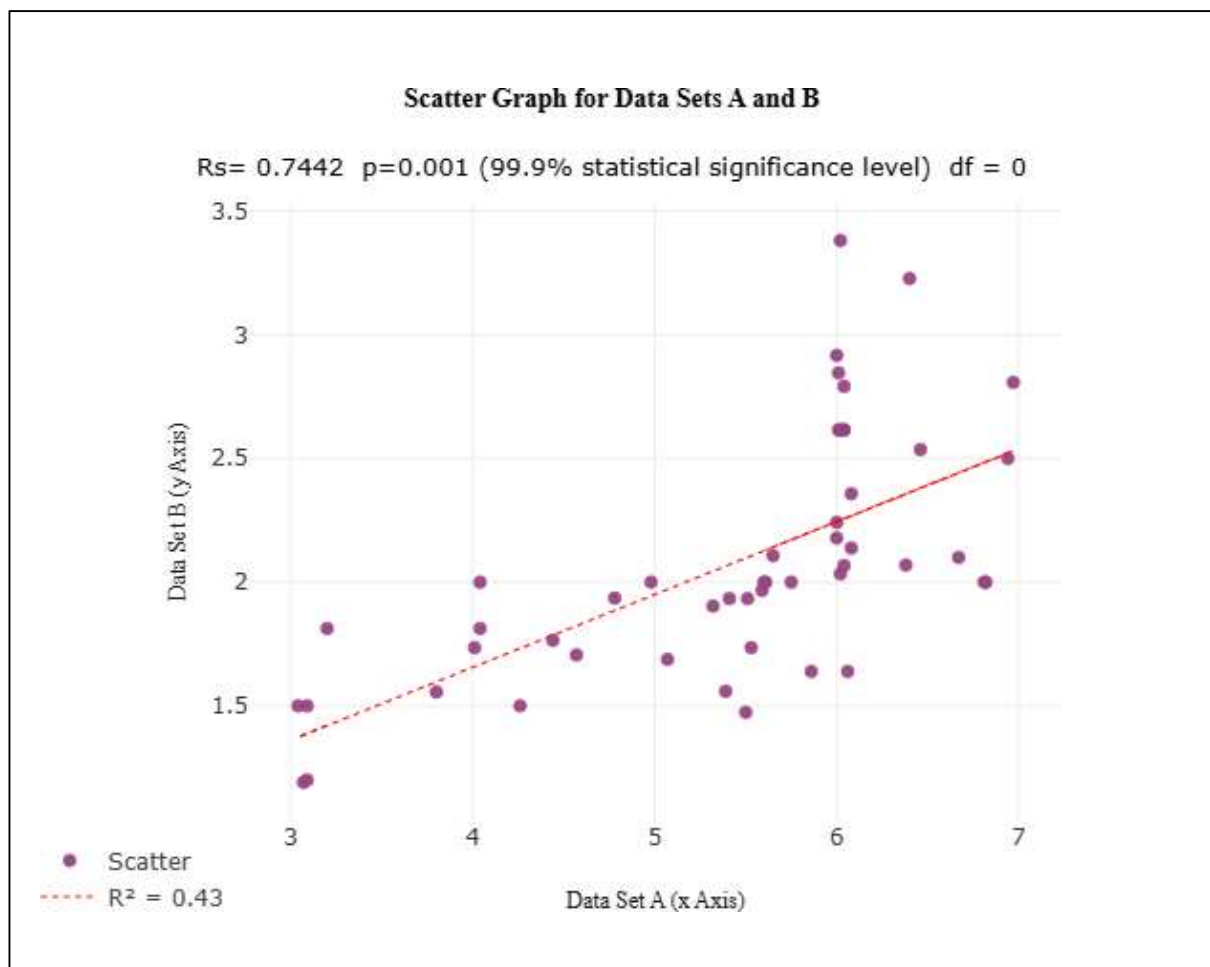


Table 3 shows the results of Spearman's correlation of all significant parameters of PCOS group.

Chart4: Indicates Spearman's correlation test Between The values of AMH and NLR of PCOS group (Data set A AMH and Data set B is NLR)

Chart 4 shows the results of Spearman's correlation test between the values of AMH (Data Set A) and NLR (Data Set B) in the PCOS group. The test evaluates the strength and direction of the relationship between these two variables. The trend line in the graph indicates an upward slope, suggesting that as AMH values increase, NLR values also tend to increase. This illustrates a positive correlation between AMH and NLR in the PCOS group. Therefore, the graph suggests that higher AMH levels are associated with higher NLR values, indicating a positive relationship between these two biomarkers in this population.

Discussion

A cohort study conducted by Malhotra et al. (2023) demonstrated AMH to be the most reliable diagnostic indicator for PCOS. The study showed a positive correlation between higher levels of AMH and more severe clinical, hormonal, and metabolic abnormalities in women with PCOS. Similarly, this case-control study showed a marked increase in the level of AMH in patients with PCOS [11]. Compared to the control group, it confirmed its utility as a diagnostic marker identifying the condition of PCOS. Rudnicka et al. (2020) proved that Polycystic Ovary Syndrome (PCOS) is linked to elevated white blood cell (WBC) counts, supporting the hypothesis that the condition is associated with low-grade inflammation. Besides, this study further suggests that such inflammation might not solely be attributed to adiposity and might instead be a contributory factor that results from an elevated level of androgen; it suggests complicated pathophysiology behind PCOS. Though a marginal elevation of WBC count in patients with PCOS as compared to controls has been seen, no clearcut relationship could be established between the level of WBC count and the concentrations of AMH [12].

NLR and PLR are documented as good indicators of inflammation, and abnormal levels typically correlate with the severity of a disease. Al-Dahhan et al., 2021 presented results in the study which said that the ratio between neutrophils and lymphocytes was found to be altered in PCOS. Results from the current study also

confirmed that there was a highly significant increase in NLR in PCOS patients in comparison with the control group. However, the PLR value was not found to be highly statistically significant though mild increase was noted in the PCOS group. Besides, a direct correlation between NLR and AMH was observed, which reflects that both of these markers tend to increase in individuals with PCOS, which makes these markers important for diagnostic purposes as well [13].

Similar to our results, a case-control study by Almaeen et al., (2022) analyzed 176 blood samples from age-matched women of reproductive age, including 88 PCOS cases and 88 healthy controls. It also identified NLR as an effective indicator, with its potential being a reliable marker. Moreover, the increased WBC count observed in PCOS patients may be associated with chronic low-grade inflammation. These results underscore the importance of using cost-effective biomarkers in assessing chronic low-grade inflammation in PCOS [14].

Conclusion

Our study results show marked differences between the PCOS and control groups with respect to biomarkers like AMH, NLR, and WBC count. The analysis reveals that AMH and NLR are closely linked in PCOS patients, with a positive correlation observed between AMH and NLR, suggesting that these biomarkers may provide useful diagnostic insights into the condition. While other markers, such as PLR, Hb, and WBC count, vary but can be taken as indicative markers together with other diagnostic tests, it is only AMH and NLR which have a very strong correlation supporting their potentiality as significant markers of inflammation and hormonal imbalances in PCOS, though further studies are required to confirm the larger clinical implications of these finding.

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Conflict of Interest

No conflict of interest to disclose

Funding Source

Nil

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Abbreviations:

- **PCOS:** Polycystic Ovary Syndrome
- **NLR:** Neutrophil-to-Lymphocyte Ratio
- **PLR:** Platelet-to-Lymphocyte Ratio
- **WBC:** White Blood Cell
- **Hb:** Hemoglobin
- **RBC:** Red Blood Cell
- **PCV:** Packed Cell Volume
- **MCV:** Mean Corpuscular Volume
- **MCH:** Mean corpuscular haemoglobin
- **MCHC:** Mean Corpuscular Hemoglobin Concentration
- **AMH:** Anti-Müllerian Hormone
- **rs:** Spearman's Rank Correlation Coefficient
- **Pvalue:** Probability value