

Exploring hematological parameters for the prediction of malaria: A focus on neutrophil to lymphocyte ratio and monocyte to lymphocyte ratio

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ABSTRACT

Objectives: The aim of this study is to observe variations in various hematological parameters in malaria, to compare these variations between cases (malaria positive patients) and controls and to determine association of NLR and MLR with malaria.

Material and Methods: It was a prospective cross-sectional study conducted in the department of Pathology, Pakistan Institute of Medical Sciences Hospital, Islamabad between 1st July 2024 to 30th December 2024. A total of 105 malaria positive-cases and 105 control cases were included in the study. Consecutive non- probability sampling was done. For each case total white cell count, differential leucocyte count, absolute counts, hemoglobin and platelet counts were observed. From the above parameters NLR and MLR were obtained. To analyze the data SPSS 20 was used.

Results: Out of 105 malaria cases, 99 were of *Plasmodium vivax*, 4 of *Plasmodium falciparum*, and 2 of mixed infections. It was observed that total leukocyte count, neutrophil percentage, lymphocyte percentage, absolute lymphocyte count, absolute monocyte count, platelet count, NLR and MLR showed significant association with malaria while Hb, and absolute neutrophil count didn't show a significant association with malaria.

Conclusion: NLR and MLR can play an important role in predicting malaria as inflammatory marker. Also, in malaria cases, altered platelet, WBC, and lymphocyte counts are vital indicators.

Keywords: Malaria, Monocyte to lymphocyte ratio, Neutrophil to lymphocyte ratio, Platelets.

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INTRODUCTION

Malaria is a parasitic infection caused by *Plasmodium* spp. It is highly prevalent in tropical and subtropical areas, causing a significant burden on health care. As per the World Health Organization's data, in 2021, the disease affected 247 million individuals worldwide and resulted in 619,000 deaths, showing a significant increase from 2019 when there were 229 million cases and 409,000 deaths [1].

There exist five main *Plasmodium* species capable of infecting humans. These include *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi*. It is transmitted in humans by the bite of female *Anopheles* mosquito. However other routes like blood transfusion and maternal transmission are also observed [2].

Malaria is endemic in Pakistan. Currently Pakistan is fighting with malaria epidemic, with approximately 3.4

million suspected malaria cases reported between January and August 2022, in contrast to 2.6 million during the previous year [3]. According to a study by Khattak *et al*, the prevalence for Malaria in Islamabad capital territory is 4.6% [4].

Laboratory tests have been successful in identifying over 170,000 cases, with majority being attributed to *Plasmodium vivax* accounting for 77% and second in line is *Plasmodium falciparum* accounting for 23%, the latter is associated with more severe forms of the disease and has comparatively high fatality rate [3]. Various diagnostic tests like microscopy, rapid diagnostic tests, and polymerase chain reaction (PCR) assays are used for diagnosis of malaria. Microscopy is the gold standard for diagnosing infection. However, microscopy has lower sensitivity, and it requires skilled professionals. Pakistani government has employed various strategies to combat malaria like early detection, timely management, targeted vector control, and epidemic prevention [5]. Peripheral blood samples are routinely analyzed for complete blood counts and differential white cell percentages and are used for the diagnosis of various infectious diseases, including malaria. However, their utilization as markers of disease risk and inflammation has not been explored much. The monocyte to lymphocyte ratio (MLR) and neutrophil to lymphocyte ratio (NLR) have

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emerged as potential indicators of inflammation in various disease states, such as tumors and cardiovascular disorders [6]. The neutrophil to lymphocyte ratio (NLR), is simply calculated as a ratio between neutrophil and lymphocyte counts in peripheral blood and serves as a biomarker that combines two different elements of the immune system, encompassing the innate immunity driven by neutrophils and adaptive immunity supported by lymphocytes [7]. Nevertheless, many researchers have expressed reservations about the sufficiency of data regarding the predictive value of NLR. On the contrary, a high monocyte to lymphocyte ratio (MLR) in peripheral blood has been found to have a good predictive value of an individual's immune response to *Plasmodium falciparum* infection [8].

The diagnosis of malaria poses significant challenges, particularly in economically less developed regions like Pakistan, where the need for rapid, cost-effective, and accurate testing methods is paramount. These differences in hematological parameters can serve as valuable markers for laboratory personnel, possibly indicating the existence of parasite and facilitating its swift detection. The objectives of this study are to determine the various hematological alterations in malarial illness, to compare the variations in hematological parameters between malaria positive patients and controls and to determine association of NLR and MLR with malaria positivity.

MATERIAL AND METHODS

It was a Prospective Cross-sectional study since data was collected to observe associations at one specific point of time and patients were not followed up over time. It was conducted at the Department of Pathology, Pakistan Institute of Medical Sciences (PIMS). The duration of study was six months from July 2024 to December 2024. Sample size was calculated by WHO Calculator with a 95% confidence level, 4.6% prevalence [4] and 5% margin of error. The sample size was estimated to be 73; however, 105 cases and 105 controls who met the inclusion criteria were enrolled for better result generalizability. The sampling technique used was consecutive non-probability sampling. Cases were defined as patients with fever, of any age-group and gender with clinical features of malaria and who were smear positive for any species of malaria. Patients of malaria who were already anti-malarial drugs were excluded from the study. Controls were defined as patients of any age group and gender with acute febrile illness, who were clinically suspected to have malaria but were found to

be smear-negative for malaria and had not taken anti-malarial drugs for their illness. The study was conducted after approval from the hospital ethics committee [ERB No: F.1-1/2015/ERB/SZABMU/1209]. The hematological parameters under study included total leukocyte count, hemoglobin, platelet count, absolute neutrophil count, absolute lymphocyte count, absolute monocyte count. NLR and MLR were derived from the absolute values. About 3 ml of each patient and control samples for complete blood count was collected by clean venipuncture in a vial containing Tri-Potassium ethylenediaminetetraacetic acid (K3-EDTA). These samples were analyzed on automated hematology analyzer BC-600 from Mindray according to manufacturer's instructions and after ensuring the reliability of testing by daily quality controls. The presence of malaria parasite as well as species of *plasmodium* was determined on Giemsa-stained peripheral blood smear by two trained hematologists. Demographic data, registration number, CBC parameters and type of plasmodium species were recorded. NLR and MLR values were calculated from the CBC data. SPSS version 20 was used to analyze the data. Qualitative variables such as gender, number of patients, and type of species were expressed in terms of frequency and percentage n(%). Quantitative variables such as age of patient, CBC parameters (TLC, Hb, platelet count, ANC, ALC, AMC) and calculated parameters NLR and MLR were measured as Mean (SD). In this study, hemoglobin value of <10 g/dl was considered as anemia [8] and a platelet count of less than $150 \times 10^3/\mu\text{L}$ was considered as thrombocytopenia. Thrombocytopenia was further subcategorized as mild (platelet count $100\text{--}149 \times 10^3/\mu\text{L}$), moderate (platelet count $50\text{--}99 \times 10^3/\mu\text{L}$) and severe (platelet count $<50 \times 10^3/\mu\text{L}$). For normality assumption, Kolmogorov-Smirnov test was used for each variable and most show p value <0.05, highlighting non-parametric data. Non-parametric test Mann-Whitney U-Test was applied to study and compare the parameters in cases and control groups. A p value < 0.05 was taken as statistically significant.

RESULTS

A total of 210 subjects were included in our study which comprised of 105(50%) cases and 105(50%) controls. Among the cases, the mean age was 20.17 (18.42) years and among controls it was 14.26 (16.65) years. The male to female ratio among cases was 1.7 :1, whereas in control group, it was 1.2 :1 (Figure-I). The order of frequency of malarial parasite species identified microscopically on blood smears was

Plasmodium vivax 99(94.3%), *Plasmodium falciparum* 4(3.8%) and mixed *falciparum* and *vivax* 2(1.9%). Table-1 depicts the comparison of Haematological abnormalities among cases and controls (n=105 in each group). Among cases with thrombocytopenia, 19(20.2%) cases showed mild thrombocytopenia, 37(39.3%) had moderate thrombocytopenia and 41(43.6%) had severe thrombocytopenia. Table-II

shows the comparison of hematological parameters in cases and control groups. Leukocyte count, Neutrophil %, Lymphocyte %, ALC, AMC and Platelet count are significantly different in both groups (p<0.05). Table-III highlights the comparison of NLR and MLR among cases and controls. Both these ratios are significantly different between the cases and control groups (p<0.05).

Table-I: Comparison of Haematological abnormalities among cases and controls (n=105 in each group)

Haematological Abnormalities	Cases	Controls
Leukopenia	18(17.1%)	18(17.1%)
Neutrophilia	13(12.4%)	18(17.1%)
Lymphopenia	27(25.7%)	16(15.2%)
Anemia	61(58.0%)	57(54.3%)
Thrombocytopenia	94(89.5%)	39(37.1%)

Table-II: Comparison of means of Haematological parameters among cases and controls (n=105 in each group)

Haematological Parameters	Cases	Controls	P value*
Leukocyte count (x 10 ³ /ul)	7.26 (4.20)	9.94 (8.17)	0.008
Neutrophil%	61.03 (19.38)	51.96 (21.82)	0.003
Lymphocyte %	29.45 (17.32)	38.14 (20.37)	0.002
Monocyte %	7.63 (7.31)	7.11 (4.26)	0.96
Absolute Neutrophil count (x 10 ³ /ul)	4.56 (3.45)	5.02 (4.18)	0.60
Absolute Lymphocyte count (x 10 ³ /ul)	2.02 (1.89)	3.99 (5.87)	<0.001
Absolute monocyte count (x 10 ³ /ul)	0.46 (0.35)	0.63 (0.48)	0.008
Haemoglobin (g/dl)	10.43 (2.70)	10.90 (2.11)	0.18
Platelet count (x 10 ³ /ul)	76.23 (61.19)	235.37 (170.73)	<0.001

* p-value computed by Mann-Whitney U test

Table-III: Comparison of means of NLR and MLR among cases and controls (n=105 in each group)

	Cases	Controls	P value*
NLR	4.13 (5.30)	3.81 (10.14)	0.003
MLR	0.31 (0.27)	0.29 (0.50)	0.025

* p-value computed by Mann-Whitney U test

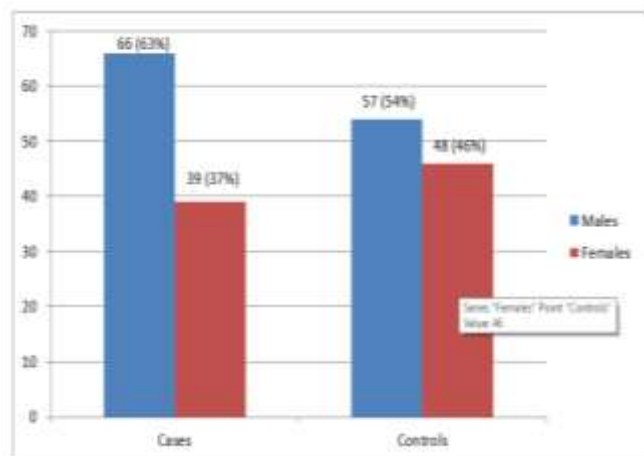


Figure-I: Gender distribution in cases and control groups (n=105 in each group).

DISCUSSION

For effective management of malaria quick and accurate diagnosis is most important [8]. The mean age of the malarial patients in our study was 20.17 (18.42) years. In another study, the mean age of these patients was 29.2 years and the highest proportion of cases (46%; 76 cases) was seen in the 20-30 years age group [9]. Although data about the prevalence of malaria with respect to age and gender is limited but it has been observed that malaria is more prevalent in males than in females [10]. Our study also showed similar results with a male to female ratio of 1.7:1. This may be because males as compared to females spend more time outside, where mosquitoes are prevalent. In the current study most of cases were of *Plasmodium vivax* 99 (94.3%), followed by *Plasmodium falciparum* 4 (3.8%). Only 2(1.9%) were of mixed *falciparum* and *vivax* infection. Similar results were observed in other

studies [11,12] however few studies reported *Plasmodium falciparum* as the most common malarial infection [13]. Studies have shown that on blood complete examination, hemoglobin, platelet counts, white cells, lymphocytes are usually low in malaria patients while monocytes and neutrophils are higher in malaria patients [14]. We compared hematological parameters among cases and controls in current study. It was observed that leukocyte count (P value=0.008), lymphocyte percentage (P value =0.002) and platelet count (P value = <0.001) were significantly low in the malaria patients as compared to controls. Percentage of neutrophils (P value= 0.003) was significantly higher in the malaria patients as compared to controls. We could not observe statistically significant association in monocyte percentage and in hemoglobin levels among cases and controls. Study done by omarine and colleagues showed statistically significant decrease in white cell counts (p value=0.0001) and platelet counts (P value=0.0164), like our study. However, they didn't observe significant differences in the means of lymphocyte percentage and neutrophil percentage, unlike current study [15]. Another study done by Awoke N. and colleagues showed Significant low white cell count (P=0.027), and lymphocyte count (P=0.004) in malaria patients than controls, thus complementing current study [16]. Thrombocytopenia was a significant finding in the current study. Association between thrombocytopenia and malaria is observed in other studies too and low platelet count is considered as a predictor of severe malaria. [17,18,19]. The neutrophil-to-lymphocyte ratio (NLR) is a ratio between the neutrophil and lymphocyte counts in the peripheral blood. This ratio represents the two arms of the immune system. Neutrophils are responsible for innate immune system while lymphocytes are indicative of adaptive arm of the immune system. Thus, NLR can be used as an indicator of systemic inflammation [20,21]. It is also investigated as a prognostic marker of parasitemia in malaria. Positive correlation between NLR and parasitemia in malaria has been observed in various studies and this ratio also tells the disease severity in malaria patients [22]. Monocyte to lymphocyte ratio is a simple ratio between monocytes and lymphocytes in the peripheral blood. This ratio also tells us the ability of the immune system to fight against an infection. After exposure, if a person is protected against malaria depends on a balance between pro-inflammatory and anti-inflammatory responses. In case of plasmodium falciparum infection, pro-inflammatory cytokines are

used as an initial immune response. However, to control this infection and for proper immune function this initial response needs to be balanced by adaptive immune system [23]. In present study when we compared the NLR and MLR between cases and controls, we found a significant relationship (P value for NLR=0.003 and for MLR=0.025). This finding in current study is similar to other studies. Study done by Kotepui, M et al showed high NLR ratio (P value < 0.0001) and high MLR ratio (P value < 0.0001) in malaria patients than controls in their study [24]. Similarly, Wolfswinkel *et al.* demonstrated a weak but statistically significant positive correlation between parasitaemia and NLR in malaria patients (rs = 0.165, p = 0.03). [25].

CONCLUSION

Differences in hematological parameters between cases and controls were significant in present study. Thrombocytopenia, leukopenia, lymphopenia and neutrophilia were most significant findings in malaria cases than controls. Ratios NLR and MLR also showed a significant association with malaria, suggesting utility in predicting malaria in this study population.

LIMITATIONS

It was a single center study with limited sample size which may limit the findings of other regions with different prevalence of malaria. Other inflammatory markers like CRP were not included in this study. Future multicenter research with larger sample size and addition of other markers in future studies may help in having more insight into immune responses in malaria.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Sundas Ali: Concept, literature search, data collection, data analysis, data interpretation, drafting, final approval, responsible for every aspect of the research

Maha Tariq Kiani: Literature search, data collection, drafting, final approval, responsible for every aspect of the research

Amna Imtiaz, Farah Hanif: Data Collection, final approval, responsible for every aspect of the research

Ayesha Siddiq: Drafting, final approval, responsible for every aspect of the research

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