

Neutrophil-to-lymphocyte ratio as a diagnostic marker: A comparative study in dengue and malaria patients

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ABSTRACT

Objective: To evaluate the diagnostic utility of neutrophil-to-lymphocyte ratio in distinguishing dengue from malaria and its association with thrombocytopenia.

Material and Methods: A prospective cross-sectional study was conducted at Creek General Hospital Laboratory from 1st June to 31st August 2025, including 105 confirmed cases of dengue (n = 50) and malaria (n = 55). Hematological indices, including platelet count and neutrophil-to-lymphocyte ratio, were assessed. Statistical analysis was performed using SPSS version 26. Mann–Whitney U test and Fisher's exact tests, with significance set at $p < 0.05$.

Results: Majority (50.5%) of patients were young adults, 16–30 years with male predominance (61%). Thrombocytopenia was present in both groups, with severe thrombocytopenia in 48% of dengue and 36.4% of malaria cases, showing no significant association with infection type ($p = 0.242$). Neutrophil-to-lymphocyte ratio differed significantly between malaria and dengue cases. Malaria patients had a higher mean value (5.43 ± 4.56 ; median 3.45, IQR 1.16–6.22) compared to dengue patients (2.72 ± 2.52 ; median 2.02, IQR 1.30–3.38) ($p < 0.001$). High neutrophil-to-lymphocyte ratio was more frequent in malaria (58.2%), low ratio predominated in dengue (84%) and $p < 0.001$.

Conclusion: Platelet count alone cannot reliably differentiate dengue from malaria, neutrophil-to-lymphocyte ratio provides strong discriminatory value. Elevated ratio is associated with malaria, and low ratio with dengue, making it a simple, cost-effective diagnostic and prognostic marker in resource-limited endemic settings.

Keywords: Dengue, Diagnostic marker, Malaria, Neutrophil-to-Lymphocyte ratio, Thrombocytopenia

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INTRODUCTION

Dengue and malaria are the two most prevalent insect-borne illnesses in tropical regions, particularly in Southeast Asia, including Pakistan [1,2]. Both diseases pose a significant public health challenge due to their endemicity, overlapping clinical symptoms, and the burden they place on healthcare systems. Malaria is a parasitic infectious disease caused by *Plasmodium* species and transmitted by the bite of infected female Anopheles mosquitoes. Among the five *Plasmodium* species that infect humans, *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*. *P. falciparum* is responsible for the most severe form of the disease. According to the World Malaria Report there were 263 million malaria cases globally in 2023 [1]. In

Pakistan alone, suspected malaria cases increased from 2.6 million in 2021 to over 3.4 million between January and August 2022 [3]. Dengue, caused by the dengue virus (DENV), an Orthoflavi-virus in the Flaviviridae family, is transmitted by *Aedes spp.* Mosquitoes [4]. It can present as classic dengue fever (DF) or progress to a more severe form such as dengue hemorrhagic fever (DHF), which is marked by plasma leakage, thrombocytopenia, and bleeding tendencies [5]. The World Health Organization (WHO) estimates that around 50 million cases of dengue occur globally each year [6]. In 2022, there were 25,932 confirmed dengue cases and 62 reported deaths in Pakistan [3].

Co-infection and misdiagnosis are significant issues, as both diseases often present with similar symptoms, including high fever, severe headache, joint and muscle pain, sore throat, nausea, rash, and general malaise [7]. These overlapping features make clinical diagnosis challenging, especially in resource-limited setting. Consequently, patients may be misdiagnosed, leading to delayed or inappropriate treatment. One emerging diagnostic aid is the neutrophil-to-lymphocyte ratio (NLR), a simple, cost-effective biomarker derived from routine blood counts. NLR, along with platelet count and other

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hematological indices, is being explored for its potential to distinguish between viral and parasitic infections and to assess prognosis [8]. Previous studies indicate that dengue patients generally exhibit lower white blood cell counts, neutrophils, monocytes, eosinophils, and NLR values compared to those with malaria [9,10]. Elevated NLR has been associated with various infectious diseases and inflammatory states, correlating with disease severity and poor clinical outcomes. In malaria, elevated NLR levels have shown promise in predicting severe manifestations such as cerebral malaria and acute respiratory distress syndrome (ARDS), highlighting its potential utility as a prognostic indicator and therapeutic monitoring tool [11].

The findings of Agrawal et al suggested that elevated NLR on admission was significantly associated with severe dengue and poorer clinical outcomes, such as prolonged hospitalization, hemorrhagic complications, or progression to shock [12]. Similarly, a cross-sectional study from Southern India revealed that elevated neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) levels were significantly associated with severe dengue manifestations, including plasma leakage, hemorrhagic complications, and organ involvement [11].

The findings of another study demonstrated that both lymphocytopenia and an elevated neutrophil-lymphocyte count ratio (NLCR) at admission were strong independent predictors of severe malaria, outperforming traditional markers like parasite load and C-reactive protein (CRP) [13]. The NLCR, in particular, showed high sensitivity and specificity, suggesting its utility as a rapid, accessible, and cost-effective tool for early risk stratification in malaria cases, aiding timely clinical intervention.

This study aimed to investigate the utility of NLR in differentiating between dengue and malaria, given their clinical similarities and the increasing need for efficient diagnostic tools in resource limited settings. Establishing NLR thresholds in the local population could improve patient stratification, early intervention, and prognosis, reducing morbidity and mortality. Moreover, integrating NLR with biomarkers such as platelet count, CRP, and procalcitonin may enhance its diagnostic accuracy, making it a valuable tool in distinguishing between dengue and malaria. If NLR is found to be a significant differentiator, it could reduce dependency on costly and time-consuming laboratory tests, making diagnosis more efficient in resource-limited settings. As a non-invasive and widely available marker, NLR holds promise for improving

the accuracy and affordability of infectious disease diagnosis in Pakistan.

MATERIAL AND METHODS

This quantitative, prospective cross-sectional study was conducted at the Creek General Hospital (CGH) Laboratory over a duration of three months, from 1st June to 31st August 2025, following the approval of institutional review board (IRB) reference no. UMD/Ethics/2025/05/21/387 dated 21st May 2025. A convenient sampling technique was used until the required sample size was achieved. Using the WHO sample size calculator, a total sample of 105 participants was determined. The calculation was based on a mean difference of 2 in the neutrophil-to-lymphocyte ratio (NLR) among dengue patients, as reported in a previous study.¹¹ As no study had documented the mean difference in NLR for malaria, the same value was used for both groups. Initially, the sample size was calculated as 32 participants per group; however, to achieve a higher statistical power of 94.3% and reduce the risk of Type II errors, the sample size was increased to 50 participants per group (total 100 cases) and further adjusted to 105 to account for potential non-compliance. The study included patients diagnosed with malaria (confirmed through microscopy or rapid diagnostic tests) and dengue (confirmed through positive NS1 and positive IgM or IgG serology). Exclusion criteria included patients with co-infections, those with chronic inflammatory conditions, and individuals who had taken antibiotics within 2–3 days prior to diagnosis. Informed consent was obtained from the patients prior to sample collection. Blood samples were collected and analyzed at the CGH Laboratory. Comparative analysis was carried out between the two disease groups (dengue and malaria) with respect to thrombocytopenia and NLR. In addition, NLR was compared with severity of disease based on degree of thrombocytopenia (moderate and severe) to evaluate any possible association. For this purpose, the Chi-square test was applied to assess categorical associations, while correlation analysis (Pearson or Spearman, depending on distribution) was used to determine the strength of relationship between NLR values and platelet counts. Data was analyzed using SPSS version 26, with mean and standard deviation calculated for age and gender distribution expressed in percentages. Data distribution was assessed using Shapiro-Wilk test. Mann-Whitney U test was used to compare the neutrophil-to-lymphocyte ratio

(NLR) between malaria and dengue patients, with statistical significance set at $p < 0.05$.

Ethical considerations were strictly maintained, with confidentiality ensured through coding and secure storage of data. Data ownership remained with the researchers and the institute, and sample selection was transparent and non-discriminatory. The IRB reviewed and scrutinized the study to ensure adherence to ethical standards and the integrity of the research.

RESULTS

A total of 105 patients were included in this study. Among these 64 (61.0%) were males and 41 (39.0%) were females. The majority of patients were in the 16–30 years age group, $n = 53$ (50.5%), followed by 31–45 years, $n = 21$ (20.0%) and 46–60 years, $n = 19$ (18.1%). Gender-stratified analysis showed that most males were in the 16–30 years category, $n = 34$ (53.1%), followed by 46–60 years, $n = 13$ (20.3%). Similarly, in females, the largest proportion belonged to the 16–30 years age group, $n = 19$ (46.3%), followed by 31–45 years, $n = 10$ (24.4%). Only 2 (1.9%) patients were above 60 years in either gender group as shown in Figure-I.

A total of 105 patients were evaluated for the presence of thrombocytopenia in study group. Among malaria cases ($n = 55$), 20 (36.4%) had severe thrombocytopenia and 35 (63.6%) had moderate thrombocytopenia. In comparison, among dengue cases ($n = 50$), 24 (48.0%) presented with severe thrombocytopenia and 26 (52.0%) with moderate thrombocytopenia as shown in Figure-II. Overall, severe thrombocytopenia was more frequent in dengue patients, whereas moderate thrombocytopenia predominated in malaria cases. No statistically significant association was observed between the type of infection and severity of thrombocytopenia as shown in Table-II. Assessment

of normality using the Shapiro–Wilk test showed significant deviations for both platelet count ($p = 0.017$ and $p = 0.003$) and NLR ($p < 0.001$); therefore, non-parametric tests were applied to study association between platelet count and NLR.

Platelet counts were reduced in both malaria and dengue patients, with greater variability observed among dengue cases. The mean platelet count was 60.69 ($SD \pm 25.5$) $\times 10^3/\mu L$ in malaria and 63.28 ($SD \pm 34.0$) $\times 10^3/\mu L$ in dengue. In contrast, neutrophil-to-lymphocyte ratio (NLR) values differed significantly between the two groups. Malaria patients demonstrated a higher mean NLR, 5.43 ($SD \pm 4.56$) compared to dengue patients, 2.72 ($SD \pm 2.52$). The stacked bar chart shown in Figure-III shows the distribution of NLR categories among malaria ($n=55$) and dengue ($n=50$) patients. In malaria cases, high NLR values were observed in 32 (58.2%) patients, low values in 21 (38.2%), and normal values in 2 (3.6%) patients. In dengue cases, low NLR values were observed in 42 (84%), while 8 (16%) patients exhibited high NLR, and none were in the normal range.

The neutrophil-to lymphocyte ratio (NLR) differed markedly when compared between malaria and dengue patients as shown in Table-II. Mann–Whitney U test demonstrated that this difference was statistically significant ($p < 0.05$). Elevated NLR values were observed more in malaria, while lower NLR values predominated in dengue. When NLR was categorized into two groups, high vs low and analyzed with the type of infection, a significant association was observed as shown in Table-III. High NLR was more common in malaria and low NLR was observed more in dengue cases.

Table-I. Association between the type of infection and thrombocytopenia.

Type of Infection	Severity of Thrombocytopenia		P-value (Fisher's exact)
	Severe($n=44$)	Moderate($n=61$)	
Malaria	20 (36.4%)	35 (63.6%)	0.242
Dengue	24 (48.0%)	26 (52.0%)	

Table-II: Comparison of neutrophil-to-lymphocyte ratio (NLR) between malaria and dengue cases.

Study groups	NLR	P-Value (Mann Whitney U)
Malaria($n=55$)	3.45 (1.16–6.22)	0.001
Dengue($n=50$)	2.02 (1.30–3.38).	

Table-III: Association of categorical neutrophil-to-lymphocyte ratio (NLR) levels with type of infection.

Type of Infection	Neutrophil-to-lymphocyte ratio (NLR)		P-value (Fisher's exact)
	High	Low	
Malaria	58.2%	41.8%	0.001
Dengue	16%	84%	

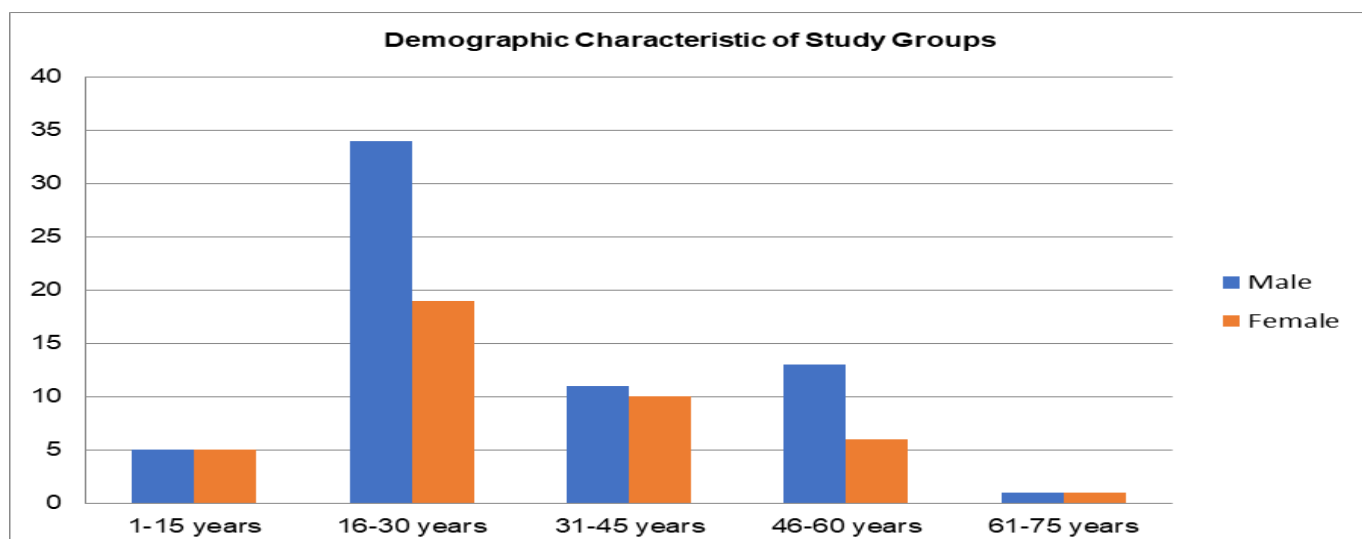


Figure-I: Age and gender-wise distribution of study participants.

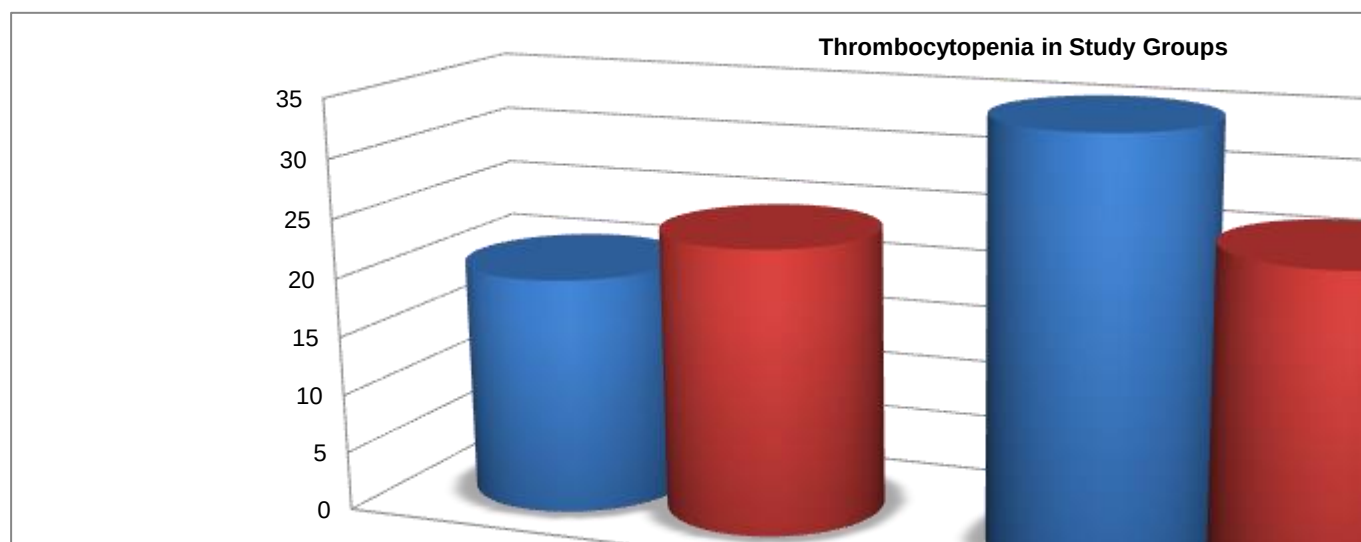


Figure-II: Number of cases with severe and moderate thrombocytopenia in study groups.

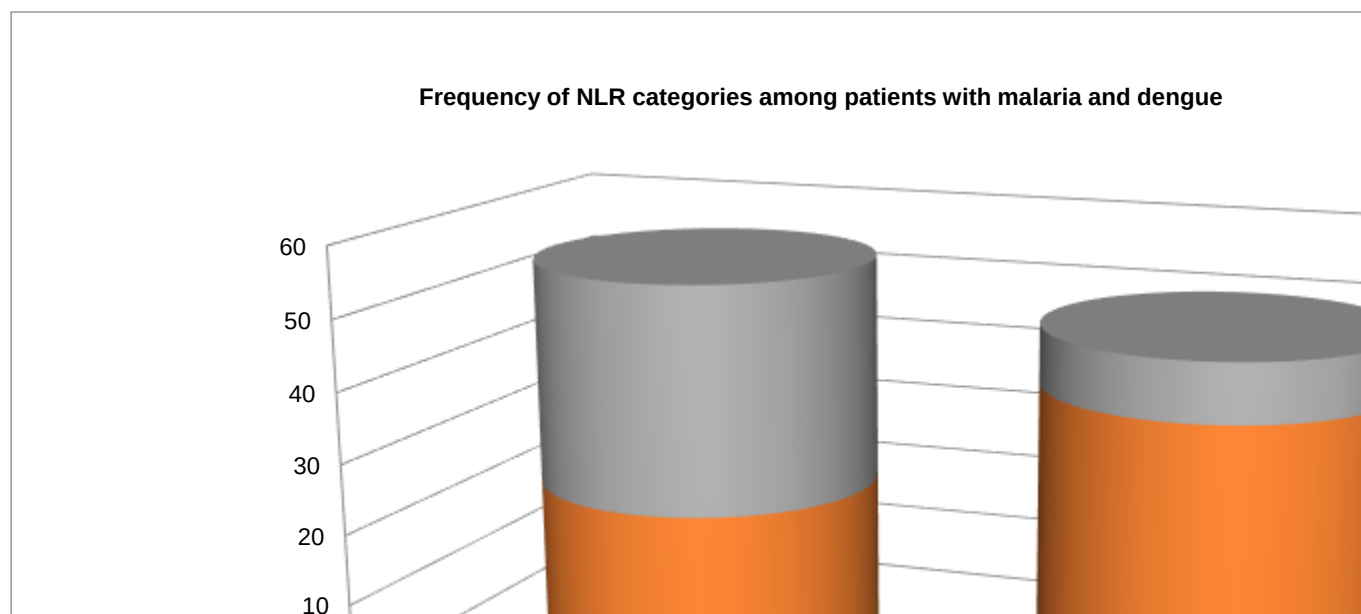


Figure-III: Distribution of malaria and dengue cases according to NLR categories.

DISCUSSION

This study compared demographic and hematological characteristics of malaria and dengue patients, with a particular focus on platelet counts, neutrophil-to-lymphocyte ratio (NLR), and disease severity. The demographic distribution of dengue and malaria cases in the present study showed important age and gender related patterns. In both dengue and malaria, the highest disease burden was observed among young adults (16–30 years). This trend aligns with previous reports highlighting that young adults are more vulnerable to mosquito-borne infections due to higher mobility, occupational exposure, and increased outdoor activities, which enhance contact with mosquitoes [14]. In both infections, very few cases were reported among the elderly, which may be attributed to partial immunity developed through repeated exposures over time or underrepresentation of older individuals in hospital-based studies [10].

Thrombocytopenia was common in both groups. Mean platelet levels were slightly lower in malaria patients ($60.69 \pm 25.5 \times 10^3/\mu\text{L}$) compared to dengue patients ($63.28 \pm 34.0 \times 10^3/\mu\text{L}$), though the difference was not statistically significant. Similar patterns have been reported in earlier studies, where dengue-associated thrombocytopenia is often more profound and linked to bleeding complications, bone marrow suppression, immune-mediated lysis, and plasma leakage while in malaria it is mediated by splenic sequestration and immune destruction [13,15]. The overlap in platelet reduction emphasizes that platelet count alone is insufficient for differentiating between the two infections, although it remains important for monitoring disease progression.

NLR values demonstrated clear differences between malaria and dengue. Malaria patients exhibited significantly higher mean NLR (5.43 ± 4.56) compared to dengue patients (2.72 ± 2.52). This elevation in malaria reflects neutrophil-dominant systemic inflammation induced by parasitemia, which induces the release of pro-inflammatory cytokines such as TNF- α , IL-6, and IFN- γ [15,16]. These mediators promote neutrophilia while concurrently suppressing lymphocyte proliferation, thereby elevating the NLR [17]. In contrast, dengue infection is characteristically associated with relative lymphocytosis, reflecting the adaptive immune activation against viral antigens, which in turn results in a lower NLR [7]. The divergent hematological profiles underscore the utility of NLR as a readily available and cost-effective biomarker to differentiate between these two common tropical infections. Previous studies have similarly reported higher NLR

values in malaria and lower values in dengue, supporting its diagnostic potential in resource-limited settings where molecular or serological testing may not be immediately accessible [18,19]. Moreover, the strong correlation of elevated NLR with malaria cases observed in this study suggests that NLR may also serve as a prognostic marker, aiding in early risk stratification and guiding timely therapeutic interventions.

When patients were stratified by NLR categories, a strong statistical association emerged between high NLR values and malaria, while low NLR values predominated in dengue. Importantly, elevated NLR was also correlated with greater clinical severity among malaria patients. More than half of the malaria cases (58.2%) were in the high NLR category, whereas 84% of dengue cases fell in the low NLR category. These findings suggest that NLR provides prognostic information. Previous studies have similarly reported elevated NLR as a predictor of severe malaria and worse clinical outcome [20,21]. Thus, NLR may serve as a simple, cost-effective biomarker for early risk stratification, particularly in resource-limited, endemic settings where advanced diagnostic facilities are not always available.

The strength of this study lies in its direct comparison of malaria and dengue within the same clinical context, integrating demographic and hematological markers. Nonetheless, some limitations must be acknowledged. The relatively small, hospital-based sample size may restrict the generalizability of findings to community populations. Future multicenter, longitudinal studies with larger cohorts are required to validate the diagnostic and prognostic role of NLR and to establish optimal cut-off values that can guide clinical decision-making.

CONCLUSION

Thrombocytopenia is a common hematological feature of both malaria and dengue. However, it does not reliably distinguish between the two. By contrast, NLR provides both discriminatory and prognostic value. Incorporation of NLR into diagnostic algorithms may enhance early differentiation between malaria and dengue and improve patient triage, particularly in endemic regions with limited laboratory resources.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Declared none

AUTHORS CONTRIBUTION

Syed Muhammad Mehdi Rizvi: Conceived the idea, designed the study and collected data, final approval, accountable for all aspects of publication

Muhammad Hassan: Conceived the idea, designed the study and collected data, final approval, accountable for all aspects of publication

Mehveen Iqbal: Manuscript writing, data analysis and result interpretation, final approval, accountable for all aspects of publication

Mahin Shams: Manuscript writing, result interpretation, corresponding author, final approval, accountable for all aspects of publication

Shahzad Ali Jiskani and Alishba Shabbir: Editing and critical revision of manuscript, final approval, accountable for all aspects of publication

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