

Original Article

Neutrophil–Lymphocyte Ratio as a Predictor of Dengue Hemorrhagic Fever Severity in Indonesian Pediatric Patients: A Single-Center Retrospective Study

I Gusti Ayu Ajeng Widia Kencana Dewi¹, Ronald Pratama Adiwinoto², Rizqi Rokhmadhoni Pikir³, Christina Rusli², Verna Biutifasari⁴, Prawesty Diah Utami⁵

¹Faculty of Medicine, Universitas Hang Tuah; ²Department of Community Medicine, Faculty of Medicine, Universitas Hang Tuah; ³Department of Pediatrics, Ramelan Central Naval Hospital, Faculty of Medicine, Universitas Hang Tuah; ⁴Department of Clinical Pathology, Faculty of Medicine, Universitas Hang Tuah; ⁵Department of Parasitology Faculty of Medicine, Universitas Hang Tuah, Komplek Barat RSAL Dr. Ramelan, Jl. Gadung No.1, Jagir, Wonokromo District, Surabaya, East Java, 60111, Indonesia

ABSTRACT

Background: Dengue hemorrhagic fever (DHF) is a significant health burden in Indonesia, necessitating reliable prognostic markers for early clinical intervention. The neutrophil-lymphocyte ratio (NLR) has emerged as a potential biomarker for disease severity assessment, although its utility in pediatric populations requires further validation.

Objective: This study aimed to determine the relationship between the neutrophil-lymphocyte ratio and DHF severity grade in pediatric patients and evaluate its potential as a prognostic indicator for clinical decision-making. **Methods:** This retrospective cross-sectional study analyzed 111 pediatric patients aged 5-14 years who were diagnosed with DHF at RSPAL Dr. Ramelan, Surabaya, between January 2022 and December 2024. Patients were classified according to the WHO severity grades (I-IV), with the NLR calculated from complete blood counts obtained during days 3-6 of illness. Statistical analysis with Spearman's rank correlation was used to examine the relationship between the NLR and disease severity. **Results:** The study population predominantly demonstrated Grade I DHF (56.8%), followed by Grade II DHF (20.7%), Grade III DHF (18.0%), and Grade IV DHF (4.5%). Patients with lower severity grades (I-II) predominantly presented low NLR values (<1) in 46.0% and 47.8% of cases, respectively, whereas those with higher severity grades (III-IV) primarily presented normal NLR values (1-3) in 65.0% and 60.0% of cases, respectively. Spearman's correlation analysis revealed no statistically significant relationship between the NLR and DHF severity ($\rho = 0.120$, $p = 0.208$). **Conclusion:** The neutrophil-lymphocyte ratio has limited utility as a standalone predictor of DHF severity in pediatric patients. These findings suggest the need for alternative biomarkers or composite indices for accurate prediction of pediatric DHF severity.

KEYWORDS

Dengue hemorrhagic fever, neutrophil-lymphocyte ratio, pediatrics, disease severity, biomarkers, Indonesia

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Dr. Budi Utomo, dr.,
M.Kes.

CORRESPONDENCE

Ronald Pratama
Adiwinoto,
Department of
Community Medicine,
Faculty of Medicine,
Universitas Hang
Tuah;
adiwinoto.ronald@hangtuah.ac.id

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INTRODUCTION

Dengue hemorrhagic fever (DHF) is a significant global public health concern, affecting more than 2.5 billion people worldwide who are susceptible to dengue virus infection. According to the Centers for Disease Control and Prevention (CDC), approximately 400 million individuals contract dengue fever annually, resulting in 40,000 fatalities due to severe manifestations of the disease [1,2]. Indonesia bears a substantial burden of this disease, with the second-highest number of dengue cases recorded globally between 2004 and 2010, surpassed only by Brazil [3]. By the end of 2022, Indonesia reported 143,000 dengue cases with 1,236 deaths, 58% of which were directly attributed to dengue fever, with West, Central, and East Java experiencing the highest mortality rates [4–6]. This geographic and epidemiological context underscores the urgent need for locally relevant predictive tools to inform the management of pediatric dengue.

Dengue hemorrhagic fever is characterized by acute dengue virus (DENV) infection manifesting as fever lasting 2–7 days, accompanied by hemorrhagic manifestations, thrombocytopenia, and hemoconcentration due to plasma leakage. This leakage leads to clinical complications, including pleural effusion, ascites, elevated hematocrit levels, and hypoalbuminemia [2]. The World Health Organization (WHO) classifies DHF into four grades of severity (I–IV) on the basis of clinical presentations and laboratory findings, with grades I and II categorized as nonshock DHF and grades III and IV classified as dengue shock syndrome (DSS) [7]. This classification system enables appropriate clinical management and improves patient prognosis. Despite its utility, this grading system may not fully capture the early immunological indicators of disease progression, particularly in the pediatric population.

The pathophysiological progression of DHF involves complex immunological responses that can be monitored via hematological parameters [8]. The WHO guidelines emphasize the importance of complete blood counts, particularly leukocyte counts, in understanding the clinical evolution of dengue infection [7]. The critical phase of plasma leakage may be predicted by leukopenia (≤ 5000 cells/mm³) and alterations in the neutrophil-lymphocyte ratio (NLR). During the initial febrile phase, patients typically present with normal leukocyte counts or neutrophilia. However, as the disease progresses, neutrophils undergo apoptosis due to direct DENV infection and bone marrow depression mediated by proinflammatory cytokines, resulting in progressive leukopenia reaching a nadir between the third and eighth days of illness [9,10]. The severity of the disease is correlated with the degree of apoptosis, and more pronounced apoptosis is associated with more severe clinical manifestations [11–13]. Given these dynamic hematological shifts, the timing of NLR measurement may significantly influence its clinical significance.

The neutrophil-lymphocyte ratio (NLR), calculated as the ratio of the absolute neutrophil count to the absolute lymphocyte count in peripheral blood, serves as a biomarker that reflects the balance between the innate immune response (neutrophils) and adaptive immunity (lymphocytes) [14,15]. This parameter has demonstrated utility in evaluating various infectious and noninfectious conditions, including malignancies [16,17], inflammatory disorders, and cardiovascular diseases [18]. In DHF, Yuntoharjo et al. [10] suggested that the NLR varies inversely with disease severity; the more severe the clinical grade is, the lower the NLR. This pattern stems from enhanced lymphocytic activity coupled with neutropenia in patients with severe DHF. During disease progression, atypical lymphocytes (blue plasma cells or transformed lymphocytes) emerge in the late febrile phase, causing NLR inversion between days 6 and 9. However, some reports have reported elevated NLR values during clinical recovery, implying that the association may be nonlinear and temporally dependent [10,19,20].

Recent investigations have indicated that the NLR may function as a prognostic biomarker for dengue infection. Patients with NLR values exceeding 2 have been observed during clinical improvement and platelet recovery, suggesting that NLR monitoring could predict disease prognosis and severity. Lower NLR values potentially correlate with increased disease severity. This relationship is particularly relevant for the pediatric population, which demonstrates heightened vulnerability to DHF. Children under 15 years of age present a disproportionate disease burden, with prevalence rates of 34.13% in the 5–14 years age group and 14.88% in those aged 1–4 years.

The 1–4-year cohort had the highest mortality rate (28.57%), which was attributed to immature immune systems and suboptimal interferon production, which inadequately inhibited viral replication and dissemination. Pediatric dengue infections frequently manifest as shock (grades III and IV DHF), which can be fatal without prompt intervention [21]. Moreover, developmental immunological differences between early childhood and adolescence may influence NLR trends, necessitating age-stratified analysis.

Despite the potential clinical utility of the NLR in prognostication, there remains a gap in understanding its specific relationship with disease severity across different DHF grades in the pediatric population. While previous studies have explored the NLR in dengue infection, comprehensive investigations examining its correlation with WHO-classified severity grades in children are limited. Furthermore, the predictive value of the NLR for pediatric DHF progression requires further validation, particularly in Indonesian healthcare settings, where the disease burden is substantial. Conflicting results in the literature, ranging from inverse to weakly positive correlations, underscore the need for clarity regarding the prognostic consistency of the NLR and its integration with other hematological markers.

This study aimed to determine NLR patterns across different DHF grades (I–IV), establish the relationship between the NLR and disease severity in pediatric patients, and evaluate the application of the NLR as a prognostic indicator in pediatric dengue fever patients. To this end, this study also examined age-specific variations, measurement timing during illness phases, and potential confounders in the interpretation of NLR trends. The findings of this investigation will provide valuable data regarding the relationship between the NLR and DHF severity in pediatric patients, potentially informing clinical decision-making and enabling appropriate early interventions to reduce mortality in this vulnerable population.

MATERIALS AND METHODS

Study design

This study employed a quantitative observational analytical design with a cross-sectional approach to investigate the relationship between the neutrophil-lymphocyte ratio (NLR) and dengue hemorrhagic fever (DHF) severity in pediatric patients. Given the retrospective nature of data collection, we acknowledge the inherent limitations in establishing causality and controlling for unmeasured confounders, as discussed below. This methodological framework allowed for the simultaneous assessment of exposure (NLR) and outcome (DHF severity) variables at a specific point in time, providing insights into their relationship without manipulating the study environment.

Study setting and population

The study was conducted at Rumah Sakit Pusat Angkatan Laut (RSPAL) Dr. Ramelan, Surabaya, Indonesia. Secondary data were collected from the medical records of pediatric patients with DHF admitted to the inpatient unit between January 2022 and December 2024. The hospital serves as a tertiary referral center for the region, receiving patients from various socioeconomic backgrounds and representing diverse clinical presentations of DHF, thereby enhancing the external validity of our study.

Variables and operational definitions

The study examined two primary variables: (a) the independent variable, the neutrophil-lymphocyte ratio (NLR), which was calculated as the absolute neutrophil count divided by the absolute lymphocyte count from peripheral blood samples. NLR categories (<1 , $1-3$, >3) were adopted from prior dengue studies that demonstrated their clinical relevance [20,22] to ensure physiological rationale and comparability across research settings. (b) Dependent variable, DHF severity in pediatric patients, classified according to the WHO criteria into four grades (I–IV), with grades I–II representing nonshock DHF and grades III–IV representing dengue shock syndrome [23,24].

Sampling technique and sample selection

This study used simple random sampling to minimize selection bias and approximate the representativeness of the target population. We selected simple random over consecutive sampling due to the large pool of eligible records and to avoid temporal clustering; this approach was implemented by assigning each eligible patient a unique identifier and using the Mersenne Twister algorithm via SPSS's random number generator, which has been validated for randomness in clinical dataset. All pediatric DHF cases during the study period were identified and assigned sequential numbers via the hospital's electronic health record system, after which a computer-generated random number sequence was used to determine the final sample.

Participants were included on the basis of the following criteria: (a) pediatric patients (5–14 years) diagnosed with DHF on the first day of hospital admission, confirmed by NS1 antigen and/or IgM/IgG serological tests, and complete blood count examinations were included; (b) patients without concurrent acute infectious diseases from nondengue viruses or parasites (e.g., malaria), as ruled out by documented negative malaria smears and respiratory viral panel results, were excluded to avoid hematological confounding; (c) patients were diagnosed with DHF between days 3 and 6 of illness, representing the critical phase when plasma leakage typically occurs. Complete medical records, including demographic information and comprehensive hematology results, particularly differential leukocyte counts, including neutrophils and lymphocytes, are available.

Data collection procedure

Data collection was conducted from August to November 2024 following a systematic protocol: (a) Procurement of institutional permissions and ethical clearance from the Ethics Committee of RSPAL Dr. Ramelan, Surabaya; (b) Retrieval of medical records of pediatric patients with DHF hospitalized during the specified timeframe (2022–2024); (c) Application of the inclusion criteria and execution of the described random sampling procedure; and (d) Extraction and documentation of relevant data points: demographic characteristics (age and sex), clinical parameters (DHF grade), and laboratory findings (complete blood count with differential), with particular attention to neutrophil and lymphocyte counts for NLR calculation. All data extraction was performed by two independent researchers, with discrepancies resolved by a third reviewer to ensure data integrity.

Laboratory methods

Blood samples were collected upon admission via standardized venipuncture techniques in EDTA-anticoagulated tubes. Complete blood counts and differential leukocyte analyses were performed via an automated hematology analyzer (Sysmex XN-1000; Sysmex Corporation, Kobe, Japan) at the hospital's central laboratory, which adheres to international quality control standards. The NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count obtained from these analyses.

Statistical analysis

Data analysis was performed via SPSS software (version 26.0; IBM Corp., Armonk, NY, USA). The analytical approach comprised the following steps. Univariate/descriptive statistics, categorical variables (DHF grades, NLR categories) are presented as frequencies and percentages; continuous variables are summarized as the means (SDs) or medians (IQRs) on the basis of normality assessed via the Shapiro–Wilk test. Bivariate analysis was performed, and the relationship between the NLR and DHF severity was examined via the Spearman rank correlation test, which was selected on the basis of the ordinal nature of both variables. Spearman's rank correlation test—chosen because both variables are ordinal and Spearman's method does not assume normal distributions, unlike Pearson's test—with significance set at $\alpha = 0.05$. We also considered multivariate regression controlling for age, illness duration, and prior dengue exposure but limited our analysis here to Spearman's test because of the exploratory nature and sample size constraints; future analyses will address these confounders in depth. Statistical significance was established at $\alpha = 0.05$, with correlation coefficients interpreted as follows:

0.00-0.19 (very weak), 0.20-0.39 (weak), 0.40-0.59 (moderate), 0.60-0.79 (strong), and 0.80-1.00 (very strong).

Ethical considerations

This study was conducted in accordance with the Declaration of Helsinki and received ethical approval from the Ethics Committee of Rumah Sakit Pusat Angkatan Laut (RSPAL) Dr. Ramelan Surabaya, Indonesia (ethical clearance number: 94/EC/KEP/2024). Patient confidentiality was strictly maintained through data anonymization, with all identifiers removed and replaced with coded identifiers during the data extraction process. Access to the dataset was restricted to authorized research staff. This study utilized existing medical records and did not require additional interventions or direct patient contact, thereby minimizing potential harm and maximizing scientific value. No personally identifiable information, such as residential addresses, birthplaces, or precise birth dates, was collected for this study.

RESULTS

Distribution of DHF severity grades

The distribution of DHF severity in the study cohort demonstrated a predominance of lower-grade disease. As presented in Table 1, Grade I DHF represented the majority of cases (56.8%, n=63), followed by Grade II DHF (20.7%, n=23), Grade III DHF (18.0%, n=20), and Grade IV DHF (4.5%, n=5). This distribution pattern aligns with the typical epidemiological profile of pediatric DHF, where severe manifestations (grades III and IV) constitute a smaller proportion of the total case burden but are associated with higher morbidity and mortality. The relatively lower prevalence of Grade IV cases (n=5) warrants consideration when interpreting statistical analyses involving this subgroup owing to limited statistical power.

Table 1. Incidence rate of pediatric patients with dhf based on grades I–IV

Severity Level	Frequency (n)	Percentage (%)
Grade I	63	56.8
Grade II	23	20.7
Grade III	20	18
Grade IV	5	4.5

Neutrophil–lymphocyte ratio distribution across DHF severity grades

The analysis of the neutrophil-lymphocyte ratio (NLR) distribution across different DHF severity grades revealed distinct patterns (Table 2). For lower severity grades (I and II), the predominant NLR category was low (<1), which was observed in 46.0% and 47.8% of the patients, respectively. This predominance of low NLR values suggests relative lymphocytosis compared with neutrophil counts during disease progression. Conversely, in patients with higher severity grades (III and IV), the normal NLR category (1-3) was most frequently observed, representing 65.0% and 60.0% of patients, respectively. The proportion of patients with high NLR values (>3) remained relatively consistent across all severity grades, ranging from 10.0% to 20.0%, with no clear increasing or decreasing trend in disease severity. This observation challenges the hypothesized direct inverse relationship between the NLR and disease severity suggested in previous studies.

Table 2. NLR distribution across pediatric patients with DHF by severity grade

DHF Grade	NLR	Frequency (n=111)	Percentage (%)
Grade I	Low (<1)	29	46
	Normal (1–3)	26	41.3
	High (>3)	8	12.7
Grade II	Low (<1)	11	47.8
	Normal (1–3)	9	39.1
	High (>3)	3	13

DHF Grade	NLR	Frequency (n=111)	Percentage (%)
Grade III	Low (<1)	5	25
	Normal (1–3)	13	65
	High (>3)	2	10
Grade IV	Low (<1)	1	20
	Normal (1–3)	3	60
	High (>3)	1	20

Relationship between the NLR and DHF severity

The statistical relationship between the NLR and DHF severity was assessed via Spearman's rank correlation coefficient, which is appropriate for evaluating associations between ordinal variables. As presented in Table 3, the analysis yielded a correlation coefficient (ρ) of 0.120, indicating a positive but weak relationship between the variables. The p value of 0.208 exceeded the predetermined significance threshold ($\alpha=0.05$), indicating that the observed correlation was not statistically significant. Although the positive correlation coefficient (0.120) suggested a slight tendency for the NLR to increase with increasing DHF severity, the magnitude of this association was insufficient to establish a meaningful predictive relationship. The absence of statistical significance ($p=0.208$) further underscores the limited utility of the NLR as a standalone predictor of DHF severity. This observed positive trend contrasts with our hypothesis and prior studies suggesting an inverse relationship, highlighting the need for further inquiry into the contextual and temporal factors affecting NLR dynamics in pediatric DHF patients.

Table 3. Spearman correlation between the NLR and severity of DHF

Relationship Test	Spearman's Rho	NLR	DHF Grade
NLR	Correlation coefficient	1.000	0.120
Sig. (2-tailed)	.	0.208	
N	111	111	
DHF Grade	Correlation coefficient	0.120	1.000
Sig. (2-tailed)	0.208	.	
N	111	111	

Remarks: Data are normally distributed if $p>0.05$.

Subgroup analysis of NLR distribution

Further examination of the data revealed noteworthy transitions in the NLR distribution patterns between nonshock DHF (grades I and II) and shock syndrome (grades III and IV). The proportion of patients with a low NLR (<1) decreased substantially from approximately 46–48% among those with nonshock DHF to 20–25% among those with shock syndrome. Concurrently, the proportion of patients with a normal NLR (1–3) increased from approximately 39–41% among those with nonshock DHF to 60–65% among those with shock. This shift suggests potential alterations in immune response mechanisms between these clinical stages, although the overall correlation was not statistically significant. When the absolute values within each NLR category across severity grades were examined, substantial intracategory variation was observed, suggesting that factors other than the NLR alone might influence disease progression and clinical manifestations. The heterogeneity within each NLR category may partially explain the weak overall correlation between the NLR and DHF severity.

DISCUSSIONS

This study investigated the relationship between the neutrophil-to-lymphocyte ratio (NLR) and dengue hemorrhagic fever (DHF) severity in pediatric patients. Our findings revealed that children with Grade I and II DHF predominantly presented low NLR values (<1), with 46% with Grade I DHF and 47.8% with Grade II DHF. Conversely, patients with more severe manifestations (grades III and IV) presented normal NLR values (grades 1–3), with 65% of patients with Grade III manifestations and 60% with Grade IV manifestations within this range. Despite these

observed patterns, statistical analysis via Spearman's correlation test yielded a p value of 0.208 ($p > \alpha = 0.05$), indicating that there was no significant relationship between the NLR and DHF severity in this cohort. Our results align with those of previous studies conducted in similar clinical settings. A comparable study at Ibnu Hospital in Makassar similarly reported no significant association between the NLR and disease severity in pediatric dengue patients [25]. Although the correlation between the NLR and DHF severity was not statistically significant in this study, it is essential to recognize the complexity of immune responses during dengue infection, which may confound straightforward biomarker correlations. The weak positive Spearman's correlation ($r = 0.120$) observed here contradicts earlier studies suggesting an inverse trend [10]. This discrepancy may stem from differences in patient populations, the timing of NLR measurements, or methodological variances across studies.

Additionally, research from the RSUD Prof. Dr. Soetomo in Surabaya demonstrated no significant correlation between the NLR and dengue infection severity ($p = 0.630$) [7]. These consistent findings across multiple Indonesian healthcare institutions suggest that regional factors, including viral serotypes, host genetic factors, and clinical management protocols, may contribute to the lack of correlation between the NLR and disease progression in the pediatric population. However, contrary evidence has been reported. A study conducted at Dr. Wahidin Sudirohusodo Hospital in Makassar revealed significant differences in NLR values between patients with and without DHF [26]. This discrepancy highlights the potential heterogeneity of the NLR's predictive value across different clinical presentations and patient subgroups [27].

These divergent findings may indicate that the utility of the NLR as a prognostic marker may be confined to specific clinical scenarios, such as differentiating between shock and nonshock states, rather than serving as a linear predictor across the entire spectrum of disease severity. Notably, the timing of sample collection during days 3–6 of illness captures a transitional phase from febrile to critical periods, during which NLR fluctuations may reflect immune instability. According to Kamsom et al. [9], neutrophil apoptosis during dengue infection varies temporally and may not align with standard clinical phases. Therefore, cross-sectional snapshots of the NLR may not fully capture the prognostic trajectories. Furthermore, our findings echo the conclusions of Koundinya et al. [20], who reported that NLR values may increase during clinical improvement, particularly in patients experiencing platelet recovery. These findings support the hypothesis that the NLR does not act as a linear marker of severity but rather shifts dynamically in response to immune restoration.

The pathophysiological basis for the observed NLR patterns in our study can be understood through the complex immunological cascade triggered by dengue virus infection. During the initial febrile phase, patients typically present with normal leukocyte counts or neutrophilia. As infection progresses, neutrophils undergo apoptosis, and bone marrow suppression, either directly mediated by the virus or through proinflammatory cytokines, leads to a progressive reduction in the leukocyte count, reaching a nadir between the third and eighth days of illness [11]. The magnitude of neutrophil apoptosis correlates with disease severity, establishing a cellular mechanism that potentially links hematological parameters to clinical outcomes. Moreover, the small sample size in the Grade IV DHF group ($n = 5$) may have limited the statistical power to detect significant correlations at the extremes of disease severity. Similar limitations were noted by Serdar et al. [28], who suggested that sample size imbalances can obscure otherwise clinically meaningful trends. This process reflects the direct cytopathic effects of the virus and the host immune response, both of which contribute to the observed hematological alterations.

The immunopathogenesis of DHF involves the activation of tissue-resident cells (mast cells and macrophages) and phagocytes (monocytes and dendritic cells), which release proinflammatory cytokines, chemokines, and proteases. While some of these mediators exhibit antiviral properties against dengue virus-infected cells, excessive inflammatory responses, often termed "cytokine storms", contribute significantly to vascular leakage, the hallmark of severe dengue [8,29]. This dysregulated inflammatory response is a critical determinant of disease progression, with endothelial activation serving as a mechanistic bridge between systemic inflammation and clinical manifestations. When our results are compared with the international literature, it becomes clear that the utility of the NLR may vary according to geographic,

immunogenetic, and age-related factors. Salazar Flórez et al. [30] discussed polymorphisms in immune system genes that influence dengue severity in Latin American populations. It is plausible that similar host genetic factors in the Indonesian pediatric population modulate immune responses in ways that affect NLR behavior. The variable impact of this process on different cell lineages may partially explain the inconsistent relationship between the NLR and disease severity observed in our study.

Systemic inflammation activates endothelial cells, leading to the contraction of capillary endothelial actin filaments, widening of intercellular junctions, and consequent plasma leakage. The increased expression of adhesion molecules, including ICAM-1, VCAM-1, and P-selectin, facilitates leukocyte adhesion to the endothelium, promoting localized inflammation and endothelial damage, which further exacerbates plasma extravasation [2,12,31]. The differential expression of these adhesion molecules in patients with varying disease severities may contribute to the heterogeneous neutrophil responses observed in our cohort, potentially confounding the relationship between the NLR and clinical outcomes.

Additionally, neutrophil suppression and the formation of neutrophil extracellular trap (NET) structures composed of DNA, histones, and antimicrobial proteins that capture and neutralize pathogens have been implicated in DHF pathogenesis [24,32]. The dual role of NETs in both pathogen containment and collateral tissue damage represents a complex immunological balance that may influence hematological parameters without a straightforward correlation with clinical severity. The variability in NET formation across patients might contribute to the inconsistent relationship between the NLR and disease outcomes.

Dengue virus-induced suppression of bone marrow progenitor cells and the subsequent release of inflammatory cytokines such as IL-6 and IL-8 disrupt normal hematopoiesis, contributing to neutropenia [33]. This phenomenon, coupled with increased neutrophil apoptosis, results in decreased neutrophil counts and is associated with leukopenia, which is frequently observed in patients with DHF [15]. The magnitude of bone marrow suppression varies considerably between individuals and is influenced by factors such as preexisting immunity, viral strain virulence, and host genetic determinants, potentially explaining the variable neutrophil responses observed across our study population [34].

Lymphocytosis, characterized by atypical lymphocytes (blue plasma cells or transformed lymphocytes), commonly occurs in the later stages of fever. These atypical lymphocytes, distinguished by dense nuclear chromatin and dark blue cytoplasm, typically appear from the third day of fever onward [35,36]. These features are supportive diagnostic features of DHF. Notably, patients with dengue shock syndrome (DSS) often present increased numbers of these atypical lymphocytes, suggesting that the degree of lymphocyte transformation correlates with disease progression [37].

This temporal evolution of lymphocyte populations from initial suppression to subsequent reactive proliferation introduces time-dependent variability in NLR measurements that may obscure potential correlations with disease severity when single time-point assessments are analyzed [15,35,38]. In addition, although multiple studies have confirmed the role of atypical lymphocytes in the later stages of dengue [22,39,40], our cross-sectional design precluded longitudinal observation of these hematologic shifts. Future research should incorporate serial measurements of hematological parameters, particularly lymphocyte subsets, platelet counts, and hematocrit, to delineate more nuanced prognostic profiles.

Several factors may explain the lack of a significant relationship between the NLR and disease severity in our pediatric cohort. The developing immune system in children may mount responses that differ qualitatively and quantitatively from those in adults. Specifically, children demonstrate distinct cytokine profiles and T-cell responses during dengue infection, with potentially different kinetics of neutrophil and lymphocyte mobilization than adults do. This developmental immunological variance may contribute to the limited prognostic utility of the NLR in pediatric patients. The duration of fever typically differs between children and adults. Children's immune systems, which are still in developmental stages, may not sustain prolonged inflammatory responses comparable to those of adults [25].

This abbreviated fever course potentially modifies the temporal dynamics of neutrophil apoptosis and lymphocyte proliferation, thereby affecting the timing of NLR fluctuations relative to disease progression. Consequently, the optimal timing for NLR measurement may differ in pediatric populations, potentially requiring serial assessments rather than single determinations [35,41]. Finally, our study population demonstrated distinct age-related characteristics. Sandinirwan et al. [42], the immune response of children under 10 years of age may be qualitatively different than that of adolescents, potentially affecting NLR trends. Although our subgroup analysis did not yield significant age-related differences, future stratified studies may provide additional insights.

Thrombocytopenia, a cardinal feature of DHF, may influence the NLR independently of disease severity. The mechanisms underlying decreased platelet production and enhanced platelet destruction in DHF patients involve common inflammatory pathways that simultaneously affect leukocyte populations [18]. The interdependence between megakaryocytes and myeloid lineages within the bone marrow compartment suggests that factors causing thrombocytopenia may concurrently impact neutrophil production, potentially introducing confounding influences on NLR measurements that are unrelated to clinical severity. The relatively small sample size, particularly within the Grade IV category (n=5), may have limited the statistical power to detect significant correlations.

The inherent variability in hematological parameters, coupled with potential confounding factors such as the timing of blood sampling relative to disease onset and preexisting comorbidities, could have further obscured potential relationships between the NLR and disease severity [43–45]. Genetic factors influencing susceptibility to severe dengue manifestations may modulate immune responses independent of the NLR [46–49]. Polymorphisms in genes encoding cytokines, pattern recognition receptors, and antigen-presenting molecules have been associated with differential risk for severe dengue outcomes, potentially introducing variability in disease progression that operates through mechanisms not reflected in simple hematological ratios [36,46,50].

Clinical implications

Clinicians should exercise caution when interpreting NLR values as predictors of disease progression in pediatric patients with DHF. While patterns were observed, with lower grades (I–II) predominantly showing NLRs <1 and higher grades (III–IV) showing NLRs ranging from 1–3, the lack of statistical significance suggests that the NLR alone may not provide reliable prognostic information for clinical decision-making in children with dengue infection. A comprehensive clinical assessment is paramount for managing pediatric patients with DHF. Rather than relying on isolated laboratory parameters, physicians should integrate multiple clinical indicators, including vital signs, fluid status, bleeding manifestations, and organ function parameters, when assessing disease severity and making management decisions. Serial monitoring of multiple hematological parameters, rather than single NLR measurements, may offer greater clinical utility. The dynamic nature of immune responses in pediatric dengue patients suggests that trends in laboratory values over time may provide more valuable prognostic information than isolated measurements alone. These findings underscore the need to develop pediatric-specific clinical prediction tools for dengue severity that account for the unique immunological responses in children.

Limitations

The study was conducted at a single center, which may reduce the generalizability of the findings to other populations. In particular, patients admitted to tertiary referral hospitals may differ systematically in terms of disease severity, referral bias, or comorbidities compared with those managed in primary or secondary care settings. While we focused on the NLR during the acute phase of illness, other biomarkers or clinical factors that could provide additional insights into disease severity were not assessed in this study. Moreover, although we analyzed NLR values during days 3–6 of illness, the precise timing of sample collection within this window varied between patients, potentially introducing inconsistencies given the dynamic nature of immune

responses during dengue progression. Further studies with larger sample sizes and longitudinal designs are needed to better understand the clinical utility of the NLR in DHF management. Future investigations should explore age-stratified immune dynamics and incorporate composite indices (e.g., the SII and PLR) for a more holistic understanding of disease progression in pediatric dengue patients.

CONCLUSIONS

This study revealed no significant relationship between the NLR and DHF severity in this pediatric cohort. The complex immunopathological mechanisms underlying dengue infection, including neutrophil apoptosis, lymphocyte transformation, and developmental variations in pediatric immune responses, may contribute to this inconsistency. Consequently, the clinical management of pediatric DHF patients should rely on a comprehensive assessment incorporating multiple clinical and laboratory parameters rather than relying on isolated NLR values. Our findings underscore the need for caution when using NLR trends without considering disease timing, prior exposure history, or age-specific immune profiles. Future research should explore alternative biomarkers or combinations of indicators that might more accurately predict disease progression in this vulnerable population. Integrating novel immunoinflammatory markers into predictive models may further enhance clinical decision-making for pediatric DHF patients.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: IGAAWKD, RPA; Methodology: IGAAWKD, RPA; Software: IGAAWKD, PDU; Validation: IGAAWKD, RPA; Formal analysis: IGAAWKD, RPA; Investigation: IGAAWKD, RPA, RRP; Resources: RRP, PDU; Data Curation: RRP, CR; Writing - Original Draft: IGAAWKD, RPA, RRP; Writing - Review & Editing: IGAAWKD, RPA, CR; Visualization: VB, RRP, PDU; Supervision: RPA.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

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