A Retrospective Observational Study to Evaluate the Neutrophil-to-Lymphocyte Ratio as a Predictor of Dengue Severity in Indian Pediatric Patients

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Abstract

Background: The neutrophil-to-lymphocyte ratio (NLR) is a potential marker for predicting dengue severity, yet its use in pediatric populations remains under-researched. This study aimed to determine the optimal cutoff value of NLR for predicting the severity of dengue infection in children under 14 years in India. **Material and Methods:** Retrospective data collection was conducted for this observational study over 1 year, from May 2023 to April 2024. Sixty-three children aged 14 years or younger with confirmed dengue fever were included, excluding those with chronic illnesses or immunodeficiencies. NLR on days one and five of hospitalization was noted and associated with the Severity of dengue fever. For analysis, chi-square test, independent t-tests, and the Area under the Receiver operating characteristic (ROC) curves were applied. **Results:** Enrolled children had a mean (SD) age of 6.1 (3.5) years, with 57.1% being males. On day one, the mean (SD) NLR for mild dengue, moderate dengue, and severe dengue were 1.64 (1.60), 1.81 (1.22), and 1.61 (0.87) respectively (p=0.882). On day five, these values were 1.34 (1.34), 1.29 (0.71), and 1.14 (0.53) respectively (p=0.719) without any significant difference. NLR had an AUC of 0.632 (95% CI: 0.487 to 0.777) for day one and 0.477 (95% CI: 0.389 to 0.661) for day five (p=0.074, not significant). **Conclusion:** In conclusion, NLR did not significantly differ with dengue severity in the pediatric population during the observed period and was not a statistically significant predictor of dengue severity.

Keywords: Dengue fever, neutrophil-to-lymphocyte ratio, pediatric, severity prediction.

INTRODUCTION

Dengue fever (DF) is one of the most common viral diseases transmitted by mosquitoes in humans. Annually, around 50 million dengue infections are reported, with the highest burden reported in South Asia.[1] Not only has it increased incidence, but it also carries a high fatality rate, making it a significant health issue.[2]

Dengue fever can occur in any age group, but the 5-15 years age group remains the frequently affected age group, with high mortality in children under 5.[3] The Indian subcontinent accounts for one-third of global dengue infections.[1] Moreover, data remain underreported in India due to a lack of laboratory confirmation of cases, with an overall seroprevalence rate of 56.9%.[4]

Conventionally, it was classified into four categories of ―classical dengue fever, non-classical dengue fever, dengue hemorrhagic fever, and dengue shock syndrome‖. However, this conventional classification lacks association with disease severity, management protocols, and specialized care in hospitals. Because of this, the World Health Organisation (WHO) established a new classification, dividing dengue into non-severe (with or without warning signs) and severe cases.[5] The same has been adopted by the ―National

Guidelines for Clinical Management of Dengue Fever‖ and categorized into mild, moderate, and severe.[6] Such classification enabled better triage of patients, leading to improved management and better outcomes, and reducing health care costs.[5]

Identification of illness severity remains significant in the health care setting, where milder cases of dengue fever can be managed in the outpatient department (OPD), with hospitalization required only for severe cases.[5]

For prediction of DF severity, studies have found clinical markers in the form of the presence of secondary infection, kidney disease, hepatic involvement, diabetes, or being

children,[7] and biochemical markers – aspartate aminotransferase, alanine aminotransferase, C-reactive protein, IL-8, decreased albumin levels, or vascular cell adhesion protein one and syndecan 1.[8] In addition, the neutrophil-lymphocyte ratio (NLR) is a novel marker routinely performed in healthcare laboratories and has proven to be a prognostic biomarker in various infections, cardiovascular diseases, cancers, and inflammatory diseases.[9] The ratio of neutrophil to lymphocyte count is affected in dengue fever.

There is a notable gap in the literature regarding the use of NLR to predict dengue severity.[9] Thus, the present study was conducted with the aim of assessing the role of NLR in predicting the severity of dengue according to the new WHO classification, which may allow for a more targeted approach and earlier identification of disease severity, thereby defining further clinical strategies for managing patients. The objectives of the study were to determine the cut-off value of NLR for predicting DF severity.

MATERIALS AND METHODS

An observational study was conducted after ethical approval from the Institutional Ethical Committee from May 2023 to April 2024, comprising 1 year, in a tertiary care setting in India. Patient data were collected retrospectively.

Inclusion criteria

Children aged 14 years or younger hospitalized with dengue fever, having positive test results of non-structural antigen-1 (NS1) or immunoglobulin IgM assay, were included in this study.

Exclusion criteria

Children who had chronic renal failure, malignancy, chronic liver diseases, and pre-existing immunodeficient status.

Sample size: Based on the results of a previous study by Prayon KM et al,[9] where NLR had a sensitivity of 75.6% and a specificity of 38.4% for predicting dengue severity, the sample size calculation for the present study was found to be 56 cases with 25% precision, 80% study power, and an alpha value of 5%. To further reduce the margin of error, 63 patients were enrolled in the study.

A venous blood sample was tested for complete blood count on day one of hospitalization and repeated on day five. Analysis was performed using a Sysmex Five-Part Analyzer (XS series, Japan) with Fluorescent Flow Cytometry. It reported total leucocyte count, absolute neutrophil and lymphocyte counts, and NLR.

National guidelines for the clinical management of Dengue were used, with clinical cases classified as mild, moderate, or severe.[6] Mild cases of Dengue were without any warning signs, which were managed at home or in the OPD with follow-ups. Those cases had only fever, with normal platelet counts, without evidence of capillary leakage or other complications or comorbidities. At the same time, moderate cases of Dengue had warning signs or a comorbidity. Warning signs were in the form of bleeding manifestations, abdominal pain, lethargy, persistent vomiting, syncope, accumulation of clinical fluid, liver enlargement, or a decrease in the platelet count with an increase in hematocrit. It required close monitoring under hospitalisation, by regular sample collections every 6 hours, and the patient was under observation. These were considered comorbid cases; any patient on steroids, immunosuppressants, or anticoagulants was also regarded as comorbid, but such cases were excluded from the study. Severe cases of Dengue included cases that had bleeding or had shock or had involvement of the organs in the form of dyselectrolytemia, acidosis, impaired consciousness, increased GST or ALT more than 1000 IU per litre, which required tertiary level care management in the hospital. The detailed organ involvement features are attached as Supplementary file I.

Statistical analysis: Data were analyzed as shown in [Figure 1].

RESULTS

In the study cohort of 63 children, the mean (+ SD) age was 6.1 (+ 3.5) years. There were 36 (57.1%) males and 27 (42.9%) females. [Table 1] describes the clinical and laboratory parameters of the study cohort.

Based on the severity of dengue fever, there were 16 cases of severe dengue, 28 of mild, and 19 of moderate. Demographic and clinical characteristics were associated with dengue severity [Table 2]. The mean age was comparable across groups (p = 0.591). Gender distribution was also statistically similar, with males comprising 23.8% in the mild group, 19.0% in the moderate group, and 14.3% in the severe group (p = 0.806), while females constituted 20.6%, 11.1%, and 11.1% respectively. Hemoglobin levels were not significantly different among the groups: 11.2 g/dL, 11.9 g/dL, and 11.5 g/dL in mild, moderate, and severe cases, respectively (p = 0.485). Platelet counts showed a trend toward significance, with the mild group at 112.5×10^9/L, and the moderate and severe groups at 71.7×10^9/L and 90.7×10^9/L, respectively (p = 0.051). On day one, the mean (SD) NLR for mild dengue, moderate dengue, and severe dengue were 1.64 (1.60), 1.81 (1.22), and 1.61 (0.87), respectively (p=0.882). On day five, these values were 1.34 (1.34), 1.29 (0.71), and 1.14 (0.53) respectively (p=0.719) without any significant difference. The ROC curve analysis yielded an AUC of 0.632 (95% CI: 0.487 to 0.777) for day one and 0.477 (95% CI: 0.389 to 0.661) for day five, according to which NLR was not found to be statistically significant to predict the severity of DF (p=0.074). [Figure 2]

Table 1: Demographic, clinical and hematological parameters of the study population	
Variables	**Study population (n=63)**
Age (years)	6.1 (3.5)
0-5	28 (44.44%)
6-10	26 (41.27%)
>10	9 (14.29%)
Male	36 (57.1%)
Female	27 (42.9%)

Hemoglobin (gm/dl)	11.5 (1.9)
Platelet count (× 109/L)	95 (57)
NLR on day 1	
Mean (SD)	1.69 (1.32)
Median (IQR)	1.42 (0.73)
Range	0.22 - 8.80
NLR on day 5	
Mean (SD)	1.27 (0.79)
Median (IQR)	1.30 (0.91)
Range	0.27 - 5.53

SD: standard deviation, IQR: Interquartile range, NLR: Neutrophil to lymphocyte ratio.

Table 2: Comparison of patient parameters with severity of dengue infection.

Variables	Mild (n=28)	Moderate (n=19)	Severe dengue (n=16)	p- value
Age (years)	6.6 (3.8)	5.6 (3.5)	5.7 (3.1)	0.591
Male	15 (23.8%)	12 (19.0%)	9 (14.3%)	0.806
Female	13 (20.6%)	7 (11.1%)	7 (11.1%)	
Hemoglobin (gm/dl)	11.2 (1.9)	11.9 (1.8)	11.5 (2.1)	0.485
Platelet count (×109/L)	112.5 (0.63)	71.7 (19.3)	90.7 (67.8)	0.051
NLR on day 1	1.64 (1.60)	1.81 (1.22)	1.61 (0.87)	0.882
NLR on day 5	1.34 (1.34)	1.29 (0.71)	1.14 (0.53)	0.719

NLR: Neutrophil to lymphocyte ratio. Independent t-test.

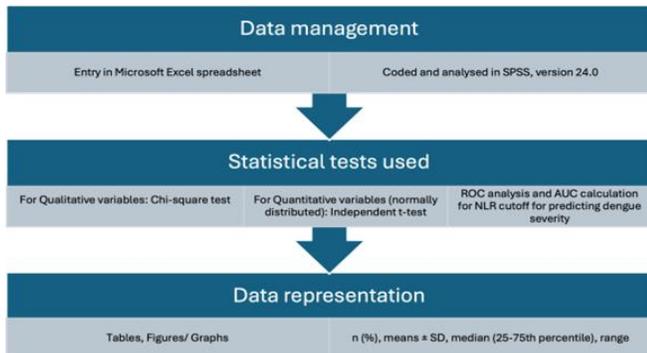Figure 1: Statistical analysis. Data normality was assessed by Shapiro-Wilk test. Statistical significance: p< 0.05. SPSS: Statistical package for Social Sciences, IBM manufacturer, Chicago, USA, ROC: receiver operating characteristic curve, AUC: area under the curve.
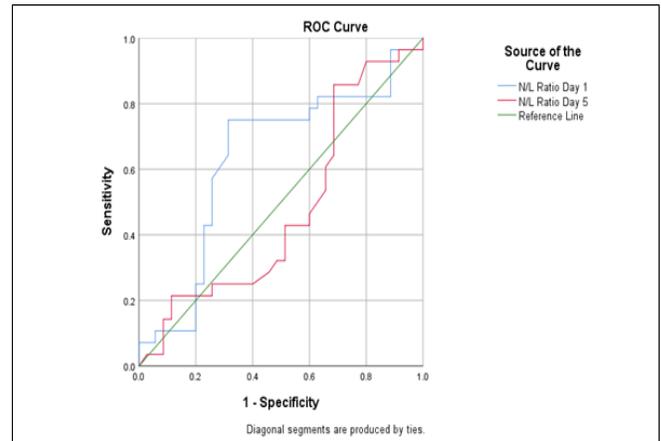

Figure 2: ROC curve for NLR on day 1 and day 5 for differentiating dengue severity.

Supplementary file I: Organ Dysfunction in Severe Dengue

Organ/System	Manifestations
Nervous System	Febrile seizures in young children Encephalopathy Encephalitis/aseptic meningitis Intracranial haemorrhages/thrombosis Subdural effusions Mononeuropathies/polyneuropathies/Guillain- Barré Syndrome Transverse myelitis
Gastrointestinal System	Hepatitis/fulminant hepatic failure Acalculous cholecystitis Acute pancreatitis Hyperplasia of Peyer's patches Acute parotitis
Kidney	Acute renal failure Hemolytic uremic syndrome (HUS)
Heart	Conduction abnormalities Myocarditis
	Pericarditis
Lungs	Acute respiratory distress syndrome (ARDS) Pulmonary haemorrhage
Musculoskeletal System	Myositis with raised creatine phosphokinase (CPK) Rhabdomyolysis
Lymphoreticular System	Infection-associated haemophagocytic syndrome IAHS or Haemophagocytic lymphohistiocytosis (HLH) Idiopathic thrombocytopenic purpura (ITP) Spontaneous splenic rupture Lymph node infarction
Eye	Macular haemorrhage Impaired visual acuity Optic neuritis
Others	Post-infectious fatigue syndrome Depression Hallucinations Psychosis Alopecia

DISCUSSION

The present study is significant because it was conducted on children and aimed to assess the utility of NLR in predicting Dengue severity. Severe cases of Dengue in children may need hospitalization, and their early identification and prediction may allow for better management.[9-11]

Overall, our study results were negative in determining NLR as a predictor of Dengue severity. At a cut-off level of 1.435, we found that the AUROC for NLR was 0.632 on day one and 0.477 on day five, with p-values not statistically significant.

Among other studies conducted on children, Prayon KM et al,[9] found that among 584 children with dengue, high NLR was present in 255 (43.7%) and normal NLR in 329 (56.3%). In corroboration, they also found that NLR was not significantly associated with dengue severity (p = 0.88). At the cut-off value of 1.97, NLR demonstrated a sensitivity of 75.6% and a specificity of 38.4% for predicting dengue severity.[10] Deshapande et al,[11] also found no statistical correlation between dengue severity and NLR among 100 children with dengue, of whom 42% had high NLR. To date, only these two studies have assessed the utility of NLR as a marker of severity, thereby negating its significant role in predicting dengue severity.

However, in addition to studies conducted on children, NLR has been used in adults, where it is a useful predictor. Navya PT et al,[12] reported that the mean values of NLR in dengue fever were 2.47±1.32, in dengue haemorrhagic fever were 1.013±0.8, and in dengue shock syndrome were 0.41±0.18 (P=0.02). NLR showed a diagnostic accuracy of 63% to predict dengue severity, with sensitivity of 77.8%, specificity 94.4%, PPV 77.8%, and NPV 33.3%.

In another study of adult patients with dengue, Yuditya DC et al,[13] showed a negative correlation between NLR and dengue severity (r = -0.733, P = 0.000). Similarly, in the study by Irmayanti et al,[14] the mean NLR was 2.19 in grade I DHF (n=52) and 0.80 in grade II DHF (n=44), indicating a significant association between NLR and dengue severity (p <0.01).

These differences may be because the severity of dengue in children may not be reflected in changes in blood cell counts. Moreover, this shows that dengue presents differently in children compared to adults. While children may be asymptomatic or experience multiple symptoms at once, adults typically report muscle pain, retro-orbital pain, nausea, and joint pain as the most common symptoms. In children, vomiting and skin rashes are more common. These distinct clinical features in children suggest that their immune systems respond differently—possibly because they have not developed immunity to the virus's various serotypes, leaving them more susceptible.[15]

Limitations: The primary limitation of our study was the small sample size resulting from recruitment at a single center. However, as dengue is an epidemic disease in this region, the expected number of patients cannot be predicted in a defined study period. Secondly, the children's nutritional status was not addressed. Lastly, the follow-up and outcomes, including detailed patient management and mortality/discharge status, were not reported in the study.

CONCLUSION

In conclusion, the neutrophil-to-lymphocyte ratio showed no meaningful differences across severity levels in DF in the pediatric population, suggesting it may have limited value as a standalone marker for differentiating disease severity. Broader, multicenter research is warranted to clarify whether and how NLR might contribute to clinical assessment in children with dengue.

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Conflicts of interest

There are no conflicts of interest.

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