

Evaluation of the efficacy of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in prognosis and severity of dengue fever in children

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Abstract

Introduction: The present study aimed to compare the haematological and biochemical indices between Dengue patients, and assess the relationship of these indices in prognosis and severity of Dengue fever.

Materials and Methods: A total number of 40 children with confirmed diagnosis of Dengue fever who visited and some of them were admitted Sri Sathya Sai Medical College and Research Institute in Chennai were selected in this case-control study. Haematological, biochemical and neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and outcome of the disease were assessed in this study.

Results: The number of children confirmed diagnosed with Dengue fever were 40, they were further divided into three different groups based on severity of the disease. Haematological and biochemical variables were statistically significant in these three different groups. Neutrophil count, platelet count, lymphocyte count, and haemoglobin concentration were also higher in the dengue fever with warning signs patient group ($P=0.001$). NLR and PLR were significantly higher in severe dengue patients compared to the mild patients ($P=0.01$). NLR had a significant relationship with the severity of the disease. Multivariable analysis for diagnostic values of NLR and PLR showed that the NLR and PLR were significant at 2.336 (95% CI: 1.636–3.336) and 1.4 (95% CI: 0.28–0.29), respectively.

Conclusion: Platelet-to-Lymphocyte Ratio (PLR) and Neutrophil-To-Lymphocyte Ratio (NLR) can be used as a prognostic marker for Dengue disease given the significant difference of PLR and NLR between mild Dengue patients and severe Dengue patients.

Keywords: Dengue, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, prognosis, severity

Introduction

Dengue is the most extensively spread mosquito-borne disease, transmitted by infected mosquitoes of *Aedes* species. Dengue infection in humans results from four dengue virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4) of Flavivirus genus. As per the WHO 1997 classification, symptomatic dengue virus infection has been classified into dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). The revised WHO classification of 2009 categorizes dengue patients according to different levels of severity as dengue without warning signs, dengue with warning signs (abdominal pain, persistent

vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, increasing haematocrit with decreasing platelets) and severe dengue^[1-3].

Dengue infection is a disease caused by the dengue virus. Dengue fever, dengue haemorrhagic fever, and dengue shock syndrome have become international health problems. This infection can affect all ages. The majority of these cases occurs in children under the age of 15 as many as 95% and about $\geq 5\%$ occur in infants^[2-4]. Dengue virus infection causes high mortality and morbidity throughout the world due to the very dynamic journey of dengue virus infection. The critical phase of dengue infection accompanied by increased capillary permeability and plasma leakage only lasts 24-48 hours, so that both clinical and laboratory monitoring is needed to evaluate any changes that occur in the phases of dengue virus infection.

Several studies have been conducted using different clinical and laboratory parameters as early predictive markers of severe dengue. Those who utilized the presence of specific warning signs as early predictors of dengue infection severity found support that the presence of vomiting, abdominal pain and bleeding were associated with the development of severe dengue^[3-7]. Others have linked demographic features such as female sex and younger age to higher severe dengue incidence. Majority have considered laboratory parameters such as LDH and albumin, levels of CRP, elevated AST, high NS1 levels, WBC and WBC differentials, hematocrit levels and cfDNA as early predictors of severe dengue^[5-9]. However, all these studies need further validation in other regions and age groups.

Neutrophils are the first leukocytes to migrate to sites of inflammation and infection where they recognize and phagocytose invading microorganisms. Leukopenia and absolute neutropenia have been described as a feature of dengue infection due to bone marrow suppression by dengue virus^[9]. In general, individuals with severe neutropenia are at higher risk of secondary bacterial infections, however, it is still uncertain whether dengue patients with severe neutropenia are more prone to secondary bacterial infections. Studies on the correlation of neutropenia with progression to severe dengue disease are scarce. One study showed that any change in the values of total leukocyte count points towards the progression of dengue towards severity^[10]. Another useful biomarker of inflammation is the Neutrophil to Lymphocyte Ratio (NLR). It has proven its prognostic value in cardiovascular diseases, infections, inflammatory diseases and in several types of cancers. A similar study done in the paediatric population reported that a rise in NLR helps in predicting the mortality in the paediatric intensive care unit^[11]. Another study assessed the ability of NLR to predict sepsis in children and concluded that high NLR values should alert clinicians to the possibility of sepsis and to initiate or change antibiotic treatment^[12]. To date, only few studies have attempted to establish a correlation of neutrophil counts as an early indicator of severe dengue in children^[9-12]. Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Alkaline Phosphatase (ALP) were used to assess organ involvement, such as hepatocellular lesions, in severe dengue, they are closely related to the severity of the disease and prognosis^[13-15].

The aim of present study was to assess the prognostic value of rise in NLR and PLR in paediatric intensive care as markers of mortality. The outcomes measure is the trend of NLR and PLR may help in prognosis and assessment of severity in children suffering from Dengue fever.

Materials and Methods

Patient recruitment: This study involved a total number of 40 children confirmed diagnosed with Dengue fever. These children were admitted in a Sri Sathya Sai Medical College and Research Institute in Chennai from June 2021 to February 2022. A cohort study was conducted after obtaining permission from the Institutional Ethical Committee.

Inclusion criteria

- All patients who are confirmed diagnosed with Dengue fever and admitted to PICU between the age of 1 month and 18 years were considered for the study.

Exclusion criteria

- Patients were excluded from the study if all the study parameters were not retrievable for them, if their duration of stay was less than 5 days or if patient was admitted for postoperative care.

Laboratory Findings

- A patient with positive serology for dengue (Positive NS1 or positive IgM, or both).
- Non-severe dengue - dengue case classified as Dengue Fever with Warning Signs and Dengue Fever without warning signs^[3,4].
- Severe dengue - dengue case presenting with severe plasma leakage, severe haemorrhage, and severe organ impairment^[3,4].

Blood sample collection

After routine informed consent from patient, 5 ml venous blood was collected from antecubital vein under aseptic precautions in a EDTA vacutainer was collected on the sixth day of illness. Samples were processed within an hour on automated 5 part differential cell counter. Peripheral smears were stained with Leishman's stain. Smears were examined by pathologist and hematologic parameters with special emphasis on WBC differentials and platelet count were reconfirmed on microscopy. Dengue serology was performed by Rapid test Immunochromatographic testing and confirmed by ELISA.

Once the patients were identified, information regarding demographic parameters like age, gender and relevant clinical history were collected. Biochemical and haematological parameters at day 5 which included the total white blood cell count, neutrophil count, lymphocyte count, and platelets were retrieved for the calculation of NLR and PLR. The data was then analyzed to determine NLR and PLR for on the day 5.

The values were determined as per the formula given below

- Neutrophil lymphocyte ratio = Neutrophil% / Lymphocyte% x 100
- Platelet lymphocyte ratio = Platelet count / Lymphocyte% x 100

Statistical Analysis: Variables were presented as mean value \pm standard deviation (SD). Complete statistical analysis of the data was conducted with the statistical software package SPSS Statistics 18 (Chicago, Illinois, USA).

Result

In our study, we had taken a total number of 40 patients who had fulfilled the inclusion criteria. Among them 23 patients were male and 17 were female (Table 1). The mean age of the children in the dengue fever subgroup was 9.5 ± 4.8 years. The study cohorts were predominantly children more than five-year-old with older age significantly associated with dengue fever. No significant association was observed between girls and boys in this age group, for dengue fever.

Table 1: Demographics characteristics of Dengue patients

Parameters	Number of Patients (N=40)	
	Frequency	Percentage
Age (Years)		
4-8	4	10
8-12	24	60

>12	12	30
Gender		
Male	23	58.07
Female	17	42.5
Severity of Dengue		
Dengue with warning signs	14	35
Dengue without warning signs	16	40
Severe Dengue	10	25
Signs/ symptoms		
Fever at admission	32	80
Diarrhoea	4	10
Vomiting	3	7.5
Erythematous rash	8	20
Swelling of hand and feet	5	12.5
Respiratory distress	9	22.5
Myalgia	21	52.5

As expected, fever was the most common presentation and no significant difference in the fever days was observed between the different severity group of dengue.

The mean platelet count of the study group was 2,10,615 (150000 – 350000/ μ l) and the mean WBC count of the study was 5890 (4000 – 10000/ μ l). The mean N:L ratio of the study group was 2.16. Among 40 patients, 13 patients had bleeding manifestations and in them 6 had shock (Table 2).

Table 2: Comparison of haematological profiles among different dengue patients groups

Parameters	Normal Range (Unit)	Dengue with warning signs (N=14)	Dengue without warning signs (N=16)	Severe Dengue (N=10)	p Value
Haemoglobin	12 – 16.5 (g/dl)	14.9 \pm 2.34	12.96 \pm 1.47	11.54 \pm 1.21	0.64
RBC	4.5 – 5.5 ($10^{12}/l$)	5.21 \pm 0.82	4.53 \pm 0.24	3.97 \pm 0.61	0.91
WBC	4000 – 10000 (μ l)	8128 \pm 452.67	7587.14 \pm 347.29	4487.14 \pm 264.32	0.74
Platelets	150000 – 350000 (μ l)	304827.42 \pm 1022.93	222428.61 \pm 2843.57	156319.27 \pm 6341.57	0.34
Neutrophils	1700–7900 (μ l)	5891.45 \pm 621.14	5063.94 \pm 236.74	4663.94 \pm 466.74	0.57
Basophils	0–200 (μ l)	28.56 \pm 6.64	22.84 \pm 4.84	16.79 \pm 2.49	0.38
Eosinophils	20–550 (μ l)	109.35 \pm 14.25	79.46 \pm 16.34	43.57 \pm 8.64	0.43
Lymphocytes	1500–4500 (μ l)	2259.43 \pm 68.47	1897.53 \pm 38.44	1597.56 \pm 62.48	0.92
Monocytes	100–900 (μ l)	654.38 \pm 64.73	859.28 \pm 54.28	1084.49 \pm 38.64	0.96
Delta Neutrophil Index (DNI)	-	0.18 \pm 0.04	0.15 \pm 0.07	0.12 \pm 0.05	0.83
Neutrophil-to-Lymphocyte Ratio (NLR)	-	1.92 \pm 0.89	2.34 \pm 0.37	2.87 \pm 0.49	0.08
Platelet-to-Lymphocyte Ratio (PLR)	-	124.61 \pm 10.67	231.52 \pm 21.69	279.38 \pm 38.21	0.84

All values were expressed as Mean±SD, $p < 0.05$ highly significant.

The white blood cell count was significantly decreased in Severe Dengue patients group, and there were also elevations in NLR and PLR. Although the values of lymphocytes were not significantly higher in Severe Dengue patients group. We also noted a significant association of leukopenia and thrombocytopenia in Severe Dengue patients group.

The biochemical parameters evaluated, transaminases (AST, ALT and ASP) were uniformly elevated in Severe Dengue patients group (Table 3). The mean differences of the levels of ESR, CRP were statistically significant among these three different dengue patient groups. Almost all biochemical parameters findings were significantly higher in children with severe dengue (Table 3). Serum ferritin levels in children with severe dengue fever showed 336.63 ± 66.28 ng/ml as compared to 69.28 ± 18.37 ng/ml in dengue with warning signs patient group.

Table 3: Comparison of biochemical profiles among different dengue patients groups

Parameters	Normal Range (Unit)	Dengue with warning signs (N=14)	Dengue without warning signs (N=16)	Severe Dengue (N=10)	P Value
Erythrocyte Sedimentation Rate (ESR)	1-29 (mm/hr)	18.34±4.67	24.68±3.29	29.89±4.94	0.36
C-Reactive Protein (CRP)	9.5-10.5 (mg/dl)	9.67±1.52	10.92±1.39	12.52±2.07	0.62
Alanine Transaminase (ALT)	4-36 (U/L)	32.42±6.48	73.61±14.89	106.49±24.37	0.98
Aspartate Transaminase (AST)	8-48 (U/L)	43.64±4.21	79.43±13.63	119.29±22.37	0.42
Alkaline Phosphatase (ALP)	44-147 (IU/L)	57.91±8.52	121.84±27.69	182.29±24.51	0.69
Ferritin	12-145 (ng/ml)	69.28±18.37	197.62±36.74	336.63±66.28	0.49
Lactate Dehydrogenase (LDH)	105-335 (IU/L)	128.37±11.84	324.67±46.29	529.84±74.28	0.94

All values were expressed as Mean±SD, $p < 0.05$ highly significant.

The demographic, clinical and laboratory findings among the dengue fever groups were evaluated to identify significant indicators of clinical severity. The clinical and laboratory values are represented in [Table 1, 2, 3]. Based on the WHO (2009) case classification criteria, 14 (35%) were clinically identified as dengue with warning signs, 16 (40%) as dengue without warning signs and the rest 10 (25%) as severe dengue. No significant differences were observed in age and gender with dengue severity in this study. Interestingly, a higher proportion (53%) of the paediatric cases were clinically identified to be in their convalescent phase of illness based on the fever duration and almost 25% of the patients in the critical phase of illness were clinically classified as 'severe' dengue. No significant association was found between the phase of illness and dengue severity.

Table 4: Variables distinguished among different dengue patients groups with multivariable analysis

Variables	Odds Ratio (95% CI)	p Value
Ferritin	1.000 (1.000–1.001)	0.16
Erythrocyte Sedimentation Rate (ESR)	1.022 (0.999–1.047)	0.08
C-Reactive Protein (CRP)	1.013 (0.891–1.152)	0.92
Delta Neutrophil Index (DNI)	2.361 (1.582–3.462)	0.97
Neutrophil-to-Lymphocyte Ratio (NLR)	2.336 (1.636–3.336)	< 0.01

Platelet-to-Lymphocyte Ratio (PLR)	1.4 (0.28-0.29)	0.67
Constant	0.004	< 0.01

Standardized odds ratio (OR) with 95% CI were calculated for each variable. Forward stepwise multivariate logistic regression model was performed in order to determine the independent predictors of severity outcome, without the effect of possible confounders (Table 4). Multivariate logistic regression analysis demonstrated that haematological parameters remained statistically highly significant independent predictor of severity outcome when compared to Delta Neutrophil Index (DNI), Neutrophil-to-Lymphocyte Ratio (NLR) and Platelet-to-Lymphocyte Ratio (PLR) at 2.361 (95% CI: 1.582-3.462), 2.336 (95% CI: 1.636–3.336) and 1.4 (95% CI: 0.28-0.29), respectively.

Discussion

Dengue fever is endemic in more than 100 countries with most cases reported from the Americas, South-East Asia and Western Pacific regions of WHO [1]. In India, dengue is endemic in almost all states and is the leading cause of hospitalization. Dengue fever had a predominant urban distribution a few decades earlier, but is now also reported from peri-urban as well as rural areas [7, 8].

The present study is perhaps the first to investigate the trend in NLR and PLR independently to predict severity of dengue fever in paediatric patients. Early diagnosis of dengue fever does improve the outcome of the patients especially those admitted to PICU. Hence, specific haematological and biochemical parameters estimation are much needed to tailor the clinical management of the patient.

As per expectations the fever, myalgia and rash were frequently observed in severe dengue cases. Similar findings were also reported by M K Malthesaet *al.*, in their study on dengue patients and by Agarwal R *et al.*, [8, 9]. Typical hallmark laboratory findings of dengue fever like leukopenia and thrombocytopenia showed significant association in our study too. Like reported elsewhere, Leukopenia was observed in 22.3% of dengue fever cases while thrombocytopenia was reported in 29.8% of the dengue fever group [10, 11]. We also observed a significant increase in monocyte count in paediatric dengue patients and it was significantly associated with increased severity. Usually, in severe cases like DHF and DSS, haemorrhagic manifestations with increased vascular permeability and fluid loss from vascular space into the chest and abdominal cavities are observed. These life-threatening symptoms usually occur in the critical or defervescence phase of illness were hypotension, weak pulse pressure (≤ 20 mm Hg) with cold, clammy skin is observed [12-14].

The present study has shown the NLR and PLR follows a rising trend with worsening in the clinical condition and increase in the severity of dengue fever in the children. PLR and NLR are inexpensive and quick to obtain and help in determining high risk patients and also to follow up closely the clinical improvement objectively as well as to predict the severity in the patients. Agarwal J *et al.*, [14], had assessed the NLR, PLR and platelet counts for predicting long term outcomes in dengue patients and concluded that survival was worse in those with a higher NLR values. This study is limited by being single centre cohort study and requires further randomized control studies to establish the range of values for NLR and PLR, in helping to further stratify patients into risk of mortality, and thereby tailoring care to individual case-based level. By comparing NLR and PLR to other known mortality predictors may improve the study.

The involvement of liver, brain, kidney, and heart were previously reported in severe dengue cases [15-17]. Hepatic dysfunctions are also commonly observed in dengue cases with severe dengue characterised by elevated liver enzyme levels [18-21]. Several studies have reported elevation in both the AST and ALT, as the predictors of severe dengue. Our results also indicate that the mean levels of the liver enzymes like ASP, AST and ALT were uniformly elevated among the paediatric dengue patients.

Barua A *et al.*, [21]. Opined that plasma ferritin levels in severe dengue cases were elevated and

may be the potential predictive biomarker for disease severity in the acute phase of infection. A systemic review and meta-analysis stated that elevated levels of serum ferritin could be an effective diagnostic method for dengue fever [2-4]. The present study findings were consistent with above findings which reported significant association between high serum ferritin levels and severe dengue fever in children.

Our findings herein demonstrate that differential clinical and laboratory features can prognosticate dengue fever from other febrile illnesses. Surprisingly, age was significantly associated with dengue fever with the mean age of children with dengue predominantly being higher (>5 years). This could possibly be explained as older children are more likely to be exposed to the environment and therefore more likely to experience mosquito bites. It may be noted however that the overall study cohort size was small and larger studies need to be done to validate the significant association of older age with dengue infections.

In children, early recognition of dengue fever and its severity in a dengue endemic region like different states of India would be very helpful in reducing not only the mortality and morbidity of dengue illness but also the overall cost of hospitalization. In this study, we attempted to find out possible biomarkers in prognosis and severity of dengue fever.

However, the findings of this study cannot be generalized due to a small sample size of 40 children. There is a need for further studies including cases of severe dengue with complications to assess the role of NLR, PLR and hepatic enzyme levels as a marker of prediction of severity dengue fever.

Conclusion

Our present study gives an insight into the fact that simple and inexpensive markers such as rise in Neutrophil Lymphocyte Ratio (NLR) and Platelet Lymphocyte Ratio (PLR) helps in predicting the prognosis and severity of dengue fever. Our study findings highlight the heterogeneous clinical presentations of dengue fever in children and identify routine clinical and laboratory parameters like leukopenia, thrombocytopenia and elevated NLR and PLR significantly discriminating dengue from other febrile illness.

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