



NEUTROPHIL-TO-LYMPHOCYTE RATIO: A RELIABLE DIAGNOSTIC AND PROGNOSTIC BIOMARKER FOR DENGUE.

Microbiology

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ABSTRACT

Background: Varied clinical presentation and associated severe complications, usually affects the outcome of the disease. Therefore, meticulous laboratory support is appreciated for both establishing diagnosis and monitoring prognosis of the disease. In most of healthcare setup, laboratory workup for diagnosis of dengue usually relies on serological tests. However, at times serological tests may be not reliable during first few days of the infection. Hence there is ever lasting demand of reliable diagnostic biomarker for dengue. In the present study, the reliability of neutrophil-to-lymphocyte ratio (NLR) as a diagnostic and prognostic biomarker for dengue was assessed. **Methods:** A total of 108 patients with febrile illness were included in the study. Dengue viral infection in these patients screened by rapid test. ELISA test was used to confirm dengue infection. NLR and platelet-to-lymphocyte ratio was estimated in patients with dengue infection confirmed by ELISA test. **Results:** Dengue was confirmed in a total of 90 cases by ELISA test. When the NLR and PLR was compared in the patients, it was noted that there was decrease in NLR in patients with severe dengue fever, as its level was found to be declined in cases with grade II and grade III dengue hemorrhagic fever. **Conclusion:** Low NLR was seen in 73.3% patients with dengue fever. In patients with severe forms of dengue infection, low NLR was the marked feature. On the other PLR remained largely inconclusive. Therefore, from the present study, it can be concluded that NLR can be used as a diagnostic biomarker and additionally its estimation will help to understand the prognosis of dengue to more severe forms. Hence NLR can be used to identify patients in whom intensified supportive therapy and intensive care is required.

KEYWORDS

Dengue, platelets, lymphocytes, neutrophil-to-lymphocyte ratio, platelets -to-lymphocyte ratio

INTRODUCTION.

Even in the 'golden era' of the modern medicine, infectious diseases continue to haunt mankind. Infectious diseases are major contributors to increased morbidity and mortality especially in tropical and subtropical regions. In recent years, India is experiencing an upsurge in incidence of tropical fevers.¹

Tropical fevers are unique to tropics and subtropics. These febrile infections are caused by a wide range of pathogens including bacteria, viruses and fungi.² Dengue, chikungunya, influenza A and Japanese encephalitis are examples of viral tropical fever whereas enteric fever (typhoid) and rickettsial fever (scrub typhus) are important tropical fevers attributed to bacterial etiologies. Malaria is an example of tropical fever of parasitic origin.²⁻⁴

Most, if not all, tropical fevers are transmitted by bite of a hematophagous insects like mosquitoes.² As multitude of factors including geographical region, sanitation and seasonal variability greatly influences the tropical fevers, there is notable variability seen the proportion of these fevers in both hospitalized patients and patients receiving treatment on OPD basis.²

Most of Indian studies have documented dengue as the most common tropical fever. Dengue, a viral infection caused by Flavivirus is a vector-borne infection transmitted by mosquito of the genus Aedes. There are total of four antigenically distinct but closely related serotypes of Dengue viruses: DENV1 to DENV4.⁵ The clinical spectrum of dengue infection is varied and ranges from asymptomatic to severe haemorrhagic fever.⁵ Additional dengue fever may lead to several complications that at times may be fatal.

Both precise and rapid diagnosis of dengue plays a crucial role in both treatment and management of the patients as delay in initiation of appropriate therapy is usually associated with poor outcome of the patients in terms of high morbidity and mortality. In most of healthcare setup, laboratory workup for diagnosis of dengue usually relies on serological tests.⁶ However, at times serological tests may be not reliable during first few days of the infection. Hence there is ever lasting demand of reliable diagnostic biomarker for dengue. In the present study, the reliability of neutrophil-to-lymphocyte ratio (NLR) as a diagnostic and prognostic biomarker for dengue was assessed.

MATERIAL AND METHODS.

The present descriptive, cross-sectional study was conducted in Department of Microbiology of a tertiary care teaching hospital. A total of 108 patients admitted to the hospital with febrile illness were

included in the study.

From these patients, 5 mL of venous blood was collected from ante-cubital with single use disposal needle and syringe under all aseptic condition. 2 mL of blood was transferred to EDTA vacutainer whereas 3 mL of blood was placed in plain vacutainer.

The serum sample from plain vacutainer obtained after centrifugation was used for performing rapid immunochromatography test and ELISA for dengue. As per the guidelines of Ministry of Health and Family Welfare (MOHFW) and Government of India, positive result by ELISA test (Robonik ELISA apparatus) was used for confirmation of dengue.

The blood from EDTA vacutainer was processed in Mindray BC-300 plus differential cell counter. Additionally, a blood smear was prepared, stained by Leishman stain and examined by Pathologist.

The NLR ratio was obtained by calculating the percentage of neutrophil distribution as a numerator and the percentage of lymphocytes as a denominator. It was expressed in the unit of $10^3/L$.

Platelet-lymphocyte ratio (PLR) was calculated by using the percentage of platelets as a numerator and the percentage of lymphocytes as a denominator. Similar to NLR, PLR was also expressed in the unit of $10^3/L$. These NLR and PLR and compared in grade I and grade II dengue hemorrhagic fever (DHF) patients. Patient's demographic and clinical features were recorded and analyzed.

RESULTS.

Out of 108 patients admitted with febrile illness, dengue was confirmed in a total of 90 cases by ELISA test. As shown in the figure 1, males were predominately affected.

The mean age of the dengue patients was 38.56 ± 28.2 years (range 9-65 years). The mean duration of febrile illness was 6.4 ± 1.2 days (range 3-7 days). The mean hemoglobin of the patients was 13.4 ± 3.1 . The mean hematocrit was 44.4 ± 4.4 . The mean total leucocyte count was 4.1 ± 0.8 and the mean minimum platelet count was 48.1 ± 24.2 .

As per the World Health Organization criteria, out of 90 dengue patients, 27 (30%) patients presented without any warning signs of dengue (Grade I), whereas 34 (37.8%) cases had warning signs like abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, elevated haematocrit with

declining platelet counts (Grade II). A total of 29 (32.2%) patients had severe dengue (Grade III). These patients presented with severe plasma leakage, bleeding or organ failure.

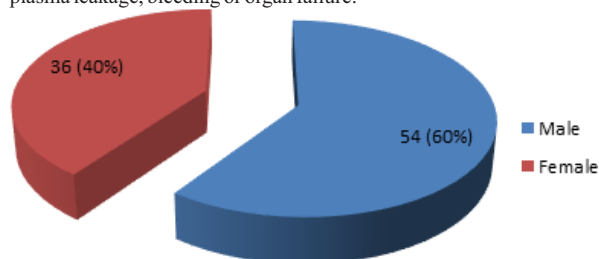


Figure 1. Gender Wise Distribution Of Dengue Patients.

When the NLR and PLR was compared in the patients, it was noted that there was decrease in NLR in patients with severe dengue fever, as its level was found to be declined in cases with grade II and grade III DHF. On comparison of PLR, it was increased in the severe form of dengue infection.

The comparison of NLR and WHO criteria for assessing the severity of dengue infection is shown in figure 2. The low NLR was noted in 40.7% of grade I patients whereas 82.4% belonging to grade II had low NLR. Among grade III patients, 93.1% had low NLR. Therefore, low NLR is significantly associated with severity of the disease.

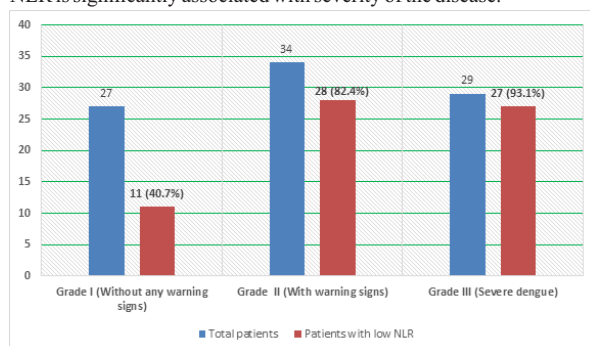


Figure 2. Comparison Of Low NLR And Stage Of Dengue Fever.

DISCUSSION.

Viruses, the ultramicroscopic beings, have always terrified human beings starting from the small pox infection to the most recent awful pandemic of COVID-19. Viral infections usually pose as a baffling scenario, as a single virus may cause variety of symptoms whereas a single symptom may be due to variety of illness. Hence laboratory diagnosis remains the cornerstone in establishing the cause of infection.

In addition to supplementing/and confirming clinical diagnosis, laboratory workup provides guidance for selecting therapeutic approach relevant for the specific condition. Therefore, there it is necessary to evaluate known biomarkers and alongside turn our focus for investigating new modalities for diagnosing and assessing the prognosis of viral infection. In the current study, the utility of NLR as an efficient marker for diagnosis and prognosis of dengue fever was evaluated.

In this study, as reported by other researchers, dengue fever was predominant in males (60%) as compared to female patients (40%). This finding may be due to the fact that outdoor activities are performed predominately by men and hence they are more prone to mosquito bite during the day time.⁷⁻⁹ The finding also highlights the importance of educating and motivating masses to use mosquito repellents and wearing long sleeved shirts and pants to prevent mosquito bites.

In this low NLR was noted in a total of 66 (73.3%) patients with dengue fever. Similar finding was noted in the study of Sadgir *et al* (2023) where 76% of dengue patients had low NLR. The low NLR may be attributed to the existence of large number of lymphocytes.¹⁰

In dengue fever, initially the neutrophil count is elevated for few days and therefore, the NLR is high during early febrile phase. Upon

progression of disease from an acute febrile phase to critical phase there is increase in the number of lymphocytes because of reactive lymphocytosis leading to reversal of NLR. The low NLR indicates the onset of critical phase, that is associated with leakage of plasma.¹⁰ The high NLR is suggestive of increase in the level of inflammation and inflammatory cellular markers. Elevated NLR signifies the increase in thrombocytosis.¹¹⁻¹³ In this study, low NLR coincided with the severe form of the disease as 82.4% of patients with grade II and 93.1% of patients with grade III dengue fever showed low NLR.

In the present study, on comparison of PLR, it was noted to be increased in the severe form of dengue infection. There are controversial findings related to PLR in dengue fever. Navya *et al*. (2024)¹⁰ reported PLR to be an accurate marker for assessing the prognosis of dengue whereas Agarwal *et al* (2023) reported a contrast finding.¹⁴ Therefore more studies are warranted to explore the utilization of PLR as a marker for establishing diagnosis and monitoring prognosis of patients with dengue fever.

CONCLUSION.

Low NLR was seen in 73.3% patients with dengue fever. In patients with severe forms of dengue infection, low NLR was the marked feature. On the other PLR remained largely inconclusive. Therefore, from the present study, it can be concluded that NLR can be used as a diagnostic biomarker and additionally its estimation will help to understand the prognosis of dengue to more severe forms. Hence NLR can be used to identify patients in whom intensified supportive therapy and intensive care is required.

Conflict Of Interest: Nil.

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REFERENCES.

- Singhi S, Chaudhary D, Varghese GM, Bhalla A, Karthi N, *et al*. The Indian Society of Critical Care Medicine Tropical Fever Group. Tropical fevers: Management guidelines. *Indian J Crit Care Med*. 2014; 18:62-69.
- Singhi S, Rungta N, Nallasamy K, Bhalla A, Peter J, Chaudhary D, *et al*. for Indian Society of Critical Care Medicine Research Group. Tropical Fevers in Indian Intensive Care Units: A Prospective Multicenter Study. *Indian J Crit Care Med*. 2017; 21:811-818.
- Punjabi N, Taylor W, Murphy G, Purwaningsih S, Picarima H, Sisson J, *et al*. Etiology of acute, non-malaria, febrile illnesses in Jayapura, Northeastern Papua, Indonesia. *Am J Trop Med Hyg*. 2012; 86:46-51.
- Susilawati T, McBride W. Acute undifferentiated fever in Asia: A review of the literature. *Southeast Asian J Trop Med Public Health*. 2014; 45:719-26.
- Trivedi S, Chakravarty A. Neurological Complications of Dengue Fever. *Curr Neurol Neurosci Rep*. 2022; 22:515-529.
- Ramadevi D, Shoban Kumar D, Hari Priya D. Evaluation and comparison of neutrophil-to-lymphocyte ratio (NLR) as a diagnostic marker for dengue infection. *EJ/MCM*. 2023; 10:752-758.
- Agarwal R, Kapoor S, Nagar R, Misra A, Tandon R, Mathur A, *et al*. A clinical study of the patients with dengue hemorrhagic fever during the epidemic of 1996 at Lucknow, India. *Southeast Asian J Trop Med Public Health*. 1999; 30: 735-740.
- Banerjee M, Chatterjee T, Choudhary GS, Srinivas V, Kataria VK. Dengue: A Clinico-Haematological profile. *Medical Journal Armed Forces India*. 2008; 64:940: 333-336.
- Modampuri Akhil Koundinya, Dhanunjaya Dasari, S Ashok Kumar, BSV Manjula, Devi vinaya. Neutrophil to lymphocyte ratio as prognostic and predictor factor for severity of dengue fever - a retrospective observational study in a tertiary care centre. *IAIM*. 2021; 8: 46-52.
- Navya P, Begum R, Thajudeen A, Hussain M, Vijayashree R. Navigating the haematological maze: Unraveling the role of NLR and PLR as predictors of dengue severity- A cross-sectional study from Southern India. *J Clin Diagn Res*. 2024; 18:EC23-EC26.
- Sadgir A, Durge K, Masavkar S. Neutrophil-lymphocyte ratio as a prognostic indicator in dengue fever patients at tertiary care hospital in Northwest-Maharashtra. *Int. J. Adv. Res*. 2023; 11:1198-1202.
- Barde P, Shukla M, Kori B, Chand G, Jain L, Varun B. Emergence of dengue in tribal villages of Mandla district, Madhya Pradesh, India. *Indian J Med Res*. 2015; 14:584-589.
- Chandra A. Dengue hemorrhagic fever: Epidemiology, pathogenesis, and risk factors for transmission. *Aspirator J Vector-Borne Dis*. 2010; 2:110-119.
- Agrawal V, Prusty B, Reddy C, Reddy G, Agrawal R, Bandaru V. Clinical profile and predictors of severe dengue disease: A study from South India. *Casp J Intern Med*. 2018; 9:334.