Original Research Article

Neutrophil To Lymphocyte Ratio As Prognostic And Predictor Factor For Severity Of Dengue Fever - A Retrospective Observational Study In A Tertiary Care Centre

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International Archives of Integrated Medicine, Vol. 8, Issue 12, December, 2021.
Available online at http://iaimjournal.com/
ISSN: 2394-0026 (P) **ISSN: 2394-0034 (O)**
Received on: 22-11-2021 **Accepted on:** 29-11-2021
Source of support: Nil **Conflict of interest:** None declared.

How to cite this article: 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(12): 46-52.

Abstract

In India dengue virus infection is epidemic and still a major health problem. It is a cause of death in young population in India. Neutrophil to lymphocyte ratio is used as prognostic factor in ICU settings for various disease. It is easy to calculate and in this study the relation between NLR and severity of dengue disease is studied. The Aims and Objectives of the study were to evaluate the NLR as prognostic marker and for severity of disease in patients with dengue fever. Around 30 patients who fulfilled the inclusion criteria were taken in to the study. The NLR was calculated on the sixth day of illness as it marks the shift from acute febrile phase to critically ill phase of the disease. The study group population age was from 18 to 48 years of age with male to female ratio of 2:1. Among them 18 (60%) patients were Ns1 antigen positive, 9 (30%) patients were IgM positive and 3 (10%) patients had both Ns1 Ag and IgM positive. Among 30 patients, 20 patients had bleeding manifestations and in them 8 had shock. In the study most of the patients had platelet count of 20,000 to 50,000 per mm^3.

In our study there was no significant relation between platelet count and NLR. There was significant relation between NLR ratio and the severity of the disease as evident by our study in which patients with NLR ratio of less than 0.9 all had bleeding manifestations except one patient and among total 8 patients of shock in study 6 had NLR of less than 1. Among 10 patients with NLR of more than 2 only one had bleeding manifestations. So there was a significant relation between neutrophil to lymphocyte ratio to the severity of dengue fever in adult patients and by keeping an eye on the NLR we can predict the prognosis and the severity of the disease in patient. Lower the NLR ratio severe the disease.

Key words

Neutrophil, Lymphocyte, Ratio, Prognostic factor, Predictor, Dengue fever, Severity.

Introduction

In tropical countries like India, dengue virus infection is still a public health problem because of the hyperendemicity of four virus serotypes (DENV1, DENV2, DENV3 and DENV4) [1]. It is still one of the main infectious cause of death in young population in India. Dengue Virus (DENV) is a mosquito-borne single-stranded RNA virus belonging to the family Flaviviridae. Human transmission occurs from bites of infected mosquitoes like Aedes aegypti and Aedes albopticus [2]. Dengue causes a wide spectrum of disease. We categories dengue fever according to the 2009 WHO criteria classify dengue according to levels of severity: (A) dengue without warning signs; (B) dengue with warning signs (abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, increasing hematocrit with decreasing platelets); and (C) severe dengue (dengue with severe plasma leakage, severe bleeding, or organ failure) [7].

Severe dengue was first recognized in the 1950s during dengue epidemics in the Philippines and Thailand. Today, severe dengue affects most Asian and Latin American countries and has become a leading cause of hospitalization and death among children and adults in these regions. A vast majority of cases are asymptomatic or mild and self-managed, and hence the actual numbers of dengue cases are under-reported. Many cases are also misdiagnosed as other febrile illnesses [3].

Because of increased burden of the disease in developing countries there is need for a easily done and interpreted prognostic marker to predict the progression and severity of the disease. Neutrophil-to-lymphocyte ratio (NLR) can be easily calculated based on a complete blood count by dividing the Neutrophil percentage by Lymphocyte percentage. There is enough literature to suggest that NLR has prognostic significance in various diseases. However, no studies have focused on NLR as a prognostic marker for dengue. This study aims to look into the possibility of NLR as an easily accessible marker for the prognosis of dengue fever and for the severity of the disease. In this study the NLR is calculated on the sixth day of illness as it marks the transition between febrile phase of disease to critical illness stage.

Aim and Objectives

- To evaluate the NLR as prognostic marker in patients with dengue fever.
- To evaluate the NLR for grading of severity of disease in patients with dengue fever.

Materials and methods

It was a retrospective observational study of 30 patients with diagnosis of dengue fever admitted in Malla Reddy institute of medical sciences, Hyderabad, Telangana. Study period was from July, 2021 to October, 2021. The statistical analysis was done on Microsoft Excel and P

value or Chi Square test was done and its significance noted.

Sample Processing: After routine informed consent from patient, 2 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 Sysmex 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.

Next, the neutrophil/lymphocyte count ratio was measured. The neutrophil/lymphocyte count ratio is the result of the percentage of neutrophil distribution divided by the percentage of lymphocytes in dengue fever patients in a unit of $10^3/\mu L$.

Inclusion criteria:

- Age > 18 years old admitted to our hospital,
- Patients with confirmed diagnosis of dengue by ELISA method for Ns1Ag or IgM or IgG or a combination of these.

Exclusion criteria:

- Age < 18 years old , > 60 years old admitted to our hospital,
- Patients with a known history of hematologic malignancy, immunosuppression, HIV positive status, known infection with other viral pathogens were excluded.

Statistical analysis:

All the data were collected in approved proforma and data were entered in MS EXCEL 2007, and was subjected to statistical analysis.

Descriptive data were analyzed using percentages and central tendency. Chi Square Test was applied for the proportions and paired t test was applied for mean values. P value < 0.05 was considered as statistically significant.

Results

In our study, we had taken a total number of 30 patients who had fulfilled the inclusion criteria. Among them 20 patients were male and 10 were female. The study group population age was from 18 to 48 years of age and the mean age of the study population was 29.43. Among them 18 (60%) patients were Ns1 antigen positive, 9 (30%) patients were IgM positive and 3 (10%) patients had both Ns1 Ag and IgM positive. The mean platelet count of the study group were 42,060 (5,000 to 1,20,000) and the mean WBC count of the study was 4,069 (1,800 to 8,200). The mean values of neutrophil and lymphocyte of the study group were 48.9 (12 to 82) and 41.6 (10 to 80) respectively. The mean N:L ratio of the study group was 2.12 (0.1 to 8.2). Among 30 patients, 20 patients had bleeding manifestations and in them 8 had shock.

In the **Table - 1**, the relation between platelet count and number of patients with bleeding manifestations and shock was seen. Only one patient (3%) had platelet count less than 5000 per mm^3 with both bleeding manifestations (5%) and shock (12.5%). 4 (13%) patients were having platelet count of 5,000 to 10,000 among them 3 (15%) had bleeding manifestations with shock in 2 (25%) patients. 4 (13%) patients were having platelet count of 10,000 to 20,000 among them 4 (20%) had bleeding manifestations with shock in 2 (25%) patients. Among study group 10 (33%) patients who had platelet count of 20,000 to 50,000 eight (40%) patients had bleeding manifestations with shock only in 2 (25%) patients among them. 6 (20%) patients had platelet count of 50,000 to 75,000 per mm^3 with bleeding manifestations in 4 (20%) patients and shock in 1(12.5%) patient. 3 (10%) patients and 2 (6%) patients were present in study group with platelet count of 75,000 to 1,00,000 and 1,00,000 to 1,50,000 per mm^3 respectively with no bleeding manifestations and shock.

Table – 1: Relationship of platelet count with bleeding manifestations and shock / organ damage.

Platelet count	**No. of patients**	**Bleeding manifestations**	**Shock**
<5,000	1 (3%)	1 (5%)	1 (12.5%)
5,000-10,000	4 (13%)	3 (15%)	2 (25%)
10,000- 20,000	4 (13%)	4 (20%)	2(25%)
20,000-50,000	10 (33%)	8 (40%)	2(25%)
50,000-75,000	6 (20%)	4 (20%)	1 (12.5%)
75,000-1,00,000	3 (10%)	0	0
1,00,000-1,50,000	2 (6%)	0	0
Total	30 (100%)	20 (100%)	8 (100%)

Table -2: Relationship of platelet count with neutrophil to lymphocyte ratio.

	Neutrophil To Lymphocyte Ratio				
Platelet count	**≤ 0.1**	**0.2 - 0.4**	**0.5 – 0.9**	**1 – 2**	**>2**
<5,000	-	-	-	1(3.3%)	-
5,000-10,000	1 (3.3%)	-	3 (10%)	-	-
10,000- 20,000	-	2 (6.6%)	1(3.3%)	1(3.3%)	-
20,000-50,000	-	1(3.3%)	3(10%)	3(10%)	3(10%)
50,000-75,000	-	1(3.3%)	1(3.3%)	2(6.6%)	2(6.6%)
75,000-1,00,000	-	-	-	-	3(10%)
1,00,000-1,50,000	-	-	-	-	2(6.6%)
Total (100%)	1(3.3%)	4 (13.3%)	8 (26.6%)	7 (23.3%)	10(33.3%)

Table 3: Relationship of N:L ratio with bleeding manifestations and shock.

Neutrophil To Lymphocyte Ratio	**No of patients**	**Bleeding manifestations**	**Shock**
≤ 0.1	1	1(5%)	1(12.5%)
0.2 - 0.4	4	4(20%)	3(37.5%)
0.5 – 0.9	8	7(35%)	2(25%)
1 – 2	7	7(35%)	2(25%)
>2	10	1(5%)	0
Total	30	20 (100%)	8 (100%) **P value- 0.001**

In the **Table - 2**, the relation between platelet count and Neutrophil to Lymphocyte Ratio was seen. Only one patient with platelet count less than 5000 per m m^3 had NLR ratio of 1(3.3%). 4 patients with platelet count of 5,000 to 10,000 were present among them 3 (10%) had NLR ratio of 0.5 to 0.9 and 1(3.3%) patient had ratio of less than 0.1. 4 patients were having platelet count of 10,000 to 20,000 among them 2 (6.6%) had NLR ratio of 0.2 to 0.4 and one patient (3.3%) had ratio of 0.5 to 0.9 and other one patient (3.3%) had ratio between 1 to 2. Among study group 10 patients had platelet count of 20,000 to 50,000 in whom 1(3.3%), 3(10%), 3(10%) and 3(10%) patients had NLR ratio of 0.2 to 0.4, 0.5 to 0.9, 1 to 2 and more than 2 respectively. 6 patients had platelet count of 50,000 to 75,000 per mm^3 with NLR ratio of 0.2 to 0.4, 0.5 to 0.9, 1 to 2 and more than 2 in 1(3.3%), 1(3.3%), 2(6.6%) and 2(6.6%) patients' respectively. 3(10%) patients with platelet count of 75,000 to 1,00,000 had NLR ratio of more than 2 and 2 (6.6%) patients with 1,00,000 to 1,50,000 per mm^3 platelet count had NLR ratio of more than 2. Among the total

study population one patient (3.3%) had NLR ratio of $\leq$ 0.1, 4 patients (13.3%) had ratio of 0.2 to 0.4, 8 patients (26.6%) had ratio of 0.5 to 0.9, 7 patients (23.3%) had ratio of 1 to 2 and 10 patients (33.3%) had ratio of more than 2.

From **Table – 3**, we can see the relation between NLR ratio and the severity of the dengue fever. In one patient with NLR ratio of less than 0.1 both bleeding manifestations and shock was seen. Among 4(20%) patients with ratio between 0.2 to 0.4 all had bleeding manifestations and 3(37.5%) had shock. Among 8 patients with NLR ratio of 0.5 to 0.9, 7 (35%) had bleeding manifestations and 2(25%) had shock. Among 7 patients with ratio between 1 to 2 all have bleeding manifestations(35%) and shock in 2(25%) among them. The most of the patients in the study i.e. 10 patients had ratio of more than 2 among them none had shock and only one (5%) had bleeding manifestations. Among total 30 patients, 20 had bleeding manifestations and 8 had shock with significant P value of 0.001.

Discussion

Dengue fever is an important arboviral disease of global concern causing major outbreaks with mortality and morbidity in endemic countries [6]. One modelling estimate indicates 390 million dengue virus infections per year (95% credible interval 284–528 million), of which 96 million (67–136 million) manifest clinically (with any severity of disease) [4]. Another study on the prevalence of dengue estimates that 3.9 billion people are at risk of infection with dengue viruses. Despite a risk of infection existing in 129 countries [5], 70% of the actual burden is in Asia [4]. The number of dengue cases reported to WHO increased over 8 fold over the last two decades, from 505,430 cases in 2000, to over 2.4 million in 2010, and 5.2 million in 2019. Reported deaths between the year 2000 and 2015 increased from 960 to 4032. The bleeding manifestations which are considered in the study population are malena, bleeding gums, heamaturia, epistaxis, petechiae, purpura and positive tourniquet test.

In the study group among 30 patients, 10 are female and 20 are male with ratio of 1:2. This correlates with, Vibha, et al. [8]; Fu Xi Qui, et al. [9]; Agarwal, et al. [10]; Malathesha, et al. [11] and Banerjee, et al. [12] in which the male to female ratio is 2:1. This is due to the fact that males predominantly form the outdoor working population and more prone to infection by mosquito bite in a day time. Majority of patients in the study are in the age group of 18 to 48 years and majority of patients had thrombocytopenia with platelet count between the range of 20000-50000/µl. This correlates with the study of Vibha, et al. [8].

From our study we found that there is no significant relation between platelet count and NLR ratio as evident by case with 5000 platelet count having ratio of 1. But there was significant relation between NLR ratio and the severity of the disease as evident by our study in which patients with NLR ratio of less than 0.9 all had bleeding manifestations except one patient and among total 8 patients of shock in study 6 have ratio of less than 1. In our study 66.7% patients have NLR ratio of less than 2 due to reactive lymphocytosis in dengue fever. In a study by Padmanabhan P Athira, et al., an arbitrary cut off of NLR was established as 2 and was found that on admission, a significantly higher proportion of dengue fever patients had NLR values < 2.0 (p=0.035) and monocyte lymphocyte ratio (MLR) $\geq$ 0.20 (p=0.0095) 12 compared to children suffering from non-dengue febrile illness [13].

In initial days of dengue infection there will be increase in neutrophil percentage so there will be higher NLR ratio but as diseases progress from acute febrile phase to critical phase there will be increase in lymphocytes due to reactive lymphocytosis and there will be reversal in NLR ratio on day 6 to day 9. Because of initiation of reversal of ratio on day 6 of disease we have taken day 6 counts in our study. Higher amount of lymphocytosis is mostly seen in young age group due to active immune system. In the

present study, a significantly higher proportion of dengue cases (10 patients) demonstrated NLR 2 was seen as the platelets improved and disease got better suggesting that NLR may be a prognostic indicator of dengue disease. A higher NLR indicates a higher level of inflammation [14] and also NLR can be used to predict the severity of inflammation and prognosis [15].

The limitations of the study were it was done in a small sample population and done in a limited age group and in a single hospital.

Conclusion

From our study we found that there is a significant relation between neutrophil to lymphocyte ratio to the severity of dengue fever in adult patients and by keeping an eye on the NLR we can predict the prognosis and the severity of the disease in patient. Lower the NLR ratio severe the disease.

So still large group multicentre studies have to be done to know more about the uses of NLR in dengue and other infectious diseases.

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