(RESEARCH ARTICLE)

Significance of neutrophil to lymphocyte ratio evaluation as a prognostic indicator in dengue patients.

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Abstract

Dengue is the most important arthropod borne re-emerging tropical viral infection in human beings resulting in more illness and deaths. Platelet count and hematocrit estimations are the most common parameters to assess the severity of dengue. However, due to the heterogeneous nature of the clinical presentation of dengue patients, it's difficult to identify sensitive clinical or laboratory predictor to assess dengue severity. Neutrophil - Lymphocyte ratio (NLR) has been proved as a prognostic marker in various diseases and may emerge as a potential predictor for the dengue severity and its prognosis. This study aims to evaluate the NLR in dengue patients and to assess its role in relation with patients clinical and serological profile as well as platelet counts. A retrospective observational study was conducted in which 100 cases (≥12 years of age) of dengue which were diagnosed at our laboratory were studied and their clinical and laboratory data were analysed. Test of significance was done by Chi Square test with consideration of P value ≤ 0.05 as statistically significant. Male predominance was observed with dengue infection. NS1Ag (+) was the most common dengue serological profile. NLR ≤2 significantly correlates with thrombocytopenia. A significant relation was found between NLR and severity of the dengue patients. In conclusion, NLR ≤2 correlates with low platelet count which corresponds to more severity of the dengue disease. NLR >2 indicates more platelet count and less severity of the disease. Hence NLR evaluation can be used as a prognostic indicator in dengue patients.

Keywords: Neutrophil- lymphocyte ratio; Dengue; NLR; Platelet count; Prognostic indicator

1. Introduction

Dengue, also known break bone fever, is considered as the most important arthropod borne re-emerging tropical and subtropical viral infection in human beings [1]. It has been re-classified as the most important mosquito-borne viral disease in the world and emerges as a public health hazard resulting in more illness and deaths. Dengue Virus (DENV) is a single stranded RNA virus and belongs to Flaviviridae family. DENV is transmitted to human beings by infected mosquitoes more commonly Aedes aegypti (A. aegypti) and Aedes albopticus (A. albopticus) mosquitoes [2]. DENV1, DENV2, DENV3 and DENV4 are the four known antigenic dengue serotypes affecting the human beings [3].

The global burden of dengue has increased from 2000 to 2019 [4]. 70% of dengue virus infections are estimated to occur in Asia [5]. Dengue has also become a major health concern in India, posing challenges for healthcare system to manage dengue patients in resource limited areas, where the at-risk population is often not aware of its warning signs and potential complications [6]. To mitigate the mortality and morbidity associated with dengue infection, it is important to understand the heterogeneous nature of the clinical presentation of dengue along with its laboratory parameters. World Health Organisation (WHO) (2011) has defined that leukocytes, platelets, and haematocrit are the important parameters for determining the clinical development as well as the severity of dengue disease [7]. Neutrophils and lymphocytes play an active role in the body's response to infection as well as inflammation, allowing the proportion of these two cell types to infer the body's immune response [8]. Neutrophil – Lymphocyte ratio (NLR) is

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also an important marker of inflammation and sepsis and may be used in dengue disease. However, limited studies are there on the use of the NLR as an indirect marker of inflammation and sepsis, particularly in viral infections [9, 10].

This study aims to look into the possibility of NLR as a potential marker for the prognostication of dengue disease.

2. Materials and methods

It was a retrospective observational study of 100 patients diagnosed with dengue disease at Department of Pathology, Akshaya Health Care, Bangalore, India, over the period of 1 year (March 2023 – March 2024). The blood samples were collected from patients referred from different hospitals and clinics for blood investigations for dengue profile as well as haemogram/ complete blood count (CBC) and dengue positive patients were included in the study.

2 ml venous blood samples were collected for all the patients in K2- ethylenediaminetetraacetic acid (K2EDTA) vacutainers tube and were stored at room temperature until they were analyzed within 2 hour. The inadequate samples, haemolysed samples and clotted samples were rejected.

After mixing each blood sample properly with automated mixer for ten minutes, complete blood count was estimated by processing the sample in an automated hematology analyzer (UNITRON BIO-MEDICALS (UBM) Fx-19T). The quality control, calibration and maintenance of the analyzer were done as recommended by the manufacturer. The same blood samples were also used to prepare air dried peripheral blood smear (PBS) and was stained with Leishman's stain. Peripheral smears were examined by the Pathologist and WBC differentials as well as platelet counts were reconfirmed under microscope as suggested by previous studies [11-13]. Dengue serology (NS1Ag or IgM and IgG] was performed by Rapid kit (Immunochromatographic method) and confirmed by Enzyme linked immunosorbent assay (ELISA) method.

Following inclusion and exclusion criteria were followed:

- **Inclusion criteria:** Patients with age ≥ 12 years of age and confirmed with diagnosis of dengue by ELISA method for Ns1Ag or IgM or IgG or a combination of these.

- **Exclusion criteria:** All other sero-negative viral infections, pregnant patients, patients with chronic renal failure, malignancy, chronic liver diseases, immunodeficient states and other autoimmune and inflammatory diseases were excluded from the study.

Clinical parameters of each of the eligible patients including age, gender, symptoms, and laboratory results with emphasis on absolute neutrophil count, absolute lymphocyte count and platelet counts were retrieved and tabulated. Neutrophil – Lymphocyte ratio (NLR) was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count.

2.1. Statistical analysis

All the data were collected and were entered in MS EXCEL (2007) for further statistical analysis. Descriptive data were statistically analyzed using percentages and central tendency. Test of significance was done by Chi square test with consideration of P value ≤ 0.05 as statistically significant.

3. Result and discussion

It was a retrospective observational study of 100 patients diagnosed with dengue disease at Department of Pathology, Akshaya Health Care, Bangalore, India, over the period of 1 year (March 2023 – March 2024).

Out of 100 patients, 71% were male and rest were female (29%) with male: female ratio of 2.4:1(Figure1). Most of the patients were belonged to 21-29 years of the age group i.e. 34% followed by 12-19 years of the age group (23%) (Figure 2).
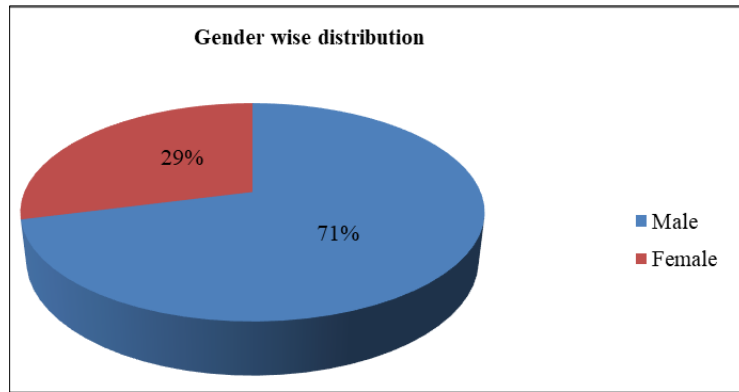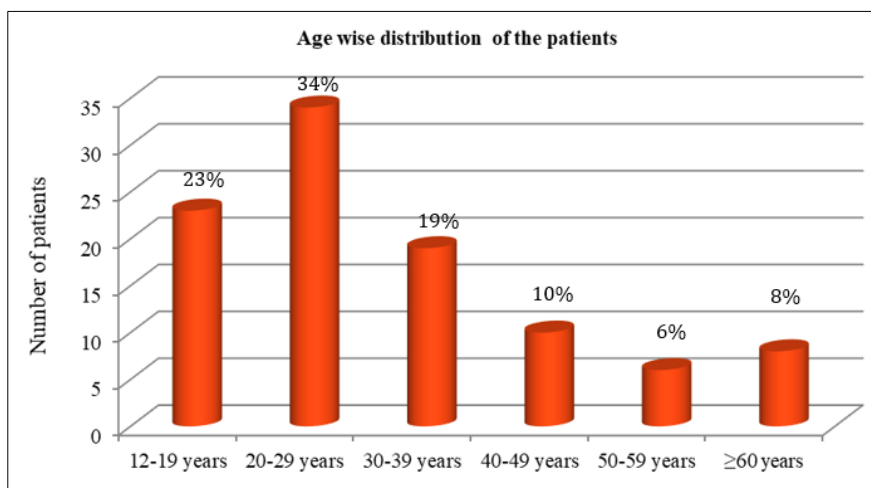
Figure 1 Gender wise distribution of the patients

Figure 2 Age wise distribution of the patients

61 % of the total patients were thrombocytopenic i.e. platelet count < 150 x1000/ μl and rest 39% with normal platelet count i.e. ≥ 150 x1000/ μl (Figure 3). 38% of the total patients were mild thrombocytopenic (i.e. < 50 x1000/ μl), 17% moderate thrombocytopenic (i.e. 50 -100 x1000/ μl) and 6% severe thrombocytopenia (i.e. 100-150 x1000/ μl) (Figure 4).
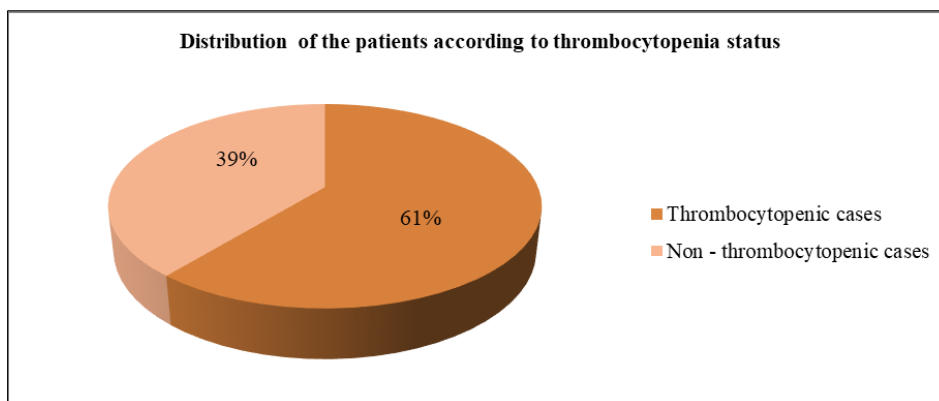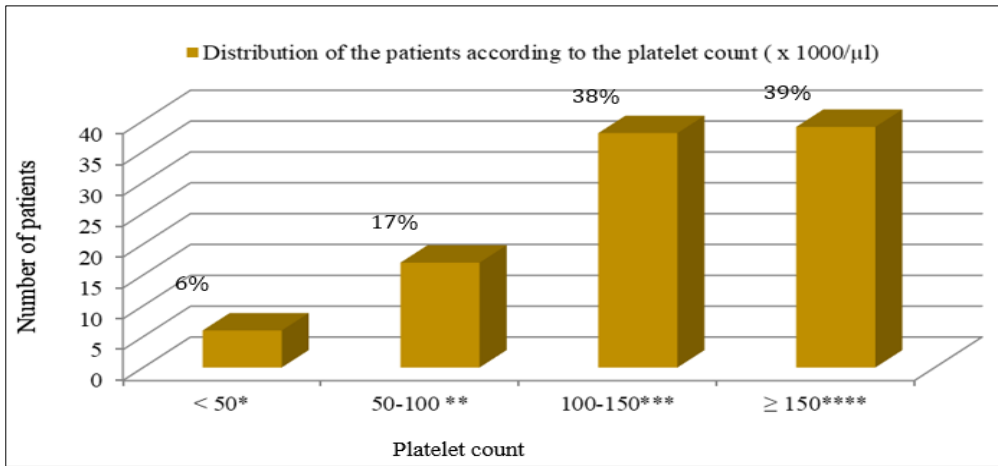

Figure 3 Distribution of the patients according to the thrombocytopenia status

Note:- * Mild thrombocytopenia, ** Moderate thrombocytopenia, *** Severe thrombocytopenia, **** Normal platelet counts

Figure 4 Distribution of the patients according to the platelet count (x 1000/ μl)

Most of the patients were NS1Ag (+) IgM (-) IgG(-) i.e. 58% followed by NS1Ag(-) IgM (+) IgG(-) i.e. 14%. No patient had the dengue serum profile of NS1Ag (+) IgM (+) IgG(+) in the study (Figure 5).
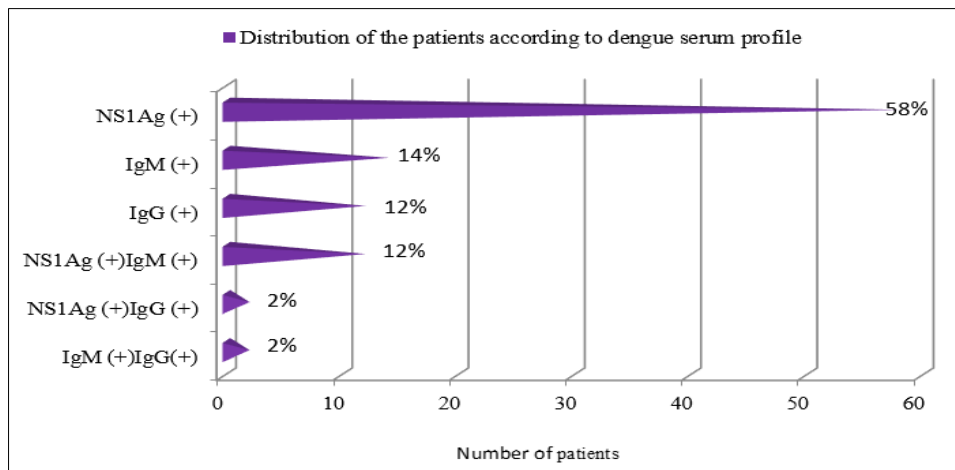

Figure 5 Distribution of the patients according to the dengue serum profile

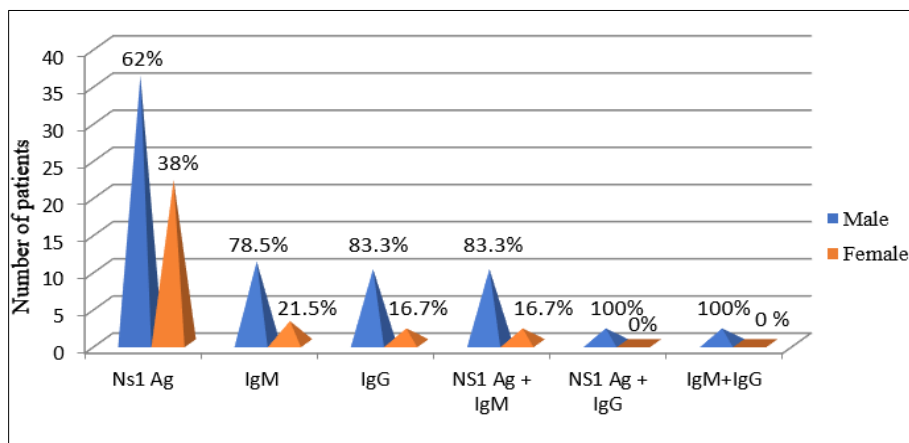Male preponderance was observed with dengue serological profile distribution (Figure 6).

Figure 6 Distribution of the dengue serological profile positivity result according to gender

In this study, NLR was also studied in each patients.25% of the patients were having NLR ≤2 while 75% had NLR >2 (Figure 7).
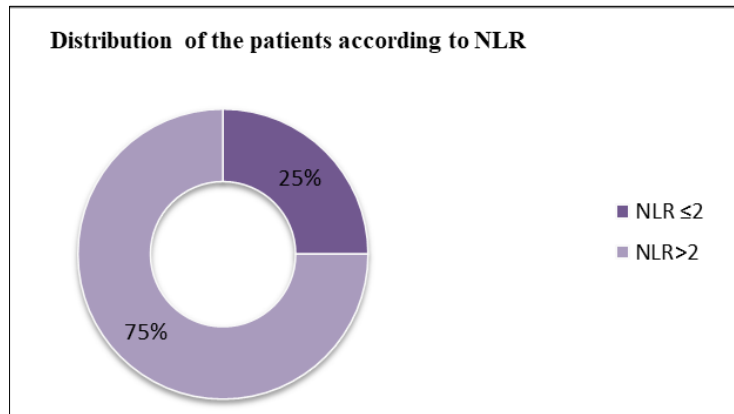

Figure 7 Distribution of the patients according to NLR

NLR has been correlated with the various demographic and clinicopathological parameters and been reported. NLR ≤2 as well as NLR >2 were mostly present in males i.e. 72% and 71% respectively without any significant difference when compared to females (p = 0.89). NLR ≤2 was most commonly found i.e. 40%, in patients with age group of 12-19 years while NLR >2 (36%) in 21-29 years age group. However, no significant difference was observed among various age groups (p = 0.23). (Table 1)

Table 1 NLR relation to demographic profile of the patients

NLR relation to gender			
Gender	NLR ≤2	NLR >2	P value (Chi square test)
Male	18 (72%)	53 (71%)	0.89
Female	7 (28%)	22 (29%)	
Total	25 (100%)	75 (100%)	
NLR relation to age groups			
Age group (years)	NLR ≤2	NLR >2	P value (Chi square test)
12-19	10 (40%)	13 (17.4%)	0.23
20-29	7 (28%)	27 (36%)	
30-39	3 (12%)	16 (21.3%)	
40-49	1 (4%)	9 (12%)	
50-59	2 (8%)	4 (5.3%)	
≥ 60	2 (8%)	6 (8%)	
Total	25 (100%)	75 (100%)	

88% of the patients with NLR ≤2 were thrombocytopenic while only 52% of the patients with NLR>2 were thrombocytopenic. A significant difference was observed between the two groups (p = 0.001). Most of the patients (52%) with NLR ≤2 were mildly thrombocytopenic while most of the patients (48%) with NLR > 2 were showing normal platelet count with significant difference among the groups (p = 0.01). NLR value distribution between thrombocytopenic cases and among various platelet groups were tabulated. (Table 2)

Table 2 NLR relation to the thrombocytopenia status and platelet count groups

NLR relation to thrombocytopenic status			
Thrombocytopenia	**NLR ≤2**	**NLR >2**	**P value (Chi square test)**
Yes	22 (88%)	39 (52%)	**0.001**
No	3 (12%)	36 (48%)	
Total	25 (100%)	75 (100%)	
NLR relation to platelet count groups			
Platelet count (x10^3/ul)	**NLR ≤2**	**NLR >2**	**P value (Chi square test)**
<50	3 (12%)	3 (4%)	**0.01**
50-100	6 (24%)	11 (14.7%)	
100-150	13 (52%)	25 (33.3%)	
≥ 150	3 (12%)	36 (48%)	
Total	25 (100%)	75 (100%)	

Most of the patients (56%) with NLR ≤2 were NS1Ag positive followed by NS1Ag + IgM positivity (16%).Most of the patients (58.7%) with NLR >2 were NS1Ag positive followed by IgM positivity (16%). No significant difference was observed among different serology result groups (p = 0.133). (Table3)

Table 3 NLR relation to the dengue serology positivity result groups

Serum positivity (Dengue profile)	NLR ≤2	NLR >2	P value (Chi square test)
NS1Ag	14 (56%)	44 (58.7%)	0.133
IgM	2 (8%)	12 (16%)	
IgG	2 (8%)	10 (13.3%)	
NS1Ag + IgM	4 (16%)	8 (10.7%)	
NS1Ag + IgM	1 (4%)	1 (1.3%)	
IgM + IgG	2 (8%)	0 (0%)	
Total	25 (100%)	75 (100%)	

Dengue fever is a medically important arboviral disease of concern globally, resulting major outbreaks with increased mortality and morbidity in endemic areas [2]. Male preponderance was observed in the present study (male: female = 2.4:1). Similar observation was shown by Agrawal et al., (2023) [14], Afsar et al., (2020) [15] and Malathesha et al., (2014) [16] with male: female ratio of 1.6:1, 2.5:1 and 1.4:1. This can be explained by the fact that males predominantly work outdoor and more prone to get infection by mosquito bite in a day time.

Majority of the patients were of between 20-29 years age, similar to Afsar et al., (2020) [15] with more patients between 21-30 years of age. Agrawal et al., (2023) [14] also showed significantly more patients below the age of 30 years. It's due to people of higher outside activity in younger age group.

Most of the patients were thrombocytopenic in the present study similar to Afsar et al., (2020) [15] and Vibha et al., (2016) [17]. Dengue virus not only affects the bone marrow directly resulting in low platelet production but also the antibodies produced against the platelets in immune response to dengue infection, causes the platelet destruction in blood and thus thrombocytopenia. Other postulated mechanisms is complement-mediated platelet destruction [18].

Most of the patients were NS1Ag positive which correlates to Afsar et al., (2020) [15]. However, in contrast to Afsar et al., (2020) [15], no patient was observed with NS1Ag (+) IgM (+) IgG(+) serum profile in the present study. Both the studies evidenced that primary dengue infection (NS1Ag+IgM) was more dominant in males.

In a study by Athira et al, (2018) [19], an arbitrary cut off of NLR was suggested as 2 and was observed significant higher proportion of dengue fever patients with NLR values < 2.0 (p=0.035) on admission compared to children suffering from non-dengue febrile illness .

In this study, 25% of the patients were having NLR ≤2. In contrast, Koundinya et al., (2021) [20] and Afsar et al., (2020) [15] reported 66.7% and 76%, respectively, with NLR<2. This contrast can be explained on the basis of testing of most of the the patients in early stage of the dengue infection, in the present study. In initial days of dengue infection there will be increase in neutrophil percentage as a result of inflammation, resulting higher NLR ratio but as diseases progress from acute febrile phase to critical phase, lymphocytes increase in number due to reactive lymphocytosis with reversal in NLR ratio by day 6 to day 9. Neutropenia in patients results from apoptosis of neutrophils caused by viral infection. Another theory suggests that neutrophils encapsulate dengue viral antigen positive platelets through neutrophil extracellular traps suggesting that neutropenia may be a defense mechanism of the host against the dengue virus in patients [21].

No significant difference was observed among various age groups with consideration to NLR (p = 0.23). However, a significant higher proportion of dengue patients demonstrated <2 in 0-20 age groups was observed by Afsar et al., (2020) [15].

A significantly higher proportion of the thrombocytopenic patients demonstrated NLR ≤2 (p = 0.001) in comparison to non – thrombocytopenic patients in the present study. Also, a significant relation was found between mild thrombocytopenia and NLR ≤2 as well as between normal platelet count and NLR >2 (p = 0.01). Afsar et al., (2020) [15] also found significant association between low platelet count and NLR <2 (p<0.05). However, Koundinya et al., (2021) [20] didn't observed any significant relation. Above finding suggests that NLR can be used as a prognostic indicator of dengue disease with NLR ≤2 indicating severity of the disease.

No significant relation was observed among serology result groups with NLR which correlates with Afsar et al., (2020) [15].

A higher level of NLR has been reported with higher level of inflammation suggesting its role to predict the inflammation severity as well as prognosis in the patients [22-24]. However, in the present study, in contrast, lower NLR were found in relation to lower platelet count i.e. more severity of the patients. It's probably because of the lymphocytosis and neutropenia seen in viral infections which were ameliorated with improvement of platelet counts.

The limitations of the present study were it was done in a small sample population and done in a single hospital. Also, NLR changes with platelet count in each patient during the course of the dengue infection was not studied. Study with more sample size representing bigger geographical region with serial changes in NLR with platelet counts in dengue patients is recommended.

4. Conclusion

This study concludes that NLR may be used as prognostic marker in management of dengue infection as NLR ≤2 significantly correlates with lower platelet counts indicating severity of the disease. NLR >2 is significantly observed in patients with normal platelet counts. Hence, NLR with cut off 2 may be used to prognosticate the disease and its therapeutic management.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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