

Neutrophil to Lymphocyte Ratio as the Predictor of Severity of Dengue Viral Fever: A Cross Sectional Study at a Tertiary Care Centre in Central Rajasthan

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Received: 30-07-2022 / Revised: 29-08-2022 / Accepted: 28-09-2022

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Conflict of interest: Nil

Abstract

Introduction: Dengue is a viral febrile illness, which present with a wide spectrum of disease including haemorrhagic disorders and shock. Neutrophil to lymphocyte ratio, proved to be a good predictor of stress and morbidity in various clinical scenarios. Our study aims at finding out the correlation between NLR and thrombocytopenia and complications in patients suffering from dengue viral fever.

Method: This is a prospective cross-sectional study at a tertiary care center. We included a total of 100 cases in this study with the history of fever of more than 38.5 degree Celsius and positive for NS1 antigen or Dengue IgM positive. All the patients were investigated with all routine investigations (CBC, NS1 antigens, IgM dengue, LFT, KFT) and followed from day 1 of admission till the time of recovery or discharge according to WHO discharge criteria whichever is earlier. Clinically patients are monitored, and CBC was repeated daily. For the purpose of this study the patients were categorised according to signs and occurrence of haemorrhage and shock into 3 groups I – without any haemorrhage, II – with haemorrhage and III – suffered with shock.

Results: Out of 100, 58 patients were males and 42 were females with male to female ratio of 1.4: 1. The mean age was 29.84±6.4 years. Maximum patients were in age group of 26 – 35 years. Most patients were diagnosed as Dengue Fever with 65% patients, 21% suffered from Dengue Haemorrhagic Fever (DHF) grade I, 15% patients suffered from DHF grade II, and 8% patients suffered from DHF grade III and IV. The mean platelet count of the study group was 140,000 (92000 to 3,60,000) on day 1 and 89540 (15000 – 306000) on day 7. The mean neutrophil count was 5531.2 (2700 to 8,200) on day 1 and 3416.8 (1800 – 7120). The mean values of lymphocyte were 48.9 (12 to 82) and 41.6 (10 to 80) respectively on day 1 and day 7. The mean N:L ratio of the study group was 2.12 (0.1 to 8.2) on day 1 and 0.95 (0.23 to 2.20) on day 7. Out of 100 patients, 26 patients had bleeding manifestations and in them 8 had signs of shock. NLR does not show statistically significant reduction with the reduction of platelet counts on day 7 of the patients. Statistically highly significant relation between occurrence of bleeding and shock with reduction in NLR in patients with dengue. (P <0.001).

Conclusion: In our study it is evident that there is haematological disturbance with the progression of dengue fever in the form of decrease in platelets and NLR from day 1 to day 7 of illness. NLR may prove to be a good predictor of occurrence of complications like

haemorrhage and shock however it's not significantly associated with decrease in platelet count.

Keywords: Dengue fever, bleeding tendency, NLR, thrombocytopenia

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Introduction

Dengue fever is an acute febrile illness presented with sudden onset of fever which may persist for 3 to 7 days, along with severe headache, myalgia, GI upset and in some cases retro-orbital pain and rash. Dengue viruses come under flavivirus, and include four serotypes 1, 2, 3 and 4 [1]. The emergence of these viruses is said to be from sylvatic strains in the forests of South-East Asia [2]. The dengue viruses are transmitted to man by the bite of infective female *Aedes aegypti* mosquito. The disease is endemic in many tropical and subtropical countries including Indian subcontinent.

Dengue virus can cause a spectrum of illness ranging from asymptomatic dengue infection to dengue fever (DF) to dengue haemorrhagic fever (DHF) to dengue shock syndrome (DSS) [3]. In 2008, a global expert consensus meeting at WHO accorded on a new classification of DF. According to the revised criteria, dengue was divided into two categories, non-severe and severe dengue (SDF); the non-severe dengue is further divided into dengue with warning signs (D+W) and dengue without warning signs (D-W). The new classification was developed based on the level of clinical severity [4].

The neutrophil to lymphocyte ratio or NLR has shown promising correlation with stress on body. Under physiologic stress, the number of neutrophils increases, while the number of lymphocytes decreases. NLR increases rapidly following acute physiologic stress (<6 hours).[5]. A normal NLR is roughly 1-3. NLR indicates the balance between innate and adaptive immune responses, and it is an excellent indicator of inflammation and stress together. The opposite dynamics in neutrophil and lymphocyte counts is a

multifactorial process depending on various immunologic, neuroendocrine, and humoral processes. The imbalance be it increase or decrease in NLR can be a useful predictor in diagnosis and establishing prognosis of various inflammatory diseases. This study aims at establishing the relation of NLR on severity of dengue.

Methods

This is a prospective cross-sectional study conducted at JLN Medical College and associated group of hospitals, Ajmer, Rajasthan. The patient enrolment was started on 1st Aug 2021 and continued till the sample size is achieved. We included a total of 100 cases in this study. Informed consent was taken from all the patients during the study. The required permission was taken from the ethics and research committee.

Study Design: It is a prospective cross-sectional study. A total of 100 patients admitted to the Medicine ward, with the history of fever of more than 38.5 degree Celsius and positive for NS1 antigen or Dengue IgM positive were selected using purposive sampling techniques. All the patients were investigated with all routine investigations (CBC, NS1 antigens, IgM dengue, LFT, KFT) and followed from day 1 of admission till the time of recovery or discharge according to WHO discharge criteria whichever is earlier.

Clinically patients are monitored and CBC was repeated daily. For the purpose of this study the patients were categorised according to signs and occurrence of haemorrhage and shock into 3 groups I – without any haemorrhage, II – with haemorrhage and III – suffered with shock.

Inclusion Criteria

1. All the patients admitted in JLM medical college and hospital having fever more than 38.5 °C.
2. NS1 or IgM dengue positive.

Exclusion Criteria

1. Age less than 18 years or more than 60 years.
2. Pre-existing chronic liver, kidney, or heart disease.
3. Patients with history of haematological disorders or deranged haematological profile.

Data Analysis

Data collected will be analysed by frequency, percentage, mean, standard deviation (S.D).

Results

In the present study, we included a total of 100 patients who had fulfilled the inclusion and exclusion criteria. Out of 100, 58 patients were males and 42 were females with male to female ratio of 1.4: 1.

The age ranged from 18 years to 40 years with mean age of 29.84±6.4 years. Maximum patients were in age group of 26 – 35 years. (Image 1). The NS1 antigen and dengue IgM positive status is shown in Table no. 1. According to the dengue severity grade, maximum patients were diagnosed as Dengue Fever with 65 patients (65%), 21 suffered from Dengue Haemorrhagic Fever (DHF) grade I, 15 patients (15%) suffered from DHF grade II, and 8 patients (8%) suffered from DHF grade III and IV.

The mean platelet count of the study group was 140,000 (92000 to 3,60,000) on day 1 and 89540 (15000 – 306000) on day 7. The mean neutrophil count was 5531.2 (2700 to 8,200) on day 1 and 3416.8 (1800 – 7120). The mean values of lymphocyte were 48.9 (12 to 82) and 41.6 (10 to 80) respectively on day 1 and day 7. The mean N:L ratio of the study group was 2.12 (0.1 to 8.2) on day 1 and 0.95 (0.23 to 2.20) on day 7. Out of 100 patients, 26 patients had bleeding manifestations and in them 8 had signs of shock.

Table 1: Status of NS1 antigen and dengue IgM antibody in study group

		Count	Percent
NS1 Ag Status	Negative	23	23
	Positive	77	77
IgM Antibody	Negative	70	70
	Positive	30	30

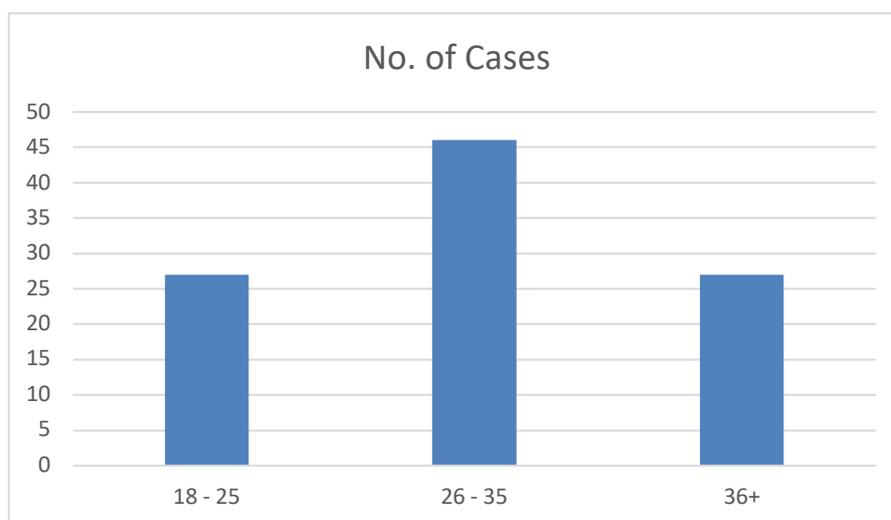


Figure 1: Distribution of cases in different age groups

Table 2 shows the relation between platelet count and NLR range on day 7 of admission of patients. The data shows that there is no statistically significant relation between the platelet count and the NLR ($P = 0.42$). This is evident with the fact that the NLR does not show significant reduction with the reduction of platelet counts on day 7 of the patients.

Table 2: Relation between platelet count and NLR in the study group

NLR Range	≤ 0.400	0.401 - 0.800	0.801 - 1.200	1.201 - 1.600	1.601 - 2.000	2.001+	Total
Platelet Count	No. of Cases						
≤ 20000	0	1	1	0	0	0	2
20001 - 50000	1	4	7	12	6	0	30
50001 - 100000	0	4	9	6	11	2	32
100001 - 200000	0	3	8	9	8	3	31
200001+	0	1	0	2	0	2	5
Total	1	13	25	29	25	7	100
Chi-squared = 23.56, DF = 20, $P = 0.42$ (NS)							

Table 3 show relation between NLR and number of patients with bleeding manifestations and patients with features of shock. It shows statistically highly significant relation between occurrence of bleeding and shock with reduction in NLR in patients with dengue. ($P < 0.001$)

Table 3

NLR Range	≤ 0.40	0.401 - 0.80	0.801 - 1.20	1.201 - 1.60	1.601 - 2.0	2.001+	Total
Dengue Fever	0	3	17	20	21	5	66
Haemorrhagic manifestations	0	4	7	9	4	2	26
Signs of Shock	1	6	1	0	0	0	8
	1	13	25	29	25	7	100
Chi Squared = 46.89, DF = 10, $P < 0.001$							

Discussion

Dengue viral fever is an important disease of global concern causing endemic outbreaks with significant mortality and morbidity in tropical countries [6]. Literature shows the prevalence of dengue at 3.9 billion people at risk of infection in 129 countries [7], and among the total exposed population, 70% of the actual burden is in Asia [8].

Dengue infection commonly causes leukopenia with total leucocyte count reduces with the severity of infection. Relative lymphocytosis with increasing atypical lymphocytosis is usually found in the febrile phase or in the recovery phase (WHO, 2011). The possible reason of neutropenia is apoptosis of neutrophils

caused by the virus infection as shown in the study of Galani et. al. 2015 [9].

In our study the male: female ratio was 1.4: 1 showing males are more affected by dengue. Similar results were reported by Vibha, et al. [10], Fu Xi Qui, et al. [11] and MA Koundinya et. al. [12] which also showed males affected more than females. This may be due to the fact that males are more involved in outdoor work.

In our study relatively younger patients were involved more as compared to older; this may be again since young generation stay outdoors more.

In our study thrombocytopenia (platelets $< 100,000$) was seen in majority of the patients on day 7 of the febrile illness.

Similar results are also published by Vibha, *et al.* [10], and MA Koundinya *et al.* [12].

In our study we found statistically significant correlation between reduction in NLR (neutropenia and relative lymphocytosis) and increasing severity of dengue viral fever and NLR correlates directly with the increased morbidity and mortality of infection. Irmayanti *et al* (2017) [13] in their study showed that, there was a significant relation between NLCR and dengue infection ($p < 0.05$).

The mean NLCR on DHF grade I is 2.19 and DHF grade II is 0.80 showing that lower the NLCR, more severe is the dengue infection, which is concordance with our study. Another cross-sectional study done by Kariadi *et al* [14] showed that there was a statistically significant difference between NLCR and DF and DHF.

MA Koundinya *et al.* [12] also reported that there was a significant relation between NLR and the severity of the disease as in their study, patients with NLR of less than 0.9 had bleeding manifestations except one patient and out of total 8 patients of shock 6 had NLR of less than 1. However few studies also reported a contrasting result. Nusa, Karla & Mantik [15] concluded that, there was no significant relation between NLCR and dengue severity grade ($p = 0.63$), however she used samples from 3rd and 4th day of fever whereas most other studies including our study analysed the findings on 6th and 7th day of illness.

In initial days of dengue infection there will be increase in neutrophil count making 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. There are few limitations of the current study, as in small sample population which may not

represent the entire population of dengue endemic area.

Conclusion

In our study it is evident that there is haematological disturbance with the progression of dengue fever in the form of decrease in platelets and NLR from day 1 to day 7 of illness. NLR may prove to be a good predictor of occurrence of complications like haemorrhage and shock however it's not significantly associated with decrease in platelet count.

References

1. Westaway EG, Brinton MA, Gaidamovich S, Horzinek MC, Igarashi A, Kaariainen L, *et al.* Flaviviridae. *Intervirology*. 1985; 24: 183–92.
2. Wang E, Ni H, Xu R, Barrett AD, Watowich SJ, Gubler DJ, *et al.* Evolutionary relationships of endemic/epidemic and sylvatic dengue viruses. *J Virol*. 2000; 74:3227–34.
3. World Health Organization. Dengue guidelines for diagnosis, treatment, prevention and control: new edition. Geneva PP - Geneva: World Health Organization; 2009. Available from: <https://apps.who.int/iris/handle/10665/44188>.
4. Ajlan BA, Alafif MM, Alawi MM, Akbar NA, Aldigs EK, Madani TA. Assessment of the new World Health Organization's dengue classification for predicting severity of illness and level of healthcare required. *PLoS Negl Trop Dis*. 2019 Aug 20; 13(8): e0007144.
5. Zahorec R. Ratio of neutrophil to lymphocyte counts—rapid and simple parameter of systemic inflammation and stress in critically ill. *Bratisl Lek Listy*. 2001;102(1):5-14.
6. Wilder-Smith A, Gubler DJ. Geographic expansion of dengue; the impact of international travel. *Med Clin North Am.*, 2008; 92(6): 1377-90.
7. Brady O.J., *et al.* Refining the global spatial limits of dengue virus

- transmission by evidence-based consensus. PLOS Neglected Tropical Diseases, 2012; 6(8): e1760.
8. Bhatt S., et al. The global distribution and burden of dengue. Nature, 2013; 496(7446): 504–507.
 9. Galani I. E., & Andreakos E. Neutrophils in viral infection: Current concepts and caveats. 2015.
 10. Vibha V. Gajera, Shilpi Sahu, Reeta Dhar. Study of Haematological pro c Group of e-Journals (PaGe). Annals of Applied Fever and its Clinical Implication. Paci Bio-Sciences, 2016; 3(3).
 11. Qui FX, Gubler DJ, Liu JC, Chen QQ. Dengue in China. A clinical review. Bull World Health Organ, 1993; 71(3/4): 349-59.
 12. 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.
 13. Irmayanti I., Nurulita A., & Sennang N. Rasio neutrophil/limfosit pada demam berdarah dengue. 2017.
 14. Dinas Kesehatan Kota Kediri. Data Infeksi Virus Dengue Kota Kediri Penyakit Demam Berdarah Tahun 2018.
 15. Nusa, Karla & Mantik, Max & Rampengan, Novie. Hubungan ratio neurtofil dan limfosit pada penderita penyakit infeksi virus dengue. E-clinic. 2015; 3. 10.35790/ecl.3.1.2015.6764.