

Utilization of Neutrophil to Lymphocyte Ratio to Assess Recovery in Patients with Dengue

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Abstract

Objectives: This study aims to assess recovery in patients with dengue by utilizing Neutrophil-to-Lymphocyte ratio (NLR) reversal i.e increase in atypical lymphocytes in peripheral blood, for early detection of transformation to DHF.

Methodology: A cross-sectional study was conducted from 26th October, 2021 to 26th November, 2021 by consecutive sampling of patients from Outdoor and Indoor of National Hospital, Lahore. Ethical approval was taken from Chughtai Institute of Pathology, Lahore before initiation of research involving 141 patients diagnosed with dengue fever. Quantitative variables like mean age, Hb, TLC, haematocrit, platelet count and degree of platelet fall were summarized as the appropriate means and standard deviations. The association NLR reversal with DHF was determined using Chi-square and Fisher's Exact test.

Results: There was no significant difference in mean Haemoglobin, Haematocrit and Total Leukocyte Count of Early-Reversal Group (14.2 ± 1.7), (43.1 ± 5.2), (3.8 ± 1.2) and of Late-Reversal group (14.7 ± 2.1), (45.1 ± 6.1), (4.5 ± 1.4). Mean of minimum platelet count observed in Early-Reversal Group was higher (49.2 ± 28.3) than Late-Reversal Group (19.3 ± 22.1) (p-value <0.05). Significant number of patients developed DHF in Late-Reversal Group (80.9%) than Early-Reversal Group (8.3%) (p-value < 0.05).

Conclusion: There is no significant difference in age, haemoglobin, haematocrit and total leukocyte count of DF and DHF patients. Serial NLR can predict the haemorrhagic complications in dengue fever patients. Severe thrombocytopenia occurs in patients with late reversal of NLR.

Keywords: Dengue fever, Dengue haemorrhagic fever, Neutrophils, Lymphocytes.

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Introduction

Dengue fever, also known as break bone fever, is a mosquito borne tropical disease caused by the mosquito bite, most commonly *Aedes Aegypti*. Its severity varies from dengue fever (DF) to dengue hemorrhagic fever (DHF) and ultimately dengue shock syndrome (DSS).¹ The mortality is 1-5% without treatment and less than 1% with adequate treatment. However, severe disease carries a mortality of around 26%.²

Since 2010, Pakistan has been experiencing dengue epidemics. In recent times, according to the National Institute of Health (NIH) Islamabad, 22,938 dengue fever cases were reported in 2017, more than 3,200 in 2018, 24,547 cases were reported in 2019 and 3,442 cases in

2020.³ In 2021, Pakistan is witness yet another epidemic for which the data regarding the total number of cases is unavailable at the moment.

Initially, the clinical presentation in majority of dengue patients is acute febrile illness without any localizing signs and symptoms.⁴ In the current era of COVID-19, it is a challenge to differentiate between these two diseases based on symptoms and history alone. Therefore, laboratory tests (e.g., CBC, serological tests, molecular tests etc.) are the mainstay modalities to confirm the diagnosis.⁵

Currently, dengue NS1 antigen and dengue IgM antibody are the only serological tests done for confirming the diagnosis of dengue infection.⁴ These serological tests are not available in underdeveloped areas or small local hospitals of Pakistan, and because of this, history, physical examination and CBC are the only dependable modalities. Keeping all this in mind, Pakistan is a developing country, and a vast majority of the population cannot afford these serological and molecular tests especially in the current COVID-19 pandemic.

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Neutrophil-to-lymphocyte ratio (NLR) is an established inflammation marker. Normal NLR values in an adult, non-geriatric, population in good health are between 1.0 and 3.5.⁶ It has been used to assess both infectious and non-infectious diseases such as cardiovascular diseases, inflammatory diseases and in several types of cancers.

The complete blood count parameters of dengue patients change almost every day after the onset of symptoms, specifically from Day 3 to Day 7.⁷ Progressive leukopenia is the earliest abnormality in dengue infection which is followed by an abrupt decline in platelet count and reversal of neutrophil-to-lymphocyte ratio (NLR) around Day 4-5 with the appearance of atypical lymphocytes.⁸ Thrombocytopenia persists even after the symptoms subside. Currently, there are no parameters to predict severity or recovery in patients with dengue before progression to DSS. Platelet count alone cannot predict whether the disease will progress to DHF or DSS or even whether the recovery phase has started or not.

Neutrophil to lymphocyte ratio has been linked to predict the severity of disease in COVID-19 patients and is being successfully used in the management of these patients.⁹ There is no such CBC parameter to predict severity in dengue patients. The aim of this study was to establish a relationship between serial NLRs and progression of patients to DHF or DSS. We hypothesized that late reversal i.e. after day 4-5 of illness can predict progression to DHF. An early predictive marker can help us to hospitalize these patients early and give timely treatment. Moreover, availability of such marker can aid the physicians in peripheral areas of third world countries because expensive specialized testing is not available there. Mostly the diagnosis of dengue in epidemics is made solely on the basis of progressive thrombocytopenia in febrile patients.

Methodology

A cross-sectional study was conducted from 26th October, 2021 to 26th November, 2021 by consecutive sampling of patients from Outdoor and Indoor of National Hospital, Lahore. Ethical approval was taken from Chughtai Institute of Pathology, Lahore before initiation of research.

All those patients who tested positive for dengue NS1 antigen and/or dengue IgM antibodies by Enzyme linked immunosorbent assay (ELISA) on Day-1 of fever were

included in the study.¹⁰ One hundred and forty one patients were selected on this basis and were followed for the development of DHF or DSS.

Serial complete blood counts (CBC) samples of these patients were analyzed from Day 1 to Day 8 of patient's illness at Chughtai Institute of Pathology by taking 2mL of blood in EDTA vials. These samples were run on Sysmex-XN9000 Automated haematology analyser (after proper quality control and calibration). The laboratory reference values for Total Leukocyte Count (TLC), Absolute Neutrophil Count (ANC) and Absolute Lymphocyte Count (ALC) were 4-11, 2-7 and 0.8-4 x 10³/μL. Neutrophil to lymphocyte ratio (NLR) of 1-3 was taken as normal and >3 as high and <1 as low. Peripheral blood smears of all these samples were also made and examined under microscope for the presence and percentage of "atypical lymphocytes". On the basis of reversal day of NLR, either within 4-5 days or after, these patients were grouped into "Group Early-Reversal" and "Group Late-Reversal" accordingly.

Patients who had previously documented TLC count less than 5.0 x 10³/μL, platelet count less than 150 x 10⁹/μL, other hematological disorder, chronic liver disease causing cytopenias, chronic kidney disease, patients with malignancy during chemotherapy, and patients who were receiving any immunosuppressive drugs were excluded from this study.

Quantitative variables like mean age, Hb, TLC, haematocrit, platelet count and degree of platelet fall were summarized as the appropriate means and standard deviations. Comparison of two groups i.e. Group Early and Group Late was made using independent sample t-test. Percentage of patients developing DHF were compared using Chi-square and Fisher's exact tests. P < 0.05 was recognized as statistically significant. All these statistical calculations were performed using the SPSS 20.0.

Results

The mean ages of early (Group A) and late (Group B) reversals were (40.5 ± 17.4) and (45.6 ± 21) (p-value > 0.05) respectively. There was no significant difference in mean Hb, HCT and TLC of Early Group (14.2 ± 1.7), (43.1 ± 5.2), (3.8 ± 1.2) and of Late group (14.7 ± 2.1), (45.1 ± 6.1), (4.5 ± 1.4) (p-value > 0.05).

Mean of minimum platelet count observed in Early group was higher (49.2 ± 28.3) than Late Group (19.3 ± 22.1) (p -value < 0.05). Significant number of patients developed DHF in Late Group (80.9%) than Early Group (8.3%) (p -value < 0.05)

P values comparing means of both groups are from Independent sample t-test. P values comparing Early and Late Reversal groups are from Chi-square (indicated by *) and Fisher's exact test. (Table I)

Table I: Demographic and Laboratory characteristics of both groups

	Early-Reversal (Group A) (n=120)	Late-Reversal (Group B) (n=21)	P value
Mean age	40.5 ± 17.4	45.6 ± 21	0.307
Mean Haemoglobin	14.2 ± 1.7	14.7 ± 2.1	0.278
Mean Haematocrit	43.1 ± 5.2	45.1 ± 6.1	0.158
Mean Total Leukocyte Count	3.8 ± 1.2	4.5 ± 1.4	0.036
Mean Minimum Platelet Count	49.2 ± 28.3	19.3 ± 22.1	0.00
Progression to DHF	10 (8.3%)	17 (80.9%)	0.00*
Day 1 N/L Ratio	7.37	4.94	0.505
Day 2 N/L Ratio	3.63	3.65	0.98
Day 3 N/L Ratio	1.82	2.58	0.51
Day 4 N/L Ratio	0.83	2.31	0.01
Day 5 N/L Ratio	0.73	1.76	0.00
Day 6 N/L Ratio	0.88	1.41	0.01
Day 7 N/L Ratio	1.16	1.17	0.965

Discussion

Dengue Haemorrhagic fever caused by dengue virus infection remains a health problem in Pakistan. Every year thousands of people are affected by it and some of them die as a result of the haemorrhagic complications. A previous study published in Indonesia, a dengue infection prevalent country, showed that there is no significant association of age, haemoglobin, haematocrit and total leukocyte count between patients of Dengue Fever and Dengue Haemorrhagic Fever.¹¹ Our study showed similar results. There was no marked difference in these parameters in both our groups. This shows that these parameters cannot predict the severity or the progression of dengue fever to haemorrhagic fever in infected patients.

Dengue infection presents as a febrile illness like any other bacterial, viral or arthropod borne infection like malaria. Without specialized testing it is difficult to differentiate it from these infections in the early febrile

phase. Neutrophil/lymphocyte ratio is useful in differentiating between these infectious diseases.¹² As previously studied this ratio follows a specific pattern with increased neutrophils in the early stage and predominant atypical lymphocytes in the later stage.⁷ The average time of this reversal of NLR previously determined was from Day 6-9.⁸ In this study, we found that in 85% of the patients this change in NLR pattern was more commonly seen between Days 3-5. This reversal of NLR marks the end of febrile phase of dengue infection. The platelet count decreased a day or two even after the febrile phase ended. A significant finding was that these patients recovered without any haemorrhagic complications. In comparison, the patients in other group whose NLR reversed after Day 5, 81% of them developed dengue haemorrhagic fever and required longer stay at the hospital. Mean minimum platelet count was seen to be significantly lower in this group also explaining the progression to dengue shock syndrome.¹³

In the recent era with a pandemic like COVID-19 it is difficult to establish a diagnosis of dengue fever without the expensive specialized testing. NLR has been used successfully as an indicator of severity in COVID19 patients.¹⁴ This study shows it can also predict severity in dengue patients by its pattern and at the same time help physicians in the periphery to differentiate between the two illnesses. Serial CBCs of dengue patients are already being done in Pakistan to monitor the disease progression. In addition NLR should be added to Dengue CBC to assess recovery in these patients. It can help in the early hospitalization, proper management and reduce mortality rate in these patients.

Conclusion

There is no significant difference in age, haemoglobin, haematocrit and total leukocyte count of DF and DHF patients. Serial NLRs can predict the haemorrhagic complications in dengue fever patients. Severe thrombocytopenia occurs in patients with late reversal of NLR.

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