ORIGINAL ARTICLE

Correlation of Total Leucocyte Count, Neutrophil to Lymphocyte Ratio and Platelet Count with Duration of Hospital Stay in Dengue Fever

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ABSTRACT

Objective: To assess the correlation of total leucocyte count (TLC), neutrophil to lymphocyte ratio (NLR), and platelet count with the duration of hospital stay in dengue patients.

Study Design: A cross-sectional study.

Place and Duration of Study: The study was conducted at the Department of Haematology and Dengue Ward, Pak Emirates Military Hospital (PEMH) Rawalpindi, Pakistan from January 2023 to February 2023.

Methods: Comprised 136 confirmed dengue-positive patients who were admitted to PEMH with symptoms of fever. All the participants were requested for their informed consent before their enrolment in the study. A 3 ml venous sample was taken, and complete blood counts were determined by Sysmex XN-3000.TLC, Hematocrit, platelet count, and differentials were measured while Neutrophil the neutrophil-to-lymphocyte ratio was calculated. Patients were followed in hospital and their total hospital stay was noted. Correlation of total leucocyte count, neutrophil to lymphocyte ratio, and platelet count in dengue patients with duration of hospital stay was noted.

Results: TLC and the neutrophil-to-lymphocyte ratio did not show any significant correlation with hospital stay in dengue patients. However, platelet count was found to be negatively correlated with hospital stay duration (r = -0.864, *P* = 0.00).

Conclusion: Platelet count has a significant negative correlation with hospital stay in dengue patients and is found to be a good predicting factor for indoor stay of dengue patients.

Keywords: *Dengue, Leucocyte Count, Platelets*.

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Introduction

Dengue fever is a deadly disease that occurs due to a single strand of RNA virus, and its human transmission is through mosquitos, Aedes aegypti, but also by Aedes albopictus.[1] According to an estimation, approximately 100 million cases of dengue are occurring worldwide, with more than 10,000 death tolls reported in around 125 different countries around the globe annually.[2] The tropical and sub-tropical species of Aedes Aegypti are widely distributed. According to a report by WHO, a total of 25,932 cases of dengue fever were diagnosed between Jan and Sept 2022 in Pakistan and the number of reported deaths this time was around 65.[3]

The Flaviviridae family of viruses comprises four

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different serotypes (DENV1, 2, 3, and 4), which cause dengue fever. Each one of the serotypes is responsible for disease ranging from mild disease (fever) to more severe symptoms caused by dengue shock syndrome and dengue hemorrhagic fever (DHF). Dengue shock syndrome occurs because of plasma leakage as a result of endothelial cell injury and endothelial activation which is a functional disorder.[4] Apart from the plasma leakage, the endothelial dysfunction may also be caused by the activation of T-cells, monocytes, complement system, and different inflammatory mediators, which include cytokines, monokines, and soluble receptors. Thrombocytopenia is caused not only due to peripheral destruction but also due to dysmegakaryopoiesis. As a result, patients may present with bleeding which is associated with platelet function defects and disseminated intravascular coagulation (DIC). The disease progression involves three phases, starting with the febrile phase, followed by the critical phase, followed by the critical phase, and, in the absence of complications, the recovery phase.[5]

Monsoon season is notorious for dengue outbreaks in Pakistan. Non-structural protein-1 (NS-1) and IgM are the most common tests done for diagnosing dengue. NS-1 is the most sensitive and specific test in the first 3 days of the disease while IgM is not as clinically useful as the NS-1 because the antibodies tend to appear later on during the progression of the disease.[6] Therefore most physicians rely on clinical diagnosis by monitoring the hematological parameters. Commonly used parameters are hematocrit, (TLC) total leucocyte count and platelet count.

Blood counts vary as the course of illness progresses from initial dengue fever leading to dengue hemorrhagic fever (DHF) and finally to the most complicated dengue shock syndrome (DSS). Leukopenia, thrombocytopenia, and raised hematocrit (as evidence determining plasma leakage) are commonly observed parameters in dengue fever.[7]

Complete blood counts have been documented to play a significant role not only in early diagnosis but also in prognostic significance. The common hematological findings observed in dengue fever are leucopenia and low platelet counts but neutropenia is also widely reported.

Methods

The cross-sectional study was planned and conducted at the Department of Haematology and Dengue Ward, Pak Emirates Military Hospital (PEMH) Rawalpindi, Pakistan from January 2023 to February 2023. The study was initiated after getting approval from the Ethical Committee of the Army Medical College Rawalpindi, Pakistan held on dated: 13th January 2023 vide letter no: ERC/ID/258. The total sample size of this study was calculated to be 113 using the WHO calculator, taking a 95% confidence level and expected cases of dengue thrombocytopenia in 8 % of cases.[8] The data of all the dengue patients who were admitted to the hospital during above-mentioned duration was analyzed.

The study encompasses all patients of both genders ages above 12 years with clinical features of dengue, which were confirmed by NS-1 positivity.

All those patients having cytopenias due to causes other than Dengue fever e.g infections, sepsis and also patients on drugs affecting the blood counts were excluded. Patients with suspected dengue but not confirmed on NS-1 were also excluded from the study.

3ml of blood sample was taken from the Dengue positive (NS-1 antigen) patients after taking their consent. The total leucocyte count, platelet count, and all the differential counts were analyzed by Sysmex XN 3000. Neutrophil to lymphocyte ratio was calculated. Data was entered and saved in HMS (hospital management system). The Data is now used with the permission of the head of the department of the Hematology Department, Army Medical College Lab in Pak Emirates Military Hospital.

The following definitions were used while interpreting the data:

Anemia: Hb is < 10 g/l in females and Hb < 12 g/l

in males.[9]
Raised hematocrit: > 0.49 (males) and > 0.48 (females)[9]
Leucocytosis: TLC $> 11 \times 10^9/L^9$
Leucopenia: TLC $< 4 \times 10^9/L^9$
Thrombocytopenia: Plt $< 100 \times 10^9/L^9$
Significant Neutrophil/Lymphocyte ratio (NLR): > 3.0 and < 1[10]
The information was entered and interpreted using Statistical Package for Social Science (SPSS) version 22. Outcomes were framed as frequencies. Normality of data was estimated by Shapiro-Wilk test. The correlation of TLC, NLR, and platelet count with a duration of hospital stay was assessed by the Spearman correlation coefficient. A *P*-value of less than 0.05 was taken as significant.

Results

Data from 136 patients with NS-1 confirmed cases admitted in PEMH from January 2023 to February 2023 was used in the study. Estimation by the Shapiro-Wilk test showed the non-parametric distribution of data. Out of 136 patients, 105 were male, and 31 were females. The median age of the population was 35 years, with IQR of 24. Ages of patients ranged between 11 to 75 years. The median day of illness (since the onset of fever) on admission was 4 days. The median length of stay (LOS) in the hospital was 5.1 (3).The mean total leucocyte count was

Table-1: Clinical and demographic data of dengue patients

Characteristics		Frequency (n)=136	Percentage %
Gender	Male	105	77.2
	Female	31	22.8
Median age (yrs)		35.1	
Symptoms	Fever	136	100
	Headache	105	77.2
	Arthralgias	122	89.7
	Myalgias	99	72.8
	Retro-orbital pain	49	36
	Bleeding	10	0.13

Table-2: Complete blood count findings during the hospital stay (n=136)

Findings	No of patients (n)	Percentage	Median (IQR)
Anemia	2	1.5	13.9 (2.3)
Raised hematocrit	21	15.4	0.41 (0.06)
Leucocytosis	4	3	3.8 (2.3)
Leucopenia	70	51.9	3.8 (2.3)
Neutropenia	54	39.7	48 (29)
Decreased NLR	56	41	1.06 (1.58)
Thrombocytopenia	85	63	80 (60)

Table-3: Relationship of hospital stay with total leucocyte count, neutrophil to lymphocyte ratio and platelets

	Spearman correlation Coefficient	
Variable	**(r)**	***P*-value**
TLC	- 0.072	0.46
NLR	- 0.071	0.414
Platelet count	- 0.864	0.000

found to be 3.8 x 10^9/l. The average hospital stay was 5.1 days. The relationship between total leucocyte count and hospital stay duration was studied. There was no correlation found between the two parameters.

Correlation of NLR and hospital stay:

The median NLR was 1.06. The relation between NLR and number of hospital days was studied, which was also not correlated.

Correlation of platelet count, and duration of indoor hospital stay:

A *P*-value of 0.00 showed a significant correlation between platelet count and duration of indoor hospital stay. The number of days of hospital stay increased as the platelet count decreased.

Discussion

Dengue fever has a highly diverse disease pattern and outcome. The purpose of our study was to determine the parameters which can be used to predict disease course in future. Since the research was not conducted during the dengue season, a sample size of 136 patients was obtained.

We noted that most patients had a history of fever, with the vast majority presenting with headache, arthralgias, and myalgias; however, bleeding was less significant. (Table-1). Non-specific symptoms like severe body aches were reported less from Bangladesh in 2018 and from New Guinea in 2016.[11-13] While a study among children from Indonesia and Bangladesh showed not only increased incidence of myalgias and backache but bleeding was also quite significant in around one-quarter of the children.[14,15] This could be due to the fact that immunity against dengue increases with increasing age.

In our study, out of 136 patients, male to female ratio was 3:1. According to many researchers, males are working in outside environment, so they are more prone to mosquito bites and thus dengue and malaria. This correlates with a study by Quintero et al. conducted in Colombia and Yashaswini in India, both of which revealed more males infected with dengue as compared to females, i.e in the ratio of 2:1.[16,17] However, another research conducted in India in 2016 revealed the reversed male to female ratio. It was 1: 1.1. The research even implied to maintain a higher index of disease severity in female patients as compared to males.[18]

In this study, we aim to provide a list of CBC parameters, which will help us to determine which patients need more care than others in terms of prognosis. Leucopenia in dengue fever could be as a result of virus induced cell destruction or caused due to decreased and suppressed production of the myeloid progenitors in bone marrow.[19] According to our study results, 51.9% of patients revealed leukopenia. (Table-2). This finding was comparable to a similar study performed by Cardinas et al. in which leukopenia was observed in 65 % of dengue patients.[20]

The neutrophil to lymphocyte ratio (NLR) rises during the initial days of dengue fever, followed by a drop due to increased lymphocyte count. Low NLR was seen in 76 % of the study group in a study by Martenez with mean of 2.12 (0.1 to 8.2) while 41% of our patients had low NLR with mean of 2.2.[21] Mean neutrophil percentage and lymphocyte percentage was 49.1 (13-90) and 42.9 (2-75) respectively which significantly correlates with mean values of neutrophil and lymphocyte percentages in a study by Koundinya Modampuri et al in which mean neutrophil and lymphocyte percentages were 48.9 (12 to 82) and 41.6 (10 to 80) respectively.[22]

39.7 % of patients in our study showed neutropenia. (Table-2). In another study by Thein et al. neutropenia was reported to be quite more than our results. In his research, 82.2% of patients were neutropenic; however, it was not found to be associated with either bacterial infections, prolonged hospital admission, or poor outcome.[23]

Thrombocytopenia is an important finding in dengue and occurs due to the peripheral destruction of platelets. In our study, thrombocytopenia was observed in 63 % of the patients, and the mean platelet count was 87 x 10^9/l. A similar study was conducted by Ojha et al. in 2017 according to which thrombocy-

topenia was observed in 79 % of cases with a mean platelet count of 78 x 10^9/l.[24] However, no correlation was noted between platelet count and NLR.

The correlation was also studied between TLC, NLR, platelet count, and hospital stay. No significant correlation was found between TLC, NLR, and hospital stay; however, a negative correlation was found between platelet count (Table-3) and number of hospital days, which is proved by the fact that a decrease in a number of platelets leads to an increase in the duration of hospital stay. Similar are the results in a study conducted in Srilanka, which also shows a negative correlation between hospital stay days and platelet count, therefore it can be taken as an important parameter to predict the prognosis and duration of indoor hospital stay in dengue patients.[25]

The most significant limitation of our study was that we were unable to follow the hematological parameters of the patients, which would help us more in studying the development of disease progression or improvement. In addition, this study lacks the medical record of patients, which may have an impact on the parameters determined for prognosis. Further research is needed to validate whether the proposed indicators can differentiate between different stages of disease severity in dengue and other viral illnesses.

Conclusion

In dengue fever, platelet count is found to be an important prognostic marker to predict the number of indoor hospital days as it is found to have a negative correlation with it. Although leukopenia is seen in a significant number of patients no correlation was determined between leucocytes and NLR with hospital stay.

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Authors Contribution

ZS: Idea conception, data collection, manuscript writing and proofreading

ST: Study designing

SS: Data collection

AB: Data analysis, results and interpretation

RI: Study designing

UN: Manuscript writing and proofreading