A Comparative Study of Red Blood Cell Parameters in Patients of Dengue Fever with Thrombocytopenia in a Tertiary Care Hospital

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ABSTRACT

Objective: To assess the significance of red blood cell parameters, including Haemoglobin, Haematocrit, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, Mean Corpuscular Haemoglobin Concentration, and Red Cell Distribution Width in patients of Dengue fever with thrombocytopenia.

Study Design: Cross-sectional study.

Place and Duration of Study: Pathology Department of Army Medical College, and Pak Emirates Military Hospital, Rawalpindi Pakistan, from Nov to Dec 2022.

Methodology: Two hundred Dengue-positive serology patients were admitted and included in this study via non-probability consecutive sampling. Informed consent was taken from the patients, and a complete blood count was performed by a semi-automated haematology analyser, XP-100. Dengue fever patients were segregated into four Groups according to their platelet counts, and results were noted.

Results: Out of 200 patients, 177(88.5%) of Dengue fever patients had thrombocytopenia. The analysis found a significant relationship between Haemoglobin, Mean Corpuscular Volume, and Haematocrit and platelet Groups 2 and 3 (*p*-values= 0.029, 0.025, and 0.008, respectively). Also, a significant association was found between Mean Corpuscular Volume and Mean Corpuscular Haemoglobin Concentration and Groups 1 and 3 (*p*-values =0.006 and 0.021 respectively).

Conclusion: Red cell parameters, including Haemoglobin, Haematocrit, Mean Corpuscular Volume and, Mean Corpuscular Haemoglobin Concentration, can be considered early predictors of developing vascular leakage and dengue fever complications and showed significant association with the degree of thrombocytopenia.

Key Words: Dengue fever; Red blood cell parameters; Thrombocytopenia.

INTRODUCTION

Dengue is a common mosquito-borne infection in Tropical countries, especially during and after monsoon season, due to significant growth of mosquito larval populations. Previously, it was considered sporadic; however, it has been occurring as an epidemic, and now it is endemic in Southeast Asian countries.[1] World Health Organization (WHO) reported that Dengue fever is endemic in 128 countries, and approximately 390 million people are infected by Dengue each year.[2] This upsurge has increased the hospital burden tremendously, rendering third-world countries under pressure due to limited resources. It is estimated that 70% Dengue disease global burden strikes Asian countries.[3,4]

Dengue infection usually causes mild symptoms, including high-grade fever, headache, retrobulbar pain, and myalgias, called classical dengue fever.[5]

However, in a few cases, it may progress from mild to severe illness with haemorrhagic manifestations and shock called Dengue haemorrhagic fever (DHF) and Dengue shock syndrome (DSS), respectively. These clinical manifestations have a high mortality rate. The main target of Dengue fever is a haematological system that leads to haematological derangements, including thrombocytopenia, leukopenia, and increased haematocrit levels. Various factors play a role in the disease pathogenesis, and one of the leading factors is IL-1b. It is released from platelets during disease advancement, leading to increased vascular permeability, plasma leakage, lowering of platelet numbers and increasing haematocrit with the worsening of disease.[6]

Complete blood counts are used worldwide because of their easy availability and proven value in Dengue disease progression and monitoring.[7,8] Dengue is endemic in our country, and during monsoon season, there is a tremendous increase in hospital workload. Being an under-resourced country with financial constraints, simple red cell parameters

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acting as an early predictor of disease progression will support our system's financial limitations by decreasing hospital stays with early evaluation and timely management of patients. This study has assessed the association and significance of red blood cell parameters, including Haemoglobin (Hb), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and Red cell Distribution Width (RDW) in patients of Dengue fever with thrombocytopenia and also emphasised the role of red cell parameters acting as early marker for patients timely management to halt the disease progression in comparison with platelet count.

METHODOLOGY

The cross-sectional study was conducted at the Pathology Department of Army Medical College and Pak Emirates Military Hospital (PEMH), Rawalpindi Pakistan, from November to December 2022 during the Dengue outbreak season, after approval of ERC (ERC/ID/240). The sample size was calculated using the OpenEpi sample size calculator with the prevalence of raised Hct of 16% in Dengue patients.[9]

Inclusion Criteria: Dengue fever-positive patients of either gender, confirmed on serology by either Dengue non-structural protein-1(Dengue NS-1) or Dengue Ig M antibodies, were included.

Exclusion Criteria: Patients with concomitant Malaria along with Dengue fever, COVID-19, Typhoid, fever of unknown origin, and paediatric population (<12 years) were excluded.

Two hundred patients who were confirmed cases of Dengue fever and admitted to PEMH were included in this study via non-probability consecutive sampling. These patients reported at Medical OPD, PEMH, Rawalpindi, complaining of fever, headaches and body aches. They were admitted to the Dengue isolation ward after confirmation by Dengue serology for isolation and management. Informed consent was obtained from all Dengue patients included in the study at the time of admission. 3ml venous blood was collected from these patients on their first visit in an Ethylene Diamine Tetra Acetic acid container and mixed gently to ensure complete anticoagulation. A complete blood count was performed by a semi-automated haematology analyser XP-100, and all test parameters included in our study, along with demographic variables, including age and gender, were collected. Peripheral smears were made of all patients with thrombocytopenia to verify the platelet count manually and excluding platelet clumps and micro clots.

Statistical Package for Social Sciences (SPSS) version 25.0 was used for the data analysis. Quantitative variables with normal distribution were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages. One-way analysis of variance (ANOVA) was applied to gauge the mean differences among the Groups. The Group differences were calculated using Post Hoc test (Tukey HSD). The *p*-value of 0.05 or less was taken as significant.

RESULTS

We analysed red cell parameters of 200 Dengue-positive patients who presented with fever from November to December 2022. The average age of Dengue-positive patients observed was 41.76±13.83 years, with a minimum age of 14 years and a maximum age of 86 years. Male gender 191(95.5%) was predominant compared to females 9(4.5%) in the study Group.

Patients were segregated into four Groups according to their platelet counts, which was an independent variable, i.e., Group-1 (Severe thrombo-cytopenia): <50 x 10^9/l, Group-2 (Moderate thrombo-cytopenia): 51-100 x 10^9/l, Group-3 (Mild thrombo-cytopenia): 101-150 x 10^9/l and Group-4 (Normal): >150 x 109/l.[9] Out of these 200 patients, 65(32.5%) were in Group-1, 81(40.5%) were in Group-2, 31(15.5%) were in Group-3 and 23(11.5%) were in Group-4. Our analysis of the study population showed that more than three-quarters of the patients 177(88.5%) had thrombocytopenia (platelet count <150x10^9/l). Mean values of red cell parameters along with Standard Deviation (SD), which were dependent variables including TRBC, Hb, HCT, MCV, MCH, MCHC and RDW, were calculated in these four platelet Groups and endorsed in Table-I. ANOVA was applied to assess the presence of significant difference between the red cell parameters of these four platelet Groups. Significant p-value was obtained with Hb (*p*-value=0.026), HCT (*p*-value=0.004), MCV (*p*-value =0.012) and MCHC (*p*-value=0.023) between these platelet Groups. Table-II describes a Post-hoc analysis between the platelet Groups to further identify the Groups with significant *p*-values. Our statistical analysis revealed a significant association between Hb, MCV and HCT of Group-2 and Group-3 with *p*-values of 0.029, 0.025 and 0.008, respectively.

Table-I: Red Cell Parameters in Platelet Groups of Dengue Fever (n=200)

Red cell parameters	Group-1	Group-2	Group-3	Group-4	p-value
TRBC(1012/l)	4.91±0.70	5.12±0.65	4.94±0.46	5.01±0.54	0.227
Hb (g/dl)	14.3±1.8	14.8±1.3	13.9±1.0	14.2±1.04	0.026
MCV (fl)	86.0±5.3	85.1±8.5	80.8±7.4	82.2±5.4	0.012
MCH (pg)	29.2±1.85	29.1±2.85	28.3±2.19	28.5±1.96	0.226
MCHC (g/dl)	34.1±1.57	34.2±1.28	35.2±2.9	34.7±2.54	0.023
HCT (l/l)	0.42±0.053	0.43±0.044	0.39±0.047	0.41±0.044	0.004
RDW-SD(fl)	42.7±7.28	43.3±5.18	40.5±4.01	41.6±3.23	0.123
RDW-CV(%)	13.3±1.78	13.3±1.26	12.9±1.04	13.3±1.0	0.623

TRBC = Total Red Blood Cell, Hb = Haemoglobin, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, HCT = Haematocrit, RDW = Red Cell Distribution Width, SD = Standard Deviation, CV = Coefficient of Variation

Table-II: Intergroup Comparison of Platelet Groups with Red Cell Parameters in Dengue Fever (n=200)

Platelet Groups (109/l)	Intergroup Comparison	TRBC	Hb	MCV	MCH	MCHC	HCT	RDW(SD)	RDW(CV)
						p-value			
Group-1	Group-2	0.203	0.215	0.883	0.987	0.934	0.500	0.922	0.995
	Group-3	0.997	0.611	0.006	0.292	0.021	0.167	0.313	0.594
	Group-4	0.910	0.992	0.128	0.551	0.446	0.937	0.861	1.000
Group-2	Group-3	0.531	0.029	0.025	0.401	0.058	0.008	0.106	0.684
	Group-4	0.895	0.346	0.308	0.677	0.683	0.389	0.595	0.998
Group-3	Group-4	0.973	0.889	0.901	0.995	0.757	0.637	0.906	0.752

TRBC = Total Red Blood Cell, Hb = Haemoglobin, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, HCT = Haematocrit, RDW = Red Cell Distribution Width, SD = Standard Deviation, CV = Coefficient of Variation

Enrolled dengue patients belong to a wide age range with min. 14 years and max. 86 years. None of these patients develop any bleeding, vascular leakage or haemorrhagic complications. Most patients presented with fever, headache, myalgias and non-specific symptoms. In our hospital, patients were monitored by their HCT levels and fluid accumulation in body spaces (pleural and peritoneal) by ultrasonography and fluid management was done accordingly.[16] We analysed these patients by segregating them into four Groups according to their platelet count. Our analysis revealed that 88.5% of Dengue fever patients had

DISCUSSION

Dengue fever significantly impacts underdeveloped countries due to lack of resources and increased disease burden.[10] The disease is transmitted quite easily by mosquitoes biting multiple people in close vicinity, so preventive measures are of great importance in hinderance of Dengue transmission.[11] Dengue fever has a variable disease course. It can progress into DHF and DSS, usually managed according to the platelet count and HCT level.[12] The typical haematological derangements in dengue fever are haemoconcentration, leucopenia and thrombocytopenia.[13,14] Patients of Dengue fever should be evaluated thoroughly to cease the progression of mild to severe life-threatening conditions with high fatality. One of the leading causes of mortality is due to late diagnosis and detection of worsening blood parameters. These haematological parameters are helpful in the early prediction of disease progression and monitoring.[15] This study aimed to assess the correlation between Dengue fever positive patient's degree of thrombocytopenia with red blood cell parameters.

Two hundred Dengue fever patients were included in our study, and the majority, i.e. 191(95.5%) of patients, were male due to more males' outside movements for personal and professional chores.

thrombocytopenia, i.e., their platelet count was <150 x 10^9/l and 73% had a platelet count <100 x 10^9/l.

Furthermore, a comparative analysis of platelet Groups with red cell parameters was done, and it was observed that patients in Group-2, followed by Group-1, showed significant findings in red cell parameters, including raised Hb, MCV and HCT and decreased MCHC value. Groups 3 and 4 were observed to have no significant association with any red cell parameters. It is also a significant finding that patients with moderate thrombocytopenia, i.e., platelet count between 51-100 x 10^9/l, are at increased risk of developing increased vascular permeability, like in patients with platelet count <50 x 10^9/l. So, starting fluid management and resuscitative measures is immensely important once the platelet count drops below 100 x 10^9/l. Raised Hb and HCT are well-known outcomes observed in patients with clinically severe Dengue fever leading to DHF and DSS.[17]

Based on the research, it was found that HCT, Hb and MCV showed a significant relationship with vascular leakage in dengue fever patients with moderate to severe thrombocytopenia.[18] HCT and Hb levels are early predictors of disease progression compared to platelet count, as red cell parameters showed a significant increase in patients with moderate thrombocytopenia.

Haemoconcentration is an early predictor and sensitive marker of plasma leakage compared with platelet count. Therefore, periodic HCT and Hb measurements and monitoring of patients with HCT and Hb level changes are necessary. Our study will help physicians make early decisions about fluid management and will also help reduce morbidity and mortality in dengue fever patients.

LIMITATION OF STUDY

The limitations of this study was the predominance of the male gender.

CONCLUSION

There is a significant relationship between the degree of thrombocytopenia and red cell parameters, including Hb, HCT, MCV and MCHC. Mean Hb, MCV and HCT levels are significantly raised in Group-2, followed by Group-1, and this guides clinicians that patients of Dengue fever with moderate thrombocytopenia (platelet count <100 x 10⁹/l) can develop plasma vascular leakage leading to haemoconcentration and need fluid support to prevent complications. Therefore, Hb and HCT can be considered early predictors of the development of plasma leakage and complications in Dengue fever patients.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

ST & SS: Data acquisition, data analysis, drafting the manuscript, critical review, approval of the final version to be published.

JZ & SB: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

AE & IAM: Conception, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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