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ORIGINAL ARTICLE

Relationship of Hematological Indices with the Outcome of Dengue Infection

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ABSTRACT

Background: Dengue infection poses a significant global health challenge, necessitating a comprehensive understanding of its hematological dynamics for effective clinical management. This study aimed to investigate the hematological parameters of dengue-infected patients, emphasizing platelet dynamics and their correlation with disease severity.
Methodology: This longitudinal study, conducted at Ayub Teaching Hospital, spanned August 2022 to February 2023. Employing consecutive sampling, 146 dengue patients were enrolled after obtaining approval from the ASRB/IRB IPDM KMU. Patients of all ages and both genders were included, except those with known ITP, hematologic malignancy, or any chronic disease. Data collection encompassed demographic characteristics and clinical symptoms, gathered through a proforma after obtaining informed consent. Hematological parameters were meticulously assessed at defined intervals Data analysis was done using SPSS V.25 and Graph-Pad Prism software. A p value ≤ 0.05 was deemed statistically significant.
Results: The mean age of participants was 38.58 ± 18.53 years. Thrombocytopenia classification revealed 50% mild, 32.9% moderate, and 17.1% severe cases at diagnosis, with 76.02% achieving normal platelet counts by the 10th day. Platelet counts and indices exhibited temporal normalization significantly quickly in a non-hospitalized group of patients, signifying recovery. Crucially, the platelet count at diagnosis was found to be a robust predictor for hospitalization, with lower counts correlating significantly with an increased likelihood of hospital admission on multivariate logistic regression [Exp(B) = 0.964, p<0.001].
Conclusions: We emphasize the predictive power of the platelet count at diagnosis for dengue severity. Trends, like the rapid normalization of platelet morphology and indices in non-hospitalized patients, provide valuable insights for clinicians, guiding more effective clinical decisions.
Keywords: Dengue Virus, Dengue fever, Epidemiology, Disease severity, Clinical Outcomes

INTRODUCTION

The dengue virus affects over 400 million individuals globally, with a significant impact on tropical and subtropical regions[1]. Inefficient vector control has led to the virus's endemicity in more than a hundred nations, causing 50 to 100 million illnesses and over 20,000 deaths annually[2]. The virus, belonging to the Flaviviridae family, is transmitted by Aedesaegypti or Aedesalbopictus mosquitoes, with four distinct serotypes and varying clinical manifestations[3].

Aedes mosquitoes, biting during the day, serve as primary vectors [4]. Dengue symptoms range from mild flu-like manifestations to severe hemorrhagic fever or fatal shock syndrome, with a variety of clinical features [5]. Platelet count is crucial in assessing dengue severity, displaying an inverse correlation with disease progression[6-8].

Thrombocytopenia is a key prognostic indicator for severe manifestations like Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS)[9].

Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) are notable in dengue. Early infection stages show increased MPV, possibly indicating heightened platelet production. PDW, reflecting platelet activation, is also reported to be higher in severe cases, indicating extensive platelet destruction[10].

Abnormal platelet morphology, increased PDW, and higher MPV levels are associated with progression to DHF and DSS, providing diagnostic and prognostic significance [11].

Through this research, we endeavor to comprehensively observe the trajectory of the disease and analyze diverse hematological indices in individuals diagnosed with dengue fever. Additionally, we seek to compare and contrast hematological parameters between the hospitalized patients and those treated as outpatients. A key focus is placed on assessing the risk factors that contribute to the progression of severe dengue, prompting the need for hospitalization.

By addressing these objectives, we aims to provide valuable insights into the clinical dynamics of dengue infection, facilitating more accurate prognoses and tailored treatment strategies for improved patient outcomes.

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METHODOLOGY

This longitudinal cross-sectional study spanned from August 2022 to February 2023 and received the necessary approvals from the Advance Study and Research Board (ASRB) and Institutional Review Board. The settings for the study included the Institute of Pathology and Diagnostic Medicine (IPDM) at Khyber Medical University (KMU), Peshawar and Ayub Teaching Hospital Abbottabad. The sample size for this study was determined to be 146 patients, taking into account that 5% of dengue fever patients require admission [12], with an absolute precision of 4%, a 95% confidence interval, and considering a 20% loss to follow-up. Purposive sampling was employed as the sampling technique for participant selection. Inclusion criteria comprised patients of all genders and ages presenting with fever and positive Dengue Serology (IgM, NS1). On the other hand, patients taking anticoagulant medications, known cases of hematological malignancies, idiopathic thrombocytopenic purpura, and other disorders affecting hematological indices.

Participants meeting the inclusion criteria were enrolled into the study after explaining the purpose of the study and associated risks and benefits. Patients were diagnosed with dengue based on WHO criteria and positive serology results. After obtaining written informed consent, and their information, including demographic, clinical, and hematological profiles, was recorded in questionnaires. Samples were collected from the outpatient department of Ayub Teaching Hospital and clinics in Abbottabad.

All the patients, including both hospitalized and non-hospitalized patients were followed on 4th day, 7th day and 10th day of diagnosis to obtain blood samples for analysis.

For data analysis, the collected data were entered into Excel and SPSS version 25, GraphPad Prism were used for the statistical analysis of the data. Means and standard deviations were used to describe continuous variables including age, and the various hematological parameters, while frequencies and percentages were used to describe categorical variables such as gender and hospitalization. Data was stratified into hospitalized and non-hospitalized patients groups. Post stratification, student's t test and MANOVA were performed, considering a p-value of less than 0.05 as statistically significant. To assess the prognostic ability of the different parameters to predict hospitalization, logistic regression analysis was performed.

RESULTS

In the current study, a total of 146 participants were enrolled. The mean age of the patients included in this study was 38.58 ± 18.53 years. The demographic and clinical parameters of our patients are shown in table 1. There was a male predominance 88(60.3%) in our sample. Most common symptom was headache identified in (98.6%) of cases. This was followed in decreasing frequency by fever 142 (95.2%) and body aches 134 (91.8%).

Table 1: Demographic and clinical characteristics of the study sample (n=146)

Parameters		n (%)
Age Categories	0-10 Years	3 (2.10%)
	11-20 Years	24 (16.40%)
	21-30 Years	33 (22.60%)
	31-40 Years	21 (14.60%)
	41-50 Years	24 (16.40%)
	51-60 Years	20 (13.70%)
	>61 Years	21 (14.40%)
Gender	Male	88(60.3%)
	Female	58(39.7%)
Signs and Symptoms	Fever	142 (95.2%)
	Body aches	134 (91.8%)
	Headache	144 (98.60%)
Dengue Hemorrhagic Fever	Present	5 (3.4%)
	Absent	141 (97.3%)
Hospitalization	Yes	82 (56.2%)
	No	64 (43.8%)

The patients were sub-classified into mild (platelet count 100-149 x 10^9cells/L), moderate (99 -50 x 10^9 cells/L) and sever (0-49 x 10^9 cells/L) thrombocytopenia groups on the basis of platelet counts as shown in figure 1. On diagnosis, 73(50%) cases had mild, 48(32.9%) moderate and 25(17.1%) had severe thrombocytopenia with none of the patients having normal platelets count. There was a progressive improvement in the platelet counts over time and thus the severity of the thrombocytopenia improved over time such that at the 10th DOD, 111 (76.02%) patients were having normal platelets count.

The hematological parameter that is greatly affected by the dengue infection is the platelets. The platelets level was screened out and recorded at different stages of the infection. As a whole, at four different stages of the infection the platelets count was recorded, i.e., at the day 1, day 4, day 7 and day 10 as shown in the table 2. The mean platelet count at day 1 was 92.81 x 10^3 cells/µL. This was lower than standardized lower limit of normal range for population. Over the following days, the mean platelet count showed improvement and the final count at 10th day was 189.11±82.29 10^3 cells/µL. The mean of platelet count day 10 was within the normal standardized range for population. TLC also showed a similar temporal increasing trend over time. Conversely, a decreasing temporal trend was observed in the mean MPV, PDW and HB. The temporal trends for all these parameters was statistically significant on repeated measures multivariate analysis of variance (MANOVA) for time effect indicating a significant change in these parameters with time.

Figure 2 visually depicts the hematological indices of individual patients both Non-hospitalized and Hospitalized at the day 1 of diagnosis, day 4, day 7, and day 10. A gradual linear increasing trend can be observed. A repeated measures multivariate analysis of variance (MANOVA) with a "Time Effect" factor, focusing on the hematological indices over the course of the disease was highly significant ($p < 0.001$) for all the parameters. It indicates that there is significant difference in this parameter across the different time points. As the patient gains recovery, all the hematological indices starts to increase to the normal level more quickly in the non-hospitalized group of patients.

Logistic regression analysis: Nominal Regression Analysis aimed to predict hospital admission based on various factors as shown in tale 8. The Case Processing Summary indicates that out of 146 cases, 82 (56.2%) were admitted, and 64 (43.8%) were not admitted; being the reference category. The Model Fitting Information shows a significant improvement in model fit, with a final model providing better fit than the intercept-only model (Chi-Square = 53.824, df = 7, $p < 0.001$).

The Likelihood Ratio Tests further assess the significance of each variable. Platelet count at the day of diagnosis stands out as a significant predictor (Chi-Square = 40.271, df = 1, $p < 0.001$), emphasizing its importance in determining hospital admission. The Parameter Estimates offer insights into the impact of each variable. Notably, a lower platelet count at the day of diagnosis (Exp(B) = 0.964, 95% CI [0.952, 0.977]) is associated with an increased likelihood of hospital admission. Other variables, such as age, hemoglobin, white blood cell count, mean platelet volume, platelet distribution width, and gender, do not show significant effects on hospitalization.

Figure 1: Temporal trend of categories of severity of thrombocytopenia

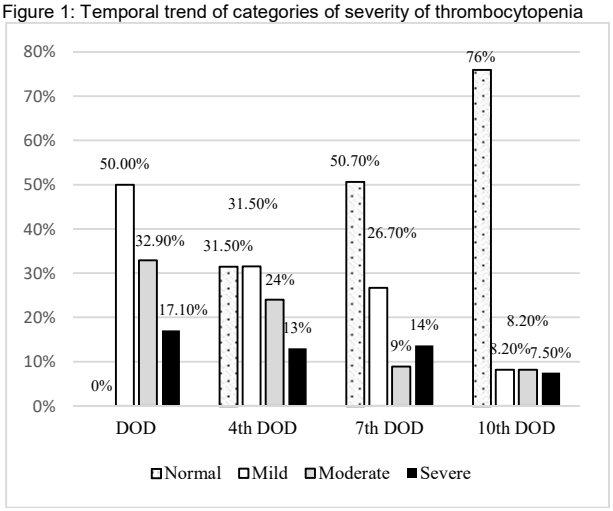


Figure 2: (2.1a-e) Hematological Indices of individual patients of Non-hospitalized and (2.21-e) Hospitalized groups during study duration

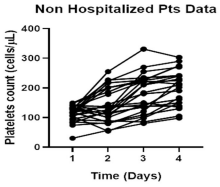
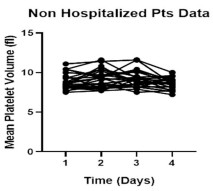


Figure: 2.1a



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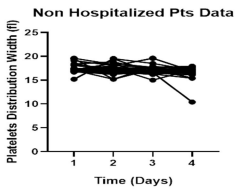

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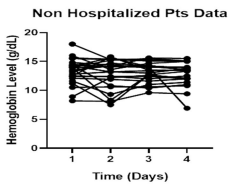

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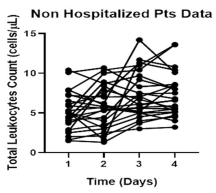

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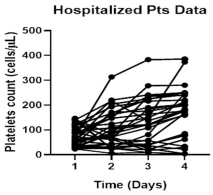
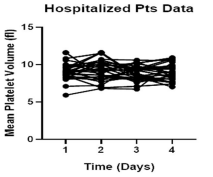


Figure: 2.2a



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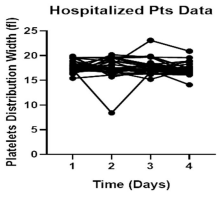

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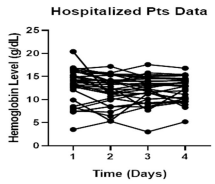

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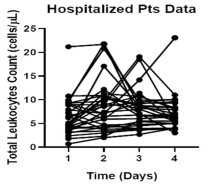

2.2e

Table 2: Mean and SD of hematological indices over the course of the disease

Parameters	Day 1 Mean±SD	Day 4 Mean±SD	Day 7 Mean±SD	Day 10 Mean±SD	P Value
Platelet (cells/µL)	92.82±3.21	125.17±5.50	159.28±6.74	189.12±6.81	<0.001
MPV (fl)	9.01±1.02	9.27±1.21	8.97±0.97	8.71±1.01	<0.001
PDW (fl)	17.59±1.13	17.45±1.76	17.55±1.31	16.87±1.40	<0.001
HB (g/dl)	13.29±2.91	12.47±2.75	12.64±2.38	12.56±2.30	<0.002
TLC (cells/µL)	5.88±3.39	7.63±4.79	7.69±3.35	7.88±6.30	<0.001

Table 3: Logistic Regression Analysis of the Predictors of Hospitalization

Factors	Hospitalization		Multivariate logistic regression		
	Yes (Mean ± SD)	No (Mean ± SD)	p-value	Adjusted Odds Ratio	95% CI for AOR
Intercept			0.660		
Age	31.52 ± 9.85	30.35 ± 9.68	0.522	0.993	0.971-1.015
Platelets	74.68 ± 37.86	116.05 ± 25.39	0.000	0.964	0.952-0.977
MPV	9.13 ± 1.03	8.85 ± 0.99	0.289	1.246	0.830-1.868
PDW	17.78 ± 1.17	17.35 ± 1.02	0.469	1.178	0.757-1.832
HB	13.35 ± 3.44	13.21 ± 2.05	0.274	1.091	0.933-1.276
TLC	6.34 ± 3.95	5.30 ± 2.39	0.230	1.113	0.935-1.325
Gender					
Female	35 (60.34%)	23 (39.65%)	1		
Male	47 (53.41%)	41 (46.59%)	0.175	0.547	0.229-1.307

This analysis underscores the predictive power of platelet count at the day of diagnosis in determining hospital admission for dengue patients. Understanding such predictors can aid clinicians in making informed decisions about patient care and resource allocation.

DISCUSSION

Our study encompassed a diverse age range, with a mean age of 38.58±18.53 years, consistent with the broad impact of dengue fever across all age groups, from children to the elderly. Notably, the majority of our patients fell within the 3rd to 6th decades of life (18–62 years). The observed male-to-female ratio of 1.52:1 echoes findings suggesting gender-related variations in dengue susceptibility and outcomes. Previous studies have reported a male predominance and attributed it to factors like hormonal differences, genetics, or differential virus exposure[10,11].

In our study, headache emerged as the most prevalent symptom, affecting 98.6% of cases, consistent with existing literature where headache is frequently reported in dengue cases[5]. Following closely, fever affected 95.2% of patients, aligning with the typical presentation of dengue fever characterized by high-grade fever, as supported by other studies[13]. Musculoskeletal involvement, reflected in body aches reported by 91.8% of patients, mirrors the common manifestation of myalgia in dengue cases, as reported in a study where 91% of patients experienced musculoskeletal pain[14].

The temporal evolution of thrombocyte morphology in our study reveals a transient impact of the virus on blood cells. Abnormalities such as anisocytosis and giant platelets, prevalent at diagnosis, decrease over time, indicating their transient nature and resolution during patient recovery [15]. While abnormal morphologies were initially observed in 15.1% of patients, the proportion decreased to 38.4% by the 10th day, highlighting the progressive improvement in platelet health and morphology during the course of dengue infection. The classification of dengue patients based on platelet counts aids in assessing disease severity and prognosis. At the time of diagnosis, none of our patients had a normal platelet count. Approximately 50% exhibited mild thrombocytopenia, 32.9% had moderate, and 17.1% had severe thrombocytopenia. This distribution aligns with previous studies and supports the proposal that platelet count serves as a prognostic indicator for disease severity and progression[7,8,16]. Notably, our results show a progressive improvement in platelet counts, with 76.02% returning to normal levels by the 10th day, indicating a favorable prognosis for most dengue patients. The recovery of normal platelet morphology parallels the improvement in platelet counts, emphasizing the positive trajectory of patient health. Initial abnormalities, such as anisocytosis and giant platelets, decreased over time, with a notable increase in patients exhibiting normal

morphology from 15.1% at diagnosis to 38.4% at the 10th day, signifying the restoration of platelet health during the recovery phase.

The platelet count emerged as a pivotal hematological parameter profoundly influenced by dengue infection. At the point of diagnosis, patients exhibited a mean platelet count of 92.81x10^3 cells/µL, falling below the normal range, indicating the substantial impact of the virus on platelet levels. As the infection progressed, a noteworthy improvement in platelet counts was observed, reaching a mean of 189.11±82.29 x 10^3 cells/µL by the 10th day, well within the normal range. These findings resonate with existing research, which consistently underscores the pronounced influence of dengue on platelet counts[16-18]. In a study involving 334 dengue-positive cases and 101 controls, significant reductions in platelet counts and elevated PDW were observed in dengue patients compared to controls [7]. The admitted patients displayed an initial platelet count of 36.89 x 10^3 cells/µL on the day of diagnosis, gradually rising to 179.66 x 10^3 cells/µL upon discharge. Another study from India corroborated these findings in admitted patients, noting a platelet count of 50.16 x 10^3 cells/µL upon admission, escalating to 143.71 x 10^3 cells/µL at the time of discharge [13]. These studies underscore the sensitivity of low platelet counts and high PDW in diagnosis and prognosis of dengue fever.

Several studies have delved into the significance of Mean Platelet Volume (MPV) in dengue cases, revealing elevated levels associated with disease severity. Our study aligns with these observations, pointing to increased MPV in dengue patients. However, it is crucial to note the existing controversy, as certain studies report no significant MPV differences. Similarly, Platelet Distribution Width (PDW) has been implicated in severe dengue cases, in line with our findings demonstrating a non-linear decreasing trend over time.

Previous studies on MPV in dengue infection reveal varying findings. Studies from Pakistan, India, and Bangladesh reported a significant increase in MPV levels in dengue patients compared to the healthy population and controls, correlating with disease severity [13-16]. Similarly, a study from Hyderabad, India, highlighted elevated MPV in dengue cases, especially in those with thrombocytopenia, suggesting it as an early marker for dengue[14]. However, a retrospective study in Kerala emphasized significant variations in platelet indices, including PDW and MPV, in dengue patients with varying platelet counts[13].

Various platelet indices, such as MPVxPDW\PLCxPCT, MPV\PLC, MPV\Platecrit, PDW\PLC, and PDW\Platecrit ratios, displayed significant differences among different platelet groups, indicating their potential value in monitoring disease severity and progression [15]. Conversely, studies from Saudi Arabia and India reported no significant differences in MPV levels between dengue patients and healthy controls[17,18]. The controversy surrounding MPV's role in dengue infection necessitates further research for a conclusive understanding.

The discernible discrepancy in mean platelet counts between hospitalized and non-hospitalized patients emphasizes the pivotal role of platelet counts in both diagnosing and managing dengue. Logistic regression analysis reinforces the importance of platelet count at diagnosis as a decisive factor influencing hospital admission. Notably, the progressive improvement in platelet counts over time, with the majority returning to normal levels, suggests a transient nature of thrombocytopenia in the course of dengue infection. Significant differences in platelet counts between hospitalized and non-hospitalized dengue patients align with existing literature, supporting the crucial role of platelet counts in diagnosing and managing dengue, as noted by various studies [19-23]. Notably, severe thrombocytopenia was identified as a significant factor associated with hospitalization and death in dengue-positive patients, emphasizing its predictive value[19]. Studies further revealed that platelet counts below 50,000/µL were linked to a ten-fold higher risk of severe dengue, including hemorrhagic fever and shock[6, 23].

Logistic regression analysis underscores the importance of platelet count at the time of diagnosis as a key determinant of hospital admission, in line with established literature highlighting platelet count as a hallmark of severe dengue, particularly dengue hemorrhagic fever[24]. These findings emphasize the clinical significance of early platelet count assessments for risk stratification and timely intervention in dengue cases, aligning with well-established evidence demonstrating the association between decreased platelet counts and disease severity[20-23].

Hemoglobin (Hb) levels in our study remained within the normal range, with a slight initial decrease at the 4th and 7th days and a gradual increase by the 10th Day of Diagnosis (DOD), indicating effective patient recovery and management. Consistent with the literature, dengue did not consistently affect Hb levels[20-22]. Some studies reported associations with anemia, but our findings do not align[24]. Studies from south India linked low hemoglobin levels at admission to hospitalization, emphasizing its potential as a marker for disease severity[11]. Total Leukocyte Count (TLC) showed a noticeable increase during the infection, signifying an active immune response against the virus. Conflicting findings in previous studies highlight the need for further research to understand the indirect effects of dengue fever on hemoglobin levels and TLC. Significant differences in Hb and TLC between hospitalized and non-hospitalized patients in our sample, persisting until the 10th DOD, suggest their utility as markers for assessing disease severity and monitoring treatment response. The higher TLC in hospitalized patients aligns with an inflammatory response, emphasizing the importance of these parameters in evaluating disease severity in dengue cases[25].

CONCLUSIONS

Our study highlights the predictive strength of the platelet count at diagnosis for dengue severity, aiding clinical decision-making. The observed trends, including the linear increase in platelet count and normalization of platelet morphology and indices significantly quickly in a non-hospitalized group of patients, contribute valuable insights for clinicians. These findings enhance our understanding of Dengue infection dynamics, guiding more effective clinical management strategies.

Author Contribution:

MZM: Contribution: Conceptualization of study design, literature search, write-up Proof reading and approval of the final manuscript, Accountable for the work

YMY: Contribution: Conceptualization of study design, literature search, write-up Proof reading and approval of the final manuscript, Accountable for the work

MZH: Conceptualization of study design, literature search, write-up Proof reading and approval of the final manuscript, accountable for the work

RA: Data Collection, Data analysis, data interpretation, Proof reading and approval of final manuscript, Accountable for the work

SQK: Data Collection, Data analysis, data interpretation, Proof reading and approval of final manuscript, Accountable for the work

HJ: Data Collection, Data analysis, data interpretation, Proof reading and approval of final manuscript, Accountable for the work

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