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Original Article

Peripheral Hematological Predictors of Severity and Mortality in Current Dengue Outbreak in a Hospital-Based Study at Nowshera

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Abstract

Objectives: To determine the predictive role of peripheral hematological markers for severity and mortality during the 2024 dengue outbreak in Nowshera.

Methodology: This prospective observational study included 178 patients who tested positive for Non-structural protein 1 (NS1) using an immunochromatographic test (ICT). Out of these, 116 NS1-positive samples were sent for serotyping to the National Institute of Health (NIH), with the support of the Public Health Research Laboratory at Khyber Medical University (PHRL-KMU), Peshawar. Various statistical tools were applied to assess the predictive value of hematological and biochemical markers for mortality in dengue infection.

Results: A total of 178 cases were analyzed, comprising 96 (53.8%) males and 82 (46.1%) females. Of the 116 samples sent for serotyping, 72 (62.1%) were PCR-positive, and among these, 66 (91.7%) were identified as DENV-2. There were 10 reported deaths. Significant differences in age, hemoglobin (Hb%), and platelet counts were noted between deceased and surviving patients with p-values of 0.09, 0.01, and 0.001 respectively, indicating statistical significance in predicting mortality. An increase in hematocrit levels was also observed in deceased patients. Thrombocytopenia emerged as a red flag in the deceased group. Notably, 9 out of the 10 deaths occurred in patients with DENV-2 serotype.

Female gender appeared to be relatively protective compared to male gender. Lower platelet and leukocyte counts demonstrated high clinical sensitivity for predicting mortality, with areas under the ROC curve (AUC) of 0.9 and 0.7, respectively.

Conclusion: Peripheral blood parameters, including decreased platelet count, elevated hematocrit, and low total leukocyte count (TLC), were found to be significant predictors of mortality with 95% confidence. Female patients had better survival outcomes across all age groups. Although age and hemoglobin levels showed trends, they were not statistically significant predictors of mortality. Recognizing these hematological markers in clinical settings can aid in early identification of high-risk patients and potentially reduce dengue-related mortality.

Key words: Dengue Fever, Mortality, Platelet count, Hematological markers.

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Introduction

The dengue fever is a tropical disease which is caused by single stranded RNA virus of Flaviviridae family. This is a deadliest seasonal viral disease. This virus has 4 subtypes i.e. (DENV-1, 2, 3, and 4), and is transmitted by Aedes mosquito. The humid and hot temperature season in Pakistan favors its spread and emerged as endemic in 2024 in our country.¹ This hot temperature supports the probation of the dengue vector to harbor and transmit this deadly disease in multiples. Highest number of cases were reported from Sept to Nov 2024 in Pakistan. Many studies reported males as victims in

number more than females and they reported with more causalities and mortality than female patients². Dengue fever is a public health challenge in tropical and subtropical regions. Its presentation varies from high grade fever to rashes and thrombocytopenia and further hemorrhagic sequences.³ Ali H et al⁴ reported 100 cases that were sent for serotyping and type 2 DENV was reported in 63% cases that shows its predominance in the current outbreak in Pakistan. This DENV -2 shared genetic similarity with Pakistan outbreak sequence in 2022 and >99% with China and Singapore out breaks in 2018-19.^{5, 6}

Hematological predictors of severity have always been under discussion for better outcome of the management of this disease. Many studies have reported Thrombocytopenia was present and elevated hematocrit (HCT) levels in seriously ill dengue patients at time of admission.^{7,8}

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With an increasing research in the field of infectious disease, the importance of simple non invasive tests like base line investigations like peripheral blood findings have the potential to predict the severity of the disease, in cost effective, affordable and accessible way to manage such patient on to categorize them on the basis of the severity.⁹

Present study was designed to determine the clinical sensitivity of various peripheral blood markers to predict the severity and mortality in dengue disease.

Methodology

This prospective observational study was conducted in the Department of Pathology at Qazi Hussain Ahmed Medical Complex (QHAMC), Nowshera, in collaboration with the Department of General Medicine, QHAMC, and with the support of the Institute of Pathology and Diagnostic Medicine, Khyber Medical University (KMU), from July 31, 2024, to November 30, 2024.

All patients admitted to the General Medicine Ward and High Dependency Unit (HDU) with a diagnosis of dengue infection were enrolled, regardless of age and gender. The initial diagnosis was made using an immunochromatographic test for the detection of non-structural protein 1 (NS1). Out of 178 NS1-positive samples, 116 were sent for serotyping through the Public Health Research Laboratory of KMU (PHRL-KMU), Peshawar, to the National Institute of Health (NIH), Islamabad.

Five milliliters of venous blood were collected from each patient using sterile techniques. Of this, 3 mL was transferred into a gel tube (yellow top) for the detection of IgM and IgG, and 2 mL into an EDTA tube (purple top) for NS1 detection. The immunochromatographic method was used for the detection of NS1, IgM, and IgG.

Randomization was performed, and every alternate NS1-positive patient, identified through the immunochromatographic method, was selected from both indoor and outpatient (OPD) settings. These samples were sent to the reference laboratory at KMU Peshawar for further transportation to NIH Islamabad for PCR and serotyping. The envelope gene was successfully amplified using nested PCR, validated via gel electrophoresis, and sequenced using the Sanger method. This three-step diagnostic process was

conducted with the support of the Department of Diagnostic Pathology and the Public Health Research Reference Laboratory at KMU Peshawar.

Data were entered into SPSS version 26 for analysis. The demographic variables included age, gender, and address of the patients. The research variables were categorized as categorical (e.g., gender, outcome, virus type, and PCR results) and numerical/quantitative (e.g., age, hemoglobin, leukocyte count, platelet count, hematocrit, etc.).

An independent t-test was used to compare age and other hematological variables across groups based on gender and disease outcome. Categorical variables were presented as percentages to show frequencies, while numerical variables were summarized using mean, median, and range. Various statistical tools were applied to hematological and biochemical markers to predict adverse outcomes in dengue infection.

A Receiver Operating Characteristic (ROC) curve was used to determine the clinical sensitivity of different quantitative hematological variables in predicting severity and mortality in dengue patients. In this study, a p-value of less than 0.05 was considered the benchmark for statistical significance.

Results

A total of 178 cases with 96(53.8%) males and 82(46.1%) females were included. The mean with standard deviation of numerical variables were; Age (31+7 years) and hemoglobin (g/dl) (10.94+ 2.27 g/dl). The median values of total Leukocytes count and platelet were 8330/cmm3 and 189000/cmm3 respectively (Table I). Out of 116 NS1 positive samples sent for serotyping, 72 were positive by PCR (62.1%). Out of 72 positive samples by PCR, 66(91.7%) were DENV-2 type of dengue infection.

Table I: Descriptive statistics.

	Age	Hb%	Hematocrit (HCT)	TLC	PLT
Number of patients	178	178	178	178	178
Mean	31	10.9	47.1		
Median				8330.0	189000
Std. Deviation	6.67	2.27	5.3		
Minimum	6.0	4.0	43.0		
Maximum	75.0	18.0	32.0		

noted in the deceased vs survivors ($p=0.09$), ($p=0.01$) and ($p=0.07$) respectively and were statistically significant to predict mortality. An increased in hematocrit and hemoglobin values were noted in the deceased vs survivors. Thrombocytopenia was prominent in the deceased due to dengue infection. (Table II). Female gender was proportionally more protective as compared to male gender. We observed 9/10 deaths with serotype DENV-2. Lower Platelet Platelets and leukocytes count showed a higher clinical sensitivity to predict worst outcome with an Area Under Curve (AUC) of 0.9 & 0.7 respectively on Receiver Operating Curve (ROC). Table III. Many of the studies as mentioned in table IV are in consistent with our findings.

Discussion

The key findings of this research is to underscore the importance of the hematological markers and their

association with severity and mortality in dengue fever. The warning signs like anemia, thrombocytopenia and age factor as indicated by statistical significance in the groups of deceased vs survivors ($p<0.05$). We reported 96(53.8%) males and 82(46.1%) females as patients and further noted that female gender was proportionally more protective as compared to male gender in term of surviving in dengue infection.

A study from India reported that male patients presenting with low platelet counts, elevated hematocrit, and low total leukocyte count (TLC) at the time of admission had higher mortality rates. Thus, male gender itself was identified as a warning sign for severity and mortality during the current outbreak.¹⁰ In our study, we observed that mortality in dengue patients was more common among older individuals compared to younger ones; however, this difference was not statistically significant ($p = 0.09$). The mean age $\pm$ standard deviation was 31 ± 7

Table II: Group Statistics (difference in deceased vs survivors) independent t test.

Outcome		N	Mean/Median	Std. Deviation	p value
Age	Survived	168	31	7.2	0.09
	Expired	10	45	3.4	
HB	Survived	168	10.9	2.2	0.01
	Expired	10	11.4	3.5	
TLC	Survived	168	9174.0		0.88
	Expired	10	5740.2		
PLT	Survived	168	231351.2		0.07
	Expired	10	68600.0		
Hematocrit (HCT)	Survived	168	43.3	3.4	0.001
	Expired	10	48.0	5.4	

Table III: Area Under the Curve (ROC CURVE) for clinical sensitivity of different hematological markers to predict mortality.

Test Result Variable(s)	AUC	Cut off values	Clinical Sensitivity %	Specificity %	Std. Error ^a	Asymptotic 95% Confidence Interval	
						Lower Bound	Upper Bound
PLT	0.9	<100k	90	85	0.1	0.8	1
TLC	0.7	<4.5k	82	70	0.1	0.6	0.9
HB	0.4	>11g/dl	60	55	0.1	0.1	0.6

Table IV: Comparison of the hematological indices in the sever/fatal dengue disease vs survivors.

Hematological predictor	National and international studies showing the hematological indices in the sever/fatal dengue disease as compared to the survivors				
	Present study	Haq FU et al14	Roy SK et al15	Nandwani S et al16	Poottasane N et al17
Hb%	11.9g/dl ($p<0.01$)	10.2 g/dl	--	7.49g/dl	P:0.23
PCV/HCT	Raised	Raised ($p<0.001$)	Raised ($p<0.001$)	>45%	Raised ($p<0.001$)
TLC	($5.7*103/cmm3$), ($p<0.88$)	($3.7*103/cmm3$)	Leukopenia ($p<0.02$)	($5.3*103/cmm3$)	Leukopenia ($p<0.01$)
Platelets	($68*1011/cmm3$) ($p<0.07$)	($49*1011/cmm3$) ($p<0.01$)	Thrombocytopenia ($p<0.03$)	Thrombocytopenia ($53*1011/cmm3$)	Thrombocytopenia ($p<0.001$)

years among survivors, whereas it was 45 ± 3 years among deceased patients. A study from a teaching hospital of Postgraduate medical institute Hayatabad Medical Complex Peshawar reported that age over 45 years, leukocytosis, high hematocrit and acute kidney injury at time of presentation to outdoor patient department predicts higher mortality than expected.¹¹ Other studies also reported an inverse relation of the age with outcome of the disease in current outbreak. These findings are consistent with the earlier studies that also suggest that adults are at higher risk of mortality in dengue as compared to younger children.^{12,13.}

Regarding the serotyping, 116 NS1 positive samples were sent to KMU, 72 were reported positive by PCR (62.1%). Out of 72 positive samples by PCR, 66(91.7%) were DENV-2 as reports shared by the National institute of Health (NIH) Islamabad through reference lab KMU. An increased in red cell mass values were noted in the deceased vs survivors and thrombocytopenia was prominent in patient who met the worst outcome in term of mortality in dengue outbreak in present study like Haq FU et al¹⁴ & Roy S et al¹⁵ presented cases with DENV-2 and showed the significant impact of the raised hematocrit, leukopenia and thrombocytopenia in predicting the worst outcome of the disease. Nandwani S et al¹⁶ reported that hematological parameters as observed at time of presentation can predict outcome of the disease. Poottasane N et al¹⁷ also reported monocytes distribution width along with

thrombocytopenia and leukopenia as predictor of mortality in DENV-2 current outbreak.

Regarding the clinical sensitivity of the hematological markers we observed that lower Platelets count and leukocytes count showed a higher clinical sensitivity to predict worst outcome with an Area Under Curve (AUC) of 0.9 & 0.7 respectively on Receiver Operating Curve (ROC). The clinical sensitivity of thrombocytopenia to predict mortality in severe dengue infection was 90% using ROC curve with a specificity of 85%. Srisuphanunt M et al¹⁸ reported the clinical sensitivity of the hematological predictor in concordance to our findings. Correct prediction of the severity of the disease at early stage contributes to faster diagnosis and reduces unnecessary treatments and mortality in DENV-2.

Conclusion

It was concluded that the role of peripheral hematological markers i.e. decreased platelet and TLC counts predict mortality with 95% confidence. Survival favors Female gender more as compared to male gender in all age categories. No significant relationship was noted for Age, Hb% to predict mortality in Dengue infection. Keeping in view the importance of these values in clinical practice can reduce severity and mortality in the current dengue outbreak.

There were some limitations of the study. This study was conducted on low sample size. Future studies executed with larger sample size are recommended to represent a population for implementation of finding in decision making to combat this deadly disease. Therefore, studies covering large population with representation of all sectors are required to predict/suggest the findings for decision making by the competent authorities to prevent future disastrous endemic.

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