Association of Dengue Severity with Host Biomarkers

Muhammad Farooq, Amna Saleem, Majida Farooq, Mariam Ejaz

Department of Medicine, Combined Military Hospital Nowshera /National University of Medical Sciences (NUMS) Pakistan

ABSTRACT

Objective: To determine the association of dengue severity with various host biomarkers and clinical parameters.
Study Design: Cross-sectional study.
Place and Duration of Study: Department of Medicine, Combined Military Hospital, Nowshehra Pakistan, from Sep to Dec 2022.
Methodology: Patients of both genders who fulfilled the inclusion and exclusion criteria were admitted for Dengue fever in this study. Sampling was done via a non-probability convenient sampling technique. Demographic and clinical information of patients was obtained. Biochemical investigations were monitored daily till the 10th day of illness.
Results: One hundred patients were part of this study, out of which 66 were males and the rest were females. 07 patients had their platelet count above $150,000 \times 10^9$ while 13 had below $20,000 \times 10^9$. Hematocrit was raised in 26 and low in 23. The rest of the tests showed high LDH (>1000U/L) in 19(19%), high CPK in 15(15%), high AST in 45, high ALT in 33 and deranged coagulation in 9% of patients. Clinically, 66% of patients had ascites, 18% had pleural effusion, and 10% had active bleeding. Ninety-seven patients recovered, while 3 died.
Conclusion: We concluded that there is a significant association of dengue severity with host biomarkers like low platelets, elevated hematocrit, high transaminases, high LDH, CPK and deranged coagulation as well as clinical parameters like ascites, pleural effusion and active bleed.

Keywords: Biomarkers, Dengue, Platelets.

INTRODUCTION

Dengue fever is a vector-borne illness caused by a flavivirus (DENV). The four distinct serotypes are from DENV-1-4, having different genotypes.[1] Various threatening elements of dengue-related illness are stored in water in holders. During the initial febrile phase, the viral nucleic acid can be detected by non-structural protein (NS1). From day 4- 14, IgM will detect dengue illness, and from day 14 onwards, IgG will help identify infection. Serological markers also differentiate between primary and secondary infection.[2-3] In case of secondary infection, circulating dengue serotypes should be carefully monitored.[4] Severe dengue is thought to be associated with high viral load, which has a positive correlation with hematocrit and negative association with platelets count.[5]

Dengue fever sometimes has a biphasic fever pattern. After the febrile phase, the patient enters the critical phase, during which a third space plasma leakage occurs. The condition may progress to dengue hemorrhagic fever and then dengue shock syndrome.

Correspondence: Dr Muhammad Farooq, Department of Medicine, Combined Military Hospital, Nowshera Pakistan
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High ALT levels suggested a strong possibility of progression to severe dengue fever, so assessment of transaminases should be done on a priority basis.[6] Ascites and pleural effusion can be diagnosed earlier through abdominal ultrasound, even when hematocrit levels are unchanged. Abdominal ultrasound has a much higher sensitivity in detecting as little as 5 ml of fluid.[7] It is well accepted that the severity of a second dengue infection depends on the age of the person at the first infection and the duration between the first and second infection. The longer the duration, the more theory increases increases, causing a slight and steady decrease in heterotypic antibody titers. There are more chances of adults to bleed if they are infected with dengue for the second time.[8] Careful fluid resuscitation is the mainstay of treatment for dengue fever. Crystalloids are the first line of fluid therapy. Blood products are only needed to be transfused in dengue hemorrhagic fever.[9] Currently, five different types of dengue vaccines are in trial according to the immune response against particular serotypes.[10]

Some host biomarkers and clinical parameters have shown promise in predicting dengue severity, but their validation across different populations and geographic regions, such as in our setup of Khyber

Pakhtoon Khuwa, is limited. Therefore, the main rationale for conducting the current study was to validate the efficacy of these biomarkers and clinical parameters in predicting the severity of disease.

METHODOLOGY

The cross-sectional study was carried out at the Department of Medicine at CMH Nowshera, Pakistan from September and December 2022 after the formal approval of the Ethical Committee (ERC #21/7 Sep 2022).

Inclusion Criteria: All dengue NS1 or IgM-positive patients of either gender were included.

Exclusion Criteria: Patients with acute myocardial infarction, acute hepatitis due to any other cause, complicated diabetes mellitus and acute stroke were excluded.

The first hundred patients admitted with dengue fever were part of this study. Sampling was done via non-probability convenient sampling. Informed consent was taken from study participants, ensuring confidentiality and that there would be no breach of privacy. All dengue NS1 or IgM-positive patients who were admitted to us inward were part of this study. Patient demographics, including name, age, sex and other biochemical parameters of study like TLC, platelets count, ALT, CPK levels, LDH levels, CRP (quantitative), PT, and APTT, were recorded in a predesigned proforma. Patients were assessed daily via vital monitoring, biochemical parameters and clinical during their hospital stay. Dengue severity was assessed through a combination of raised biomarkers with clinical parameters like ascites, pleural effusion and active bleeding. Documentation was done regarding presentation and discharge.

Dengue fever was diagnosed as per WHO guidelines 2022 in any patient with a high-grade fever (40°C/104°F) and 2 of the following symptoms occurring during the febrile phase (2-7 days): severe headache, myalgia and arthralgia, retro-orbital pain, nausea, vomiting, swollen glands, and rash. Worsening dengue symptoms, signs of plasma leakage in the third spaces, and any active bleed indicate severe dengue.[11]

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. Chi-square test was applied to explore the inferential statistics

RESULTS

The patients' ages ranged from 17-70. The mean age was 37.29±13.67. 28% of the patients presented during the first to third day of illness, 46 during the third to fifth day, and 23 presented after the fifth day of disease. Among all, 44 were febrile at presentation. 26 patients had raised hematocrit, while 23 had hematocrit in the lower range. Patients with high LDH (> 1000U/L) were 19(19 %), while 48 had LDH in the range of 500-1000 U/L. Patients with CPK > 800 U/L were 15. Raised AST was found in 45 patients, while raised ALT was found in 33 patients. Coagulation was arranged in 9% of patients, while it was normal in 91 patients.

Abdominal ascites were seen in 66% of patients, pleural effusion in 18%, while 10% of the patients had active bleeding (Figure).97 % of patients made a full recovery and were discharged home, while 3% died. There was a significant association of dengue severity with low platelets count, hematocrit, and deranged biomarkers like AST, LDH, CPK, ALT and coagulation. In addition, dengue severity was strongly correlated with clinical parameters like ascites, pleural effusion and active bleeding. Moreover, there was a statistically significant association of hematocrit (*p*-value 0.012), coagulation (*p*-value<0.001), ascites (<0.001), pleural effusion (<0.001)and active bleed (<0.001) with poor outcome of the patients (death) (Table). 7 % patients had their platelets above 150,000x109/L, 42 % had between 50,000-150,000 x109/L, and 35% had between 20,000-50,000 x109/L, while in 13% platelets count was below 20,000 x109/L.
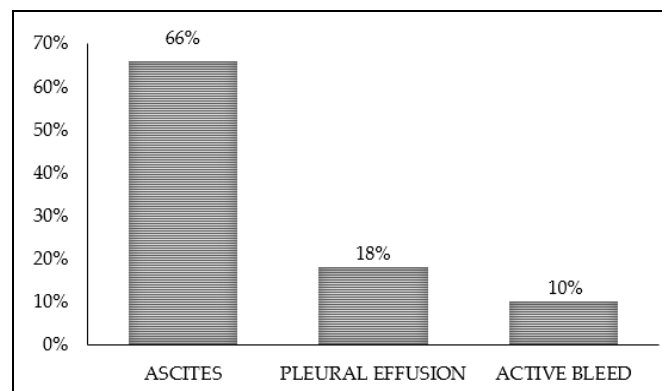

Figure : Frequency Of Dengue Complications (n=100)

DISCUSSION

The current study looked at two different aspects: the different host biomarkers involved in assessing dengue severity and the rising and falling trends of

these biomarkers with the duration of illness. In addition, combining host biomarkers with clinical parameters could enhance dengue severity assessment. Complete blood count and various other haematological biomarkers showed varying trends in patients with dengue during their illness.

We observed that patients who presented during the initial three days of their illness mainly had pyrexia/fever (temp> 98.6˚F), which settled after day 3 in most cases. After the febrile phase was over, it was seen that hematocrit(HCT) was on the higher side in most patients while low in a few during the critical phase of illness. Patients with low HCT had the worst outcome of Dengue illness. A retrospective study carried out in 2018 also explained similar results that patients with dengue fever had elevated hematocrit from day 3 to day 8 of their illness in most patients, which was indicative of plasma leakage.[11]

Table: Parameters of Dengue severity (n=100)

Biochemical Parameters	GROUP-A Normal Biochemical Parameters	GROUP-B Raised Biochemical Parameters	*p*-value
LDH	67(67%)	33(33%)	<0.001
CPK	40(40%)	60(60%)	<0.001
AST	45(45%)	55(55%)	<0.001
ALT	67(67%)	33(33%)	0.019
Coagulation	91(91%)	9(9%)	<0.001
Clinical Parameters			
	Present	Absent	
Ascites	66(66%)	34(34%)	<0.001
Pleural effusion	18(18%)	82(82%)	<0.001
Active bleed	10(10%)	90(90%)	<0.001

Platelet count significantly dropped during the critical phase (days 3-8 of illness), which is comparable with another study done in 2020, concluding that thrombocytopenia is an important host parameter for assessing dengue severity.[12]

Liver function tests, including AST and ALT, were also raised in this study population during the critical phase of dengue. The levels started settling down in the recovery phase. We did not study ALT and AST individually. However, a study quoted here also quantified AST/ALT specifically to look for their importance as an early predictor of dengue severity. It showed that AST levels rise earlier in dengue fever than ALT, so it was a more reliable marker in dengue severity than ALT. Overall, the ratio of AST and ALT

was very helpful in identifying patients with severe dengue on admission.[13]

Host inflammatory biomarker serum lactate dehydrogenase (LDH) was studied, and levels were extremely high in our dengue patients during the critical phase. The decreasing trend of LDH was noticed in the recovery phase of dengue patients from day 7 onwards. A similar study conducted to assess the association of dengue severity with LDH also concluded that patients with dengue fever had their serum LDH levels raised twice the normal value; however, if it was more than four times the normal value, it was indicative of severe dengue fever in the form of DHF and DSS.[14]

We did not manage any patients with severe dengue with steroids, even the patients with significant elevation of all host biomarkers. All patients in our study were managed according to fluid resuscitation guidelines. However, a case report was published in 2020 based on hemophagocytosis syndrome (HPS) in dengue patients. It stated that a dengue patient who presented initially with stable vital signs and mildly deranged LFTs and LDH had her condition deteriorated with abrupt marked elevation of AST and LDH with a deranged coagulation profile. She was labelled as having severe dengue with HPS and multiorgan dysfunction. She was treated with systemic steroids and was improved.[15]

Creatine Phosphokinase (CPK) was also found to be elevated in many dengue patients along with LDH. A study was done to look for serum activity of CPK in dengue patients who had no previous cardiac illness or myositis. Nevertheless, the levels were raised in dengue patients, suggesting possible involvement of skeletal and myocardial cells.[16]

Clinically, we assessed the patients daily during their admission and found out that patients with raised LFTs, LDH and CPK, and decreased platelets had ascites and pleural effusion indicative of plasma leakage. A study was done to understand better the association of plasma leakage in dengue patients with endothelial degradation. It was postulated that increased heparanase activity was responsible for the destruction of a protective coat of endothelium in dengue patients.[17]

It was also seen that the coagulation profile was deranged in 9% of our patients. Similar results were seen in another study, which showed a high magnitude of deranged coagulation in patients with

dengue fever. Recognizing this derangement earlier helps prevent the deterioration of dengue.[18]

In a nutshell, our study validates existing host biomarkers of dengue severity. Combining these biomarkers with clinical parameters such as ascites, pleural effusion, and deranged coagulation could enhance the accuracy of dengue severity assessment. Furthermore, undiscovered biomarkers may provide valuable insights. Future studies could focus on identifying new host biomarkers that are specific, sensitive, and reliable indicators of dengue severity.

LIMITATIONS OF STUDY

This study had certain limitations. We could not study the gender-specific association of dengue fever and its severity because there were fewer female patients in our study.

CONCLUSION

We concluded that there is a significant association of dengue severity with host biomarkers like low platelets, elevated hematocrit, high transaminases, high LDH, CPK, and deranged coagulation, as well as clinical parameters like ascites, pleural effusion, and active bleed.

Conflict of Interest: None.

Authors Contribution:

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

MF & AS: Conception, study design, drafting the manuscript, approval of the final version to be published.

MF & ME: Data acquisition, data analysis, data interpretation, critical review, 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.

REFERENCES

1. Roy SK, Bhattacharjee S. Dengue virus: epidemiology, biology, and disease aetiology. Can J Microbiol 2021; 67(10): 687-702. https://doi.org/10.1139/cjm-2020-0572
2. Awan NJ, Chaudhry A, Hussain Z, Baig ZI, Baig MA, Asghar RJ, et al. Risk Factors of Dengue Fever in Urban Areas of Rawalpindi District in Pakistan During 2017: A Case Control Study. JMIR Public Health Surveill 2022; 8(1): e27270. https://doi.org/10.2196/27270
3. Kularatne SA, Dalugama C. Dengue infection: Global importance, immunopathology and management. Clin Med 2022 ; 22(1): 9-13. https://doi.org/10.7861/clinmed.2021-0791
4. Damodar T, Dias M, Mani R, Shilpa KA, Anand AM, Ravi V, et al. Clinical and laboratory profile of dengue viral infections in and around Mangalore, India. Indian J Med Microbiol 2017 ; 35(2): 256-261. https://doi.org/10.4103/ijmm.IJMM_15_423
5. 5) Pathak B, Chakravarty A, Krishnan A. High viral load positively correlates with thrombocytopenia and elevated haematocrit in dengue infected paediatric   patients. J Infect Public Health 2021 ; 14(11): 1701-1707. https://doi.org/10.1016/j.jiph.2021.10.002
6. Ayaz F, Furrukh M. Assessment of Severity of Dengue Fever by Deranged Alanine Aminotransferase Levels. Cureus 2020 ; 12(9): 10539. https://doi.org/10.7759/cureus.10539
7. Dewan N, Zuluaga D, Osorio L, Krienke ME, Bakker C, Kirsch J. Ultrasound in Dengue: A Scoping Review. Am J Trop Med Hyg 2021 ; 104(3): 826-835. https://doi.org/10.4269/ajtmh.20-0103.
8. Halstead S. Recent advances in understanding dengue. F1000Res 2019; 8: F1000 https://doi.org/10.12688/f1000research.19197.1
9. Kularatne SA, Dalugama C. Dengue infection: Global importance, immunopathology and management. Clin Med 2022; 22(1): 9-13. https://doi.org/10.7861/clinmed.2021-0791
10. Deng SQ, Yang X, Wei Y, Chen JT, Wang XJ, Peng HJ. A Review on Dengue Vaccine Development. Vaccines 2020 ; 8(1): 63. https://doi.org/10.3390/vaccines8010063
11. Chaloemwong J, Tantiworawit A, Rattanathammethee T, Hantrakool S, Chai-Adisaksopha C, Rattarittamrong E, et al. Useful clinical features and hematological parameters for the diagnosis of dengue infection in patients with acute febrile illness: a retrospective study. BMC Hematol 2018; 18: 20. https://doi.org/10.1186/s12878-018-0116-1
12. Castilho BM, Silva MT, Freitas ARR, Fulone I, Lopes LC. Factors associated with thrombocytopenia in patients with dengue fever: a retrospective cohort study. BMJ Open 2020 ; 10(9). doi: https://doi.org/10.1186/s12878-018-0116-1
13. Md Sani SS, Han WH, Bujang MA, Ding HJ, Ng KL, Shariffuddin MA. Evaluation of creatine kinase and liver enzymes in identification of severe dengue. BMC Infect Dis 2017 ; 17(1): 505. https://doi.org/10.1186/s12879-017-2601-8
14. Sirikutt P, Kalayanarooj S. Serum lactate and lactate dehydrogenase as parameters for the prediction of dengue severity. J Med Assoc Thai 2014; 97 (Suppl 6): S220-31.
15. Ishak SH, Yaacob LH, Ishak A. Severe Dengue with Hemophagocytosis Syndrome. Malays Fam Physician 2020 ; 15(1): 47-49.
16. Agudelo-Salas IY, Quinceno N, Duque J, Bosch I, Restrepo BN. Actividad en suero de CK y CK-MB en pacientes con infección por el virus dengue [Serum activity of CK and CK-MB in patients with dengue virus infection]. Rev Salud Publica 2017 ; 19(4): 460-467. https://doi.org/10.15446/rsap.v19n4.39597
17. Buijsers B, Garishah FM, Riswari SF, van Ast RM, Pramudo SG, Tunjungputri RN, et al. Increased Plasma Heparanase Activity and Endothelial Glycocalyx Degradation in Dengue Patients Is Associated With Plasma Leakage. Front Immunol 2021; 12: 759570. https://doi.org/10.3389/fimmu.2021.759570
18. Adane T, Getawa S. Coagulation abnormalities in Dengue fever infection: A systematic review and meta-analysis. PLoS Negl Trop Dis 2021; 15(8). https://doi.org/10.1371/journal.pntd.0009666

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