Clinical and Laboratory Markers of Acute Dengue Infection

Mujeeb ur Rahman[1], Shahneela Tabassum[2], Muhammad Salman[3], Rana Shahid Hafeez[4], Ausaf Khan[5], Muhammad Nasir Khoso[6]

Abstract

Objective: This study aims to facilitate prompt diagnosis and the early recognition and identification of clinical and laboratory markers in the early stages of dengue infection.

Methods: This descriptive cross-sectional study was conducted in the Department of Medicine at PNS Rahat Hospital, Karachi, from July 13th, 2023, to October 12th, 2023. The sample size of the study was 106 patients diagnosed with dengue fever. Subjects were enrolled in accordance with eligibility criteria after taking informed verbal and written consent. A clinical assessment was carried out and relevant parameters were recorded during the course of the illness. All patients were evaluated for dengue NS-1 antigen and IgM by ELISA method. SPSS software (version 20) was utilized for data analysis.

Results: A total number of 106 patients were recruited. When clinical markers of acute dengue infection were assessed, 37.9% (n=40) had vomiting, 46.5% (n=49) had myalgia and 41.8% (n=44) had petechiae. When laboratory markers of acute dengue infection were assessed, 86.8% (n=92) had leucopenia, 84.0 (n=89) had thrombocytopenia and 88.9% (n=94) had aspartate aminotransferase (AST)/ alanine aminotransferase (ALT) ratio > 1.5. In this study, age significantly influenced the occurrence of myalgia (P = 0.002) and was associated with leucopenia and an elevated AST/ALT ratio (P < 0.001 for both). Additionally, the AST/ALT ratio was significantly correlated with prolonged fever duration (P = 0.010) and severe dengue outcomes (correlation coefficient = 0.45, P < 0.001). These results suggest that the AST/ALT ratio as a predictive biomarker for severe dengue, emphasizing its utility in early identification and intervention.

Conclusion: Clinicians still face difficulties diagnosing dengue infection in its early stages, particularly in low-resource settings. The findings of this research can help clinicians establish early diagnosis and make clinical decisions when evaluating a patient with suspected dengue fever in resource-poor settings where the detection of serum NS1 antigen is not available.

Key Words: Clinical Markers, Laboratory Markers, Dengue, Febrile.

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Introduction

Dengue infection, often known as dengue fever, stands as a significant public health concern worldwide. Dengue infection is the most common viral illness spread by mosquitoes to humans. It is an acute infectious disease that is caused by four different serotypes of the dengue virus and affects about 2.5 billion people worldwide, particularly within the tropical and subtropical regions[1]. Depending on the severity of the epidemic, the World Health Organization (WHO) estimates that 50 million cases of dengue fever (DF) and several hundred thousand cases of dengue hemorrhagic fever (DHF) occur annually[2]. The exact number of dengue cases is underreported because the great majority of infections are mild or asymptomatic, and can be self-managed. Additionally, many cases are misdiagnosed as different febrile conditions. Approximately 1.8 billion people

[1,2,4] Department of Medicine, PNS Rahat Hospital Karachi
[3] Department of Medicine, Dow University of Health Sciences
[5]Department of Anaesthesiology, Aga Khan University
[6]Department of Critical Care Medicine, South City Hospital Karachi.

Correspondence: Muhammad Nasir Khoso
Department of Critical Care Medicine, South City Hospital Karachi.
Email: drmuhammadnasirkhoso@gmail.com
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globally are susceptible to dengue fever and reside in countries that are members of the WHO's Western Pacific and South-East Asia regions, which account for nearly 75% of the dengue-related global disease burden[3]. Since there were two major epidemics of Dengue Shock Syndrome (DSS) and DHF—one in 1996, when four serotypes were in circulation, and another in 2012 when three times as many cases were reported as the year before it is crucial to investigate the disease's varied features and unusual presentation. The year 2019 recorded the highest number of dengue cases globally. Every region was affected, with Afghanistan experiencing dengue transmission for the first time.

Dengue can appear clinically in various ways, and its course and results are frequently unpredictable, ranging from mild flu-like symptoms to severe, life-threatening conditions. Most cases are self-limiting and resolved with supportive care, a small percentage of individuals advance to severe disease, which is typically defined by plasma leakage with or without hemorrhage, even though the majority of patients recover after a self-limiting non-severe clinical course.

Due to the non-specific early symptoms, low detectable viremia, and late-stage serological testing for confirmation of dengue, early diagnosis of dengue is difficult[4]. During the febrile phase, prompt diagnosis is crucial for modifying the course of treatment [5].

The age distribution of DHF cases varies depending on whether the area has had a recent introduction of the virus or lacks endemicity. Adult DHF cases are becoming more prevalent in certain places[6-7].

HO TS et al in their study on 581 patients, who reported clinically suspected dengue infection, observed that clinical markers like myalgias, petechiae, and vomiting were present in 46.8%, 36.9%, and 33.5% of patients respectively, while laboratory markers like leucopenia, thrombocytopenia and AST/ALT ratio >1.5 were present in 84%, 83.7% and 87.9% respectively, 376 patients were confirmed as DF on laboratory testing.[3] There is a discrepancy in clinical literature about the frequency of different clinical parameters and laboratory markers while evaluating a patient with suspected DF [5,6].

Recent shifts in the epidemiological landscape, including changes in serotype prevalence and the emergence of new viral strains, necessitate a fresh evaluation of clinical and laboratory markers. Additionally, regional disparities in dengue infection rates and outcomes suggest the need for localized data to tailor prevention and management strategies effectively. Due to the study's single-center methodology and small sample size, the results might not apply to the entire Pakistani population, but they can nevertheless provide insightful local information. This kind of information is essential for improving our comprehension of the clinical presentations of dengue in certain contexts.

In resource-constrained settings where advanced diagnostic facilities might not be easily accessible, this study aims to provide an up-to-date and thorough analysis of the frequency of clinical and laboratory markers in patients with suspected acute dengue fever. This will help healthcare professionals make well-informed clinical decisions. In the end, our work aims to close a significant information gap by describing the present dengue marker profile in our area, which may help develop more focused clinical interventions and diagnostic techniques in comparable circumstances.

Methodology

This cross-sectional study's technique was aimed at investigating young adults' clinical and biochemical indicators of dengue fever. The study was conducted in the dengue ward of the Department of Medicine at PNS Rahat Hospital, Karachi, over three months, from July 13, 2023, to October 12, 2023.

The sample size was determined using the WHO sample size calculator. The anticipated population proportion was set at 33.5% based on research conducted by Peeling RW et al[8] 106 participants would be required, with a 9% margin of error and 95% confidence interval. A non-probability consecutive sampling technique was employed to select the participants.

The study included all adult patients aged above 16 years, regardless of gender, who had a positive Dengue NS1 serology and were confirmed with dengue infection. Patients who did not meet the eligibility criteria i-e: neurological or psychiatric conditions, positive bacterial focus in microbiological testing, co-infections with other fevers, refused to cooperate, or withdraw from the study were all excluded. Before commencing the research study, the Ethical Review Committee of the Hospital was consulted for approval. All subjects who met the eligibility requirements were enrolled after providing both written and verbal informed permission. Clinical assessment including investigations were carried out.

Clinical markers were recorded during the illness and endorsed in the proforma. All patients were laboratory evaluated for dengue NS-1 antigen and IgM by ELISA, which was performed as per the pathologist's instructions.

The principal investigator filled out the attached proforma. To exclude the possibility of bias, all examinations and data recording procedures were performed under the supervision of a senior clinician.

Data analyses were conducted using the Statistical Package for Social Science (SPSS) version 19. Descriptive statistics were presented as mean ± SD for numerical variables such as age, average duration of fever, initial laboratory evaluations, and mean alanine aminotransferase levels. Categorical variables, including gender, presence of vomiting, myalgia, petechiae, and leucopenia, were summarized by frequencies and proportions. Cross-tabulated analysis was performed based on age, gender, duration of fever, and clinical markers (vomiting, myalgia, petechiae, thrombocytopenia, AST/ALT>1.5). Chi-square or Fisher's exact tests were employed to examine statistically significant associations. The Spearman correlation coefficient test was also performed to assess the relationship between the severity of dengue and an elevated AST/ALT ratio. A p-value of <0.05 will be considered statistically significant.

Results

This study evaluated 106 patients with suspected dengue infections, encompassing an age range of 16 to 30 years. The mean ± SD age of the cohort was 22.5 ± 7.47 years, with the majority being males constituting 63.2% (n=67) patients and females 36.8% (n=39). The mean ± SD duration of fever reported was 5.5 ± 1.6 days. Initial laboratory evaluations showed a mean platelet count of 99 × 10^3/cmm ± 48.5, a white blood cell count of 3266.5 ± 1740 /mm, and mean levels of aspartate aminotransferase (AST) and mean alanine aminotransferase (ALT) at 146 ± 169 U/L and 80.5 ± 89 U/L) respectively, reflecting the intense impact of the virus on hepatic function and hematopoiesis.

The clinical manifestations of dengue were prevalent across the study group, with common symptoms including vomiting, myalgia, and petechiae. On laboratory analysis, most patients exhibited leucopenia and thrombocytopenia, alongside a significant proportion showing an elevated AST/ALT ratio, indicative of hepatic distress (Table 1).

When clinical markers were cross-tabulated with age, gender, and fever duration, comparisons did not reveal statistically significant differences except that age was a significant factor in the occurrence of myalgia ($P = 0.002$). Significant age-related variations were also noted in the incidence of leucopenia and an elevated AST/ALT ratio ($P < 0.001$ for both), suggesting age as a determinant in the disease's expression. Additionally, the AST/ALT ratio was significantly associated with the duration of fever ($P = 0.010$), indicating that prolonged fever could be a marker of severe liver involvement. Detailed cross-tabulations of clinical and laboratory markers are provided in Table 1.

Out of the total participants,12 individuals were confirmed to have severe dengue, constituting 11.3 % of the study population. This specific group displayed heightened clinical symptoms and severe abnormalities in their blood parameters. The determination of severe dengue was made using both clinical observations and laboratory assessments

Table 1: Cross-tabulated Clinical and Laboratory Markers of Acute Dengue Infection with respect to Age, Gender, and Duration of Fever to ascertain associated severity. (n=106)

Clinical Markers & Laboratory Markers	Age (years) 16-24 (n=73)	>24 (n=33)	P-value	Gender Male (n=67)	Female (n=39)	P-value	Duration of Fever (days) <3 days (n=43)	>3 days (n=63)	P-value
Vomiting									
Yes	31 (42.5%)	9 (27.3%)	0.135	29 (43.3%)	11 (28.2%)	0.122	17 (39.5%)	23 (36.5%)	
No	42 (57.5%)	24 (72.7%)		38 (56.7%)	28 (71.8%)		26 (60.5%)	40 (63.5%)	0.752
Myalgia	0.002								
Yes	41 (56.2%)	8 (24.2%)	0.678	32 (47.8%)	17 (43.6%)	0.002	18 (41.9%)	31 (49.2%)	
No	32 (43.8%)	25 (75.8%)		35 (52.2%)	22 (56.4%)		25 (58.1%)	32 (50.8%)	0.456
Petechiae									
Yes	33 (45.2%)	11 (33.3%)	0.251	32 (47.8%)	12 (30.8%)	0.087	15 (34.9%)	29 (46.0%)	
No	40 (54.8%)	22 (66.7%)		35 (52.2%)	27 (69.2%)		28 (65.1%)	34 (54.0%)	0.253
Leucopenia									
Yes	69 (94.5%)	23 (69.7%)	<0.001	61 (91.0%)	31 (79.5%)	0.267	35 (81.4%)	57 (90.5%)	
No	4 (5.5%)	10 (30.3%)		6 (9.0%)	8 (20.5%)		8 (18.6%)	6 (9.5%)	0.175
Thrombocytopenia									
Yes	63 (86.3%)	26 (78.8%)	0.507	59 (88.1%)	30 (76.9%)	0.132	33 (76.7%)	56 (88.9%)	
No	10 (13.7%)	7 (21.2%)		8 (11.9%)	9 (23.1%)		10 (23.3%)	7 (11.1%)	0.057
AST/ALT > 1.5									
Yes	70 (95.9%)	24 (72.7%)	<0.001	63 (94.0%)	31 (79.5%)	0.09	34 (79.1%)	60 (95.2%)	
No	3 (4.1%)	9 (27.3%)		4 (6.0%)	8 (20.5%)		9 (20.9%)	3 (4.8%)	0.010

Table 2: Correlation Analysis of Clinical and Laboratory Parameters with Dengue Severity

Parameter	Correlation Coefficient	P-Value
Myalgia	0.35	0.002
Leucopenia	0.45	<0.001
Elevated AST/ALT	0.50	<0.001
Duration of Fever	0.4	0.010

A detailed correlation analysis of clinical and laboratory parameters with dengue severity showed a significant association between the severity of dengue and an elevated AST/ALT ratio (correlation coefficient = 0.45, $P < 0.001$). No other clinical or laboratory markers showed a significant correlation with the severity of the disease, as shown in Table number 2. This finding underscores the AST/ALT ratio as a predictive biomarker for severe dengue, aiding in early identification and intervention.

The case fatality rate within this cohort was found to be 1.9%, with two deaths reported among the 106 patients studied. This statistic highlights the critical need for vigilant clinical monitoring and prompt therapeutic intervention in patients showing signs of severe dengue.

Table 3: Clinical and Laboratory Indicators of Acute Dengue infection

Indicator	Found in no. of cases	%
Vomiting	40	37.9
Myalgias	49	46.5
Petechiae	44	41.8
Leucopenia	92	86.8
Thrombocytopenia	89	84.0
AST/ALT >1.5	94	88.7

Discussion

Effective dengue care requires early case detection. However, test confirmation was not required, according to the 2009 revised symptom-based clinical care guidelines published by the World Health Organization. The medical community is concerned about the scope of the revised dengue case classification because the WHO's 2009 recommendations, which advocate for symptom-based clinical care, do not require test proof. Many clinicians view the updated classification of dengue patients as being unduly wide. Clinicians are divided on this position, many contend that the updated classification of dengue cases is unduly inclusive [9]. Precise identification of dengue infection becomes critical since the main objectives of dengue control are to eliminate the emergence of severe cases and

validate suspected cases. Generally, it has been observed worldwide that setting up universal laboratories specifically for diagnosing dengue infections is neither efficient nor cost-effective. As a result, determining whether patients need diagnostic testing and whether to use serological or virological techniques becomes crucial in the context of local clinical practice.

This study evaluated the clinical and laboratory characteristics of 106 young people who had been diagnosed with dengue illness. Our study's average age was 22.5 years old. Similar correlations have been reported in the literature to date as also found by Thai KT et al in a study that young individuals are commonly impacted [10]. The results show a preponderance of men (63.2%), which is consistent with research conducted by Muhammad A. et al. that found the same predilection of dengue fever incidence by gender in Pakistan [11]. Our study has revealed vomiting as a prominent symptom (37.9%) which is almost the same percentage as revealed in a study carried out by Sushma M et al [12].

Our study's initial laboratory evaluations showed a mean platelet count of 99 × 10^3/cmm and a white blood cell count of 3266.5/mm, which are indicative of the leukopenia and thrombocytopenia that are commonly associated with dengue infections. The elevated liver enzymes, with mean values of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) at 146 U/L and 80.5 U/L, respectively, further demonstrate the significant hepatic involvement in our sample. Bhattarai BR et al. also found the same outcomes and some additional features concerning laboratory markers in a study on the association of hematological and biochemical parameters of Acute Dengue Infection during Dengue outbreaks in Nepal [13]. Swamy et al in a study also found significantly elevated AST and ALT levels with additional features of hypoalbuminemia and A/G ratio. All parameters were found markedly raised in severe dengue [14]. Further research is necessary to determine the relationship between the severity of the sickness and the levels of enzymes, as the elevated liver enzymes in our sample could point to a more serious dengue infection.

It might be challenging to differentiate acute dengue virus infection from other types of febrile illnesses due to the variability of the early symptom/sign set[15]. According to our study, when the clinical indicators of acute dengue infection were evaluated, 37.9% (n=40) had vomiting, 46.5% (n=49) had myalgia and 41.8% (n=44) had petechiae. Acute dengue infection laboratory indicators showed that 86.8% (n=92) had leucopenia, 84.0% (n=89) had thrombocytopenia and 88.7% (n=94) had AST/ALT > 1.5 (Table 3). One similar study revealed clinical parameters such as vomiting, persistent vomiting, abdominal pain and bleeding manifestations (petechiae, mucosal bleeding, and GI bleeding), and hepatic manifestations in the form of hepatomegaly and elevated AST and ALT levels[16]. Mohan Lal Kanojia et al in a study, recorded fever, myalgia, arthralgia, abdominal pain, vomiting, anorexia, altered taste, and skin rashes as clinical features [17]. A study conducted by A. S. Adam et al. identified clinical and laboratory features that align with those reported in various other studies[18]. The revealed features included mucosal bleeding, abdominal pain, myalgia, petechiae, headache, and vomiting. Parameters like SGOT, SGPT, PT, and aPTT were found abnormal. A prior study conducted in Thailand revealed that children who had dengue had higher rates of anorexia, vomiting, and positive tourniquet test and increased plasma AST and ALT levels, decreased absolute neutrophil and monocyte counts, and decreased total white blood cell count[19].

Notably, according to the World Health Organization's criteria, which include severe clinical symptoms and major hematological derangements, 12 participants or 11.3% of the study population were diagnosed with severe dengue. It is especially alarming because more than 10% of the cohort had severe dengue, which suggests that dengue virus infections may have detrimental effects on one's health. In a research, Tsheten et al. meta-analyzed 143 articles with total 13090 individuals that revealed risk factors of severe dengue as being a child, secondary infection, patients with pre-existing diabetes and renal disease. Warning signs strongly associated with severe disease were increased

haematocrit with a concurrent decrease in platelet count, abdominal pain, lethargy, vomiting, hepatomegaly, ascites, pleural effusion and melena[20].

The percentage of severe cases in this study is consistent with international research, which indicates that depending on the region and dengue virus serotype involved, between 5% and 20% of dengue infections escalate to severe forms [21]. This distinction highlights the unpredictability of dengue infection severity and the importance of promptly identifying and treating potentially dangerous cases. A key finding of our research is the significant association between a high AST/ALT ratio and dengue severity, with a correlation coefficient of 0.45 and a P-value less than 0.001. The significance of this study lies in the identification of the AST/ALT ratio as a critical marker for severe dengue.

Saghir et al in recent study conducted in Pakistan founded elevated ALT levels in patients with dengue fever, DHF and DSS [22]. Severe dengue can exacerbate the rise of AST relative to ALT because of substantial capillary leakage and subsequent organ involvement; this is generally more evident in cases of muscle damage or mitochondrial destruction. Singh et al in a study advocated utility of serum transaminases in predicting disease severity in dengue fever. This separates the AST/ALT ratio as a unique signal because said study highlights only utility of either AST or ALT [23]. Our findings support the value of the inclusion of the AST/ALT ratio in regular evaluations of patients with dengue fever. The strong correlation shown in our study adds to the growing body of evidence suggesting that the AST/ALT ratio should be considered a critical component of the clinical assessment of dengue fever.

The findings of our study indicate a 1.9% case fatality rate (CFR), with two deaths among the 106 people who were looked at being recorded. This CFR emphasizes the possible fatality of dengue fever, especially in cases where it progresses to more severe forms. This rate highlights the significance of effective clinical management and prompt treatment interventions, even if it is within the lower range of CFRs reported globally, which can vary greatly depending on local healthcare resources and ways to manage the condition. The CFR that we discovered in our sample agrees with other studies that were conducted in similar settings using accepted dengue control practices. One Study revealed that total of 48,906 cases including 183 deaths (case fatality ratio (CFR): 0.4%) were reported Khyber Pakhtunkhwa (KPK) from January to November 2021 [24]. In this study, CFR is somehow on lower side. The study's slightly lower CFR could be the consequence of effective early intervention strategies or differences in the virulence of the dengue strains that were observed. These factors could all affect how the illness progressed and how severe it got. According to a recent report, comparative study regarding hematological parameters in patients with dengue fever and other febrile illnesses was carried out that showed thrombocytopenia, neutropenia and lymphopenia as prominent findings in dengue patients[25]. In addition to the history and physical examination, straightforward clinical and laboratory markers can be used as an adjuvant. Though, specificity of dengue diagnostic tests is far accurate but sensitivity still falls behind so it lays potential cost of universal laboratory diagnostic screening[26].

Furthermore, our results validate ongoing attempts to improve risk stratification models that more precisely predict catastrophic outcomes in dengue patients. Healthcare providers can more effectively identify patients who are at a higher risk of serious complications or death by combining indicators such as initial clinical presentation, test results, and demographic data. These findings show how important it is for healthcare workers to have ongoing training and resources, especially in endemic areas. Improving their capacity to manage dengue successfully addresses urgent health issues and supports larger public health initiatives meant to lessen the disease's overall burden. As dengue infection is endemic in our country and two pandemics remained very catastrophic and claimed many lives, it is need of an hour that simple diagnostic tools may be adopted for early diagnosis of infection to prevent such pandemics. Pakistan shares a huge burden of disease and has many re-

resource-poor setups in far and remote areas where serological and virological detection facilities are not available, these simple tools may guide clinicians in early diagnosis of dengue infection and thus may help prevent outbreaks as well as complicated cases. Our study suggests that these clinical and laboratory results could act as predictors to encourage early dengue infection detection and can help clinicians make rational clinical decisions during the evaluation of a patient with suspected DF in resource-poor setups, where the facility of detection of serum NS1 antigen is not available.

However, our study does face limitations, including its relatively small sample size and the confinement to a single geographic area and single-center study, which may affect the generalizability of the results. The non-probability consecutive sampling technique was employed, which can hinder the generalization of the findings to the entire population. To further corroborate these results, future studies should try to include a broader, more diversified population from a variety of geographical areas.

Conclusion

Clinicians worldwide continue to face difficulties in accurately diagnosing dengue infection early due to its non-specific early symptoms. These laboratory and clinical results could act as predictive markers to encourage early detection of dengue infection and can help clinicians make rational clinical decisions during the evaluation of a patient with suspected dengue fever in resource-poor setups, where the facility of detection of serum NS1 antigen is not available.

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