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ORIGINAL RESEARCH

Study on parameters of severity in Dengue infection with both NS1 Antigen and Dengue IgM reactive on the same day, in a tertiary care hospital

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ABSTRACT

Aim: The study aimed to evaluate clinical, hematological, and biochemical parameters associated with disease severity in patients diagnosed with dengue infection, who were simultaneously reactive for both NS1 antigen and Dengue IgM antibody on the same day of testing, in a tertiary care hospital setting. **Material and Methods:** This hospital-based, observational cross-sectional study was conducted over six months in the Department of Microbiology at a tertiary care teaching hospital. A total of 125 adult patients with clinical suspicion of dengue fever and dual positivity for NS1 antigen and Dengue IgM (confirmed via ELISA) were enrolled consecutively. Clinical profiles, laboratory investigations (CBC, LFT, RFT, electrolytes), and WHO 2009 severity classification were documented and analyzed. Patients were categorized into dengue without warning signs, dengue with warning signs, and severe dengue. Data were statistically evaluated using SPSS version 25.0, with $p < 0.05$ considered significant. **Results:** The most affected age group was 26–35 years (27.20%), with a male predominance (60.80%). Based on WHO classification, 44.80% had dengue with warning signs, and 14.40% progressed to severe dengue. Fever was the most common symptom (100.00%), followed by headache (71.20%) and myalgia (61.60%). Laboratory markers correlated significantly with disease severity. Platelet count showed a significant decline ($p < 0.001$), while hematocrit, liver enzymes (ALT, AST), and creatinine levels increased with severity ($p < 0.05$). Hypoalbuminemia, hyponatremia, and hyperkalemia were also significantly associated with severe cases. **Conclusion:** Simultaneous NS1 and IgM positivity in dengue patients represents a critical diagnostic window where early identification of severity predictors is crucial. Clinical monitoring, coupled with laboratory trends in platelets, hematocrit, liver enzymes, albumin, and renal function, is vital for effective triage and management of severe dengue.

Keywords: Dengue severity, NS1 antigen, IgM antibody, thrombocytopenia, biochemical markers

INTRODUCTION

Dengue fever, a mosquito-borne viral infection, has emerged as one of the most significant public health concerns in tropical and subtropical regions across the globe. The disease is primarily transmitted by the Aedes aegypti mosquito, with Aedes albopictus serving as a secondary vector in some regions. Dengue virus (DENV), belonging to the Flaviviridae family, was historically known to exist in four antigenically distinct serotypes (DENV-1 to DENV-4), each capable of inducing a broad spectrum of clinical manifestations. The discovery of a potential fifth serotype (DENV-5) has further complicated the global landscape of dengue management, posing new challenges in diagnosis, vaccine development, and outbreak control.[1]

The global burden of dengue has risen dramatically over the last few decades, with nearly half of the world's population now at risk. Rapid urbanization, increased human mobility, climate change, and the adaptability of Aedes mosquitoes to peri-domestic environments have all contributed to the resurgence and expansion of dengue-endemic areas. The viral infection exhibits a cyclical pattern of outbreaks, often overwhelming healthcare infrastructure, particularly in densely populated, resource-constrained settings. Dengue infection typically presents as an acute febrile illness but can escalate into severe manifestations, including dengue hemorrhagic fever (DHF) and

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dengue shock syndrome (DSS), both of which can be fatal without timely medical intervention.[2]

The disease's clinical presentation varies significantly depending on host factors, previous exposure to different serotypes, and the virulence of the infecting strain. A primary infection often results in a self-limiting illness, while secondary infection with a heterologous serotype increases the risk of severe disease due to a phenomenon known as antibody-dependent enhancement (ADE). This immunopathological mechanism, wherein pre-existing non-neutralizing antibodies facilitate enhanced viral entry and replication in host cells, underscores the complexity of dengue pathogenesis and the difficulty in developing an effective, universally protective vaccine.[3]

One of the more recent public health developments concerning dengue has been the identification of the fifth serotype (DENV-5), which was first reported in a sylvatic cycle in Malaysia. While still not widely circulating in urban environments, its emergence raises important epidemiological and immunological concerns. Existing vaccines and diagnostic tools have been developed based on the four classical serotypes. The introduction of a new serotype may compromise the effectiveness of these interventions and necessitate the redesign of both therapeutic and preventive strategies.[4]

In addition to horizontal transmission via mosquito vectors, dengue virus is also capable of vertical transmission, wherein infected female mosquitoes can transmit the virus to their offspring. This mode of transmission ensures the persistence of the virus in vector populations even in the absence of active human infections. Such a mechanism complicates efforts aimed at vector control and underscores the need for continuous entomological surveillance and community-level interventions. Understanding vertical transmission dynamics is particularly crucial in regions that experience seasonal dengue outbreaks, as it may contribute to the early onset of transmission cycles during subsequent seasons.[5]

From a neuropathological standpoint, dengue is no longer viewed solely as a febrile illness confined to hematological and vascular involvement. Neurological complications such as encephalitis, encephalopathy, and Guillain-Barré syndrome are increasingly being recognized, indicating that dengue virus is neurotropic in certain cases. These manifestations can occur either due to direct viral invasion of the central nervous system or as a result of systemic inflammatory responses. Neurological involvement, though less common, often leads to prolonged hospital stays, higher healthcare costs, and increased morbidity, particularly in vulnerable populations.[6]

The pathogenesis of dengue involves complex interactions between the virus, host immune response, and genetic factors. Following inoculation by the mosquito vector, the virus primarily targets dendritic cells and macrophages, leading to widespread viremia. The host immune response, particularly the production of cytokines and chemokines, plays a central role in symptom development. However, in severe cases, dysregulated immune responses can result in plasma leakage, coagulopathy, and multi-organ dysfunction. The molecular and cellular pathways implicated in severe dengue are still being elucidated, and further research is required to identify reliable prognostic markers and therapeutic targets.[7]

Seroprevalence studies remain a critical component of understanding dengue epidemiology, particularly in endemic countries like India. They provide insight into population-level immunity, identify high-risk groups, and guide public health interventions. These findings emphasize the importance of region-specific data in tailoring effective control measures and resource allocation.

MATERIAL AND METHODS

This hospital-based, observational cross-sectional study was conducted in the Department of Microbiology at a tertiary care teaching hospital over a period of six months, following approval from the Institutional Ethics Committee. The primary objective of the study was to evaluate clinical, hematological, and biochemical parameters associated with the severity of dengue infection in patients simultaneously reactive for both NS1 antigen and Dengue IgM antibody on the same day of testing.A total of 125 patients aged 18 years and above, who presented to the outpatient or emergency departments with clinical suspicion of dengue fever and subsequently tested positive for both NS1 antigen and Dengue IgM antibody using ELISA-based kits, were enrolled consecutively after obtaining informed written consent.

Inclusion Criteria

- Patients aged ≥18 years.
- Clinical symptoms suggestive of dengue infection (fever with or without headache, myalgia, retro-orbital pain, rash, or bleeding manifestations).
- Simultaneous positivity for both NS1 antigen and Dengue IgM antibody on the same day of illness.
- Willingness to participate and provide informed consent.

Exclusion Criteria

- Patients with isolated positivity for either NS1 antigen or IgM only.
- Patients with known hematological disorders or chronic liver/kidney diseases.
- Cases of co-infection with malaria, chikungunya, typhoid, or leptospirosis.
- Pregnant women and immunocompromised individuals.

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Data Collection and Laboratory Analysis

Detailed clinical history including duration of fever, presence of warning signs (abdominal pain, persistent vomiting, mucosal bleeding, lethargy, hepatomegaly), and evidence of plasma leakage or shock was recorded at the time of admission. Physical examination and daily monitoring of vital signs and fluid status were performed.Venous blood samples were collected at admission for complete blood count, liver function tests, renal function tests, serum albumin, and hematocrit. Platelet counts and hematocrit were monitored serially during the hospital stay. All samples were processed in the hospital's central laboratory using standardized protocols.

Patients were categorized based on WHO 2009 classification into:

- **Dengue without warning signs**
- **Dengue with warning signs**
- **Severe dengue**

Clinical outcomes such as duration of hospitalization, need for ICU admission, presence of bleeding manifestations, and recovery status were recorded.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 25.0. Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages. Chi-square test and Student's t-test were used for comparison of categorical and continuous variables, respectively. A p-value of <0.05 was considered statistically significant.

RESULTS

Table 1: Demographic Distribution of Dengue Patients

The study included a total of 125 dengue-positive patients who were simultaneously reactive for both NS1 antigen and Dengue IgM on the same day. The most affected age group was 26–35 years, comprising 27.20% (n = 34) of the total cases, followed by the 36–45 years group at 24.00% (n = 30). Patients aged 18–25 years accounted for 17.60% (n = 22), while those aged 46–55 and >55 years comprised 16.80% (n = 21) and 14.40% (n = 18), respectively. Statistical analysis using the Chi-square test revealed no significant association between age group and severity of dengue infection ($p = 0.312$).

Regarding gender distribution, males constituted the majority of the cohort with 60.80% (n = 76), while females represented 39.20% (n = 49). This indicates a male preponderance among dengue-positive individuals in this study, although gender-wise comparison was not subjected to statistical significance testing in this table.

Table 2: Distribution Based on WHO 2009 Classification

Based on the WHO 2009 dengue classification, the largest proportion of patients fell into the "dengue with warning signs" category, which comprised 44.80% (n = 56) of cases. This was followed by 40.80% (n = 51) of patients categorized under "dengue without warning signs." Notably, 14.40% (n = 18) of patients developed severe dengue. This distribution highlights that nearly half of the study participants exhibited warning signs at presentation, while a significant minority progressed to severe disease, underlining the clinical importance of early detection and monitoring for severity indicators.

Table 3: Common Clinical Features Among Dengue Patients

Fever was a universal symptom, present in 100.00% (n = 125) of the study participants, making it the most consistent clinical presentation. Other commonly observed symptoms included headache in 71.20% (n = 89) and myalgia in 61.60% (n = 77) of cases. Retro-orbital pain was reported by 43.20% (n = 54), and gastrointestinal involvement was evident through vomiting in 38.40% (n = 48) and abdominal pain in 29.60% (n = 37). Signs of severity such as mucosal bleeding were seen in 16.80% (n = 21), while rash was present in 13.60% (n = 17). Hepatomegaly was detected in 12.00% (n = 15) and evidence of plasma leakage, indicated by ascites or pleural effusion, was documented in 8.00% (n = 10). These findings emphasize the wide clinical spectrum of dengue and its potential to affect multiple organ systems.

Table 4: Laboratory Parameters According to Disease Severity Classification

Significant laboratory differences were observed across the three clinical severity categories of dengue. Mean platelet count decreased progressively with disease severity: 98.6 ± 24.3 ×10⁹/L in dengue without warning signs, 75.4 ± 21.1 ×10⁹/L in dengue with warning signs, and 48.7 ± 18.5 ×10⁹/L in severe dengue ($p< 0.001$). Similarly, hematocrit values increased significantly with severity (39.1 ± 3.6%, 41.4 ± 4.1%, and 44.7 ± 4.5%, respectively; $p = 0.002$), reflecting hemoconcentration due to plasma leakage.

Serum albumin levels declined from 3.8 ± 0.4 g/dL in mild cases to 2.9 ± 0.6 g/dL in severe cases ($p< 0.001$), indicating capillary permeability and protein loss in severe dengue. Hepatic involvement was evident from elevated transaminases: ALT and AST levels were significantly higher in severe dengue (ALT: 92.4 ± 31.2 U/L, AST: 106.3 ± 34.8 U/L) compared to non-severe groups ($p = 0.008$ and $p = 0.005$, respectively).

The total leukocyte count was lowest in severe dengue (3.6 ± 1.5 ×10⁹/L), compared to higher values in dengue without warning signs (4.3 ± 1.2 ×10⁹/L), with a statistically significant difference ($p = 0.041$). Renal function, as measured by serum creatinine, showed a significant upward trend with severity ($p = 0.014$), rising to 1.4 ± 0.4 mg/dL in the severe group.

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Electrolyte imbalances were also observed. Serum sodium levels dropped significantly with increasing severity (from 135.6 ± 3.1 mEq/L to 131.2 ± 4.5 mEq/L; $p = 0.021$), suggesting hyponatremia due to plasma leakage and fluid redistribution. Potassium levels showed a rising trend (from 4.1 ± 0.4 mEq/L to 4.6 ± 0.6 mEq/L; $p = 0.038$), which could reflect renal involvement or hemolysis.

These laboratory findings underscore the critical importance of serial monitoring of hematological, hepatic, renal, and electrolyte parameters in dengue patients, as they can serve as early indicators of disease progression and guide timely intervention.

Table 1: Demographic Distribution of Dengue Patients

Variable	Category	Frequency	Percentage (%)	*p*-value
Age Group (years)	18–25	22	17.60	
	26–35	34	27.20	
	36–45	30	24.00	
	46–55	21	16.80	
	>55	18	14.40	0.312[1]
Gender	Male	76	60.80	
	Female	49	39.20	—

[1]*p*-value calculated using Chi-square test comparing age distribution with disease severity (not significant).

Table 2: Distribution Based on WHO 2009 Classification (n = 125)

Dengue Category	Frequency	Percentage (%)
Dengue without warning signs	51	40.80
Dengue with warning signs	56	44.80
Severe dengue	18	14.40
Total	**125**	**100.00**

Table 3: Common Clinical Features Among Dengue Patients (n = 125)

Clinical Feature	Frequency	Percentage (%)
Fever	125	100.00
Headache	89	71.20
Myalgia	77	61.60
Retro-orbital pain	54	43.20
Vomiting	48	38.40
Abdominal pain	37	29.60
Mucosal bleeding	21	16.80
Rash	17	13.60
Hepatomegaly	15	12.00
Ascites/Pleural effusion	10	8.00

Table 4: Laboratory Parameters According to Disease Severity Classification

Parameter	Dengue without WS (n = 51)	Dengue with WS (n = 56)	Severe Dengue (n = 18)	*p*-value
Platelet count (×10⁹/L)	98.6 ± 24.3	75.4 ± 21.1	48.7 ± 18.5	<0.001
Hematocrit (%)	39.1 ± 3.6	41.4 ± 4.1	44.7 ± 4.5	0.002
Serum albumin (g/dL)	3.8 ± 0.4	3.3 ± 0.5	2.9 ± 0.6	<0.001
ALT (U/L)	56.8 ± 20.3	71.6 ± 22.7	92.4 ± 31.2	0.008
AST (U/L)	62.5 ± 18.9	84.2 ± 25.6	106.3 ± 34.8	0.005
Total leukocyte count (×10⁹/L)	4.3 ± 1.2	4.0 ± 1.4	3.6 ± 1.5	0.041
Serum creatinine (mg/dL)	0.9 ± 0.2	1.1 ± 0.3	1.4 ± 0.4	0.014
Sodium (mEq/L)	135.6 ± 3.1	133.8 ± 3.7	131.2 ± 4.5	0.021
Potassium (mEq/L)	4.1 ± 0.4	4.3 ± 0.5	4.6 ± 0.6	0.038

WS = Warning Signs.

DISCUSSION

The demographic findings from Table 1 indicate that the age group most commonly affected by dengue was 26–35 years, followed by those aged 36–45 years. This is consistent with studies such as Shah et al. (2019) and Dhivya Lakshmi et al. (2018), who reported similar age predilections in dengue cases, suggesting that younger adults in the working population may be more exposed to mosquito vectors due to increased outdoor activity.[6,7] The observed male preponderance (60.80%) is in line with prior findings by Suresh (2021) and Patel (2018), where

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higher incidence in males was attributed to greater occupational exposure and less frequent use of protective clothing.[8,9] However, in the present study, the age distribution did not show a statistically significant association with disease severity, which echoes the findings of Elaagip et al. (2020), who also reported no strong correlation between age and progression to severe dengue.[10]

The severity classification in Table 2, based on WHO 2009 guidelines, revealed that 44.80% of patients presented with warning signs and 14.40% developed severe dengue. These proportions are clinically significant and support the observations of World Health Organization (2009), which highlighted that early warning signs are predictive of progression to severe disease.[11]The distribution in our study aligns with the seroprevalence trends reported by Chukwuma (2018) in Africa and Suresh (2021) in India, where a notable fraction of patients transitioned from non-severe to severe forms. This underscores the need for clinicians to be vigilant for early warning signs, especially in endemic regions.[12,8]

In Table 3, fever was present in 100% of cases, affirming its role as the most universal symptom in dengue infection. Headache (71.20%) and myalgia (61.60%) were also prevalent and comparable to the symptomatology reported by Shah et al. (2019) and Elaagip et al. (2020).[6,10] Retro-orbital pain and gastrointestinal symptoms like vomiting and abdominal pain were frequently observed and often coincided with the transition to warning sign stages. Interestingly, mucosal bleeding and rash were less common but important indicators of possible severe dengue, a finding also highlighted in studies by Dhivya Lakshmi et al. (2018). Hepatomegaly and signs of plasma leakage such as ascites or pleural effusion, although less frequent, were mostly associated with severe disease stages, consistent with clinical patterns described in the WHO classification.[7]

Laboratory parameters presented in Table 4 demonstrated statistically significant alterations across the severity spectrum. The progressive thrombocytopenia seen with increasing severity of illness reinforces its role as a hallmark indicator of dengue progression, corroborated by studies such as Whitehead et al. (1971) and World Health Organization (2009).[13,11] The rise in hematocrit with disease severity reflects hemoconcentration due to plasma leakage, a known predictor of severe dengue. The decline in serum albumin levels is indicative of capillary leak syndrome and correlates well with severity, as emphasized by Simon and Rangel (2021), who examined environmental and pathophysiological contributors to dengue outcomes.[14]

Elevated liver enzymes, especially AST and ALT, were prominent in the severe dengue group, suggesting hepatic involvement—a well-documented feature in dengue pathology reported by Shah et al. (2019) and Patel (2018). Leukopenia, often observed in viral infections, showed significant decline with severity, supporting its diagnostic utility. Serum creatinine showed a notable rise in severe cases, highlighting potential renal involvement, a complication that, although less common, warrants close monitoring.[6,9]

Electrolyte disturbances were also evident. The observed hyponatremia is likely due to increased vascular permeability and hemodilution, as suggested in the work of Elaagip et al. (2020). Rising potassium levels, possibly attributable to hemolysis or early renal compromise, were more frequent in severe cases, further emphasizing the systemic impact of dengue and the importance of comprehensive biochemical monitoring.[10]

Taken together, the findings from this study align closely with existing literature and WHO guidelines. They reinforce the utility of a combined clinical-laboratory approach in predicting disease severity. Early identification of warning signs, coupled with regular monitoring of platelets, hematocrit, serum albumin, liver enzymes, and renal and electrolyte parameters, can significantly improve clinical outcomes and reduce dengue-related morbidity and mortality.

CONCLUSION

This study highlights that dengue infection, particularly in individuals simultaneously positive for NS1 antigen and IgM antibody, exhibits a wide clinical and laboratory spectrum, with a significant proportion progressing to severe forms. Key predictors of severity included thrombocytopenia, rising hematocrit, hypoalbuminemia, elevated transaminases, and renal dysfunction. Early recognition of warning signs and routine monitoring of laboratory parameters are essential for timely intervention. A combined clinical and biochemical assessment can significantly reduce complications and improve patient outcomes in dengue management.

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