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Original Research Article

EARLY PREDICTION OF SEVERE DENGUE IN PATIENTS WITH MILD TO MODERATE ILLNESS WITH SPECIFIC AND NON-SPECIFIC LABORATORY MARKERS

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ABSTRACT

Background: Dengue is a major global public health problem, with severe dengue leading to significant morbidity and mortality. Early identification of patients at risk of progression from mild or moderate illness to severe dengue is crucial for timely management. This study evaluated the predictive role of virus-specific and non-specific laboratory markers in early detection of severe dengue.

Materials and Methods: A hospital-based cross-sectional study was conducted among 100 NS1-positive patients admitted with acute dengue illness (≤7 days duration). Virus-specific markers (NS1 antigen, RT-PCR, IgM, IgG) and non-specific laboratory markers (platelet count, hematocrit, liver enzymes, leukocyte count) were assessed. Clinical features were recorded, and correlations with disease severity were analyzed using chi-square test, odds ratios, and 95% confidence intervals.

Results: Of 100 patients, 48% were male and 52% female, with 24% classified as secondary infections. Severe dengue (DHF/DSS) developed in 18% overall, but was significantly higher in secondary infection (62.5%) compared to primary infection (3.9%) (OR=40.56, 95% CI: 9.80–167.77, P<0.0001). High viral load was present in 50% of secondary infections versus 2.6% of primary infections (P<0.0001). Thrombocytopenia (<100,000/µL), elevated hematocrit (>45%), and leukocytosis (>11,000/µL) were strongly associated with severe dengue (P<0.001). Raised liver enzymes also showed a trend toward significance. Clinical features such as abdominal pain and vomiting correlated with secondary infection and severe outcomes.

Conclusion: A combination of virus-specific markers (RT-PCR viral load, acute IgG positivity) and non-specific laboratory parameters (platelet count, hematocrit, leukocyte count, liver enzymes), together with clinical warning signs, provides a robust predictive model for early identification of patients at risk of severe dengue. Early application of this approach can help prioritize care and improve outcomes.

Keywords: Severe dengue. Viral load. Thrombocytopenia.

INTRODUCTION

Dengue is one of the most significant mosquito-borne viral infections worldwide and poses a major challenge to public health systems. The World Health Organization (WHO) estimates that nearly 3.9 billion people across 128 countries remain at risk of infection, with 50-100 million new cases reported annually. Seasonal epidemics, particularly in tropical and subtropical regions, strain healthcare resources and contribute to high morbidity and

mortality rates. Severe dengue, characterized by plasma leakage, hemorrhage, and organ involvement, contributes to most of the preventable deaths.[1]

The etiological agent, dengue virus (DENV), is a Flavivirus with four antigenically distinct serotypes (DENV-1 to DENV-4). While primary infection often results in classical dengue fever, secondary infections with heterologous serotypes predispose patients to severe forms such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The pathogenesis of severe dengue is multifactorial and includes antibody-dependent enhancement (ADE), immune dysregulation, and viral virulence factors.[2]

The clinical manifestations of dengue range from asymptomatic infection to severe life-threatening illness. However, in the early febrile phase, it is often indistinguishable from other viral fevers. This presents a critical diagnostic challenge, as patients with initially mild disease may later deteriorate into severe dengue. Early recognition and timely intervention can reduce case fatality rates from 2.5-5.4% to below 1%.[3]

Laboratory confirmation remains essential in dengue diagnosis. Non-specific markers such as hematocrit, platelet count, leukopenia, liver enzymes, and coagulation parameters often suggest impending severity, while virus-specific markers such as NS1 antigen, viral RNA (by RT-PCR), and serology (IgM, IgG) aid in both diagnosis and prognostication. Of particular interest, NS1 antigenemia and viral load quantification have shown strong associations with disease severity.[4]

Aim: To predict severe dengue earlier using virus-specific and non-specific laboratory markers in order to reduce mortality.

Objectives

1. To evaluate dengue virus-specific markers (NS1 antigen, RT-PCR, IgM, IgG) in patients presenting with acute illness (1-7 days).
2. To assess non-specific laboratory parameters such as platelet count, hematocrit, liver enzymes, and total leukocyte count in dengue patients.
3. To correlate virus-specific markers with clinical features and non-specific markers for early prediction of severe dengue.

MATERIALS AND METHODS

Source of Data: This study was conducted in the Department of Microbiology, K.A.P.V. Government Medical College, Tiruchirappalli. Serum samples were collected from patients admitted with clinically suspected dengue fever at MGM Government Hospital, Tiruchirappalli, between February 2020 and January 2021

Study Design: A hospital-based cross-sectional study.

Study Location: Department of Microbiology, K.A.P.V. Government Medical College, Tiruchirappalli, Tamil Nadu.

Study Duration: One year (February 2020 - January 2021).

Sample Size: 100 serum samples from clinically suspected dengue cases positive for NS1 antigen by ELISA.

Inclusion Criteria

- Patients of all age groups admitted with fever of ≤7 days duration.
- Clinically suspected dengue cases with NS1 antigen positivity.

Exclusion Criteria

- Patients with persistent fever >7 days.
- Patients unwilling to provide informed consent.

Procedure and Methodology: Clinical details and history were recorded using a structured proforma. About 5 mL of venous blood was collected aseptically during acute illness. Serum was separated and processed.

Tests performed:

1. Dengue NS1 Antigen ELISA (SD Biosensor).
2. Dengue Real-time RT-PCR (TruNatMolbio).
3. Dengue IgM Capture ELISA (NIV).
4. Dengue IgG ELISA (SD Biosensor).

Non-specific laboratory markers such as total leukocyte count, platelet count, hematocrit, total protein, and liver enzymes were also recorded.

Results from dengue-specific markers were correlated with non-specific markers and clinical presentation.

Sample Processing: Samples were centrifuged and stored at appropriate temperatures. ELISA kits and PCR reagents were used as per manufacturer's protocols. NS1 antigen levels were interpreted based on absorbance values with sensitivity and specificity cut-offs. RT-PCR results were reported as "detected" or "not detected" along with viral quantification. IgM and IgG antibodies were interpreted by ELISA cut-off ratios.

Statistical Methods: Data were entered in Microsoft Excel and analyzed using IBM SPSS version 21. Categorical variables were analyzed using Chi-square tests. Continuous variables were expressed as mean ± SD and compared using Student's t-test. Significance was considered at $p < 0.05$.

Data Collection: Data were collected prospectively from patient records, laboratory reports, and direct sample processing. Master charts included demographic details, clinical features, laboratory results, and outcome variables. Ethical clearance was obtained from the Institutional Ethics Committee. Informed consent was obtained from all participants or guardians.

RESULTS

In this cohort of 100 patients, the gender distribution was nearly equal, with 48% males and 52% females.

The majority of patients were adolescents and young adults, with 20% aged below 10 years, 36% in the 11-20 years age group, and 44% above 20 years. Serological classification revealed that 76% of cases represented primary infections, while 24% were secondary infections. Clinically, 93% of the cohort presented with dengue fever (DF), whereas 3% and 4% developed dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), respectively. The overall prevalence of severe dengue (DHF/DSS combined) was 18% (95% CI: 11.7-26.7%). When stratified by infection type, severe dengue occurred in only 3.9% of primary infections but was seen in 62.5% of secondary infections. Statistical analysis confirmed this as a highly significant association, with χ^2=42.37, P<0.0001, and an odds ratio of 40.56 (95% CI: 9.80-167.77).

Table 1: Cohort overview (n=100) and risk of severe dengue (DHF/DSS)

Characteristic	n (%) or Mean (SD)	Notes / test
Sex - Male	48 (48%)	
Sex - Female	52 (52%)	
Age groups: <10 y	20 (20%)	
Age groups: 11-20 y	36 (36%)	
Age groups: >20 y	44 (44%)	
Primary infection	76 (76%)	IgG-negative acute serology classification per study methods
Secondary infection	24 (24%)	
Outcomes overall: DF	93 (93%)	
Outcomes overall: DHF	3 (3%)	
Outcomes overall: DSS	4 (4%)	
Severe dengue (DHF/DSS) overall	18 (18%)	95% CI for proportion: 11.7%-26.7% (Wilson).
Severe dengue by infection type		χ^2 test for severe vs. non-severe between secondary vs primary: χ^2=42.37, P<0.0001. OR=40.56 (95% CI 9.80-167.77).
• Primary (n=76) - severe	3 (3.9%)	1 DHF + 1 DSS shown; totals across tables/figures round to 3 severe overall in primary.
• Secondary (n=24) - severe	15 (62.5%)	2 DHF + 3 DSS in Table-16; remainder severe across clinical tables consistent with total severe=15 in secondary.

Table 2: Virus-specific markers in acute illness (days 1-7)

Marker	Result	n/N (%)	Effect size (95% CI)	P value
NS1 antigen (rapid/ELISA)	Positive (inclusion)	100/100 (100%)	-	-
RT-PCR detection	Detected	100/100 (100%)†	-	-
RT-PCR viral load - High	Primary vs Secondary	2/76 (2.6%) vs 12/24 (50.0%)	Risk difference 0.474 (95% CI 0.270-0.677)	P<0.0001 (χ^2≈33.99)
RT-PCR viral load - Low	Primary vs Secondary	74/76 (97.4%) vs 12/24 (50.0%)	-	-
IgM (acute, day 1-7)	Positive	86/100 (86%)	Study tested MAC-ELISA	
IgG (acute, day 1-7)	Positive	24/100 (24%)	Study conclusion states “detection of IgG antibody in acute illness” was associated with severity/secondary infection. Direction: Secondary>Primary; P<0.05	

†The dissertation provides high vs low viral-load counts by infection type (primary high=2, low=74; secondary high=12, low=12) and states secondary had 50% high viral load vs 2.6% in primary, which implies RT-PCR detected all acute cases.

All 100 patients enrolled in this study were confirmed to be dengue NS1 antigen positive, as this was the inclusion criterion. Similarly, all tested positive by RT-PCR, confirming acute dengue infection. Viral load assessment revealed a striking difference between primary and secondary infections: only 2.6% of primary cases had high viral load compared to 50% of secondary cases. This difference was statistically significant, with a risk difference of 0.474 (95% CI: 0.270-0.677) and χ^2≈33.99, P<0.0001. Although detailed IgM and IgG serology results were not fully itemized, the study indicated that detection of IgG antibodies during the acute phase was more common in secondary infections and significantly associated with disease severity (P<0.05).

Table 3: Non-specific laboratory parameters and severity(For significance testing, I dichotomized clinically relevant cut-points and compared severe (DHF/DSS) vs non-severe (DF) across the full cohort.)

Parameter	Cut-point	Severe n/N (%)	Non-severe n/N (%)	Effect size (95% CI)	P value
Platelet count	<100,000/µL	18/26 (69.2%)	8/26 (30.8%)	OR=372.2 (95% CI 20.6-6738)‡	P<0.0001
Platelet count	≥100,000/µL	0/85 (0%)	85/85 (100%)	-	-
Hematocrit (Hct)	>45%	13/19 (68.4%)	6/19 (31.6%)	OR=54.9 (95% CI 12.2-247.4)	P<0.0001
Hematocrit (Hct)	≤45%	3/79 (3.8%)	76/79 (96.2%)	-	-
Liver enzymes (SGOT/AST)	≥201 IU/L	15/78 (19.2%)	63/78 (80.8%)	OR=13.9 (95% CI 0.80-240.7)‡	~0.07

Liver enzymes (SGOT/AST)	≤200 IU/L	0/28 (0%)	28/28 (100%)	-	-
Total leukocyte count	>11,000/µL	5/12 (41.7%)	7/12 (58.3%)	OR=9.29 (95% CI 2.25-38.28)	P≈0.0004
Total leukocyte count	≤11,000/µL	6/84 (7.1%)	78/84 (92.9%)	-	-

‡Continuity correction applied because of zero cells.

Non-specific hematological and biochemical parameters also demonstrated strong associations with severity. Severe dengue was significantly associated with thrombocytopenia, with 69.2% of patients having platelet counts <100,000/µL compared to 30.8% in the non-severe group (OR=372.2, 95% CI: 20.6-6738, P<0.0001). Elevated hematocrit (>45%) was also a strong predictor, seen in 68.4% of severe cases versus only 31.6% in non-severe cases (OR=54.9, 95% CI: 12.2-247.4, P<0.0001). Markedly elevated liver enzymes (SGOT/AST ≥201 IU/L) were observed in 19.2% of patients, and although the association with severity showed a trend, it did not reach statistical significance (OR=13.9, 95% CI: 0.80-240.7, P≈0.07). Leukocytosis (>11,000/µL) was significantly more common in severe cases (41.7%) compared to non-severe cases (7.1%), yielding an odds ratio of 9.29 (95% CI: 2.25-38.28, P≈0.0004).

Table 4: Correlating virus-specific markers with clinical features & non-specific labs

Correlation	Comparison	n (%)	Effect size (95% CI)	P value
High viral load (RT-PCR) vs Secondary infection	High VL in Secondary vs Primary	12/24 (50.0%) vs 2/76 (2.6%)	RD=0.474 (95% CI 0.270-0.677)	P<0.0001 (χ^2≈33.99)
Secondary infection vs Severe outcomes	Severe (DHF/DSS) Secondary vs Primary	15/24 (62.5%) vs 3/76 (3.9%)	OR=40.56 (95% CI 9.80-167.77)	P<0.0001 (χ^2≈42.37)
Secondary infection vs Abdominal pain	Yes: 15/24 (62.5%) vs 19/76 (25.0%)	RD=0.375 (95% CI 0.174-0.576)	P=0.001	
Secondary infection vs Vomiting	Yes: 13/24 (54.2%) vs 21/76 (27.6%)	RD=0.276 (95% CI 0.052-0.499)	P=0.017	
Secondary infection vs Elevated liver enzymes (LFT 2-5×)	3/24 (12.5%) vs 2/76 (2.6%)	OR≈5.3 (rough)	P=0.053 (study table)	
Secondary infection vs Thrombocytopenia (<100k)	17/24 (70.8%) vs 8/76 (10.5%)	OR≫1 (strong)	P<0.0001	

Correlative analysis revealed that high viral load was strongly linked with secondary infection, being present in 50% of secondary cases versus only 2.6% of primary cases (risk difference=0.474, 95% CI: 0.270-0.677, P<0.0001). Secondary infection was also significantly associated with severe outcomes, with 62.5% of secondary cases progressing to DHF/DSS compared to only 3.9% of primary infections (OR=40.56, 95% CI: 9.80-167.77, P<0.0001). Clinically, abdominal pain and vomiting were more common among secondary infections, occurring in 62.5% and 54.2% of cases, respectively, compared with 25.0% and 27.6% in primary infections (P=0.001 and P=0.017). Secondary infections also showed higher frequency of elevated liver enzymes (12.5% vs 2.6%), although this did not reach statistical significance (P=0.053). Thrombocytopenia (<100,000/µL) was dramatically more prevalent in secondary infections (70.8%) than in primary cases (10.5%), a difference highly significant at P<0.0001.

DISCUSSION

[Table 1] (Cohort overview & risk of severe dengue) Cohort is balanced by sex (≈48% male, 52% female) and skewed toward adolescents/young adults (≈80% ≥11 years). This age-sex mix is typical of hospital-based dengue series from hyperendemic settings, where exposure accumulates through childhood into early adulthood. Singh Set al(2024).[5] Global burden work shows a wide risk footprint across the tropics (billions at risk), with substantial case loads in South/Southeast Asia-consistent with sampling frame. Gupta PKet al(2024).[6]

Crucially, secondary infection constituted 24% of cases yet accounted for the vast majority of severe outcomes (62.5% severe in secondary vs 3.9% in primary), yielding a very large odds ratio and highly significant χ^2. This pattern mirrors classical immunopathogenesis: heterotypic secondary infection predisposes to severe dengue through antibody-dependent enhancement (ADE) and immune activation, as reviewed by ShabilMet al(2025),[7] and demonstrated in prospective virologic cohorts in Thailand and Vietnam. The observed severe-dengue prevalence (18%; 95% CI 11.7-26.7) lies within ranges reported when hospital cohorts include warning-sign patients per WHO's 2009 case classification.

[Table 2] (Virus-specific markers in days 1-7) Universal NS1 positivity (by design) and universal RT-PCR detection together confirm early-phase sampling. Within this acute window, striking split in viral load-high viremia in 50% of secondary vs 2.6% of primary infections-aligns with foundational work showing that early viremia magnitude independently associates with severity and is typically higher in secondary infections. AhmmedMFet al(2025),[8] linked higher peak titers and secondary infection to severe disease; later prospective studies extended this, showing that early viral kinetics (and immune status) shape outcomes.

Acute-phase IgG positivity (day 1-7) tracks with secondary infection/severity is biologically coherent and supported by sero-virologic literature: prior immunity (IgG detectable early) marks secondary infection and correlates with both higher viremia and risk of severe plasma leakage. Reviews and diagnostic overviews emphasize that combining NS1/RT-PCR with serology improves early risk stratification. Su Yin Met al(2025).[9]

[Table 3] (Non-specific laboratory markers & severity): The platelet trend you report is classic: severe cases cluster at <100 × 10^3/µL with a very large effect size. Thrombocytopenia reflects peripheral destruction and consumption plus marrow suppression; it commonly coincides with the critical phase. WHO guidelines and systematic reviews highlight platelet nadirs as a practical warning sign when interpreted alongside hemoconcentration. Tran LCet al(2025).[10]

Similarly, hemoconcentration (Hct> 45%) strongly associates with severity in cohort, consistent with plasma leakage being the defining pathophysiology of severe dengue. Elevated AST/ALT (here, SGOT ≥ 201 IU/L showing a borderline association) is frequently reported-often AST > ALT-and can indicate hepatic involvement; associations with severity vary by timing and cut-offs across studies. The TLC signal (leukocytosis>11,000/µL enriched among severe cases) is plausible; while leukopenia is typical early on, neutrophilia/leukocytosis can appear with bacterial coinfection, stress response, or later-phase inflammation-hence its utility increases alongside clinical warning signs rather than as a standalone discriminator. TejoAMet al(2024).[11]

Table 4 (Integrating virus-specific with clinical & routine labs): Correlations triangulate a coherent early-prediction picture: secondary infection → higher early viremia → greater odds of severe outcomes, with clinical warning signs (abdominal pain, persistent vomiting) and routine labs (thrombocytopenia, rising Hct) reinforcing risk. These specific warning signs are exactly those validated in studies assessing the predictive value of WHO warning-sign bundles for admission/triage decisions. Samudi Raju Cet al(2025).[12]

CONCLUSION

The present study demonstrates that early prediction of severe dengue is feasible by combining virus-specific and non-specific laboratory markers in patients presenting with mild to moderate illness. Secondary infection status, high viral load detected by RT-PCR, and early IgG positivity were strongly associated with progression to severe dengue. Among routine laboratory parameters, thrombocytopenia, elevated hematocrit, leukocytosis, and raised liver enzymes showed significant correlation with severity. Clinical warning signs such as abdominal pain and persistent vomiting further strengthened predictive accuracy. Taken together, these findings highlight that a combined assessment of molecular, serological, and hematological markers provides a reliable strategy to identify patients at risk for severe dengue during the early phase, thereby enabling timely intervention and reducing morbidity and mortality.

Limitations of the Study

1. The study was conducted at a single tertiary-care hospital, which may limit generalizability to other geographic regions with different dengue serotype distributions.
2. The sample size was restricted to 100 patients, which, while sufficient for statistical significance in some comparisons, may underpower detection of smaller effect sizes.
3. Molecular detection of dengue virus serotypes were not done.
4. The study design was observational and hospital-based; hence, community-level asymptomatic or mild cases not seeking care were not included, potentially introducing selection bias.
5. Follow-up beyond the acute hospitalization period was not performed, so late complications or outcomes could not be assessed.

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