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Association of Dengue Virus Serotypes with Disease Severity and Clinical-Hematological Parameters: A Hospital-Based Analytical Study

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Abstract

Background: Dengue disease severity varies by viral serotype and host factors. This study examined associations between dengue virus (DENV) serotypes, disease severity, and clinical-hematological parameters.

Aims & Objectives: To investigate the molecular epidemiology of circulating dengue serotypes, identify DENV-1 to DENV-4 by RT-PCR, determine their demographic and clinical distribution, assess associations with disease severity, and generate data to strengthen dengue surveillance and public health interventions.

Methods: A hospital-based cross-sectional analytical study was conducted among 290 clinically suspected dengue patients at Index Medical College Hospital and Research Centre, Indore, from January 2023 to January 2026. Serum samples were screened using the SD BIOLINE™ Dengue Duo Rapid Test, followed by viral RNA extraction and real-time RT-PCR with the RealStar® Dengue Type RT-PCR Kit 1.0. Dengue serotypes were identified, and clinical and hematological parameters were compared according to disease severity.

Results: Real-time RT-PCR confirmed dengue infection in 67 (23.1%) patients. DENV-2 was the predominant serotype (46.3%), followed by DENV-3 (26.9%), DENV-1 (20.9%), DENV-4 (3.0%), and mixed infections (3.0%). Serotype was significantly associated with disease severity ($p=0.012$). Mean platelet count declined significantly from 142,800/µL in dengue without warning signs to 42,300/µL in severe dengue ($p<0.001$). Thrombocytopenia was the most common hematological abnormality (88.1%). DENV-2 infection, platelet count <50,000/µL, bleeding manifestations, and abdominal pain were independently associated with severe dengue.

Conclusion: DENV-2 predominated and was significantly associated with severe dengue. Molecular serotyping combined with clinical and hematological assessment may facilitate early identification of patients at risk of severe disease.

Keywords: Dengue; DENV-2; Serotyping; RT-PCR; Disease severity; Thrombocytopenia; Hematological parameters.

Introduction

Dengue is a major mosquito-borne viral infection caused by dengue virus (DENV), an RNA virus belonging to the *Flaviviridae* family [1]. DENV exists as four antigenically distinct serotypes, namely DENV-1, DENV-2, DENV-3, and DENV-4. Infection can range from an uncomplicated febrile illness to severe dengue characterized by plasma leakage, severe bleeding, shock, and organ involvement [2].

The clinical outcome of dengue is influenced by viral, host, and immunological factors, including the infecting serotype [3,4]. Differences in serotype-specific virulence may contribute to variations in disease severity and hematological abnormalities such as thrombocytopenia, leukopenia, and hemoconcentration. Among the serotypes, DENV-2 has frequently been associated with more severe clinical manifestations, although disease patterns may vary geographically and temporally [5,7].

Molecular identification of dengue serotypes using real-time reverse-transcription polymerase chain reaction (RT-PCR), together with assessment of clinical and hematological parameters, can provide important information regarding serotype-specific disease patterns [8,9]. Therefore, the present hospital-based analytical study was undertaken to evaluate the association of dengue virus serotypes with disease severity and clinical-hematological parameters among laboratory-confirmed dengue patients.

Material and method

A hospital-based cross-sectional analytical study was conducted in the Department of Medical Microbiology, Index Medical College Hospital and Research Centre, Malwanchal University, Indore, Madhya Pradesh, from January 2023 to January 2026. The study included patients of all age groups presenting to the outpatient department, emergency department, and inpatient wards with clinically suspected dengue infection. A total of 290 eligible patients were enrolled consecutively after obtaining written informed consent. Patients presenting with acute febrile illness within 1–7 days of fever onset were included, while patients with laboratory-confirmed alternative causes of acute febrile illness, inadequate or hemolyzed specimens, refusal of consent, or unavailable baseline clinical and laboratory information were excluded. The sample size was calculated using the standard formula for estimation of a single population proportion, considering an expected prevalence of 24.49%, 95% confidence level, and 5% absolute precision.

Approximately 5 mL of venous blood was collected aseptically from each participant, and serum was separated by centrifugation at 3000 rpm for 10 minutes. Serum samples were initially tested using the SD BIOLINE™ Dengue Duo Rapid Test (Abbott Diagnostics) for detection of dengue NS1 antigen and dengue-specific IgM/IgG antibodies. Testing was performed according to the manufacturer's instructions. Samples positive by serological testing and clinically suspected acute-phase cases were subjected to molecular detection and serotyping.

Viral RNA was extracted from serum samples using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). Molecular detection and serotyping were performed using the RealStar® Dengue Type RT-PCR Kit 1.0 (altona Diagnostics GmbH, Hamburg, Germany) on a Rotor-Gene Q real-time PCR system. The multiplex real-time RT-PCR assay enabled detection and differentiation of DENV-1, DENV-2, DENV-3, and DENV-4. Master Set 1 detected DENV-1 and DENV-4, whereas Master Set 2 detected DENV-2 and DENV-3. An internal control was included to monitor extraction efficiency and PCR inhibition. Samples showing characteristic amplification in the corresponding fluorescence channel were considered positive for the respective dengue serotype.

Clinical information, including duration of fever, bleeding manifestations, abdominal pain, hypotension, and other relevant manifestations, was recorded. Disease severity was classified according to the WHO 2009 dengue classification into dengue without warning signs, dengue with warning signs, and severe dengue. Hematological parameters, including hemoglobin, total leukocyte count, platelet count, and hematocrit, were recorded and compared among different dengue serotypes and disease-severity categories. Data were analysed using SPSS version ,Associations were assessed using Chi-square tests and logistic regression. A p-value <0.05 was considered statistically significant.

RESULT

Among the 290 study participants, 67 (23.1%) were positive for dengue virus by real-time RT-PCR compared with 61 (21.0%) by rapid testing; the difference was not statistically significant ($\chi^2 = 1.92$, $p = 0.166$). Among RT-PCR-positive cases, DENV-2 was the predominant serotype (31, 46.3%), followed by DENV-3 (18, 26.9%), DENV-1 (14, 20.9%), DENV-4 (2, 3.0%), and mixed infection (2, 3.0%). RT-PCR positivity was significantly associated with age group ($p = 0.041$) and gender ($p = 0.038$), with higher positivity among males (65.7%).

Disease severity was significantly associated with dengue serotype ($p = 0.012$). Of the 67 RT-PCR-confirmed patients, 33 (49.3%) had dengue without warning signs, 24 (35.8%) had warning signs, and 10 (14.9%) had severe dengue. Mean platelet count decreased progressively with increasing disease severity, from 142,800 ± 38,400/µL in patients without warning signs to 86,700 ± 29,100/µL with warning signs and 42,300 ± 18,500/µL in severe dengue ($p < 0.001$). Overall, the mean hemoglobin, total leukocyte count, and platelet count were 13.1 ± 1.8 g/dL, 4,220 ± 1,310/mm³, and 101,600 ± 49,700/µL, respectively. Multivariate logistic regression showed that DENV-2 infection (OR 2.91, 95% CI: 1.28–6.62), platelet count <50,000/µL (OR 4.72, 95% CI: 2.01–11.08), bleeding manifestations (OR 3.18, 95% CI: 1.29–7.82), and abdominal pain (OR 2.37, 95% CI: 1.04–5.41) were independently associated with severe dengue. Among RT-PCR-confirmed patients, thrombocytopenia was the most common hematological abnormality (88.1%), followed by leukopenia (52.2%) and hemoconcentration (28.4%). No significant gender-wise differences were observed in the distribution of hematological abnormalities.

Table 1. Comparison of Positivity Rates by Rapid Test and Real-Time RT-PCR (n = 290)

Test Method	Positive n (%)	Negative n (%)	χ^2 value	p-value
Rapid Test	61 (21.0)	229 (79.0)		
Real-Time RT-PCR	67 (23.1)	223 (76.9)	1.92	0.166

Table 2. Distribution of Dengue Virus Serotypes and Association of Demographic Characteristics with Real-Time RT-PCR Positivity among Study Participants

Variable	Category	RT-PCR Positive n (%)	RT-PCR Negative n (%)	p-value
Dengue virus serotype	DENV-1	14 (20.9)	–	–
	DENV-2	31 (46.3)	–	
	DENV-3	18 (26.9)	–	
	DENV-4	2 (3.0)	–	

	Mixed infection	2 (3.0)	–	
	Total	**67 (100)**	–	
Age group (years)	<15	5 (7.5)	19 (8.5)	**0.041***
	15–24	13 (19.4)	48 (21.5)	
	25–34	25 (37.3)	63 (28.3)	
	35–44	13 (19.4)	41 (18.4)	
	45–54	7 (10.4)	30 (13.5)	
	≥55	4 (6.0)	22 (9.9)	
	Total	**67 (100)**	**223 (100)**	
Gender	Male	44 (65.7)	132 (59.2)	**0.038***
	Female	23 (34.3)	91 (40.8)	
	Total	**67 (100)**	**223 (100)**	

***p < 0.05, statistically significant.**

Table 3.Association Between Dengue Virus Serotypes and Disease Severity Among RT-PCR Confirmed Dengue Patients

Serotype	Without Warning	Warning Signs	Severe	p-value
DENV-1	10	3	1	
DENV-2	11	13	7	
DENV-3	10	8	0	
DENV-4	1	1	0	
Mixed	1	0	1	
Total	**33**	**24**	**10**	**0.012***

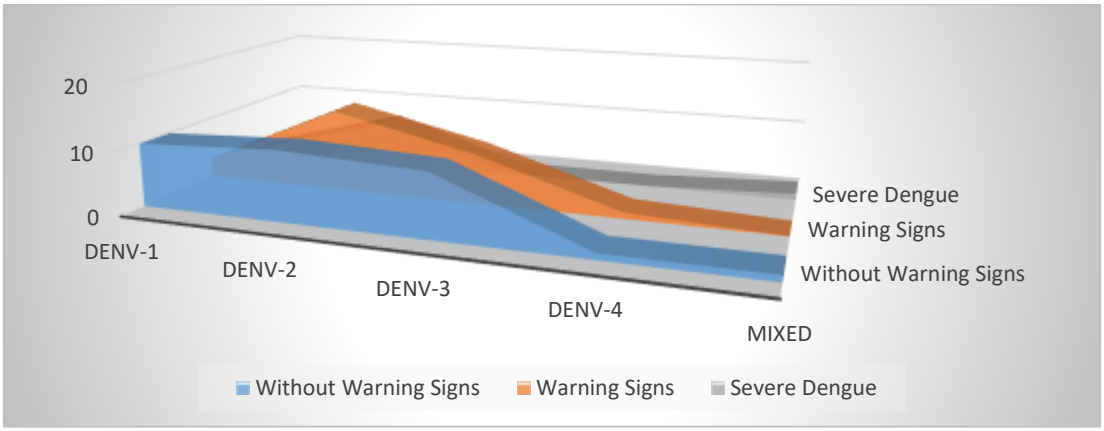

Figure 1.Association Between Dengue Virus Serotypes and Disease Severity Among RT-PCR Confirmed Dengue Patients

Table 4.Comparison of Mean Platelet Count According to WHO 2009 Dengue Disease Severity Categories

Severity	Mean Platelet Count (/µL)	SD	p-value
Without warning	142,800	38,400	<0.001*

Warning signs	86,700	29,100	
Severe dengue	42,300	18,500	

Table 5. Hematological Parameters Among RT-PCR Confirmed Dengue Patients

Parameter	Mean ± SD
Hemoglobin (g/dL)	13.1 ±1.8
Total Leukocyte Count (/mm³)	4,220 ±1,310
Platelet Count (/µL)	101,600 ±49,700

Table 6. Multivariate Logistic Regression Analysis of Factors Associated with Severe Dengue Among RT-PCR Confirmed Patients

Variable	Odds Ratio (95% CI)	p-value
DENV-2 infection	2.91 (1.28–6.62)	0.009*
Platelet count <50,000/µL	4.72 (2.01–11.08)	<0.001*
Bleeding manifestations	3.18 (1.29–7.82)	0.011*
Abdominal pain	2.37 (1.04–5.41)	0.039*
Age ≥45 years	1.69 (0.72–3.97)	0.221

Table 7. Gender-wise Distribution of Hematological Abnormalities Among RT-PCR Confirmed Dengue Patients (n = 67)

Hematological Abnormality	Male	Female	Total	p-value
Thrombocytopenia	39 (88.6)	20 (87.0)	59 (88.1)	0.842
Leukopenia	24 (54.5)	11 (47.8)	35 (52.2)	0.603
Hemoconcentration	13 (29.5)	6 (26.1)	19 (28.4)	0.771
Elevated Hemoglobin	8 (18.2)	3 (13.0)	11 (16.4)	0.589
Any hematological abnormality	41 (93.2)	21 (91.3)	62 (92.5)	0.782

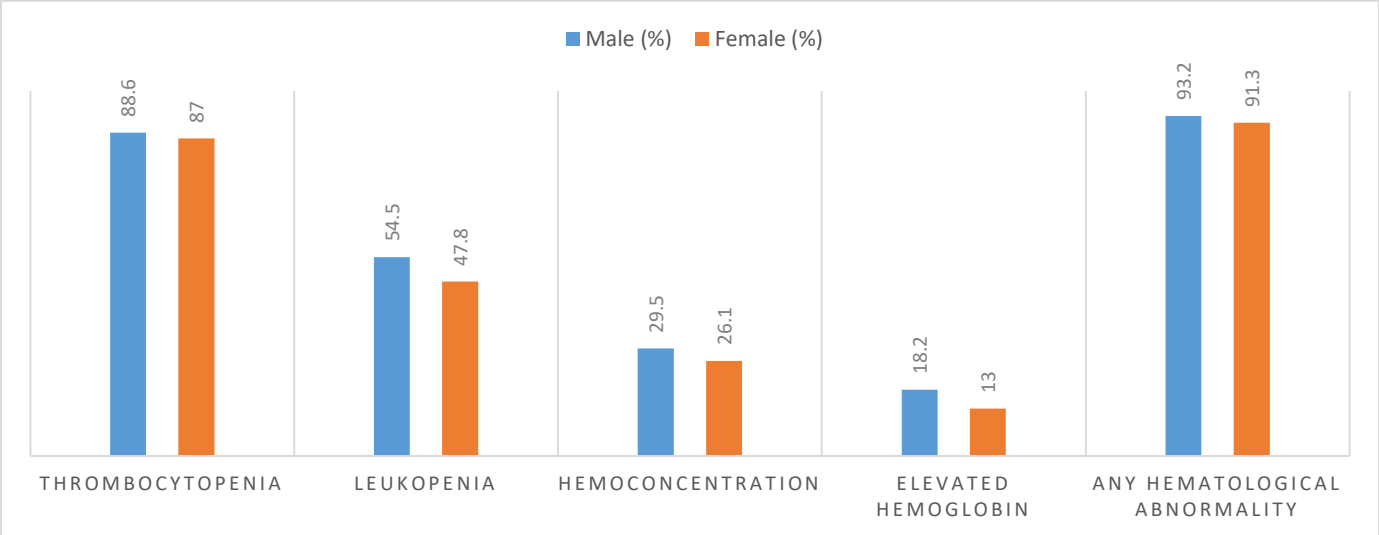

Figure 2. Gender-wise Distribution of Hematological Abnormalities Among RT-PCR Confirmed Dengue Patients (n = 67)

Discussion

The present hospital-based analytical study demonstrated a dengue virus RT-PCR positivity rate of 23.1% among 290 clinically suspected patients. The positivity detected by RT-PCR was slightly higher than that of the rapid test (21.0%), although the difference was not statistically significant (p=0.166). This finding highlights the value of molecular testing for confirmation of dengue infection, particularly during the acute phase of illness when viral RNA can be detected before or during the early development of detectable antibodies.

Among the 67 RT-PCR-confirmed cases, DENV-2 was the predominant serotype (46.3%), followed by DENV-3 (26.9%), DENV-1 (20.9%), DENV-4 (3.0%), and mixed infections (3.0%). Patil et al. (2026) [10] reported a predominance of DENV-2 in their recent molecular surveillance study from Karnataka, India. In contrast, Rao et al. (2020) [11] reported DENV-3 as the predominant serotype in their hospital-based study from Mangalore. These differences demonstrate considerable geographical and temporal variation in dengue serotype distribution, which may be influenced by changes in circulating viral strains, population immunity, climatic conditions, and local transmission dynamics.

A statistically significant association was observed between dengue virus serotype and disease severity (p=0.012). DENV-2 accounted for the largest proportion of patients with warning signs and severe dengue in the present study. Similarly, Vaughn et al. (2000) [12] demonstrated that DENV-2 infection, higher viremia, and viral serotype were associated with increased disease severity. A recent genomic study from India in 2025 also suggested that genetic variations within DENV-2 may contribute to differences in clinical severity [13]. Nevertheless, dengue severity cannot be attributed to serotype alone, as host immune response, previous dengue infection, age, viral load, and viral genetic characteristics may also influence clinical outcome.

A strong inverse association was observed between platelet count and disease severity. The mean platelet count decreased progressively from 142,800 ± 38,400/µL among patients without warning signs to 86,700 ± 29,100/µL among those with warning signs and 42,300 ± 18,500/µL among patients with severe dengue (p<0.001) [14]. Thrombocytopenia was the most frequent hematological abnormality in the present study, occurring in 88.1% of RT-PCR-confirmed patients. Sontakke et al. (2024) [15] reported a significant relationship between platelet parameters and severity of thrombocytopenia in dengue patients. Similarly, a South Indian study published in 2018 reported thrombocytopenia as an important laboratory feature associated with severe dengue. The progressive reduction in platelet count observed in the present study therefore supports its clinical usefulness as a marker for monitoring disease progression.

Leukopenia was observed in 52.2% of patients, while hemoconcentration was present in 28.4%. These findings are consistent with the characteristic hematological abnormalities described in dengue infection. Martina et al. (2009) [16] described the underlying pathophysiological mechanisms of dengue, including immune-mediated responses, vascular permeability, and hematological abnormalities associated with disease progression. Leukopenia may result from transient bone marrow suppression and peripheral destruction of leukocytes, whereas hemoconcentration may reflect increased vascular permeability and plasma leakage during disease progression.

Multivariable logistic regression demonstrated that DENV-2 infection, platelet count below 50,000/µL, bleeding manifestations, and abdominal pain were independently associated with severe dengue. DENV-2 infection was associated with approximately threefold higher odds of severe disease (OR 2.91), while platelet count below 50,000/µL showed the strongest association (OR 4.72). Similar associations between severe thrombocytopenia and severe dengue have been reported by previous investigators. Vaughn et al.

(2000) [12] also demonstrated that viral factors, including dengue serotype and viremia, were associated with disease severity. According to the World Health Organization (WHO, 2009) [19] abdominal pain and bleeding manifestations are important clinical features associated with dengue with warning signs and progression toward severe disease.

The highest number of RT-PCR-positive cases was observed in the 25–34-year age group, and a significant association was observed between age group and RT-PCR positivity (p=0.041). Male patients accounted for 65.7% of RT-PCR-positive cases compared with 34.3% females, with a statistically significant association between gender and RT-PCR positivity (p=0.038). Patil et al. (2026) [10] and Rao et al. (2020)[11] also reported a male predominance among dengue-positive patients. However, differences in age and sex distribution may reflect healthcare-seeking behavior, occupational exposure, population structure, and local transmission patterns rather than inherent biological susceptibility.

Conclusion

The present study demonstrates that DENV-2 was the predominant circulating serotype and was significantly associated with disease severity. Severe dengue was characterized by marked thrombocytopenia and was independently associated with DENV-2 infection, platelet count below 50,000/µL, bleeding manifestations, and abdominal pain. These findings emphasize the importance of molecular serotyping combined with clinical and hematological assessment for early identification of patients at risk of severe dengue. However, the relatively small number of RT-PCR-positive cases and the single-centre design should be considered when generalizing these findings to other populations.

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