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RESEARCH ARTICLE

Dynamic trends of dengue fever serotypes in northern India: Exploring clinical manifestations, serotype dissemination, and the influence of mixed infections

Pandey, P.[1], Ranjan, R.[1], Agarwal, P.[1], Gupta, R.M.[1], Pandey, A.K.[2], Das, A.[3], Yadav, V.S.[4*]

[1]Department of Microbiology, ESIC Medical College & Hospital, Faridabad 121001, Haryana, India
[2]ESIC Medical College & Hospital, Faridabad, 121001, Haryana, India
[3]ESIC Medical College & Hospital, Alwar, Rajasthan, India
[4]Department of Microbiology, ESIC Medical College & Hospital, Faridabad, 121001, Haryana, India
*Corresponding author: vishwanathyadav533@gmail.com

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ABSTRACT

Examining the co-circulation of various serotypes and finding serotypes linked to illness severity were the main objectives of this study, which sought to investigate the epidemiology and serotype distribution of dengue in Haryana, North India. The cross-sectional study, which was carried out in a tertiary care hospital between September 2021 and April 2023, enrolled participants who met WHO criteria for probable dengue fever. Blood samples underwent molecular and serological diagnostics, such as immunochromatographic testing, VIDAS® Dengue NS1 assays, and TRUPCR® Dengue Detection and serotyping kits, in addition to the collection of clinical and demographic data. Dengue was found to be present in 212 of the 536 probable cases, with serotype DENV-2 being the most common. There have also been reports of mixed DENV-1 and DENV-2 infections. Different serotypes caused different lengths of sickness; DENV-2 showed a sustained high RT-PCR positivity. The severity of the disease was linked to distinct serotypes based on significant differences in aspartate aminotransferase (AST) levels between individuals with dengue fever (DF) and dengue haemorrhagic fever (DHF). The study emphasizes how complicated dengue virus infections can be because of the co-circulation of several serotypes and the possibility of mixed infections. Serotypes and illness severity are correlated, which emphasizes the necessity of continuous surveillance and monitoring to improve outbreak prediction and management. These results are critical for guiding clinical judgments and public health policy, especially with relation to the possible introduction of a dengue vaccine.

Keywords: Dengue fever; dengue haemorrhagic fever; serotype; mixed infection.

INTRODUCTION

Dengue is a mosquito-borne viral infection caused by any one of the five dengue virus serotypes (DENV-1 to -5). In October 2013, the fifth and latest addition to the current serotypes of dengue fever virus was reported as DENV-5. It was detected in a 37-year-old farmer admitted to a hospital in Sarawak state of Malaysia in the year 2007, however, it has not been reported in India. Dengue is a significant global health threat that affects 3.9 billion people worldwide (WHO, 2023). Most dengue infections are either asymptomatic or present with mild, flu-like symptoms, while a small proportion of cases may progress into a life-threatening form of the disease known as severe dengue (Holmes & Twiddy, 2000; Bhatt *et al*., 2013; Muller *et al*., 2017). Dengue has a moderate-to-low mortality rate; however, the mortality rate among severe dengue patients may rise significantly with inadequate clinical management (Muller *et al*., 2017). DENV exists in five genetically and antigenically distinct serotypes (DENV 1-5) under the family *Flaviviridae*. Each of the DENV serotypes is further classified into 3–5 genotypes based on genetic divergence. In addition to the serotypes, even the genotypes within each dengue serotype may influence the severity of DENV infection (Vicente *et al*., 2016).

Serotypes/genotypes and lineages of DENV are associated with more severe outbreaks. Many reports from India have shown an association between change in DENV genotype/lineage and magnitude of the outbreak and disease severity (Vinodkumar *et al*., 2013; Saha *et al*., 2016; Tazeen *et al*., 2017; Shrivastava *et al*., 2018). Emergence of new serotype or lineage/clade shifts in circulating DENV genotypes led to enhanced severity during dengue outbreaks (Messer *et al*., 2003; Kukreti *et al*., 2010; Hapuarachchi *et al*., 2016; Saha *et al*., 2016). A lineage shift in DENV-3 was reported to cause severe disease in Sri Lanka (Ospina et al., 2010; Saha *et al*., 2016; Choudhary *et al*., 2017). Emergence of genotype III of DENV-3 in 2005 resulted in a dengue outbreak in Northern India (Dash *et al*., 2006). Recently, the emergence of Asian or genotype I of DENV-1 also caused a large outbreak of dengue with 12,000 cases in Tamil Nadu, South India (Cecilia *et al*., 2017).

All the four serotypes of DENV have circulated in India at different times, but generally, one serotype dominates a given outbreak. The dengue outbreak in 1996 in Delhi was caused by

genotype IV of DENV-2 replacing genotype V isolates of 1957 and 1967 (Singh *et al*., 1999) and the virus remained in circulation till 2002. The second outbreak, 2003 in Delhi, was due to the emergence of DENV-3 which remained as the dominant serotype till 2006 (Dash *et al*., 2006). Over a period from 2007-2009, DENV-1 became the predominant serotype in Delhi by replacing DENV-2 and DENV-3 (Chakravarti & Arora, 2010). Earlier dengue outbreaks were attributed to the sudden emergence of serotype or genotype that co-circulate along with existing genotype for some time before getting replaced by others in subsequent years. In recent years, co-circulation of multiple serotypes has been reported from different parts of India (Reddy *et al*., 2017). A high percentage of co-infection with more than one serotype was also observed with increased disease severity (Das *et al*., 2013; Tazeen *et al*., 2017; Bharaj *et al*., 2008). In 2017, co-circulation of all four DENV serotypes in a single outbreak was reported from Odisha (Mishra *et al*., 2017) and Hyderabad (Vaddadi *et al*., 2017). Senaratne and Sirisena (2020) reported that the consequences of DENV co-infections on illness outcomes in particular individuals are complicated, and DENV serotype co-infections have the potential to contribute to DENV genetic diversity. Therefore, comprehending DENV co-infections may aid in understanding the worldwide dengue pandemic (Senaratne & Sirisena, 2020). Despite growing research on potential severity predictors, no definitive predictors of severe dengue resulting in unwanted hospitalization and fatalities have been identified (Vinodkumar *et al*., 2013; Vicente *et al*., 2016; Shrivastava *et al*., 2018). Considering the threat that dengue poses to the country and the likelihood of a dengue vaccine being introduced soon, continuous monitoring of viruses circulating in different parts of the country is crucial. Molecular monitoring of viruses and early detection of changes in the circulation pattern may help in the prediction of DENV outbreaks and can augment disease control efforts.

MATERIALS AND METHODS

A cross-sectional study was conducted in a tertiary care hospital in North India to identify patients with suspected dengue fever. The study was conducted between September 2021 and April 2023 and included patients presenting with fever of 1-5 days to the Medicine and Paediatrics Outpatient Department from different districts of Haryana. The classification of cases was done based on WHO, 2009. All patients not fulfilling the inclusion criteria as per the case definitions were excluded.

The study protocol was approved by the Institutional Human Ethics Committee (Approval number 134 X/11/13/2021IEC/106), and demographic and clinical details of the patients were collected after obtaining written informed consent. Blood samples were collected aseptically and transported to the Laboratory for serological diagnosis. The study flow is described in detail in Figure 1.

Case definitions (WHO, 2009)

Dengue Fever (DF) was defined as fever with at least two of the following symptoms: headache, retro-orbital pain, arthralgias/ backache, myalgias, abdominal pain, rash, bleeding, and decreased urine output along with leukopenia (total leukocyte count of less than 4,000/microliter) or thrombocytopenia (platelet count less than 100,000/microliter) and either non-structural protein 1 (NS1) or dengue IgM positive. DF without any evidence of plasma leakage or shock was called classical DF. Dengue Haemorrhagic Fever (DHF) was defined as confirmed DF with evidence of plasma leakage as indicated by more than 20% increase or decrease in haematocrit from baseline or radiological evidence of plasma leakage in the form of ascites, pleural effusion, increased gallbladder wall thickness, or pericholecystic fluid.

Immunochromatographic test

The serum samples were collected from patients fulfilling the inclusion criteria. The samples were subjected to NS1 rapid test in accordance with the manufacturer's recommendations (SD Bioline).

VIDAS® Assays

VIDAS® dengue NS1 Ag is an automated qualitative two-step immunoassay combined with enzyme-linked fluorescent assay (ELFA) detection, developed for the VIDAS® family of instruments (Combe *et al*., 2017). The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strip. All steps are performed automatically by the instrument, within 40 to 60 min.

For the VIDAS® DENGUE NS1 Ag assay, the Dengue NS1 antigen is captured by a monoclonal antibody specific for the four serotypes coated on the interior of the SPR wall. During the second step, the captured NS1 antigen is detected by monoclonal antibodies specific for the NS1 antigen of the four DENV serotypes conjugated to alkaline phosphatase. During the final detection step of the VIDAS® dengue NS1 Ag assay, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyses the hydrolysis of the substrate into a fluorescent product (4-Methyl-umbelliferone). Fluorescence is measured at 450 nm and a relative fluorescence value (RFV) is generated (background reading subtracted from the final fluorescence reading). The instrument automatically calculates the results according to standard (S1) and returns index (i) (i = RFV sample / RFVS1). The test results are interpreted as negative (i < 1.0) and positive (i > 1.0) (Combe *et al*., 2017).

Dengue NS1 positivity cut-off values

The Dengue NS1 positivity limit values are determined by area under the ROC curve (AUC) and Youden index analysis using clinically characterized positive human samples and negative human samples.

Nucleic acid extraction

The samples were subjected to extraction and purification using QIAamp Viral RNA Mini Extraction Kit following the manufacturer's instructions.

TRUPCR® dengue detection kit

The RNA was then subjected to TRUPCR® Dengue Qualitative Kit is an *in vitro* nucleic acid amplification assay for the qualitative detection of Dengue viral RNA from patient samples.

In addition to the Dengue RNA-specific amplification and detection system, the assay includes oligonucleotides for the amplification and detection of the IC (Internal Control). Probes specific for Dengue RNA are labelled with the fluorophore FAM™. The probe specific for the IC is labelled with a fluorophore detectable in the TEX™ channel. The amplification conditions consisted of cDNA synthesis at 50°C for 20 min, 94°C for 10 mins, 35 cycles of 94°C for 15 sec, and 55°C for 30 sec. This was followed by a final primer extension at 72°C for 30 sec using the TRUPCR® Dengue Qualitative Kit. Briefly, to perform the standard RT-qPCR reaction, with 15 µL of RT-qPCR mix containing 10 µL of Master Mix, 2.65 µL of combined primer/probe mix, 2 µL of endogenous Internal control primer/probe mix, 0.35 µL enzyme mix, 10 µL of nucleic acid extract.

Real Time PCR for confirmation of serotypes

Samples positive by RT-PCR were further subjected to multiplex RT-PCR for serotype identification. The TRUPCR® Dengue Virus Serotyping Kit is designed to detect all four serotypes of DENV (DEN1, DEN2, DEN3, and DEN4). The fifth serotype was not included in the study. It is a two-tube multiplex RT-PCR; one tube utilizes the identification of Dengue RNA, Internal Control (IC), and Dengue

serotype 1, and the other tube utilizes the identification of DENV serotypes 2, 3, and 4. The following setting for channel selection was used: dengue-specific RNA and Dengue serotype 2 utilizes FAM, Dengue 1 & 3 utilizes HEX, Internal Control & Dengue 4 TEX Red. All samples having a Ct value of ≤35 were considered positive.

The methodology used in this study provides a detailed and comprehensive approach to identifying and confirming dengue fever cases, which may aid in developing effective prevention and control strategies.

Statistical analysis

The Mann-Whitney U test was used to determine statistical significance for continuous variables and Fischer's exact test for categorical variables. All data and figures were analysed using GraphPad Prism software version 8.0 (GraphPad Software, San Diego, CA, USA). A p-value of <0.05 was regarded as significant.

RESULTS

Demographic characteristics, clinical manifestations, and temporal variation

A total of 536 dengue-suspects were screened, 220 patients were positive for dengue NS1 Immunochromatographic card test, out of which 212 were serologically confirmed by VIDAS® NS1 assay. The current guidelines suggest that in order to confirm DENV infection, circulating DENV RNA should be detected by reverse-transcription PCR (RT-PCR) and/or the secreted viral non-structural protein 1 (NS1) antigen should be detected by immunoassay within the first 5-7 days of illness (acute phase). Therefore, no additional testing was done. 212 samples were subjected to Real Time PCR-based Dengue detection kit; all the 212 samples were positive by real time PCR. The study layout has been represented in Figure 1.

Age-wise stratification of RT-PCR positive cases revealed that most cases belonged to the paediatric age group. The age-wise distribution of the cases is depicted in Figure 2. All the Real time-PCR negative cases were excluded from the study. The common reason for NS1 rapid card positivity could be attributed to cross-reactivity with other flaviviruses, hematologic malignancy patients, etc. The patients were classified into probable dengue, dengue with warning signs, and severe dengue. In our study, we classified 201 as Dengue Fever/probable dengue cases and 11/212 as dengue with warning signs, and severe dengue.

The biochemical parameters Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) levels between Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF) over different days

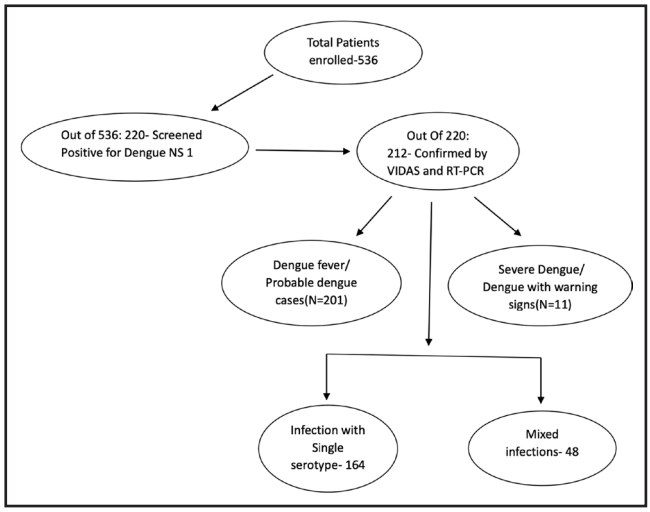

Figure 1. Algorithm for patient screening and inclusion.

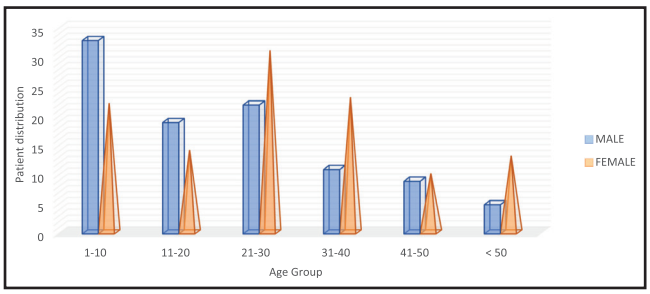

Figure 2. Age and sex wise dissemination of confirmed dengue cases.

of illness were compared. The details are illustrated in Table 1. It is apparent that in general, the median AST and ALT levels tend to be higher in DHF compared to DF. Additionally, the levels of both AST and ALT appear to show some variability over the course of illness. The Mann-Whitney U test was applied to compare differences between ALT & AST levels in DF and DHF group, it was noted that p-value was found to be significant (<0.05) on comparing the median AST levels (Table 1). The median values of WBC counts, Haematocrit levels, and Platelet counts for both DF and DHF patients on different days of illness were noted. The p-values provide a measure of the statistical significance of the differences observed in Haematocrit levels and Platelet counts between the two groups. It is evident that there are differences in these parameters between the two groups of patients. Specifically, DHF patients tend to show lower median WBC counts and Haematocrit levels compared to DF patients on various days of illness. Additionally, DHF patients generally exhibit lower median Platelet counts compared to DF patients (Table 2).

Table 1. Comparison of median Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) levels between Dengue Fever (DF) and Dengue Haemorrhagic Fever (DHF) over different days of illness

Day of Illness	Median AST (U/L)		Median ALT (U/L)	
	DF	DHF	DF	DHF
2.5	110.00	212.00	35.00	56.00
4	145.00	201.00	49.00	93.50
5	152.00	182.50	78.00	94.00
6	192.00	223.50	81.00	114.00
7	178.00	218.00	103.00	142.50
P-value	**0.0159**		**0.1508**	

Table 2. Comparison of median values for White Blood Cell (WBC) counts, Hematocrit (HCT) levels, and Platelet counts between Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF) patients across different days of illness

Day of Illness	WBC ('10^3/µL)		HCT (%)		Platelets ('10^3/µL)	
	DF	DHF	DF	DHF	DF	DHF
2.5	3.5	2.7	40.2	40.5	136.0	107.0
4	3.2	3.8	40.5	43.0	125.0	90.0
5	3.5	5.9	40.1	41.5	118.5	78.5
6	4.1	6.6	39.9	40.9	111.5	54.5
7	4.5	6.4	38.7	36.2	98.0	41.0
P-value	**0.2948**		**0.1732**		**0.0159**	

Serum calcium levels did not show a distinct pattern in either DF or DHF, and there was no clear association between serum calcium levels and the haematocrit in DHF patients. Median corrected serum calcium values were compared between DF and DHF groups and did not show a significant difference.

Serotype distribution and duration of illness

Of the 212 samples positive for viral RNA by Real Time PCR, 164 (77.36%) cases were found to be infected with a single DENV serotype, 48 (22.6%) cases documented mixed infection i.e., infected with multiple serotypes. In single serotype infections, DENV-2 was found to be the commonest serotype (68.90%), followed by DENV-3 and 1 (17.07%; 14.02%).

Among patients positive for multiple serotypes, co-circulation of DENV-1 and DENV-2 was the most common (62.50%), followed by DENV-1 and DENV-3 (25.00%). DENV-1 and DENV-4 were least prevalent (12.50%).

The serotype distribution in different forms of dengue fever was also determined. Serotype 2 was the most predominant serotype in both Dengue fever and Dengue Haemorrhagic Fever (DHF) followed by serotype 3 and serotype 1. In the mixed infection cases, the most prevalent serotype was serotype 1 & 2 followed by serotype 1 & 3. Mixed infection due to serotype 1 & 4 was seen in only dengue fever cases illustrated in Table 3.

Table 3. Disease wise stratification of data depending on infection caused by single serotype and mixed infection

Serotype Distribution	Dengue Fever (n=201)	Dengue Haemorrhagic Fever (n=11)
Serotype 1	22	1
Serotype 2	111	2
Serotype 3	27	1
Mixed infection (DENV 1 & 2)	25	5
Mixed infection (DENV 1 & 3)	10	2
Mixed infection (DENV 1 & 4)	6	0

Hematologic data on platelet count and white blood cell (WBC) count was contrasted between DENV patients who had mono-infection and mixed infection. Particularly, there was no variance in the WBC count between patients with mono and mixed infection. Serotype distribution in Dengue fever cases with respect to platelet count (< 100,000/mm^3) at 2.5 days and 5 days in mono infection and mixed infections revealed a close association (P=0.0338). Similarly, the serotype distribution in Dengue haemorrhagic fever cases with respect to platelet count (< 100,000/mm^3) at 2.5 days and 5 days revealed no significant association. This may be attributed to the low number of mixed infection cases. The details are illustrated in Figure 3.

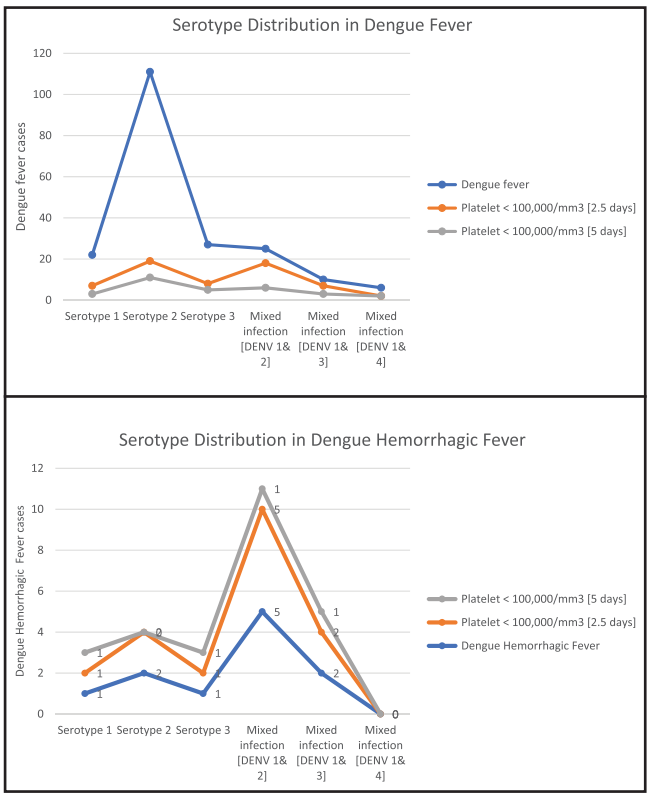

Figure 3. Disease wise stratification of data depending on infection caused by single serotype and mixed infection with special emphasis to platelets count.

We next analysed the serotype-wise duration of illness among the RT-PCR positive cases, the total number of cases for each category (single serotype-specific or mixed infection) and the corresponding RT-PCR positivity rates on different days of illness. It was noted that Serotype 1 has a decreasing RT-PCR positivity over the course of illness. Serotype 2 shows high RT-PCR positivity throughout the illness, with a slight decrease by the 5th day. Serotype 3 also exhibits a decrease in RT-PCR positivity over time on comparison with the other prevalent serotypes across the single serotype-specific or mixed infection cases (P<0.0001) (Table 4). Mixed infections involving serotype combinations generally have RT-PCR positivity rates that decrease as well. These results provide insights into the dynamics of DENV serotypes and mixed infections in terms of RT-PCR positivity over the course of illness (Figure 4).

Table 4. Correlation between dengue virus real-time PCR positivity on different days of disease and the most prevalent serotypes

RT-PCR Positivity	DENV 1	DENV 2	DENV 3	DENV 1 & 3	DENV 1 & 2	DENV 1 & 4
After 2.5 days of illness	23	113	28	12	30	6
After 5th day of illness	2	94	2	7	13	3
P-value	P < 0.0001					

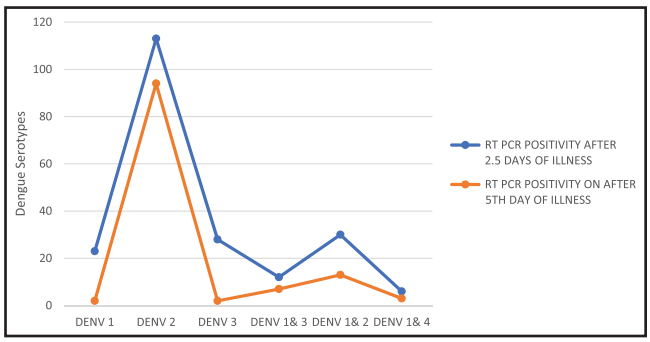

Figure 4. Correlation of viral persistence with specific serotypes of dengue.

DISCUSSION

The study's use of advanced diagnostic methods, including VIDAS® and TRUPCR® assays, contributed to accurate and comprehensive identification of dengue cases. The results highlighted the importance of early detection, particularly within the first few days of illness, to effectively manage and control dengue outbreaks. The study's findings could have significant implications for public health policy and clinical practice. Furthermore, the study shed light on the fluctuations in key biochemical parameters, such as AST, ALT, WBC counts, haematocrit levels, and platelet counts, which could serve as indicators of disease progression and severity. Ahmed *et al*. (2020) reported that clinicians can employ the AST/platelet count ratio measure to enhance their ability to make decisions in primary healthcare settings. Furthermore, they hypothesized that low ALT and MCV are predictors of DENV infection (Ahmed *et al*., 2020). Rao *et al*. (2020) revealed that to forecast the progression and prognosis of dengue fever, an assortment of prognostic markers has been identified. In our study, we also found a strong correlation of ALT & AST with respect to DF & DHF. AST levels were significantly different between the DF and DHF groups. Similar findings have been reported in other studies (de Souza *et al*.,2007; Trung & Wills, 2010). AST elevation is much more common and levels are higher than ALT Because AST has been demonstrated in several tissues other than the liver and its release from those tissues may be much greater than its release from the liver. Hepatocellular biopsies from DHF patients have revealed histologic characteristics typical of viral hepatitis (Burke, 1968). The findings indicate that because of monocyte mobility and an extensive immunological response, the liver may be involved in dengue viral infection, especially in DHF (Pang & Cardosa, 1983; Cornain *et al*., 1987). Faster recovery from dengue and shorter hospital stays are associated with larger lymphocyte percentages (Ananda Rao *et al*., 2020). In our study, similar findings were noted in terms of hematologic data between DENV patients who had mono-infection and mixed infection.

The identification of specific serotypes associated with different clinical presentations adds to the growing body of evidence linking DENV diversity to disease outcomes. The study's detailed analysis of serotype distribution and mixed infections emphasizes the need for ongoing monitoring and surveillance of circulating serotypes to better predict and mitigate outbreaks. During the surveillance investigation reported by Tchetgna *et al*. (2021), three DENV serotypes were identified: DENV-1 genotype V, DENV-2 genotype II, and DENV-3 genotype III. Since DENV-1 and DENV-2 are already known in Douala, the characterization of three dengue serotypes during the same period in one city raises concerns about severe cases of the disease, such as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), which are brought on by the development of antibody-dependent enhancement (ADE) in people who have already contracted the disease (Dejnirattisai *et al*., 2010; Halstead *et al*., 2014; Simo Tchetgna *et al*., 2021). The prevalence of various mixed infections/co-infections in different states of India are depicted in Table 5. However, Rao *et al*. (2020) observed that the severity of the illness in dengue infection was not associated with coinfections involving numerous different serotypes. The study additionally demonstrated that serum AST and ALT levels and ferritin could potentially act as better biomarkers to evaluate the severity of the illness, and repeated estimates of these indicators should be considered for attention (Rao *et al*., 2020; Riswari *et al*., 2023) suggested that the substantial percentage of asymptomatic infections and the implications of a longer duration of concealed virus transmission may prolong the presence of a potential reservoir for DENV infections, supporting the need for comprehensive and persistent vector control interventions (Riswari *et al*., 2023).

Numerous conflicting findings have been published regarding the clinical manifestations in patients who have concurrent dengue infections that range in severity from less serious to fatal. Taking into consideration the scenarios, it is conceivable that the existence of several serotypes in a single person most likely contributes to the patient's rising viremia, leading to disease severity. There were noticeably more severe disease symptoms (15%) and warning indications (90%) in patients with mixed DENV infections in research carried out in Brazil, India, and Malaysia (Chahar *et al*., 2009; Anoop *et al*., 2010; Fahri *et al*., 2013). There have also been reports on the frequency of patients with reduced platelet counts and severe thrombocytopenia in mixed infection.

A meta-analysis demonstrates that the risk of severe dengue infections has been enhanced by the presence of certain serotypes, including the initial infection with DENV-3 from the Southeast Asia (SEA) region, subsequent infection with DENV-2, DENV-3, and DENV-4 also from the SEA region, as well as DENV-2 and DENV-3 from non-SEA spots. When establishing clinical predictions about the severity of the illness, these serotypes are therefore worthy of special consideration (Soo *et al*., 2016; Murugesan & Manoharan, 2020). The documented detrimental effect of mixed infections on the severity of the disease has a major long-term influence on the

Table 5. Table depicting the prevalence of various mixed infection/co-infection cases in different states of India

Study	DENV-1	DENV-2	DENV-3	DENV-4	DENV-1 & 2	DENV-1 & 3	DENV-1 & 4	DENV-2 & 3	DENV-2 & 4	DENV-3 & 4	DENV-1, 2 & 3	DENV-1, 2, 3 & 4
Present study	23	114	28	Nil	12	30	6	Nil	Nil	Nil	Nil	Nil
Murugesan & Manoharan, 2020	49	41	8	10	5	1	Nil	Nil	3	Nil	Nil	Nil
Reddy *et al*., 2017	Nil	Nil	Nil	Nil	Nil	9	Nil	7	Nil	Nil	8	2
Vaddadi, *et al*., 2017	6	5	3	4	7	1	Nil	Nil	5	2	Nil	Nil
Shrivastava *et al*., 2018	9	26	17	12	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Vinodkumar *et al*., 2013	2	13	7	2	3	2	Nil	12	1	Nil	Nil	Nil
Bharaj *et al*., 2008	9	3	26	1	Nil	4	2	1	Nil	1	Nil	Nil
Rao *et al*., 2020	1	Nil	56	2	Nil	Nil	56	Nil	Nil	27	Nil	Nil

potential future trajectory of DENV (Descloux *et al*., 2009; Thai & Nguyen, 2012).

When viral nucleic acid persists in the urine after the fifth day of illness, the patient is likely secreting the virus, with shedding rates ranging from 10^2 to 10^5 genome equivalents per milliliter of urine. Assuming an individual sheds 1321 ± 495 mL of urine in a single day (Borghi *et al*., 1996), every patient with the infection will release 10^4–10^8 genome equivalents daily. According to Duyen *et al*., a heterologous immune response appeared to be crucial for the development of high viral loads in DENV-2 and DENV-3 infections, and a larger viral load on the third day of illness was a standalone indicator of higher hemoconcentration (Duyen *et al*., 2011). Wang *et al*. found that DHF patients had much greater amounts of DENV RNA than DF patients. The study additionally demonstrated that DENV RNA in DHF patients' plasma could remain for up to 6 days following defervescence, indicating a significant role for viral RNA in the pathophysiology of severe dengue (Wang *et al*., 2003). Similar findings were noted in our study where Serotype 2 showed high RT-PCR positivity throughout the illness, with a slight decrease by the 5th day. Serotype 3 also exhibits a decrease in RT-PCR positivity over time. It may also suggest that a high viral load that endures beyond defervescence could be a sign of DHF. These findings provide light on the importance of viral components and shed light on dengue pathogenesis.

CONCLUSION

In conclusion, this study provides valuable insights into the epidemiology, clinical presentation, and serotype distribution of dengue fever in North India. The study involves a substantial sample size of 536 dengue-suspect cases, with 212 confirmed RT-PCR positive cases, providing a robust dataset for analysis. This enhances the reliability and validity of the findings. The use of multiple diagnostic methods (NS1 Immunochromatographic card test, VIDAS® NS1 assay, and Real Time PCR) ensures the accuracy and reliability of dengue diagnosis, minimizing the likelihood of false positives. The study provides valuable data on the distribution of dengue virus serotypes and the prevalence of mixed infections. This information is crucial for understanding the epidemiology of dengue and guiding public health interventions. The analysis of RT-PCR positivity over the course of illness for different serotypes offers insights into the dynamics of viral detection, which can inform timing and interpretation of diagnostic tests. The study's examination of mixed dengue infections and their clinical implications addresses an often overlooked aspect of dengue research, providing a more nuanced understanding of the disease.

Overall, the manuscript's strengths lie in its comprehensive and detailed approach to studying dengue, from demographic and clinical characteristics to serotype distribution and temporal dynamics, all underpinned by rigorous diagnostic and statistical methods.

The findings underscore the complexity of DENV infections, with the co-circulation of multiple serotypes and the potential for mixed infections. Notably, the prevalence of different serotypes varied across different forms of dengue, suggesting a potential association between serotype and disease severity.

Overall, this research contributes to our understanding of dengue dynamics in North India and provides a foundation for informed public health policy and clinical decision-making. As efforts continue towards the development and introduction of dengue vaccines, the insights gained from this study could aid in refining disease control strategies and ultimately reducing the burden of dengue on the affected population.

Ethical approval

This study was performed in line with the principles of the Declaration of Helsinki. The study protocol was approved by the Institutional Human Ethics Committee (Approval number 134 X/11/13/2021IEC/106).

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Conflict of Interest

The authors declare that they have no conflict of interest.

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