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Research Article

Dominance of Dengue Viral Serotypes in India: A Comprehensive Study

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A B S T R A C T

Introduction: Dengue is a global health threat exacerbated by the diversity of DENV serotypes and their shifting dominance. This study investigates the changing patterns of DENV serotypes and their clinical implications in India over a 12-year period. The objective of the present study is to analyse the epidemiological trends, serotype distribution, and clinical characteristics of dengue infections in India from 2010 to 2022.

Methods and Material: A total of 3,800 patients with dengue were studied across rural and urban settings in India. Data were collected on clinical presentations, serotype distribution, and diagnostic results, including RT-PCR, NS1 antigen, and antibody tests. Patients were categorised based on serotype prevalence and infection type (single or concurrent).

Results: The serotype distribution revealed that DENV-2 was the predominant serotype from 2010 to 2018. However, a shift to DENV-3 dominance was observed from 2019 to 2022. The clinical findings showed that 63% of RTPCR-positive patients had dengue fever (DF) and 37% had dengue-like fever. NS1 antigen was present in 91% of RTPCR-positive cases. Only 8.9% had both NS1 and IgM, while none had detectable IgG or IgM + IgG. Clinical symptoms included high fever, retro-orbital pain, rashes, ascites, and hepatomegaly. Thrombocytopenia was present in 82% of patients, with additional findings of low leucocyte counts, anaemia, and elevated transaminase levels. Among the concurrent infections, various serotype combinations were observed, indicating a high incidence of concurrent infections.

Conclusion: To conclude, the study highlights a significant shift from DENV-2 to DENV-3 dominance in recent years, correlating with changes in environmental factors such as monsoon patterns. The findings underscore the need for continuous surveillance and adaptive management strategies to address the evolving dengue epidemiology effectively.

Keywords: Dengue Virus (DENV), Serotype Dominance, Concurrent Infections, Epidemiology, Clinical Characteristics

Introduction

Dengue is a mosquito-borne viral illness prevalent in tropical and subtropical countries.[1] The dengue virus (DENV) has been endemic in regions such as Africa, the Eastern Mediterranean, the Americas, Southeast Asia, and the Western Pacific for over a decade.[2] According to the World Health Organization (WHO), about half of the world's population is at risk of DENV infection, with cases increasing yearly. In 2020, the WHO reported a rise in dengue infections in countries like the Philippines, Vietnam, India, Colombia, and Brazil, making dengue a serious threat alongside COVID-19.

In India, the past decade has seen a sharp increase in cases of dengue fever (DF), dengue haemorrhagic fever (DHF), and dengue shock syndrome (DSS), with multiple DENV serotypes contributing to the outbreaks.[3] Reports of severe illness due to concurrent infections have highlighted the need for improved surveillance, training, and monitoring of dengue patients. The aim of the current study is to bridge the gap in understanding the clinical features and infection patterns of DENV in India.

DENV infection can range from mild fever to severe forms like DHF and DSS. The virus has four different serotypes (DENV-1 to DENV-4), with immunity being specific to each serotype.[4–6] Infection with one serotype does not provide immunity against others, and concurrent infections can worsen patient outcomes.[7,8] The illness can vary from asymptomatic to life-threatening, with symptoms lasting from two to seven days, including retro-orbital pain, nausea, vomiting, diarrhoea, abdominal pain, hepatomegaly, and thrombocytopenia.[9,10] A second infection with a different serotype often exacerbates the patient's condition. Predicting the movement of serotypes within a population is challenging, but many clinicians are striving to understand DENV serotype variations to provide effective treatments.

Our research studied 3800 DENV-infected patients over 12 years to understand serotype patterns and concurrent infections, and monitor dominant DENV serotypes. The study, conducted in the tropical region of Davangere, Karnataka, used data from both urban and rural patients. We observed an increase in cases and concurrent infections, along with a gradual shift in the dominant DENV serotype within the study cohort.

Material and Method

A prospective study conducted at S. S. Institute of Medical Sciences and Research Centre, Davangere, Karnataka, India, from January 2010 to December 2022

Patient Enrolment and Sample Collection

- **Study Population and Inclusion Criteria:** The study included a total of 3,800 patients presenting with fever within 5–7 days of onset and a body temperature exceeding 100 °F. Patients were selected based on specific inclusion criteria, ensuring they met the World Health Organization (WHO) case definition for DF and DHF.[1]
- **Data Collection:** Clinical and demographic data were collected through structured interviews with the patients and physical examinations conducted by their treating physicians. Information gathered included the onset and duration of fever, symptoms, and any relevant medical history.[11,12]
- **Haematological Investigations:** Blood samples were collected from each patient for haematological investigations and dengue serology. Haematological parameters included platelet count, haematocrit levels, and white blood cell counts. Thrombocytopenia was defined as a platelet count below 100,000 cells/mm³. An increase in haematocrit greater than 20% was considered significant, indicating possible plasma leakage. Leucopenia was defined as a white blood cell count of less than 4,000 cells/mm³.[15]
- **Classification of Patients:** Based on clinical presentation, laboratory results, and the WHO severity grading scale, hospitalised patients were classified into three categories: DF, DHF, and DSS.[12–15]
- **Follow-up:** Daily follow-up of hospitalised patients was conducted to monitor disease progression and changes in haematological parameters. Regular blood tests were performed to track platelet count, haematocrit, and white blood cell count. This helped assess the severity of the disease and guide clinical management.[15–17]

RNA Extraction and Dengue Virus Detection by qRT-PCR

- **RNA Extraction:** Viral RNA was extracted from the serum samples using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) following the manufacturer's protocol. Extracted RNA was either used immediately for RT-PCR or stored at −70 °C for future analysis.[2,15]
- **Reverse Transcription and PCR:** Reverse transcription (RT) and polymerase chain reaction (PCR) were conducted using a one-step RT-PCR kit (Qiagen, GmbH, Hilden, Germany) in a single reaction tube. A universal primer set was used for both RT and PCR amplification. The reaction was carried out in a thermal cycler (Eppendorf, Germany) according to optimised conditions for dengue virus detection.[15,16]
- **Nested PCR:** The preliminary PCR product was subjected to nested PCR in a separate reaction tube to increase the specificity and sensitivity of the dengue serotype detection. This step was also performed in a thermal cycler.[16,17]
- **Gel Electrophoresis:** The final PCR products were analysed using agarose gel electrophoresis. A 2%

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agarose gel (Bangalore Gene) was prepared, and the PCR products were run in Tris-borate buffer. The gel was stained with ethidium bromide and visualised under a UV transilluminator at 302 nm to detect the amplified dengue virus RNA.[17]

- **Dengue Serotype Identification:** The specific serotypes of the dengue virus (DENV-1, DENV-2, DENV-3, or DENV-4) were identified based on the size of the amplified PCR products corresponding to each serotype.[2,15,16]

Dengue Serology by Immuno-chromatographic Test (ICT) In addition to RT-PCR, an ICT kit (J Mitra and Co. Pvt Ltd, New Delhi, India) was used to detect the presence of the NS1 antigen, as well as IgM and IgG antibodies against the dengue virus.[15,17]

Other Investigations

Additional diagnostic tests performed were the Widal test for typhoid, malaria test chikungunya test the test for typhus fever.[2,15–17]

Ethical Consideration

The Institute's Ethics Review Board reviewed and approved the study protocol. Informed consent was obtained from all participating patients, ensuring that they were fully aware of the study's purpose, procedures, potential risks, and benefits. Participants were informed of their right to withdraw from the study at any time without any negative consequences. All data collected were kept confidential and anonymised to protect patient privacy.

Data Analysis

Blood indices were initially recorded on a continuous scale and later categorised using clinically meaningful cut-offs. Statistical analysis was performed to evaluate the association between clinical severity and haematological parameters, particularly focusing on thrombocytopenia, elevated haematocrit, and leucopenia.

Results

Demographic Distribution of DENV Patients

A total of 3,800 patients were included in this study, with data collected over a period of 12 years (2010 to 2022). The demographic distribution of patients spanned both rural and urban regions. The age distribution was as follows: 485 patients (13%) were below 5 years of age, 2,479 patients (65%) were between 6 and 15 years of age, and 836 patients (22%) were above 15 years of age (Table 1). Out of the 3,800 patients, 2,388 (63%) tested positive for one of the DENV serotypes at the time of testing. All tests were conducted within the first 24 hours of the patient's admission to the hospital.

Clinical Characteristics of RTPCR-Positive and RTPCR-Negative Patients

Dengue serological analysis revealed notable differences between RTPCR-positive and RTPCR-negative patients. NS1 antigen was present in 91% of the RTPCR-positive group compared to only 3% in the RTPCR-negative group (Table 2). None of the RTPCR-positive patients showed the presence of IgM + IgG or IgG alone in the dengue serology, while 8.9% of patients were positive for both NS1 and IgM serotypes (Table 2).

Most patients in the study presented with mild DF, with a smaller number exhibiting DHF and DSS. Clinical presentations varied across 23 symptoms in both RTPCR-positive and RTPCR-negative patients, with fever, retro-orbital pain, and flushing being the most common symptoms (Table 3). Gastrointestinal bleeding (melena) and swollen liver (hepatomegaly) were more prevalent in RTPCR-negative patients.

Thrombocytopenia, a hallmark of DENV infection, was observed in 82% of patients, with 3,120 patients (both RTPCR positive and RTPCR negative) having a platelet count of less than 1,00,000 platelets/mm^3 (Table 4). Additionally, patients exhibited slight anaemia (66.5%), low leucocyte count (70%), and elevated levels of SGOT and SGPT.

DENV Serotype Distribution in Single and Concurrent Infections

The changing trend in DENV serotypes within the population highlights the need for continuous surveillance of DENV outbreaks. DENV can be classified into four distinct serotypes (DENV-1 to DENV-4), each inducing different immune responses to infection. Patient data were collected and categorised based on the serotypes as shown in Figure 1. Of the 3,800 patients, 2,388 (63%) tested positive for DENV serotypes (Fig-1, Table-1).Within the RTPCR-positive group, 1,481 patients (62%) had single infections, while 907 patients (38%) had concurrent infections (Figure 1). The most prevalent DENV serotype in the single-infected population was DENV-2, with a 47.1% infection rate (Figure 2a). DENV-3 was the second most predominant serotype, while DENV-4 was the least common among single infections.

In concurrent infections, the DENV-2 and DENV-3 combination was the most common, observed in 56% of patients (Figure 2b and Table 5).

DENV-3 Serotype Outbreaks Observed in India

DENV-2 has been a dominant serotype observed in multiple outbreaks globally. In India, all four serotypes have been circulating since the 2003 DENV outbreak in Delhi. Our

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study observed a quadrennial change in dominant DENV serotypes.[20] From 2010 to 2018, DENV-2 was the dominant serotype in single infections. However, from 2019 to 2022, there was a shift in dominance from DENV-2 (36%) to DENV-3 (38%) (Figure 3a).

A similar trend was observed during the 2003–2004 DENV outbreak in Delhi, with a noticeable shift from DENV-2 to DENV-3 (Figure 3b). Since the 2003 outbreak, DENV-3 has persisted in Delhi. Subsequent years saw DENV-3 outbreaks in Kolkata, Rajasthan, Madhya Pradesh, Maharashtra, Haryana, and recently in Karnataka[5,7,9,13,15–19] (Table 6 and Figure 3c).

Comparing recent outbreaks, many studies have noted a shift in dominant DENV serotypes, with DENV-2 becoming secondary. This trend could be attributed to factors such as population movement, changes in vector patterns, or varying monsoon conditions. These changes have led to the gradual adaptation of DENV-3 as the predominant serotype in outbreaks. Therefore, the possibility of future dominant DENV-3 outbreaks cannot be ruled out.

Table 1.Demographics of the Study Population

Demography	Division	No. of Cases	Percentage
Region	Rural	2266	60
	Urban	1534	40
Sex	Male	2102	55
	Female	1698	45
RTPCR test	Positive	2388	63
	Negative	1412	37
Age (years)	< 5	485	13
	6–10	1417	37
	11–15	1062	28
	> 15	836	22

Table 2.Dengue Serology

Dengue Serology	Result	RTPCR	
		Positive	Negative
		n (%)	n (%)
NS1	Positive	2174 (91.0)	42 (3.0)
	Negative	214 (9.0)	1370 (97.0)
NS1 + IgM	Positive	212 (8.9)	33 (2.3)
	Negative	2176 (91.1)	1379 (97.7)
IgM	Positive	19 (0.8)	14 (1.0)
	Negative	2369 (99.2)	1398 (99.0)
IgM + IgG	Positive	0 (0.0)	20 (1.4)
	Negative	2388 (100.0)	1392 (98.6)
IgG	Positive	0 (0.0)	58 (4.1)
	Negative	2388 (100.0)	1354 (95.9)

Table 3.Clinical Manifestations of Patients with RTPCR Positive and Negative Results

Clinical Features	RTPCR Positive n	%	RTPCR Negative n	%
Fever	2388	100.0	1412	100.0
Retro-orbital pain	2142	89.7	1251	88.6
Flushing	1725	72.2	982	69.5
Rash	1498	62.7	526	37.3
Acute respiratory distress syndrome	174	7.3	272	19.3
Encephalopathy	68	x2.8	164	11.6
Hepatitis	196	8.2	87	6.2
Melena	98	4.1	588	41.6
Epistaxis	21	0.9	27	1.9

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Haematemesis	24	1.0	14	1.0
Hepatomegaly	188	7.9	644	45.6
Splenomegaly	54	2.3	153	10.8
Ascites	478	20.0	84	5.9
Pleural effusion	212	8.9	62	4.4
Cyanosis	29	1.2	18	1.3
Convulsion	72	3.0	188	13.3
Oliguria	19	0.8	18	1.3
Hypoglycaemia	24	1.0	11	0.8
Abscess	19	0.8	32	2.3
Pneumoniae	31	1.3	48	3.4
Haematuria	16	0.7	11	0.8
Gum bleeding	8	0.3	5	0.4
Sub-conjunctival haemorrhage	5	0.2	38	2.7

Table 4.Platelets Count Among the Study Population (per mm^3)

Age (Years)	< 20,000	20001–50000	50001–100000	> 100001	Total
< 5	41	210	142	92	485
6–10	149	572	488	208	1417
11–15	72	388	520	82	1062
> 15	24	282	232	298	836
Total	286	1452	1382	680	3800

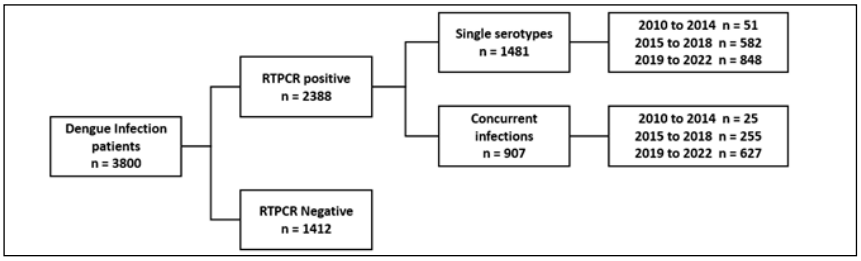

Figure 1.Categorisation of Patients' Data Collected during the Last 12 Years

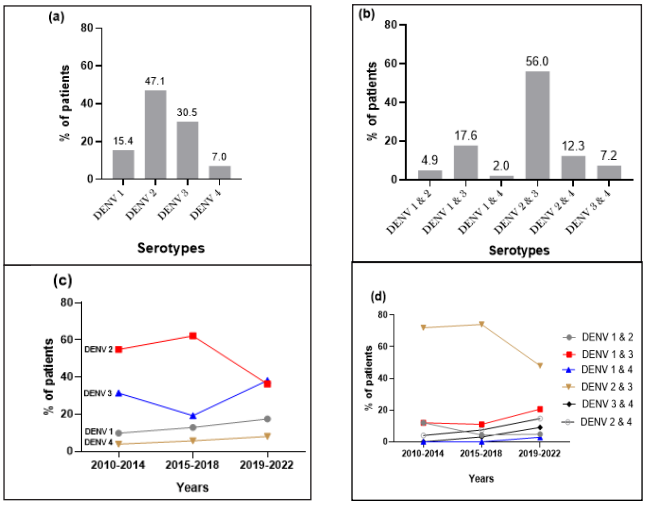

Figure 2.Serotype Distribution in the Population (a).Single Serotype Infected Patients showing 47.1% DENV-2 Infections; (b).Concurrent Infections showing 56% DENV-2 and DENV-3 Infections; (c).Single Infection Distribution; and (d).Concurrent Infection Distribution Over 12 Years

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Table 5.Distribution of DENV Serotypes Over a 12-Year Period at Central Karnataka

Incidence of Dengue serotypes (2010 to 2022)						
Single serotypes	2010–2014		2015–2018		2019–2022	
	Number	Percentage	Number	Percentage	Number	Percentage
DENV-1	5	9.8	75	12.9	148	17.5
DENV-2	28	54.9	362	62.2	308	36.3
DENV-3	16	31.4	112	19.2	324	38.2
DENV-4	2	3.9	33	5.7	68	8.0
Total	51	100	582	100.0	848	100
Concurrent infections						
DENV-1 & DENV-2	3	12.0	11	4.3	30	4.8
DENV-1 & DENV-3	3	12.0	28	11.0	129	20.6
DENV-1 & DENV-4	0	0.0	0	0.0	18	2.9
DENV-2 & DENV-3	18	72.0	189	74.1	301	48.0
DENV-2 & DENV-4	1	4.0	19	7.5	92	14.7
DENV-3 & DENV-4	0	0.0	8	3.1	57	9.1
Total	25	100.0	255	100.0	627	100.0

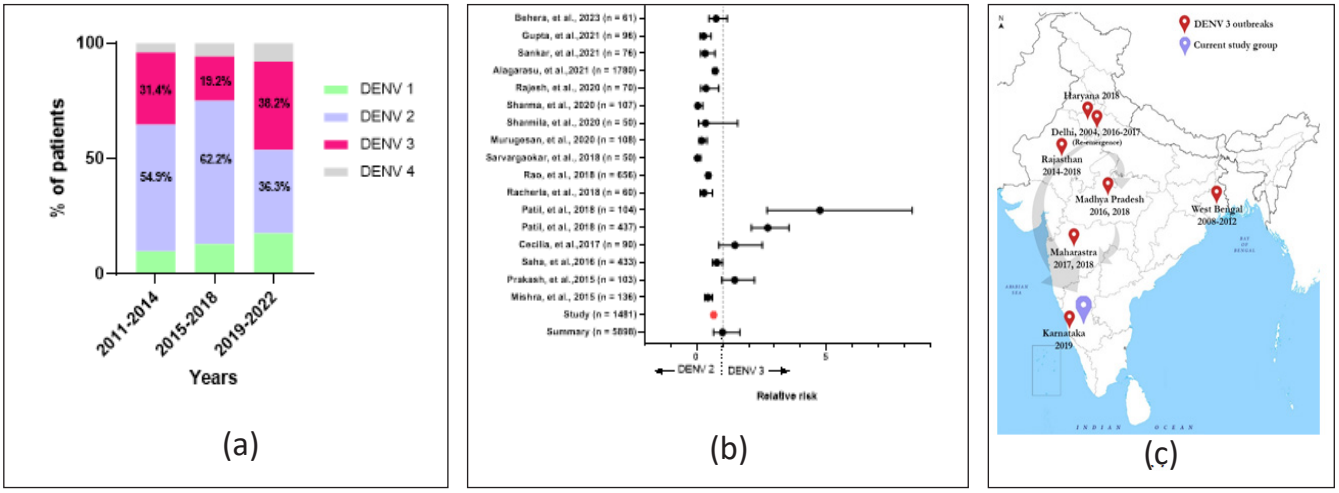

Figure 3.DENV-3 Serotype Outbreaks Observed in India in the Last Decade (a).DENV Serotype Population Observed in Four Years Window (Quadrennial); (b).Forest Plot showing Relative Risks of DENV-3 Serotype Observed since 20156–14,18,19,24,26,27 (c).DENV Infection Across India Published since 2015 showing the Possibility of DENV-3 Movement Through Indian States (India map downloaded from www.mapsofindia.com)

Table 6.DENV-3 Outbreaks in Different States of India

Study Author, Year	Cohort Year	Location (RTPCR Positive Number)
Dash et al., 2006[15]	2004	Delhi (n = 135)
Saha et al., 2016 [9]	2008-2012	Kolkata (n = 433)
Tiwari et al., 2019[16]	2016	Madhya Pradesh (n = 135)
Barde et al., 2019[17]	2016	Madhya Pradesh (n = 71)
Kalitha et al., 2021[13]	2014-2018	Rajasthan (n=735)
Patil et al., 2018[7]	2017	Nashik (n = 437)
Islam et al., 2020[5]	2016-2017	Delhi (n = 48)
Alagarasu et al., 2021[18]	2018	Madhya Pradesh (n = 109) Haryana (n = 67) Maharashtra (n = 380)
Rao et al., 2020[19]	2019	Manipal, Karnataka (n = 59)

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Discussion

Dengue remains a significant global health concern, affecting millions of individuals each year across tropical and subtropical regions.[1] The ongoing threat of dengue is further complicated by the presence of multiple serotypes of the dengue virus (DENV), which contribute to the variability and unpredictability of outbreaks. The four distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) circulate simultaneously in many endemic regions, presenting challenges not only for diagnosis and treatment but also for prevention efforts. One of the most difficult aspects of managing dengue is the frequent shifts in serotype dominance that occur over time, altering the epidemiological landscape and making it harder to predict the severity of future outbreaks.[3] These shifts can lead to increased cases of severe disease, particularly in individuals who have previously been infected by one serotype and are then exposed to another, as immunity to one serotype does not confer protection against the others.[4,20] Instead, it can increase the risk of severe outcomes, such as DHF and DSS, through a process known as antibody-dependent enhancement (ADE).

In India, where dengue is hyperendemic, the disease is primarily seasonal, with outbreaks peaking during the monsoon and post-monsoon periods, typically from August to December. This seasonal pattern aligns with the life cycle of Aedes mosquitoes, which breed in stagnant water that accumulates during the rainy season.[8–10] Our 12-year study involving 3,800 patients provides important insights into the demographics and epidemiological characteristics of dengue in the region. One notable finding was the high proportion of paediatric cases, with 78% of patients being under 15 years of age. This highlights the significant burden of dengue on children, who may be more susceptible to severe disease and complications. In addition, a slight male predominance was observed, with 55% of cases occurring in males, which is consistent with other studies suggesting that men may be at higher risk of exposure to mosquito vectors, potentially due to differences in occupational or outdoor activities. A majority of patients (60%) came from rural areas, underscoring the rural-urban divide in healthcare access and the heightened vulnerability of rural populations to vector-borne diseases like dengue.

The circulation of all four DENV serotypes in both single and concurrent infections further highlights the dynamic and complex epidemiology of dengue in India.[23,24] This hyperendemicity increases the risk of individuals being infected by more than one serotype over time, which can lead to more severe disease outcomes.[15,16] Concurrent infections with multiple serotypes were detected in a significant number of cases in our study, indicating that the epidemiological environment is not static, but rather continuously evolving as different serotypes emerge and re-emerge as dominant strains.

Early clinical signs of dengue, such as thrombocytopenia (a low platelet count) and lymphopenia (a reduction in lymphocytes), are commonly used by clinicians to diagnose the illness.[7,24,25] However, these signs are not specific to dengue, and confirmation of the diagnosis typically requires laboratory tests, such as those detecting DENV antigens. In our study, patients were, on average, 5 to 7 days into their illness at the time of recruitment, with high fever being a prominent symptom, as indicated by body temperatures above 100 °F. Out of the patients tested, 63% were confirmed to have DF, while 37% presented with symptoms resembling dengue but lacked a confirmed diagnosis (dengue-like fever). Laboratory testing using RT-PCR revealed that 91% of positive cases had detectable NS1 antigen, a marker of acute dengue infection, while 8.9% had both NS1 and IgM antibodies, and only 0.1% had IgM antibodies alone. Interestingly, none of the patients tested positive for IgG or a combination of IgM and IgG, which is likely due to the early stage of the illness when the tests were conducted. Despite the variation in serotypes, the clinical features among patients were largely consistent, with common symptoms including high fever, retro-orbital pain (pain behind the eyes), skin rashes, ascites (fluid accumulation in the abdomen), and hepatomegaly (enlarged liver). Thrombocytopenia was observed in 82% of patients, along with low white blood cell counts (leucopenia), anaemia, and elevated transaminase levels, which indicate liver inflammation.

One of the most concerning aspects of dengue epidemiology is the role of concurrent infections in exacerbating the severity of the disease.[26] Our study revealed 907 cases of concurrent infections, where patients were infected with multiple DENV serotypes simultaneously. These concurrent infections are thought to arise from individuals being bitten by mosquitoes carrying different serotypes. The presence of multiple serotypes within a single patient can lead to severe complications, including DHF and DSS, due to immune system interactions that enhance the severity of the disease.[26,27] Historically, DENV-2 has been the most prominent serotype in Asia, associated with more severe disease outcomes. However, recent outbreaks in Central and South India have shown a shift toward DENV-3 and, to a lesser extent, DENV-1. Our study found that DENV-3 had re-emerged as a dominant serotype, accounting for 30.5% of cases, while DENV-2 still represented 47.1% of infections. DENV-4, although less common, re-emerged in South India in 2017, but accounted for only 7% of cases in our study. The co-circulation of all four serotypes suggests that India remains at risk for severe outbreaks, as the presence of multiple serotypes within a community can increase the incidence of secondary infections and, consequently, severe disease.

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India's tropical climate, with its variable rainfall patterns and monsoons, plays a critical role in shaping the epidemiology of dengue.[15,18] The timing and intensity of monsoon rains directly affect mosquito breeding, influencing both the frequency and severity of outbreaks.[12] Changes in monsoon patterns, such as increased rainfall or extended monsoon seasons, can alter the transmission dynamics of DENV and potentially lead to shifts in serotype dominance.[16,22] In our study, we observed a significant shift in serotype dominance over the 12-year period. From 2010 to 2018, DENV-2 was the predominant serotype, but in the last four years, DENV-3 began to dominate. This shift occurred during a period of increased precipitation and longer monsoon seasons, suggesting that climate factors may have contributed to changes in the epidemiological profile of dengue in the region.

The movement of DENV-3 across India has been noted in previous studies, including the 2003–2004 outbreak in Delhi, where all four serotypes were detected, marking the region as hyperendemic. Since then, DENV-3 has been increasingly reported in other states, such as Kolkata, Rajasthan, Madhya Pradesh, Maharashtra, Haryana, and Karnataka.[26–29] Our findings align with these reports, as we observed a similar pattern of DENV-3 movement in Karnataka, particularly during the 2019 spike in cases. This trend underscores the evolving nature of dengue serotype prevalence in India and highlights the need for continuous surveillance to track the emergence and spread of different serotypes. By understanding these trends, public health officials can better anticipate future outbreaks and implement targeted interventions to reduce the burden of dengue in vulnerable populations.

Conclusion

This study underscores the dynamic nature of dengue epidemiology, particularly the shifting dominance of DENV serotypes over time. Our findings reveal that while DENV-2 was the predominant serotype in India from 2010 to 2018, there has been a notable shift towards DENV-3 dominance in recent years. This shift is consistent with observations from earlier outbreaks, including the significant DENV-3 presence in Delhi and other regions of India following the 2003–2004 outbreak.

The study highlights the variability in clinical presentations and serotype prevalence, with all four DENV serotypes circulating simultaneously and contributing to both single and concurrent infections. The high prevalence of concurrent infections, coupled with the observed changes in serotype dominance, emphasises the need for ongoing surveillance and adaptive management strategies to effectively address dengue outbreaks.

Additionally, the shift towards DENV-3 dominance aligns with changes in monsoon patterns and precipitation, suggesting that environmental factors may play a role in serotype dynamics. This study contributes valuable insights into the evolving epidemiology of dengue in India and reinforces the importance of continuous monitoring to anticipate and manage future outbreaks effectively.

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Conflict of Interest: None

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