



Research Article

Dengue Epidemic during COVID-19 Pandemic: Clinical and Molecular Characterization – A Study from Western Rajasthan

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Abstract

The concurrent emergence of dengue fever and the COVID-19 pandemic posed significant challenges to India's healthcare system, particularly in Western Rajasthan, a region characterized by its arid climate and unique socio-demographic conditions. This study aimed to investigate the clinical and molecular characteristics of dengue during the COVID-19 pandemic, focusing on trends, diagnostic challenges, and serotype distribution.

Conducted at Dr. S.N. Medical College, Jodhpur, in 2021, the study included 550 dengue-positive patients confirmed via rapid diagnostic tests and further analyzed using Dengue NS1 antigen and IgM antibody ELISA. Molecular characterization was performed using RT-PCR for serotyping.

The results revealed a male predominance (72.36%) and a higher incidence in the 21–30-year age group (39.09%). Urban areas accounted for 67.73% of cases, with significant NS1 and IgM positivity (p=0.042 and p=0.004, respectively). Most cases (86.91%) were managed outpatient, though IgM positivity was significantly higher among hospitalized patients (19.19%, p<0.001), indicating severe or prolonged infections. Platelet counts were above $100,000/\text{mm}^3$ in 86.91% of cases, with only 0.37% showing critically low counts (< $20,000/\text{mm}^3$). Seasonal analysis showed a peak in October (n=325), correlating with post-monsoon vector breeding. Serotyping identified DENV2 as the dominant strain (97.42%), associated with severe dengue manifestations, including Dengue Haemorrhagic Fever (DHF).

The study highlights the dual burden of dengue and COVID-19, emphasizing the need for enhanced vector control, improved diagnostic strategies and public health interventions during overlapping outbreaks. The predominance of DENV2 underscores the importance of serotype-specific surveillance and preparedness to mitigate future dengue epidemics in the region.

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Keywords: Dengue; COVID-19; Serotyping; DENV2; Western Rajasthan; Seasonal variation; Thrombocytopenia





Introduction

India continues to face significant challenges related to vector-borne diseases with vector-borne diseases for a long time, and dengue has become a major public health issue, as per the NVBDCP report of 2021 [1]. The COVID-19 pandemic further exacerbated the burden, putting a double burden on our healthcare system, as highlighted by WHO in 2021 [2]. Western Rajasthan, which is known for its dry climate and unique social conditions, a sharp rise in dengue cases was observed during the pandemic. This situation posed substantial challenges for doctors, policymakers, and researchers, according to ICMR (2021) [3].

The dengue outbreak happening at the same time as COVID-19 not only put pressure on medical resources but

also caused confusion in diagnosing patients because both diseases show similar symptoms like fever, body pain, and low platelet count [4]. This made it hard for clinicians to differentiate between the two conditions. On top of that, testing facilities were limited, and most healthcare resources were focused on COVID-19, making the situation even more critical, as mentioned by the MoHFW in 2021 [5].

Studying the genetic makeup of the dengue virus strains in the region during this time can help determine whether viral changes contributed to increased disease severity or faster spread, as pointed out by Biswas and Chattopadhyay in 2021 [6].

This research aims to look at the trends, impact, and challenges caused by dengue and COVID-19 happening together in this region. By understanding what led to the rise



in dengue cases, we hope to improve vector control methods, make diagnosis easier, understand the disease through molecular characterization, and develop better strategies to handle such outbreaks in the future.

Materials and methods

This study was conducted in the Department of Microbiology at Dr. S.N. Medical College, Jodhpur (Rajasthan), in the year 2021. It was a hospital-based study, and the study was approved by the Institutional Ethics Committee (IEC) with approval number 5166/27.03.2018. After taking consent from the patients, Socio-demographic data were collected using a structured proforma. We included patients of all age groups who showed clinical symptoms of dengue-like illness. However, patients with co-infections like malaria, typhoid, chikungunya, or other febrile illnesses were excluded from the study.

From each patient, 5 ml of blood was collected, and the serum was separated using the centrifugation technique. Out of the total samples, 550 patients tested positive using the Dengue Duo Rapid Diagnostic Test. These positive samples were further confirmed using the Dengue NS1 antigen and Dengue IgM Capture ELISA tests. The samples that were positive in the ELISA test were then processed for dengue serotyping using the CDC DENV-1-4 Real-Time RT-PCR Assay.

Results

A total of 550 patients were confirmed positive for dengue infection using the Dengue Duo Rapid Card test. These samples were further analyzed using both Dengue NS1 antigen and Dengue IgM antibody Capture ELISA. Among them, 229 (41.64%) tested positive for NS1 antigen, while 271 (49.27%) were positive for IgM antibodies. Additionally, 121 samples showed positivity for both NS1 antigen and IgM antibody. The data indicated a male predominance of 72.36%, with the highest infection rate observed in the 21–30-year age group (39.09%).

Table 1 shows a higher number of dengue cases were from urban areas (67.73%) compared to rural areas (31.27%). Dengue NS1 positivity was significantly higher in urban areas (63.76%) than in rural areas (36.24%) (p = 0.042, significant). Dengue IgM positivity was also significantly more common in urban areas (62.73%) than rural (37.27%) (p = 0.004, significant).

Table 1: Demographic distribution of cases. Dengue NS1 ELISA Dengue IgM Capture Variables Number Percentage **ELISA** positive positive Geographic distribution 378 146 (63.76%) 170 (62.73%) Urban 67.73 31.27 101 (37.27%) Rural 172 83 (36.24%) p value 0.042(S)0.004 (S) Hospital presentation OPD 478 86.91 201 (87.77%) 219 (80.81%) IPD 72 13.09 28 (12.23%) 52 (19.19%)

0.705

< 0.001 (S)

Most dengue cases were managed in the outpatient department (OPD) (86.91%), while only 13.09% required inpatient hospitalization (IPD). Dengue NS1 positivity showed no significant difference between OPD and IPD cases (p=0.705, not significant). However, dengue IgM positivity was significantly higher among hospitalized (IPD) patients (19.19%) compared to OPD cases (80.81%) (p<0.001, significant), suggesting severe or prolonged infections in admitted cases.

Table 2 shows the platelet count distribution among dengue-positive patients varied significantly. The majority of patients (86.91%) had a platelet count above 100,000 /mm³, indicating that severe thrombocytopenia was observed to be relatively uncommon. Only 2 patients (0.37%) had critically low platelet counts below 20,000 mm³, which is often associated with severe dengue and an increased risk of hemorrhagic manifestations. 12 patients (2.18%) had platelet counts between 21,000-40,000 mm³, while 30 patients (5.45%) had counts in the 41,000-50,000 mm³ range.28 patients (5.09%) had platelet levels between 51,000-100,000mm³, reflecting moderate thrombocytopenia.

Table 3 shows the distribution of dengue cases and their ELISA test positivity varied significantly throughout 2021. No cases were reported from January to July, indicating a clear seasonal pattern. The first dengue cases appeared in August (n = 6), with a slight increase in September (n = 18), before peaking dramatically in October (n = 325).October recorded the highest number of cases (n = 325), with 147 (64.19%) testing positive for Dengue NS1 ELISA and 201 (74.17%) positive for Dengue IgM Capture ELISA. This suggests that

Table 2: Clinical platelet counts of cases.					
Platelet counts	Number of cases	Percentage %			
< 20,000	2	0.37			
21,000-40,000	12	2.18			
41,000-50,000	30	5.45			
51000-1 lakh	28	5.09			
>1 lakh	478	86.91			
Total	550	100			

Table 3: Seasonal variation of dengue cases in relation to NS1 and IgM positivity.					
Month Total number of cases		Dengue NS1 ELISA positive cases	Dengue IgM Capture ELISA positive cases		
Jan. 2021	0	0	0		
Feb. 2021	0	0	0		
Mar. 2021	0	0	0		
April 2021	0	0	0		
May 2021	0	0	0		
June 2021	0	0	0		
July 2021	0	0	0		
Aug. 2021	6	3 (1.31%)	2 (0.74%)		
Sept. 2021	18	18 3 (1.31%)			
Oct. 2021	ct. 2021 325 147 (64.19%)		201 (74.17%)		
Nov. 2021	148	64 (27.95%)	54 (19.92%)		
Dec. 2021	53	12 (5.24%)	11 (4.06%)		
Total 550		229 (100%)	271 (100%)		
p value		0.006 (S)	< 0.001 (S)		

p value



dengue transmission was at its peak during this month. After October, cases declined gradually, with 148 cases in November and 53 in December. Correspondingly, Dengue NS1 and IgM positivity also decreased, indicating a waning outbreak. In August and September, case numbers were low (6 and 18, respectively), with minimal NS1 and IgM positivity. This suggests the early phase of dengue transmission before the epidemic peak. The p - values for NS1 ELISA (0.006) and IgM ELISA (< 0.001) indicate a statistically significant variation in dengue positivity across the months. This highlights the seasonality of dengue, with peak cases occurring in the postmonsoon period (October-November), emphasizing the need for enhanced surveillance and preventive measures during these months.

Table 4 shows the serotyping of dengue-positive samples revealed a significant predominance of the DENV2 serotype among infected individuals. Among the 271 ELISA-positive cases, DENV2 was the most prevalent serotype, detected in 264 cases (97.42%), indicating that it was the dominant strain in circulation during the study period.DENV3 was identified in only 4 cases (1.48%), suggesting limited circulation.DENV1 was detected in 3 cases (1.10%), showing minimal prevalence. DENV4 was not detected in any sample (0.00%), implying its absence in the study population.

These findings highlight a strong predominance of the DENV2 serotype, which has been associated with severe outbreaks and increased disease severity in previous studies. The low detection of other serotypes suggests a limited cocirculation of multiple strains during this period. This data is crucial for understanding dengue epidemiology and guiding public health interventions.

The Table 5 presents details of two dengue cases, both classified as Dengue Hemorrhagic Fever (DHF) and associated with DENV2 serotype. Patient 1: A 35-year-old male with a critically low platelet count (< 20,000cu/ mm), presenting with fever, cough, arthralgia, myalgia, and rash. The patient required hospitalization for 5 days.Patient 2: A 45-year-old

Table 4: Dengue serotyping of positive ELISA cases.					
Serotype	ELISA positive cases	Percentage			
DENV1	3	1.10%			
DENV2	264	97.42%			
DENV3	4	1.48 %			
DENV4	0	00.00%			
Total	271	100%			

Table	Table 5: Severe dengue and their associated serotypes.								
Case	Sex/Age	Platelet counts	Presentation of symptoms	Classification	Hospital stay (in days)	Serotype			
1	35/M	< 20000mm ³	Fever, cough, arthralgia, myalgia and rash	DHF	5	DENV2			
2	45/M	< 20000mm ³	Fever, cough, arthralgia, myalgia and rash	DHF	4	DENV2			

male with similar severe thrombocytopenia (< 20,000/mm³) and the same symptom profile. This patient had a hospital stay of 4 days.

Both cases were linked to DENV2 infection, a serotype known for its association with more severe dengue manifestations, including DHF. The presence of thrombocytopenia and systemic symptoms highlights the need for close monitoring and supportive care in such patients. These findings reinforce the clinical impact of DENV2 and its potential to cause severe disease requiring hospitalization.

Discussion

The findings from the study provide valuable insights into the epidemiology, clinical presentation, and serotype distribution of dengue infection during the study period.

Dengue positivity and diagnostic markers

Among the 550 dengue-positive cases, 41.64% were positive for NS1 antigen, and 49.27% were positive for IgM antibodies. The NS1 antigen is typically detected in the early phase of infection (days 1–5), while IgM antibodies appear later (after day 5). The higher IgM positivity suggests that many patients were tested during the later stages of infection or had secondary dengue infections, which are associated with a stronger IgM response (World Health Organization [2] and Premazzi Papa M, et al. [7].

The overlap of 121 cases positive for both NS1 and IgM indicates concurrent early and late-phase infections, which is consistent with the dynamics of dengue immune responses [8].

Demographic and geographic distribution

The higher infection rate among males aligns with previous studies suggesting that males may experience greater exposure to Aedes mosquito vectors due to occupational or behavioral factors [9].

This age group is often more mobile and engaged in outdoor activities, increasing their exposure to Aedes mosquitoes [10].

The higher prevalence in urban areas is consistent with the urban transmission dynamics of dengue, driven by factors such as population density, poor sanitation, and stagnant water sources conducive to Aedes breeding [11]. The significant difference in NS1 and IgM positivity between urban and rural areas (p = 0.042 and p = 0.004, respectively) further underscores the urban-centric nature of dengue transmission.

Seasonal pattern

The seasonal pattern of dengue transmission observed in the study, particularly the peak in October, can be further contextualized by considering the impact of the COVID-19 pandemic on public health measures, including vector control activities such as fogging.



During the COVID-19 pandemic, many public health resources were diverted to managing the pandemic, leading to a reduction in routine vector control activities such as fogging, larviciding, and community-based clean-up interventions. This disruption likely contributed to an increase in Aedes mosquito populations, creating favourable conditions for dengue transmission [12].

Community participation in vector control activities, such as eliminating mosquito breeding sites, remains a key component of community-based dengue prevention efforts. Public health campaigns should emphasize the importance of these measures, even during times of crisis [13]. Additionally, restrictions on movement and public health interventions aimed at controlling SARS-CoV-2 might have inadvertently influenced vector breeding dynamics by altering human behaviour and exposure patterns [14].

The absence of reported dengue cases between January and July further supports the seasonal nature of dengue transmission. The initial rise in cases from August to September indicates the early phase of virus circulation, with the epidemic peak in October (n = 325). This coincides with previous studies that highlight a lag period between the monsoon season and peak dengue transmission due to increased vector breeding [15].

Clinical management and severity

Most cases were managed in outpatient settings, reflecting the predominance of mild to moderate dengue cases. However, the significantly higher IgM positivity among hospitalized patients (19.19% IPD vs.~80.81% OPD, p < 0.001) suggests that IgM positivity may correlate with severe or prolonged infections requiring hospitalization [16].

The majority of patients (86.91%) had platelet counts above 100,000/mm³, indicating that severe thrombocytopenia was uncommon. However, the presence of critically low platelet counts (< 20,000/mm³) in 0.37% of cases highlights the risk of severe dengue, particularly Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS) [16].

Serotype distribution

The overwhelming predominance of DENV2 is significant, as this serotype has been associated with severe outbreaks and increased disease severity in previous studies [8]. The limited circulation of DENV1, DENV3, and the absence of DENV4 suggest a lack of serotype diversity during the study period, which may impact herd immunity and future outbreak susceptibility and future outbreak dynamics [17].

The two severe cases of DHF associated with DENV2, characterized by thrombocytopenia and systemic symptoms, underscore the serotype's potential to cause severe disease. This aligns with previous reports linking DENV2 to severe dengue manifestations, including DHF and DSS [8,18].

Ethical statement

This study was approved by the Institutional Ethics Committee (IEC No. 5166/27.03.2018). Informed consent was obtained from all participants prior to sample collection. All procedures were conducted in accordance with the ethical standards of the institutional and national research committees, and with the 1964 Helsinki declaration and its later amendments.

Conclusion

Epidemiological tracking plays a crucial role in monitoring dengue virus (DENV) circulation, outbreak patterns, and regional serotype prevalence. Disease severity prediction helps identify the risk of severe dengue (DHF/DSS) due to secondary infections involving a different serotype, which is linked to antibody-dependent enhancement. It also supports public health strategies by aiding in the formulation of control measures, vector surveillance, and outbreak preparedness. Additionally, clinical management benefits from understanding disease progression and guiding patient monitoring in endemic regions.

Limitations and future aspects

- Due to COVID-19 waves, fewer blood samples were tested between January and July for dengue test.
- The present study was a single-center study; hence results may not be generalizable to the entire region or state.
- This study was hospital-based, and many mild, unreported cases may have led to underestimation of the true positivity rate.

Our study contributes to the understanding of dengue epidemiology in Jodhpur, Rajasthan, and offers insights that can inform public health strategies.

By addressing these challenges, we can strive for more effective dengue control and prevention measures, ultimately reducing the burden of this disease on the local population.

The genomic analysis enhances our knowledge of Dengue virus diversity and provides a foundation for future research.

The study's findings call for increased awareness, improved healthcare-seeking behavior, and community-based interventions to effectively combat Dengue in the region.

The insights gained from this research can guide public health policies, vaccine development, and tailored strategies for Dengue prevention and control.

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