

## **A Study on Circulating Serotype Diversity of Dengue Virus During 2021-2022 in A Tertiary Care Hospital, North-West Zone, Rajasthan**

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### **Abstract**

**Introduction:** DENV-2 is associated with more severe dengue and has significant association with DHF (dengue hemorrhagic fever) in comparison to DENV-1, DENV-3 and DENV-4. So, infection with DENV-1 followed by DENV-2 is more dangerous than infection with DENV-4 followed by DENV-2. This increases the necessity of serotyping of dengue virus with every outbreak/ every year.

**Methods:** The Cross-sectional study was conducted in the Department of Microbiology, Sardar Patel Medical College, Bikaner and about 9000 blood samples were collected out of which 332 sero positive samples for NS1 antigen &/ IgM antibody were stored & studied from P.B.M. & A.G. of hospitals from April 2021 to April 2022.

**Results:** 92.47% patients were NS1 positive and 7.53% patients were NS1 negative. 85.84% patients were IgM negative, 10.84% patients were IgM positive and 3.31% patients were IgM equivocal result. 84.64% samples were of low titrate IgG and 15.36% samples were of high titrate IgG. 84.34% patients had primary dengue infection and 15.66% patients had secondary dengue infection. Out of 332 sample identified as 30(9.04%) DENV-1, 289(87.05%) as DENV-2, 9(2.71%) as DENV-3 and 1(0.30%) as DENV-4. Three (0.90%) cases were co-infected with serotypes DENV-1,2. DENV-2 was found to be predominant serotype.

**Conclusion:** In conclusion, this study reveals that all four serotypes and co-infection with multiple serotypes were found to be circulating in Bikaner Rajasthan which suggests that this could be a hyper endemic province for dengue. DENV-2 was the predominant serotype followed by DENV-1. Thus the results of the present investigation can assist in designing control strategies for future epidemics and to determine the evolutionary pattern of the emerging Dengue virus.

**Keywords:** Dengue, Serotype, DENV.

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## Introduction

Dengue virus has 4 serotypes, so a person can be infected 4 times. Recovery from infection due to one serotype can provide lifelong immunity. But cross-immunity from other serotypes is not present. Further subsequent infection due to other serotypes increases the risk of severe dengue. Dengue virus types 1, 2, and 3 have all been isolated during previous dengue outbreaks in Delhi, but a particular type has always predominated. During the 1996 outbreak of DHF/DSS, we had 26 isolates of dengue virus type 2, but only 1 isolate was identified as dengue type 1. However, we subsequently showed that dengue virus type 1 continued to circulate during the post-epidemic period and became the predominant strain. Dengue virus type 3 has recently reemerged in South Asia, including north India, [1-3]

DENV-2 is associated with more severe dengue and has significant association with DHF (dengue hemorrhagic fever) in comparison to DENV-1, DENV-3 and DENV-4. So, infection with DENV-1 followed by DENV-2 is more dangerous than infection with DENV-4 followed by DENV-2. This increases the necessity of serotyping of dengue virus with every outbreak/ every year.[4-5]

## Material And Method

### Study Period

This is a cross-sectional study, from April 2021 to April 2022.

### Place Of Study

The present study was conducted in the Department of Microbiology, Sardar Patel Medical College, Bikaner and blood samples were collected from P.B.M. & A.G. of hospitals.

### Sample Size

In the time period of one year, about 300 samples positive for NS1 antigen and/ IgM antibody archived at -80° C were taken.

## Inclusion Criteria

- Patient with clinical symptoms of dengue like fever, myalgia, arthralgia, headache, retro-orbital pain, nausea and vomiting for 1-7 days was taken.
- Patient with history of previous dengue infection was taken.
- Sample positive for NS1 antigen and IgM antibody by ELISA test was taken.
- Serum archived at -80° C was taken.

## Exclusion Criteria

- Patients with clinical symptoms suggesting different etiology was excluded.
- Negative samples were excluded.
- Inadequate amount of serum in samples were excluded.
- Haemolysed and turbid samples were excluded.

## Methodology

Collected Blood samples were centrifuged and sera were separated. Than NS1 antigen and IgM antibody positive samples from ELISA test were archived at -80° C deep freezer.

Following tests were performed on serum-

- Dengue IgG Capture ELISA test by PANBIO Dengue IgG Capture ELISA kit
- Dengue RNA extraction by MagMAX Dx Prefilled Viral/Pathogen Nucleic Acid Isolation kit
- Single step RT-PCR test for serotyping by Hi-PCR Dengue Serotyping Probe PCR kit

## Statistical Analysis

Statistical analysis was done for using chi-square test and unpaired t test.

## Results

The Cross-sectional study was conducted in the Department of Microbiology, Sardar Patel Medical College, Bikaner and about 9000 blood samples were collected out of which 332 sero positive samples for NS1

antigen & IgM antibody were stored & studied from P.B.M. & A.G. of hospitals from April 2021 to April 2022.

**Table 1: Result of NS1 antigen & IgM antibody ELISA test in study subjects (n=332)**

Result	NS 1 antigen	IgM antibody
Positive	307 (92.47%)	36 (10.84%)
Negative	25 (7.53%)	285 (85.84%)
Equivocal	-	11 (3.31%)
Total	332 (100.00)	332 (100.00)

In our study, 307 (92.47%) patients were NS1 positive and 25 (7.53%) patients were NS1 negative. 285 (85.84%) patients were IgM negative, 36 (10.84%) patients were IgM positive and 11 (3.31%) patients were IgM equivocal result.

**Table 2: Result of IgG antibody ELISA test in study subjects (n=332)**

IgG antibody titrate	No of cases	Percentage
Low titrate (<18 panbio unit)	281	84.64
High titrate (>22 panbio unit)	51	15.36
Equivocal (18-22 panbio unit)	0	0
Total	332	100.00

In our study, 281 (84.64%) patients had low titrate IgG present and 51 (15.36%) patients had high titrate IgG present.

**Table 3: Categorization of dengue into Primary or secondary infection in study subjects (Sero positive patients for NS1 & IgM (n=332))**

Type of infection	No of cases	Percentage
Primary dengue infection (low titer <18 P.U.)	281	84.34
Secondary dengue infection (high titer >22 P.U.)	51	15.66
Total	332	100.00

In our study, 281 (84.34%) patients had primary dengue viral infection and 51 (15.66%) patients had secondary dengue viral infection.

**Table 4: Interpretation of Dengue serotyping by Multiplex RT-PCR (Sero positive patients for NS1 & IgM (n=332))**

Dengue serotype	No of cases	Percentage
DENV 1	30	9.04
DENV 2	289	87.05
DENV 3	9	2.71
DENV 4	1	0.30
DENV 1,2	3	0.90
Total	332	100.00

In our study, out of 332 sample identified as 30 (9.04%) DENV-1, 289 (87.05%) as DENV-2, 9 (2.71%) as DENV-3 and 1 (0.30%) as DENV-4. Three (0.90%) cases were co-infected with serotypes DENV1,2.

DENV-2 was found to be predominant serotype.

#### Discussion

This is a Cross-sectional study which was conducted in the Department of

Microbiology, Sardar Patel Medical College, Bikaner and blood samples were collected from P.B.M. & A.G. of hospitals from April 2021 to April 2022. In the time period of one year, 332 samples archived at -80° C were included in our study.

In our study, 51 (15.66%) patients had secondary dengue viral infection. This is low in comparison to a study of North India, Chungal K.H. et.al. (2017)[6], where 66.7% patients had secondary dengue infection.

In our study, out of 332 samples 30(9.04%) were identified as DENV-1, 289(87.04%) as DENV-2, 9(2.71%) as DENV-3 and 1(0.30%) as DENV-4. Three (0.90%) cases were co-infected with serotypes DENV-1,2. So in the present study, all the four serotypes were identified along with co-infections. DENV-2 was found to be the most predominant serotype in circulation followed by DENV-1.

Another study conducted in 2010 at Ernakulam district also showed DENV-2(43.2%) as predominant serotype.[7]

Genotyping study conducted in Kerala in the year 2010[8] also had a predominance of DENV2 serotype (51.72%). However, a similar study from a tertiary care center in Central Kerala for a period of one year (January to December, 2016) had shown a predominance of DENV-1.

Our study findings are consistent with a study by Basawarajappa et. Al[9], 2021, in which majority of the infections were caused due to DENV-2 serotype in Bangalore.

In study by Rajiwani et. al.[10],2022, Jaipur, most common serotypes in circulation were DENV-2 and DENV-3 (60% cases) which is also similar with our findings.

Our study also observes that all four serotypes were found to be co-circulating, which was also seen the study by Barde et. al.[11], 2019, Jabalpur. All the four DENV serotypes with dominance of DENV-3 (n = 33; 47%) were detected. In a study by

Vanlalhmningthanpuiet et. al.[12] (2020), Aurangabad, also the predominant serotype of DENV detected was DENV-3 along with DENV-1, 2 & 4 stating the co-circulation of all 4 serotypes.

Co-infection was also seen in our study in 3 samples by DENV 1 & DENV 2. In a similar study by Rahman et. al.[13], 2018, Assam, co-infection was seen in 15.1% samples by DENV 1 & 2.

Co-infection was also stated in Barde et. al.[11], 2019 where two samples had dual infection of DENV-2 and DENV-3.

In Vanlalhmningthanpuiet et. al.[12], 2020, also one case was co-infected with DENV-2 & 3. In Rajiwani et. al.[10], 2022, 4 samples had co-infection with serotypes 1 and 3, and one patient had co-infection with serotypes 2 and 3. These Stats that concurrent infection is present in population in throughout the country by mainly DENV 1, 2 & 3.

## Conclusion

In conclusion, this study reveals that all four serotypes and co infection with multiple serotypes were found to be circulating in Bikaner Rajasthan which suggests that this could be a hyper endemic province for dengue. DENV-2 was the predominant serotype followed by DENV-1. Thus the results of the present investigation can assist in designing control strategies for future epidemics and to determine the evolutionary pattern of the emerging Dengue virus.

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