# Original Research Paper

## Microbiology

## PREVALENT DENGUE SEROTYPES IN CENTRAL, INDIA

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**ABSTRACT** **Introduction:** Dengue is an endemic arbovirus disease, and ~3.9 billion peoples from more than 125 countries (accounting 40% of world population) are at risk .The dengue virus has four genetically distinct serotypes (DENV 1–4) that can cause either mild febrile flu-like symptoms, i.e., dengue fever or severe form called dengue hemorrhagic fever and dengue shock syndrome , which is characterized by vascular permeability and plasma leakage. **Methodology:** This was a cross-sectional study done from January 2019 to december 2021 at Department of Microbiology, I.G.G.M.C, Nagpur. The serum samples were then screened for the presence of dengue NS1 antigen, using Pan Bio NS1Ag ELISA kit. Laboratory confirmed NS1 positive acute phase serum samples, were sent to NIV Pune for serotyping within 72 hours of collection. All samples were transported on dry ice and they were tested by real time RT PCR at National Institute of Virology, Pune for dengue virus RNA & serotype identification. **Results:** out of total 283 suspected fever cases were subjected for the test NS1Ag ELISA of them, NS1Ag ELISA was found to be positive in 197 (69.61%) cases and out of total 197 NS1 ELISA positive cases were subjected for the test RT-PCR of them, RT-PCR was found to be positive in 108 (54.82%) cases. The most common serotype was found to be serotype DEN 1 followed by DEN 2 in both years. male preprodance of 61(56.48%) was found than females 47 (43.52%), In the year 2019-2020, maximum dengue positivity 9 (60%) was seen in the age group of 0-10 years while in the year 2020-2021, maximum positivity 52 (55.92%) was seen in the age group of 21-30 years. maximum positivity was found to be in the month of September followed by in October in both the years. **Conclusion:** The present study concluded that all four serotypes circulate with predominant being DENV-1 type . Dengue mainly affected adult male population, and seasonal peak during monsoon and post-monsoon period. The information of predominant serotypes can guide in forecasting dengue outbreaks and improving control measures of vectors thus may be helpful in the prevention of outbreaks.

**KEYWORDS :** Dengue virus, dengue virus-1, dengue virus-2, season, serotypes

## INTRODUCTION

Dengue virus causes dengue fever which is a febrile arboviral disease.It is an enveloped RNA virus with a single positive strand It is transmitted through the bite of infected Aedes mosquito.The genome is composed of three structural protein genes, encoding the nucleocapsid or core protein (C), a membrane associated protein (M), an envelope protein (E), and seven non-structural (NS) protein genes. The non-structural (NS) proteins are NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5 (WHO, 2014). NS1 is a highly conserved glycoprotein that seems to be essential for virus viability but has no established biological activity. There are four virus serotypes, which are designated as DENV 1, DENV 2, DENV 3 and DENV 4. Although all four serotypes are antigenically similar, they are different enough to elicit cross-protection only for a few months after infection by any one of them. Infection with any one serotype confers lifelong immunity to the virus serotype.(5)All four serotypes can cause the full spectrum of disease from a subclinical infection to a mild self-limiting disease, the dengue fever (DF) and a severe disease that may be fatal, the dengue haemorrhagic fever/dengue shock syndrome (DHF/ DSS). Immunity based antibody dependent enhancement (ADE) is one of the pathogenesis process, for the severe cause of the disease. [1]

Dengue is an endemic arbovirus disease, and ~3.9 billion peoples from more than 125 countries (accounting 40% of world population) are at risk .The dengue virus has four genetically distinct serotypes (DENV 1–4) that can cause either mild febrile flu-like symptoms, i.e., dengue fever (DF) or severe form called dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which is characterized by vascular permeability and plasma leakage. In India, dengue poses an increasing burden with significant underreporting. Establishment of different serotypes and emergence of new genotype within serotypes which leads to enhanced disease severity due to the exacerbation of immune response mediated by Cytokines. [2]

Acute dengue can be diagnosed by detecting the DENV genome. A number of real-time RT-PCR assays have previously been developed, including a universal DENV real-time RT-PCR designed and validated methods detecting DENV genomes are recommended by the World Health Organization to be used for laboratory confirmation of dengue during the first five to six days after symptomatic onset and PCR-based techniques are at present the only methods for determining the infecting serotype during acute disease.

The main aim and objectives of this study to find out the prevalent dengue serotype in the region.

## MATERIALS AND METHODS

This was a cross-sectional study done from January 2019 to december 2021 in the Department of Microbiology, I.G.G.M.C, Nagpur. The patients were evaluated by clinicians for dengue virus infection based on WHO 2009 criteria to identify patients suspected to have dengue. After informed consent, 3-5ml of blood samples from clinically suspected cases (who reported within one to five days of fever) were collected in a container without anti-coagulants. Patient history was taken on structured clinical data sheet provided by NIV, Pune. Serum was separated from the blood samples by centrifugation. The serum samples were then screened for the presence of dengue NS1 antigen, using Pan Bio NS1Ag ELISA kit and processed according to manufacturer's guidelines .

Laboratory confirmed NS1 positive acute phase serum samples, were sent to NIV Pune for serotyping within 72 hours of collection. Those serum samples which could not be processed within 72 hours of collection were stored in the deep freezer at -80 C until further processed. All samples were transported on dry ice and they were tested by real time RT PCR at National Institute of Virology, Pune for dengue virus RNA & serotype identification.

## RESULTS
### Table.1 DENGUE TEST POSITIVITY BY NS1 Ag ELISA :

| Sr.no | Test | NS1 Ag ELISA | Percentage % |
|---|---|---|---|
| 1. | Positive | 197 | 69.61% |
| 2. | Negative | 86 | 30.39% |
| 3. | Total | 283 | 100% |

Total 283 suspected fever cases were subjected for the test NS1Ag ELISA of them, NS1 Ag ELISA was found to be positive in 197 (69.61%) cases and 86 (30.39%) were negative.
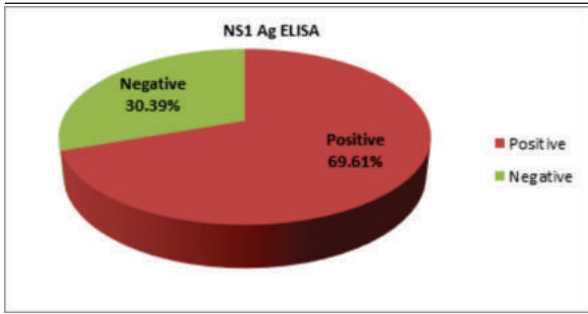
**Fig1: Dengue test positivity by NS1 Ag ELISA**

**Table.2 RT-PCR test results of NS1 positive samples :**

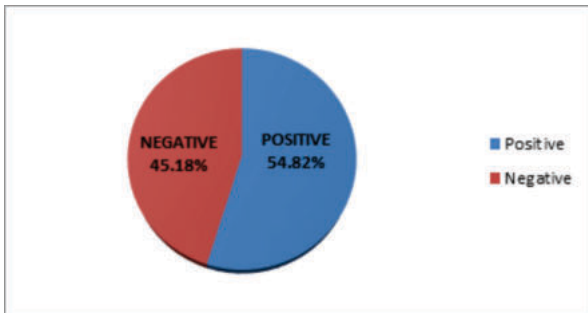| RT-PCR results | No of cases | Percentage % |
|---|---|---|
| Positive | 108 | 54.82% |
| Negative | 89 | 45.18% |
| Total | 197 | 100% |



**Fig2: Dengue test positivity by RT-PCR test**

**Table.3 DISTRIBUTION OF SEROTYPES**

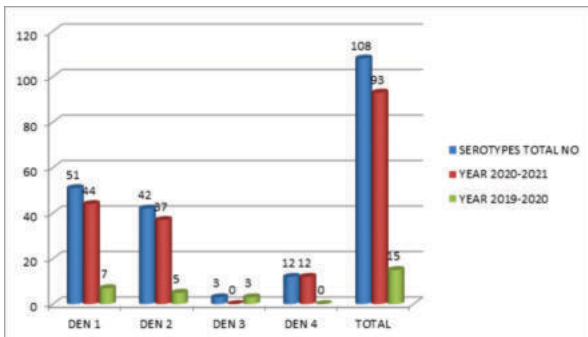| Sr. no | Serotypes | In The Year 2019-2020 | In The Year 2020-2021 | Total No. |
|---|---|---|---|---|
| 1. | DEN 1 | 7 (46.67%) | 44 (47.31%) | 51 (47.22%) |
| 2. | DEN 2 | 5 (33.33%) | 37 (39.79%) | 42 (38.89%) |
| 3 | DEN 3 | 3 (20%) | 00 | 3 (2.78%) |
| 4. | DEN 4 | 0 | 12 (12.90%) | 12 (11.11%) |
| 5 | TOTAL | 15 (100%) | 93 (100%) | 108 (100%) |



**Fig 3 :- DISTRIBUTION OF SEROTYPES**

**Table 4: GENDER WISE DISTRIBUTION OF SEROTYPES**

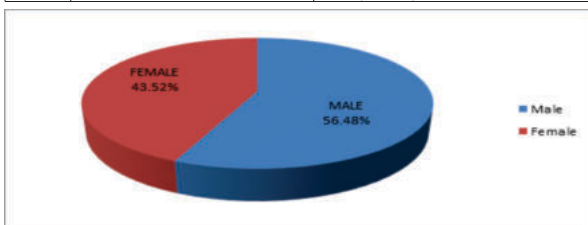| Sr. no | Gender | Positive cases, n =108% |
|---|---|---|
| 1. | Male | 61(56.48%) |
| 2. | Female | 47 (43.52%) |
| 3. | Total | 108 (100%) |



**Fig 4:- GENDER- WISE DISTRIBUTION**

**Table 5: GENDER- WISE DISTRIBUTION OF SEROTYPES:-**

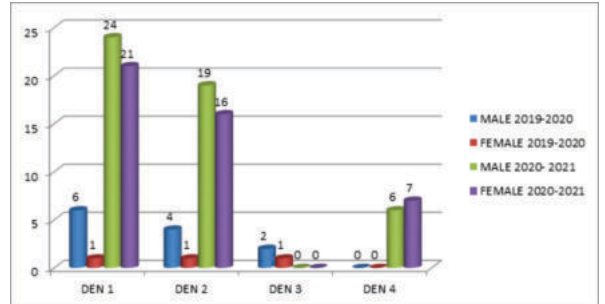| Sr.no | Years | Gender | Den 1 | Den 2 | Den 3 | Den 4 | Total | Overall (108)% |
|---|---|---|---|---|---|---|---|---|
| 1. | 2019-2020 | Male | 6 (40%) | 4(26.66%) | 2(13.33%) | 0 | 12 (80%) | 15 (13.89%) |
| | | Female | 1(6.66%) | 1(6.66%) | 1(6.66%) | 0 | 3 (20%) | |
| 2. | 2020-2021 | Male | 24(25.80%) | 19(20.43%) | 0 | 6(6.45%) | 49(52.68%) | 93 (86.11%) |
| | | Female | 21(22.58%) | 16(17.20%) | 0 | 7(7.52%) | 44(47.31%) | |



**Fig 5: GENDER- WISE DISTRIBUTION OF SEROTYPES:-**

**Table 6:- AGE-WISE DISTRIBUTION in 2019-2020 and 2020-2021**

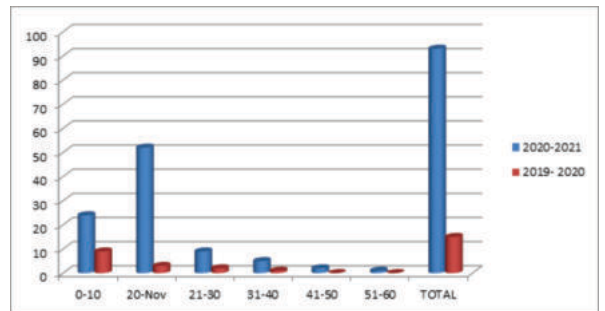| Sr.no | Age groups (years) | Positive cases (%) in 2019-2020 | Positive cases (%) in 2020- 2021 |
|---|---|---|---|
| 1. | 0-10 | 9 (60%) | 24 (25.80%) |
| 2. | 11-20 | 3 (20%) | 9 (9.68%) |
| 3. | 21-30 | 2 (13.33%) | 52 (55.92%) |
| 4. | 31-40 | 1 (6.67%) | 5 ( 5.37%) |
| 5. | 41-50 | 0 | 2 (2.16%) |
| 6. | 51-60 | 0 | 1 (1.07%) |
| 7. | Total | 15 (100%) | 93 (100%) |



**Fig 6:- AGE-WISE DISTRIBUTION in 2019-2020 and 2020-2021**

**Table 7: AGE-WISE DISTRIBUTION OF DENGUE POSITIVE CASES BY RT-PCR**

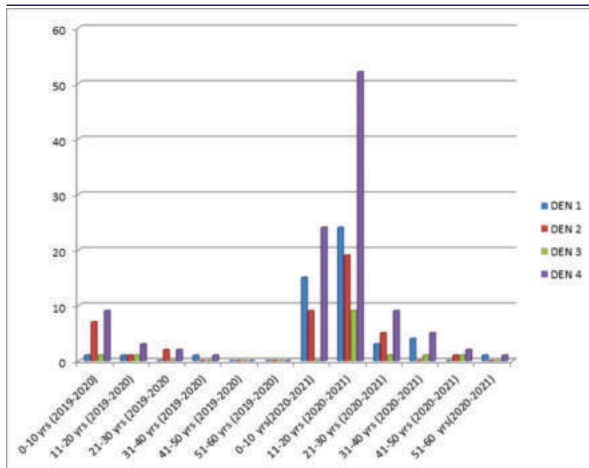| Sr.No | Years | Age groups | Den 1 | Den 2 | Den 3 | Den 4 | Total (108) % |
|---|---|---|---|---|---|---|---|
| 1. | 2019-2020 | 0-10 | 1 | 7 | 1 | 0 | 9 |
| | | 11-20 | 1 | 1 | 1 | 0 | 3 |
| | | 21-30 | 0 | 2 | 0 | 0 | 2 |
| | | 31-40 | 1 | 0 | 0 | 0 | 1 |
| | | 41-50 | 0 | 0 | 0 | 0 | 0 |
| | | 51-60 | 0 | 0 | 0 | 0 | 0 |
| | | Total | | | | | 15 |
| 2. | 2020-2021 | 0-10 | 15 | 9 | 0 | 0 | 24 |
| | | 11-20 | 3 | 5 | 0 | 1 | 9 |
| | | 21-30 | 24 | 19 | 0 | 9 | 52 |
| | | 31-40 | 4 | 0 | 0 | 1 | 5 |
| | | 41-50 | 0 | 1 | 0 | 1 | 2 |
| | | 51-60 | 1 | 0 | 0 | 0 | 1 |
| | | Total | | | | | 93 |

**Fig :7: AGE-WISE DISTRIBUTION OF SEROTYPES**

**Table 8: MONTH-WISE DISTRIBUTION OF DENGUE POSITIVITY BY RT-PCR**

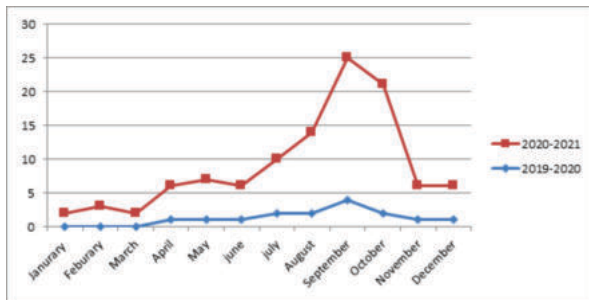| Sr.No | Months | 2019-2020 | 2020-2021 |
|-------|--------|-----------|-----------|
| 1. | Janurary | 0 | 2 (2.15%) |
| 2. | Feburary | 0 | 3 (3.22%) |
| 3. | March | 0 | 2 (2.15%) |
| 4. | April | 1 (6.67%) | 5 (5.38%) |
| 5. | May | 1 (6.67%) | 6 (6.45%) |
| 6. | June | 1 (6.67%) | 5 (5.38%) |
| 7. | July | 2 (13.33%) | 8 (8.60%) |
| 8. | August | 2 (13.33%) | 12 (12.90%) |
| 9. | September | 4 (26.66%) | 21 (22.58%) |
| 10. | October | 2 (13.33%) | 19 (20.43%) |
| 11. | November | 1(6.67%) | 5 (5.38%) |
| 12. | December | 1(6.67%) | 5 (5.38%) |
| 13. | Total | 15 (100%) | 93 (100%) |



**Fig8: Month wise distribution of dengue positivity by RT-PCR**

## DISCUSSION

In the present study, out of total 283 suspected fever cases were subjected for the test NS1Ag ELISA of them, NS1Ag ELISA was found to be positive in 197 (69.61%) cases and 86 (30.39%) were negative. Our findings were similar to R. Ganesan et al (2019) [3] NS1 Ag was positive in 51.4% patients.and Neelam Gupta et al ( 2019 ) [4] found positivity in 64.3% .

In the present study, out of total 197 NS1 ELISA positive cases were subjected for the test RT-PCR of them, RT-PCR was found to be positive in 108 (54.82%) cases and 89 (45.18%) were negative. Similar findings were reported by Tanmay K. Mehta (2009)[5] , that on the basis of RT-PCR, dengue infection was confirmed in 55.78% patients. Study done by Preeti Bharaj et al (2008 ) [6] reported 69.5% dengue positivity of RNA by RT-PCR.

In the table 3 In the present study , in the year 2019-2020 , the most common serotype was found to be serotype DEN 1 followed by DEN 2 and DEN 3 while DEN 4 was not observed and in the year 2020-2021 DEN1 was found to be the most common serotype followed by DEN 2 but the serotype DEN 3 was completely replaced by DEN 4. Study done by Rubina Paul et al (2018) [7] , DENV-1 serotype was found in 57% , DENV-2 serotype was found in 3% and DENV-3 serotype was found in 40% DENV-4 serotype was not found in any sample.

Study done by BINITA JOSEPH ARING et al (2021) [8] found that Circulation of all the four serotypes, higher monotypic infection by DENV-1 serotype 41.2%, followed by DENV-4, DENV-2 and DENV-3. While study done by K. Alagarasu et al ( 2021) [9] found serotype DENV-2 was found to be the most common serotype in many states. Another study done by Shera Chaterji et al (2011) [10] found that DENV serotype, DENV-1 is 35.1%, DENV-2 is 29.9%, DENV-3 35.1% , DENV-4 is 0%. Rajesh NT et al (2017) [11] ,Circulation of all the four serotypes of DENV has been reported from different regions of India. In 2017, DENV-1 and DENV-3 were more prevalent in Maharashtra, Western India. Persistent circulation of DENV-1 might have led to herd immunity permitting the emergence of other serotype viruses. Circulation of multiple serotypes suggests the hyperendemic nature of dengue in this region.

In the present study ,out of total 108 dengue positive cases, 61(56.48%) were males and 47 (43.52%) were females. The ratio of Male to female was found to be 1.29 :1. Similar study done Rubina Paul et al(2018) [7] shows the higher prevalence of dengue infection was noted among males 64% than females 36%. The affected male to female ratio was 1.8:1 . Nazish Parveen et al (2018) [12] also reported that more males were affected 53.57% as compared to females 33.33% with affected male/female ratio of 1.6:1. It might be due to differences in the socio-cultural environment where males are more exposed to outdoor activities and their bodies less covered as compared to females. [13]

In present study in both the consecutive year 2019-2020 and 2020-2021, male preprodance was found and the most common serotype was DEN 1 in the males than in the females. BINITA JOSEPH ARING et al (2021) [8] found that The ratio of male cases was higher than female, and in age group 21-35 year (47%) While the study done by Rishi Gowtham Racherla et al (2018) [14] found DENV-1in 5.9% female and 6.7% male, DEN 2 in 29.4% female and 22.2% male. DEN 3 in 5.9% female and 8.9% male, DEN 4 in 19.6% female and 26.7% male.

Table no 6 shows that In the year 2019-2020, Out of total 15 dengue positive cases, maximum number of dengue positivity 9 (60%) was seen in the age group of 0-10 years, followed by 3 (20%) cases in the age group of 11-20 years, 2 (13.33%) were in the age group of 21-30 years, 1 (6.67%) were seen in 31-40 years, 0 % cases above 50 years and above.

In the present study, in the year 2020-2021, Out of total 93 dengue positive cases, maximum number of dengue positivity 52 (55.92%) was seen in the age group of 21-30 years, followed by 24 (25.80%) cases in the age group of 0-10years , 9 (9.68%) were in the age group of 11-20 years , 5 ( 5.37%) were seen in 31-40 years, 2 (2.16%) cases in 41-50 years .least no of positivity were seen above 60 years i.e.1 (1.07%) years.

Our findings are similar to Ekta Gupta et al (2006) [15] found that age group most commonly affected is 21–30 yrs followed by 0-10 years. Similar results were reported by Preeti Bharaj et al (2008) [6] were the maximum number of positivity were in the age group of 20–30 years (35.4%) followed by the age group 12–20 years (20.8%).

Nazish Parveen et al (2018) [12] found the maximum numbers of dengue positive cases 52.7% were found in the age group of 21–40 years followed by 0–20 years 33.3% cases respectively similary Dr. Krishna Kumar Mani et al (2020) [16] observed more positivity of 30.90% in the age group 20 to 30 years followed by 10 to 20 years is 23.64% and 30 to 40 years is 21.82%. Rishi Gowtham Racherla et al (2018) [14] found that Adult age group was most affected amongst all the age groups This age group comprises working and student population. As dengue is mainly spread through Aedes mosquitoes which are day biters, the crowded areas such as workplaces and colleges may facilitate the spread of infection. Paediatric age group was next affected followed by geriatric age group.

Table no 7 shows: In the present study, in the year 2019-2020, serotype distribution , out of total 15 cases , 9 cases were in the age group of 0-10 years which includes, 1 cases of DEN 1, 7cases of DEN 2, 1 cases of DEN 3, 0 cases of DEN 4 . 3cases were in the age group of 11-20 years which includes, 1 cases of DEN 1, 1 cases of DEN 2, 1 cases of DEN 3, 0 cases of DEN 4 . 2 cases were in the age group of 21-30 years which includes, 0 cases of DEN 1, 2 cases of DEN 2, 0 cases of DEN 3, 0 cases of DEN 4 . 1 cases were in the age group of 31-40 years which includes, 1 cases of DEN 1, 0 cases of DEN 2, DEN 3 and DEN 4and no cases were seen above 50 years and above.

In the year 2020-2021, serotype distribution , out of total 93 cases , 24 cases were in the age group of 0-10 years which includes, 15 cases of DEN 1, 9cases of DEN 2, 0 cases of DEN 3 and DEN 4 . 9 cases were in the age group of 11-20 years which includes, 3 cases of DEN 1, 5 cases of DEN 2, 0 cases of DEN 3, 1 cases of DEN 4 . 52 cases were in the age group of 21-30 years which includes, 24 cases of DEN 1, 19 cases of DEN 2, 0 cases of DEN 3, 9 cases of DEN 4 . 5 cases were in the age group of 31-40 years which includes, 4 cases of DEN 1, 0 cases of DEN 2, DEN 3 and 1 case of DEN 4. 2 cases were in the age group of 41-50 years which includes, 0 cases of DEN 1, 1 cases of DEN 2, 0 cases of DEN 3, 1 cases of DEN 4. 1 cases were in the age group of 51-60 years which includes, 1cases of DEN 1 and 0 cases of DEN 2, DEN 3 and DEN 4 respectively.

Study done by Rishi Gowtham Racherla et al (2018) [14] found DENV-1 - 3% in ≤18years of age group , 3% in 19-59 years ,0% in ≥60years , DENV- 2- 16% in ≤18years, 9% in 19-59 years and 0% in ≥60years ,DENV-3 – 1% in ≤18years ,6% in 19-59 years and 0% in ≥60years ,DEN 4- 8% in ≤18years , 14% in 19-59 years and 0% in ≥60years

In the present study, dengue positivity in the years 2019-2020 was found to be maximum in the month of September 2019-2020 i.e. 4 (26.66%) and maximum positivity in the years 2020-2021 was found to be in the month of September 21 (22.58%) followed by in October 19 (20.43%) and august 12 (12.90%). Similar findings were observed in the study done by Rubina Paul et al ( 2018) [7] that majority of cases tested positive for dengue were obtained in the month of September and October while study done by Vanlalhmingthanpuii et al (2020) [1] found that majority of cases i.e.19 (52.7%) were seen in the months of September to November 2017 (monsoon & post monsoon season).

## CONCLUSION
This study revealed the presence of four serotypes of dengue virus, with predominance of DENV-1 followed by DEN 2. This information on predominant circulating serotypes helps us in forecasting dengue outbreaks, which will certainly strengthen our ability and preparedness to address recurrent epidemics in this part of India. This should be concerns to public health authority and should stimulate the arbovirus diagnostic method in public health laboratories.

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