Mapping the distribution and trends of co-circulating dengue virus serotypes in Odisha, India: A retrospective facility-based analysis

SALONI LABALA, ABHINAV SINHA, SAILENDRA PANDA, JYOTIRMAYEE TURUK, SANGHAMITRA PATI, PRAKASH KUMAR SAHOO

ABSTRACT

Background. Dengue, caused by mosquito bite, is an emerging disease of international concern. Evidence regarding the prevalent dengue serotypes is scarce, but essential for its management during the outbreaks. Hence, we mapped the distribution and trends of currently prevalent dengue virus (DENV) serotypes in Odisha.

Methods. We conducted a facility-based retrospective study from referral samples sent for the diagnosis/confirmation of dengue in 2018. The samples were serologically tested for enzyme-linked immunosorbent assay (ELISA) IgM antibody and NS1. Only NS1-positive samples were chosen for serotyping. A pool of 8–10 NS1-positive samples were analysed for district-wise serotypes. Ribonucleic acid extraction and nested polymerase chain reaction (PCR) was done from NS1-positive samples. The PCR products were then subjected to gel electrophoresis.

Results. A total of 2892 samples were screened for dengue virus across various districts of Odisha where 763 samples were found to be NS1-positive. Thirteen of 18 districts covering all topographies of Odisha predominantly had DENV2 serotype. Only few districts such as Balangir, Kalahandi and Rayagada had mixed serotypes.

Conclusion. Although DENV2 is predominantly prevalent, mixed serotypes too exist in Odisha. Evidence based on variations of dengue serotypes across topographies, seasons, gender and age groups may support public health efforts in preventing the disease.

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INTRODUCTION

Dengue, a member of the *Flaviviridae* family, is one of the major emerging public health concerns in India but we do not have a specific antiviral drug against it. Though, the virus prevails for a long time, only prevention and control of the virus is targeted, and eradication seems a daunting target. In a short span, the dengue infection has rapidly spread from tropical to subtropical areas with around 2.5 billion people living in the dengue-risk zone.[1]

The dengue infection among humans is caused by *Aedes aegypti* mosquito, which preferentially rests in dark and cool closets, and generally tends to bite humans indoors. They lay eggs on the sides of water-holding containers such as tyres. These eggs hatch into larvae following rains or floods. Favourable environmental conditions help in transforming larvae into pupae followed by adult mosquitoes, in little over a week. It takes a mean of 15 days (5–33 days) for the virus to multiply, mature, and migrate to the salivary glands before the mosquito can transmit it to humans.[2,3]

The dengue virus (DENV) has four predominant serotypes: DENV1, DENV2, DENV3 and DENV4;[4] though a fifth serotype that is closely related to DENV4 has also been reported in 2013.[5] Patients with dengue infection manifest a high-grade fever (40 °C/104 °F) accompanied with other flu-like symptoms such as headache, pain behind eyes, nausea, vomiting, myalgia, arthralgia and rash. The incubation period of dengue is 4–10 days. The disease is a continuum that ranges from mild to severe forms, which can also be fatal.[2,6] Repeated exposures to dengue virus may also lead to dengue haemorrhagic fever (DHF), which manifests more aggressively compared to dengue fever. The clinical presentation of DHF includes severe abdominal pain, bruising or bleeding under the skin, clammy or cold skin, nose bleeding, a sudden decrease in the blood pressure, haematemesis, black stool and capillary leakage.[7] There is a short-lived, cross-protective immunity to heterologous serotypes for months post-exposure.[8] Dengue virus-specific immunoglobulin (IgM) antibodies appear as early as around 3 days of dengue viral fever and can persist for 30–60 days, whereas IgG antibodies appear at about day 7, peak at 2–3 weeks and persist for life.[9]

Not much is known about the geographical distribution of type-specific dengue virus in Odisha as dengue serotyping is not done routinely. Additionally, each dengue serotype exhibits varied levels of severity and different symptoms. There is a need to understand the patterns of dengue spread through serotyping to facilitate knowledge of endemicity and assist public health efforts for prevention and control of dengue. We aimed to map

ICMR-Regional Medical Research Centre, Chandrasekharpur, Bhubaneswar, Odisha, India
SALONI LABALA, ABHINAV SINHA, SAILENDRA PANDA, JYOTIRMAYEE TURUK, SANGHAMITRA PATI, PRAKASH KUMAR SAHOO Division of Virology

Correspondence to PRAKASH KUMAR SAHOO; *shuvaprakash@gmail.com*

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the distribution and co-circulation of currently prevalent dengue serotypes in Odisha.

METHODS

Study design and setting

A retrospective cross-sectional study based at the Regional Medical Research Centre, Bhubaneswar was done through the dengue samples received in 2018. The Regional Virology Research and Diagnostic Laboratory (VRDL) of this institute is the diagnostic centre for various viral diseases including dengue where samples of suspected dengue cases are referred for testing from entire Odisha.

Odisha is located between 17.78°N and 22.73°N latitudes, and 81.37°E and 87.53°E longitudes. The total area of the state is 155 707 km^2 which includes coastal plains in the east and hills in the west. Four meteorological seasons: winter (January–February), pre-monsoon (March–May), southwest monsoon (June–September) and northeast monsoon (October–December) are experienced in the state. This climatic pattern favours mosquitoes to survive easily making Odisha a dengue-prone area.[10]

Topographically, Odisha is divided into four regions.[11] The first is the northern plateau, a predominantly hilly area with an elevation ranging from 2000 to 3000 feet above sea level; the second is a coastal belt consisting of a number of river deltas; the third is the central table land with flat, slightly surge and folded topography with an elevation around 1000 feet; and the fourth is the eastern ghats with elevated plateaus ranging from 900 to 2000 feet dominated by hills with valleys and plains lying between them. Our study included samples from 18 of 30 districts of Odisha covering all the four topographical regions (Fig. 1).

Serotyping investigations

Samples of the blood and cerebrospinal fluid (CSF) along with a standardized case report form were referred to this facility from different hospitals across the state for diagnosis. The samples were transported to the laboratory in cold chain. According to the onset of fever, the dengue IgM and/or NS1 test was done. Although for NS1, samples were tested individually, but we decided to pool samples for serotype analysis. We pooled 8–10 NS1 enzyme-linked immunosorbent assay (ELISA)-positive samples for further analysis. Samples were pooled for serotyping in alignment with the objective of our study, i.e. we needed serotypes for district/region and not for individuals.

The samples were categorized based on their age, gender, district and month of collection. The pooled samples were subjected to ribonucleic acid (RNA) extraction for serotyping. Further, the extracted samples proceeded for nested polymerase chain reaction (PCR) as suggested by Lanciotti *et al.*[12] Briefly, nested PCR samples underwent two rounds of test, the first used reverse transcriptase enzyme which converted RNA into deoxyribonucleic acid (DNA) and in the second round Taq polymerase enzyme was used to obtain a more specific result. Nested PCR was done to increase the specificity of DNA amplification by reducing the non-specific amplification. The nested PCR assay was conducted using two sets of primers (outer pair and inner pair) for a single locus and two successive PCR cycles were run. In the first PCR cycle, outer pair primers generated DNA products as the regular PCR does thus, their DNA products may contain non-specifically amplified DNA fragments. Further, a second round of PCR using the second set of 'inner' primers whose binding sites are completely or partially different and located after the 3′ end of each of the outer pair primer was used in the first PCR reaction. Therefore, a second PCR product shorter than the first one was produced. Nested primers were used as important control for experiments with unknown genome sequence. The PCR products along with the LADDER DNA (100bp) were analysed with gel electrophoresis to identify its size.

FIG 1. Topographical representation of Odisha

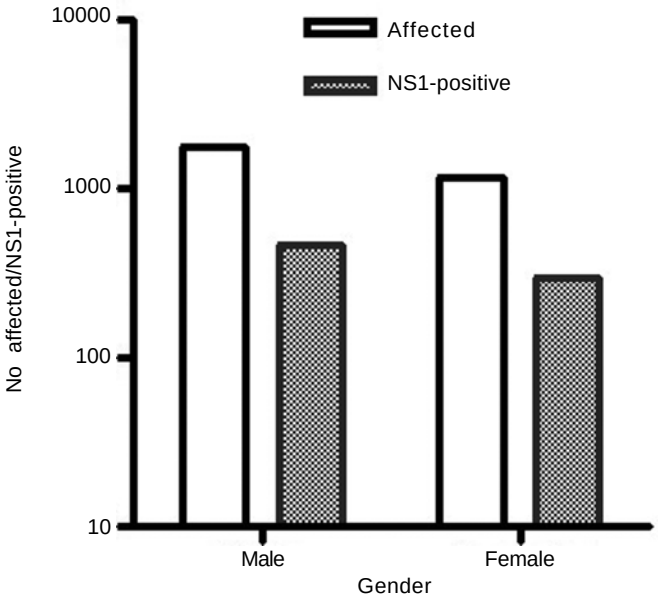

FIG 2. Gender distribution of dengue affected individuals and their NS1 antigen positivity

Ethical considerations

Ethical approval was obtained from the State Research and Ethics Committee as well as Institutional Ethics Committee of ICMR-Regional Medical Research Centre, Bhubaneswar before the study. Individual patient consent was not taken as the samples were referred to this centre.

RESULTS

A total of 2892 serum samples were received from 18 district hospitals across Odisha during January–December 2018. These samples were subjected to confirmation of dengue virus and identifying serotype. After the diagnosis, only NS1 antigen-positive samples were considered for serotyping and analysis. Of the 2892 samples tested, 763 (26.4%) were positive for NS1 antigen by ELISA. The samples received from Angul, Balangir, Baragarh, Dhenkanal (Central Table Land); Mayurbhanj (Northern Plateau), Khorda, Nayagarh, Puri, Bhadrak, Cuttack, Balasore, Jagatsinghpur, Kendrapada, Ganjam, Jajpur (Coastal belt) and Rayagada, Kalahandi, Kandhamal (Eastern Ghats) were positive for dengue virus.

Among 2892 samples received, 1742 (60.2%) patients were men and 1150 (39.8%) were women. A total of 763 individuals were found to have antibodies against NS1 dengue virus antigen and 260 patients had IgM antibodies to dengue virus. The gender distribution of patients having antibodies to NS1 antigen is shown in Fig. 2. There was no significant difference observed in NS1-positivity rate between genders.

We observed a seasonal variation as most cases of dengue virus occurred during June to November, with a peak around August. The positivity rate of dengue varied from 20% to 34% across the seasons. Additionally, the dengue-positivity rate had consistent patterns with seasonal variation (Fig. 3).

Antibodies to NS1 antigen among the infected individuals were found to be higher among those aged 11 to 50 years. The peak antigen positivity 30.5% (Fig. 4) was observed among those aged 31–40 years.

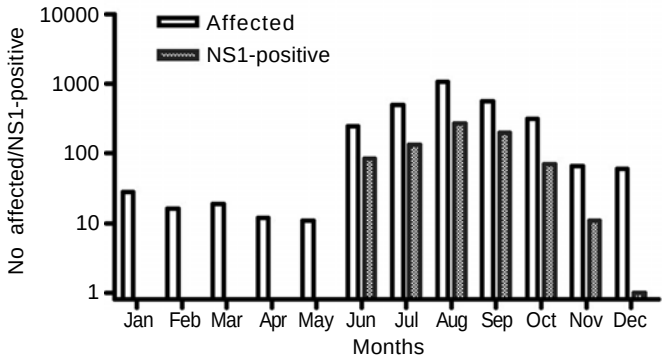

FIG 3. Month-wise distribution of dengue affected individuals and their NS1 antigen positivity

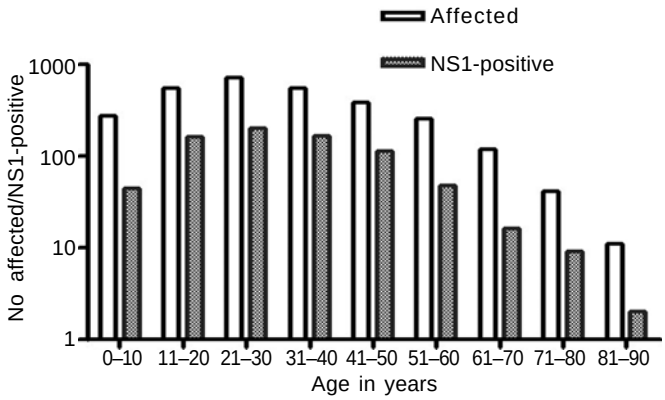

FIG 4. Age-wise distribution of dengue affected individuals and their NS1 antigen positivity

TABLE I. District-wise dengue serotype prevalence

District	Serotype-positive
Angul	DEN2
Balangir	DEN1, DEN3
Balasore	Not found
Bhadrak	Not found
Baragarh	DEN2
Cuttack	DEN2
Dhenkanal	Not found
Ganjam	DEN2
Jajatsinghpur	DEN2
Jajpur	Not found
Kalahandi	DEN1, DEN2
Kandhamal	DEN2
Kendrapada	Not found
Khorda	DEN2
Mayurbhanja	DEN2
Nayagarh	DEN2
Puri	DEN2
Rayagada	DEN2, DEN3

All four serotypes of dengue, namely DENV1, DENV2, DENV3, and DENV4, were detected in the samples. The observed base pair sizes were DENV1-482 bp, DENV2-119bp, DENV3-290bp and DENV4-392bp, respectively. Only few districts such as Rayagada, Balangir and Kalahandi had mixed serotypes (Table I). Serotyping could not be done for 5 of 18 districts as very few samples were received from these districts. Further,

among these samples, dengue-positive cases were less and few could not be processed due to low sample quantity/volume.

DISCUSSION

Dengue is an emerging disease of international concern.[13] During the past few decades it has spread across vast geographical regions including urban areas.[14] The key factors responsible for rapid spread of dengue are urbanization and frequent travel including international travel. The first dengue outbreak in Odisha was reported during 2010 from Bhubaneswar.[10] Following this outbreak, cases continued to be reported from the entire state. We screened for dengue and serotyping of the virus was done to map the prevalent dengue serotypes across the state. Previous studies on dengue serotypes in Odisha reported serotypes either in a particular district or as a part of other investigations. To the best of our knowledge, this is the first study from this region that summarizes dengue serotypes across all topographies and seasons making the evidence comprehensive.

Previous reports suggest DENV1, DENV2 and DENV3 serotypes of dengue to be prevalent in Odisha.[15,16] We observed DENV2 and DENV3 to be the most dominant circulating serotypes, with a few districts having mixed serotypes as well. These mixed serotypes DENV1 and DENV2 in Kalahandi; DENV2 and DENV3 in Rayagada; and DENV1 and DENV3 in Balangir were heterogeneously distributed, which might pose a challenge for disease control in the respective areas.

Annual seasonal variation of dengue revealed that the infection appears abruptly around July, often at the start of the rainy season with a peak in August and September, the most favourable months for mosquito breeding, which is consistent with the findings of other studies.[17] This seasonal trend needs to be considered for preparedness and public awareness programmes to control future outbreaks. Our study showed that men (61%) are more likely to have dengue infection than women. Also, young people aged 21–40 years were more prone to dengue infection in comparison to other age groups, which is consistent with the findings of other studies.[18,19] One of the reasons for this pattern could be that men and young people often work outdoors, thus exposing them to mosquito bites.

In 2018, DENV2 dengue serotype was predominant in Odisha. Further studies are required for better understanding of the epidemiology of disease. We suggest continuous surveillance to monitor trends in the characteristics of circulating dengue serotypes for better understanding of the endemicity and severity of the disease. This will help in strengthening public health strategies to control the disease including deployment of resources.

Strengths and limitations

Although this study summarized prevalent serotypes across all topographical regions and seasons of Odisha, yet samples from only 18 districts could be covered. A few samples could not be processed successfully, which led to insufficient results in those districts.

Conclusion

We summarize the dengue epidemiology in Odisha including prevalent serotypes, seasonal patterns, age and gender distribution. We have mapped the distribution of each dengue serotype in Odisha, a first attempt including all topographies and seasons. Although DENV2 serotype was predominant in 2018, mixed serotypes too prevail in the state. Evidence based on dengue serotype variations across topographies, seasons, gender, age group may support public health efforts in prevention and control of the disease.

Conflicts of interest. None declared

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