Original Article

Viral characteristics and clinical presentation in dengue co-infection- Findings from a facility based observational study in Odisha, India

Jyotirmayee Turuk[1], Subrata K. Palo[2], Sonalika Rath[1], Subhra Subhadra[1], Jyotsnamayee Sabat[1], Prakash K. Sahoo[1], Sailendra Panda[1], Sanghamitra Pati[1]

[1]VRDL, ICMR-RMRC Bhubaneswar, Nalco Square, Odisha, [2]Department of Public Health, ICMR-RMRC Bhubaneswar, Odisha, India

Abstract

Background: Dengue has affected many countries globally. Two-fifths part of the world is at risk, which can be affected by dengue disease. In India, the dengue incidence has increased in the recent past and emerged as an important health problem in many states including Odisha. Dengue disease presents with atypical clinical symptoms when associated with other co-infections. **Materials and Methods:** A facility-based longitudinal study was carried out over a period of 1 year to determine the dengue co-infection and its outcome. The suspected cases were clinically assessed following a standard case report format and serological investigations including serotyping were carried out. **Results:** 33.6% samples were dengue positive of which 78.5% were positive for NS1 Ag, 26.6% positive for dengue IgM and 5.1% to both. Among the dengue positive cases, 60.9% were male and mean age was 31.52 (±17.03) years. High occurrence of cases was during May to November with maximum in August. Among the 975 dengue positives, 57 (5.8%) were found to have co-infection. Chikungunya was the most common co-infection in 71.9%, followed by herpes simplex (HSV) (7%) and other diseases. Fever was the most common presenting symptom (98.2%), followed by myalgia (91.2%), retro orbital pain (91.2%), pain abdomen (12.3%), rash/lesion (8.8%), burning micturition (5.3%), petechiae (1.7%) and pruritus (1.7%) among the co-infected cases. **Conclusions:** All the four dengue serotypes were found to be circulating with DEN 2 as the most predominant one. About 5.8% of dengue cases have co-infection (mainly with Chikungunya) and clinically present with atypical signs and symptoms.

Keywords: Co-infection, dengue, phylogeny, serology

Highlights

1. Atypical symptoms with dengue infection may go unrecognised and therefore the confection of dengue along with other viral and bacterial diseases is under reported.
2. Co-infection of multiple serotypes was seen.
3. Co-circulation of DEN 1, 2, 3 and 4 serotypes with predominance of DEN 2 was observed among dengue cases of Odisha during 2018.

Address for correspondence: Dr. Jyotirmayee Turuk, CMR-RMRC Bhubaneswar, Nalco Square, Odisha - 751 023, India.
E-mail: drjyotirmayeeturuk@gmail.com

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Background

The global incidence of dengue is estimated to be 390 million per year, of which 96 million manifest apparently.[1] Although more than 100 countries worldwide are now affected by dengue, it is prevalent in Africa, the Americas, Asia, the Caribbean and the Pacific making it an important public health problem in these regions. In 2021,

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the five countries reporting most cases are Vietnam, Colombia, Paraguay, Philippines and Sri Lanka.[2] In India, the incidence of dengue infection has increased remarkably over the last decade, with more than 67,000 diagnosed dengue cases in 2019, as per reports by the National Vector Borne Disease Control Programme.[3] In clinically suspected patients for dengue, the prevalence of laboratory confirmed dengue positive was 38.3% and dengue seroprevalence in the general population is 56.9%.[4] In Odisha (an eastern state of India), dengue has re-emerged since 2010 and a number of dengue cases are increasingly reported thereafter from different parts of the state.[5] A study from western Odisha showed that the prevalence of dengue among febrile cases to be 25.3%, an important aetiology for non-malarial febrile illness.[6] The geo-climatic condition of Odisha favours the increased vector density for dengue, thereby making people of the state vulnerable to dengue.[7]

Symptoms in dengue vary from mild self-limiting fever to severe forms of manifestations like severe headache, petechiae, bone pain, enlargement of lymph nodes and frank bleedings.[8] In 2012, WHO expanded the horizon of classic dengue fever and named it expanded dengue syndrome.[9] Expanded dengue syndrome is usually associated with co-infection and with multiple organ involvement leading to severe shock. There are few studies, mostly case reports, of dengue co-infection with other viral, bacterial, immunological and parasitic diseases. Studies in India on dengue co-infection showed the incidence of dengue and Chikungunya co-infection to be the most common varying from 9.5% to 10.7%[10,11] followed by dengue and JE and Zika viral co-infection. Considering limited studies available on the viral characteristics and clinical manifestations in dengue co-infection, present study was intended to explore this dimension.

Materials and Methods

Study Design and Setting

A facility-based observational study was carried out at Regional Medical Research Center (RMRC), Bhubaneswar, Odisha, during January to December 2019. The dengue suspected cases referred for laboratory investigations to regional Virology Research and Diagnostic Laboratory (VRDL), Bhubaneswar from all parts of Odisha to the centre during the period studied. While the VRDL of RMRC, Bhubaneswar has facilities to diagnose more than 50 viruses along with serotyping for some viruses, RMRC centre has also facilitated for diagnosis of malaria parasites and bacterial infections. The samples referred to VRDL were accompanied with a standardized case report form (CRF) having detailed clinical information about the patient. In case of any missing information, one research assistant was engaged to contact the case and collect the detailed information over the phone.

Serological Investigation

Serum samples from patients were subjected to ELISA-based tests specific for dengue depending on fever history adhering to WHO guideline 2009.[12] Patients with history of 1–5 days were tested for dengue NS1 antigen and patients having fever for more than 5 days were tested for dengue-specific IgM antibody (MAC—ELISA). Patients having a history of 3–5 days of fever were tested for both dengue NS1 antigen and IgM antibodies in the serum. Other suspected viral co-infections were tested using appropriate methods (serology or polymerase chain reaction (PCR)). Malaria was tested using Advantage Malaria card (J. Mitra and Co. Pvt. Ltd. (RDT)) and bacterial infection was confirmed by culture and sensitivity. The dengue ELISA positive samples were serotyped by nested PCR and genomic analysis was done by sequencing.[13]

Serotyping by RT-PCR

All dengue NS1 positive samples were serotyped using reverse transcription PCR using QIAamp viral RNA kit. The dengue serotyping was performed according to standard protocol followed at VRDL. The amplified product was used for the second step using D1, TS1, TS2, TS3 and TS4 primers. The final product was visualised in 2% agarose gel in the gel doc system.[13]

Phylogenetic Analysis

A 362 bp product from the CprM region was sequenced using the first-round primers and the ABI BigDye terminator cycle sequencing ready reaction kit (DNA Sequencer, ABI, Vernon, CA, USA). Phylogenetic tree was computed among four serotypes using the Kimura 2 parameter matrix and neighbour joining (NJ) method using Mega software, version 6.[14–17]

A subset of randomly selected representative samples which were Dengue serotype 2 from each district were taken and sequenced. An NJ phylogenetic tree was constructed with available reference sequences isolated from different regions of India.

Results

Out of a total 3005 dengue suspected samples referred to the centre, 2902 were enrolled for the study with a drop out of 3.4% because of either absence of CRF or improper sample transportation. The male and female enrolled cases were 1744 (60%) and 1158 (40%), respectively. A total of 974 (33.6%) samples were tested dengue positive of which 765 (78.5%) positive for NS1 Ag, 259 (26.6%) positive for dengue IgM and 50 (5.1%) to both. Among the dengue positive cases, 593 (60.9%) were male and 381 (39.1%) were female. The mean age among positive cases was 31.52 (±17.03) years (31.53 (±17.04) years among male and 31.46 (±16.99) years among females. The detailed age and genderwise distribution of dengue positive cases is presented in Table 1. Genderwise there was no significant difference in the odds ratio of dengue cases between male and females (OR = 1.05, 0.897–1.230, $P > 0.05$). Significant difference in odds ratio was observed in dengue cases in different age groups and depicted in Table 1. Monthwise distribution of dengue cases showed the occurrence to be high during May to November with maximum in the month of August [Figure 1].

Dengue Co-Infections

Among the 974 dengue positives, 57 (5.8%) were found to have co-infection. Chikungunya was the most common

Table 1: Gender and agewise distribution of dengue cases (*n*=2902)

	Total cases (*n*)	Dengue positive *n* (%)	Odds ratio (OR)
Gender			
Female	1158	381 (39.1)	1
Male	1744	593 (60.9)	1.05 (0.89-1.23)
Total	2902	974	
Age			
0-10	277	64 (6.6)	1
11-20	547	200 (20.5)	1.92 (1.38-2.67)**
21-30	713	266 (27.3)	1.98 (1.44-2.72)***
31-40	562	207 (21.3)	1.94 (1.40-2.69)***
>40	803	237 (24.3)	1.39 (1.01-1.91)*
Total	2902	974	

*P<0.05, **P<0.01, ***P<0.001

co-infection in 71.9%, followed by herpes simplex (HSV) (7%) and other diseases. Fever was the most common presenting symptom (98.2%), followed by myalgia (91.2%), retro orbital pain (91.2%), pain abdomen (12.3%), rash/lesion (8.8%), burning micturition (5.3%), petechiae (1.7%) and pruritus (1.7%) among the co-infected cases. Detailed co-infection types and the clinical presentations are given in Table 2. An interesting feature was that fever was a late clinical presentation in cases presenting with co-infection as compared to monoinfection. Thrombocytopenia was aggravated in dengue Chikungunya co-infections, with 1–2 cases reported with sudden onset of bleeding from gums and nose.

Dengue Serotypes by Reverse Transcription PCR (RT-PCR)

Reverse transcription PCR (RT-PCR) was performed for all the 765 NS1 positive samples. All the four dengue serotypes (DEN 1, 2, 3 and 4) were found among the samples. While monoinfection with DEN 2 was observed in majority 567 (74.1%), DEN 1 in 183 (23.9%) and DEN 3 was found in 15 (2.0%) of the samples. Multiple serotypes of DEN 2 and DEN 4 were detected in 6 (0.7%) of samples.

Among the 57 co-infected dengue cases, 14 were positive to NS1. Their serotype assessment showed positive to DEN 2 serotype in 12 (85.7%) cases and positive to DEN 3 serotype in 2 (14.3%) co-infected cases.

Phylogenetic Analysis

Phylogenetic tree computed among four serotypes using Kimura 2 parameter matrix and NJ method in Mega software, version 6 was found that a 362-bp product from CprM region was identical for D2 had clustered with sequences of genotype IV with 99% identity with strains isolated from Kerala and 98% identity with North Indian strains [Figure 2].

Discussion

This is the first ever study from the state of Odisha exploring the viral characteristics and clinical presentations in dengue

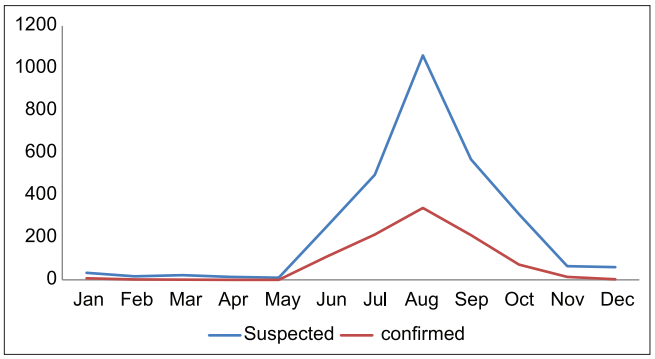

Figure 1: Monthwise distribution of suspected and dengue positive cases

co-infection. Among all the cases investigated, 33.6% were dengue positive. A study from Puerto Rico found that 18.6% of the total fever cases are because of dengue.[18] The mean age of dengue cases was 31.52 years and the proportion of males was 60% among the dengue positives. This could be due to more exposure to mosquito bites among males of this age group. The male preponderance of dengue cases is also seen in other studies from across the world. It is seen in one of the studies from Colombo, which showed that dengue was caused in majority of males of about 66.3% from the total dengue cases in the population considered.[19] The findings of the common age group affected is in contrast to other studies that found higher proportion of dengue cases in the age group 11–20 and 15–24 years.[20,21] Majority of the cases were reported during the month May to November, which shows the disease starts during the pre-monsoon with peak transmission during monsoon. Other studies have also found monsoon and post-monsoon as the favourable period for dengue transmission.[22,23] During the pre-monsoon period, if there is rain, this leads to accumulation of fresh water in the surroundings leading to multiplication of Aedes vector, which increases the probability of dengue transmission. All the four dengue serotypes DEN 1, 2, 3 and 4 were found to be present and circulating in the state of Odisha with DEN 2 as the predominant serotype. Infection with both DEN 2 and DEN 4 serotypes was found in 0.7% dengue positive cases. Similar multi-serotype dengue infection has also been reported earlier.[4] A hospital-based study from Malaysia suggested that dengue patients positive for multiple serotypes have more severe clinical manifestations than monoserotypes. However, this could not be associated in the present study.

Most common form of co-infection with dengue was found to be Chikungunya (71.9%). Both dengue and Chikungunya viruses are transmitted by Aedes mosquito, so it is highly possible that both viruses could be transmitted concurrently. Other studies have reported co-infection of dengue and Chikungunya to be around 32% among patients with fever of unknown origin (FUO).[24,25] The symptoms in cases with co-infection of dengue and Chikungunya were fever, myalgia and retro orbital pain and a gross degree of thrombocytopenia. The cases with dengue and Chikungunya co-infection were followed up and it was found that myalgia persisted for longer time in these groups of patients.

Table 2: Type of co-infection and symptoms among dengue co-infection cases (*n*=57) and dengue monoinfection (*n*=917)

Dengue with co-infections	Number of cases 974	Symptoms in number of patients with co-infection							
		Fever	Myalgia	Pruritus	Petechiae	Rash/ lesions	Burning micturition	Retro Orbital pain	Pain abdomen
Dengue with chikungunya	41 (71.9%)	41	41	-	-	-	-	18	-
Dengue with herpes	4 (7%)	4	4	-	-	4	-		-
Dengue with hepatitis	2 (3.5%)	2	-	1	-	-	-		2
Dengue with malaria	2 (3.5%)	2	2	-	-	-	-		-
Dengue with typhoid	3 (5.3%)	3	3	-	-	-	-		3
Dengue with sickle cell anaemia	1 (1.8%)	1	1	-	1	-	-		1
Dengue with measles	1 (1.8%)	1	1	-	-	1	-		1
Dengue with bacterial UTI	3 (5.3%)	2	-	-	-	-	3		-
Dengue monoinfection	917	917	917					870	
Total	974	56	52	1	1	5	3		7

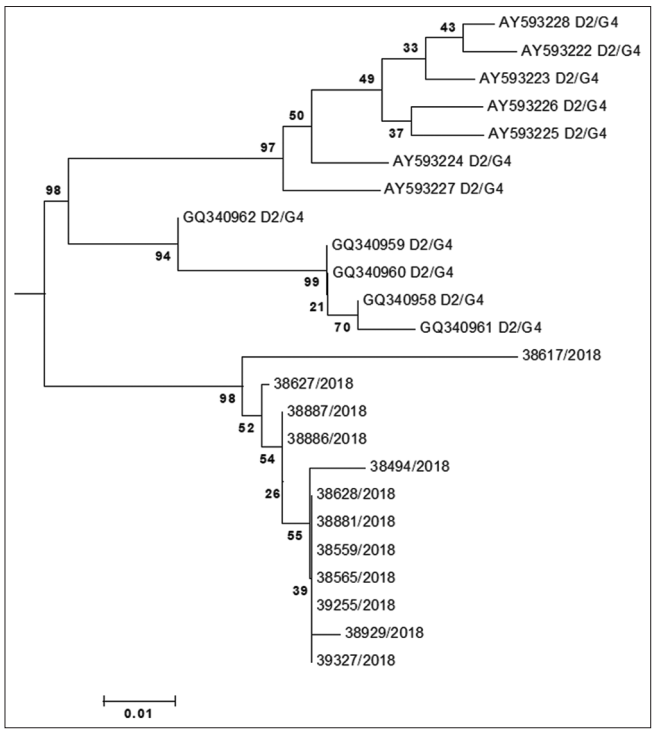

Figure 2: Phylogenetic tree of identified four dengue serotypes. Phylogenetic tree computed among four serotypes using Kimura 2 parameter matrix and neighbour joining method in Mega software, version 6 was found that a 362 bp product from CprM region were identical for D2 had clustered with sequences of genotype IV with 99% identity with strains isolated from Kerala and 98% identity with North Indian strains

Other than Chikungunya, co-infection with other viruses, such as herpes, HAV, HEV and measles, were also observed. Many case reports have shown dengue co-infection with similar viral infections.[26–30] Although the patients with co-infection, presented initially with associated clinical symptoms, it was often neglected until the onset of fever.

Dengue co-infection with other diseases like malaria, typhoid, sickle cell anaemia and urinary tract infection were also observed in the present study. Many case reports have cited concurrent dengue infection in similar patients.[22,31,32] Fever, myalgia and arthralgia were the most common presenting symptoms among the dengue cases. However, few cases also reported with atypical symptoms like rhinorrhoea, subconjunctival haemorrhage, retro orbital pain and dizziness with reduced vision. Other study shows that erythema, morbilliform rash, cutaneous hypersensitivity reactions as atypical presentations of dengue.[33]

Conclusion

The findings from the study put to a nutshell conclude that DEN 2 was most prevalent in our region, although co-circulation of other serotypes DEN 1, 3 and 4 serotypes was also observed among the dengue cases. Apart from dengue monoinfection with single serotype, few cases also had multiple serotypes. Dengue with co-infection was found in the majority of cases followed by other viruses, bacteria and parasites. Dengue transmission starts during the pre-monsoon period, which emphasises the urgent need for developing appropriate strategies on dengue control and prevention to be implemented during the pre-monsoon period. This study has put forward the status of co-infection in dengue cases and the associated symptoms that may trigger up with unusual presentation.

As India is endemic to many diseases like typhoid, leptospirosis and enteroviral infections, which also present with fever and gastrointestinal symptoms, diagnosis of dengue in these co-infection cases presenting with a wide range of atypical symptoms was quite challenging in our study. Underreporting of co-infection of dengue along with other viral and bacterial diseases has put forth a demand for skilled clinical perspective to avoid lack of timely detection and proper management, which may lead to increased number of fatalities.

Abbreviations:

VRDL: Viral Research Diagnostic Laboratory
RMRC: Regional Medical Research Centre
HAV: Hepatitis A virus
HEV: Hepatitis E virus

FUO: Fever of unknown origin
PCR: Polymerase Chain reaction
RT-PCR: Reverse transcription
RDT: Rapid diagnostic test
CRF: Case report form

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Ethical Approval and Consent to Participate

Ethical approval for this study was taken from the state research and ethics committee and RMRC Bhubaneswar Institutional ethical committee (Approval on 14.08.21). Written consent was not taken from the cases because the researchers did not come directly in contact with the cases. The samples were received along with the filled up CRF on which the tests were done.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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