Viral Characteristics and Clinical Presentation in Dengue Co-infection—Findings from a Facility Based Observational Study in Odisha, India

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Abstract

Background: Dengue is a widespread disease affecting many countries and about two fifth of the world is at risk for this disease. In India, the dengue incidence has increased in recent past and emerged as an important health problem in many states including Odisha. Cases with dengue co-infection with other diseases usually have atypical clinical presentation.

Methods: A facility based longitudinal study was carried out over a period of one 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%), pain abdomen (12.3%), Rash/lesion (8.8%), burning micturition (5.3%), Petechiae (1.7%) and Pruritus (1.7%) among the co-infected cases.While DEN-2 serotype was observed in majority (74.1%) more than two serotypes was found in 5.85% of dengue positives .

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.

Background

The global incidence of dengue is estimated to be 390 million per year, of which 96 million manifest apparently(1). More than 100 countries worldwide are now affected by dengue with about two fifth of world population at risk for this infection makes it an important public health problem(2). In India, the incidence of dengue infection has increased remarkably over last decade(3). In clinically suspected patients for dengue the prevalence of laboratory confirmed dengue positive was 38.3% and dengue sero prevalence in general population is 56.9%(4). In Odisha (an eastern state of India), dengue has re-emerged since 2010 and 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 form of manifestations like severe headache, petechiae, bone pain, enlargement of lymph nodes and frank bleedings(8). In 2012, WHO has 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 coinfection.Considering limited studies available on the viral characteristics and clinical manifestations in dengue co-infection, present study was intended to explore this dimension.

Methods

Study Design and setting: A facility based observational study was carried out at Regional Medical Research Center (RMRC), Bhubaneswar, Odisha during January–December 2018. The dengue suspected cases referred to regional Virology Research and Diagnostic Laboratory (VRDL), Bhubaneswar for investigations from all parts of Odisha to the centre during the period was studied. While the VRDL of RMRC, Bhubaneswar has facilities to diagnose more than 50 viruses with serotypes of some viruses, RMRC centre has also facilities to diagnose 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 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 five 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 infections were tested using appropriate methods (serology or 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 polymerase chain reaction and genomic analysis were done by sequencing(12).

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 CprM region was sequenced using the first-round primers and the ABI Big Dye terminator cycle sequencing ready reaction kit (DNA Sequencer, ABI, USA). Phylogenetic tree was computed among four serotypes using Kimura 2 parameter matrix and neighbour joining (NJ) method using Mega software, version 6(14–17).

A subset of randomly selected representative samples Dengue serotype 2 from each district were taken and sequenced. A neighbour joining 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 female). The detailed age and gender wise distribution of dengue positive cases is presented in Table-1. Gender wise 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. Month wise

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 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%), 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.

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 mono infection 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 was 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 neighbour joining (NJ) 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. (Figure-2).

Discussion

This is the first ever study from state of Odisha exploring the viral characteristics and clinical presentations in dengue co-infection. Among all the cases investigated, 33.6% were dengue positive. A study from Thailand found 44-73% of the total fever cases are because of dengue. 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. This finding is contrast to other studies that found higher proportion of dengue cases in the age group 11-20 and 15-24 years(18–20).

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 found monsoon and post monsoon as the favourable period for dengue transmission(20,21). 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 DEN1, 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 serotype was found in 0.7% dengue positive cases. Similar multi serotype dengue infection has also been reported earlier. A hospital based study from Malaysia suggested that dengue patient’s positive for multiple serotypes have more severe clinical manifestations than mono serotypes. 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)(22–24). Other than chikungunya, co-infection with other viruses such as Herpes, HAV, HEV and Measles were also observed. Many case reports have showed dengue co-infection with similar viral infections(25–27). 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(20,28,29). Fever, myalgia and arthralgia were the most common presenting symptoms among the dengue cases. However few cases also reported with atypical symptoms like rhinnorrhoea, subconjuctival haemorrhage, retroorbital pain, dizziness with reduced vision. Other study shows that erythema, morbilliform rash, cutaneous hypersensitive reactions as atypical presentations of dengue(30). Infections caused by typhoid bacilli, leptospira, enterovirus with fever and gastro intestinal symptoms may simulate dengue fever(31).

Conclusion

Co-circulation of DEN 1, 2, 3 and 4 serotypes with predominance of DEN-2 was observed among dengue cases. Apart from dengue monoinfection with single serotype, few cases also had multiple serotypes. The 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. Majority of dengue cases present with typical dengue manifestation, few cases also present with a wide range of atypical symptoms which makes difficulty in suspecting such cases. Therefore the co-infection of dengue along with other viral and bacterial diseases is under reported.

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

Declarations

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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.

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.

Availability of data and materials: The datasets used and/or analysed during the current study are available on request from the corresponding author .

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Competing interests: None.

Consent for publication: Not applicable

Authors contribution: S.K.P and S.R managed the OPD, S.S and J.S carried out diagnostic tests and serotyping, S.P carried out the serotyping and sequencing of the strains.S.K.P did the statistical analysis, manuscript writing, editing. J.T has build the manuscript,editing ,monitoring of investigations in the laboratory .S.P conceptualised the idea.

All the authors have read the content and approved for publishing.

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Tables

Table-1: Gender and Age wise distribution of dengue cases (N = 2902)

Gender	Total cases (N)	Dengue positive n (%)	Odds Ratio (OR)
Female	1158	381 (39.1)	1
Male	1744	593 (60.9)	1.05 (0.89-1.23)
Total	**2902**	**974**	
Age			
0 to 10	277	64 (6.6)	1
11 to 20	547	200 (20.5)	1.92 (1.38-2.67)**
21 to 30	713	266 (27.3)	1.98 (1.44-2.72)***
31 to 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

Table-2: Type of co-infection and symptoms among dengue co-infection cases (N=57)

Dengue with co-infections	Number of cases	Symptoms in number of patients with co-infection						
		Fever	Myalgia	Pruritus	Petechiae	Rash/lesions	Burning micturition	Pain abdomen
Dengue with Chikungunya	41 (71.9%)	41	41	-	-	-	-	-
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	-
Total	57	56	52	1	1	5	3	7

Figures

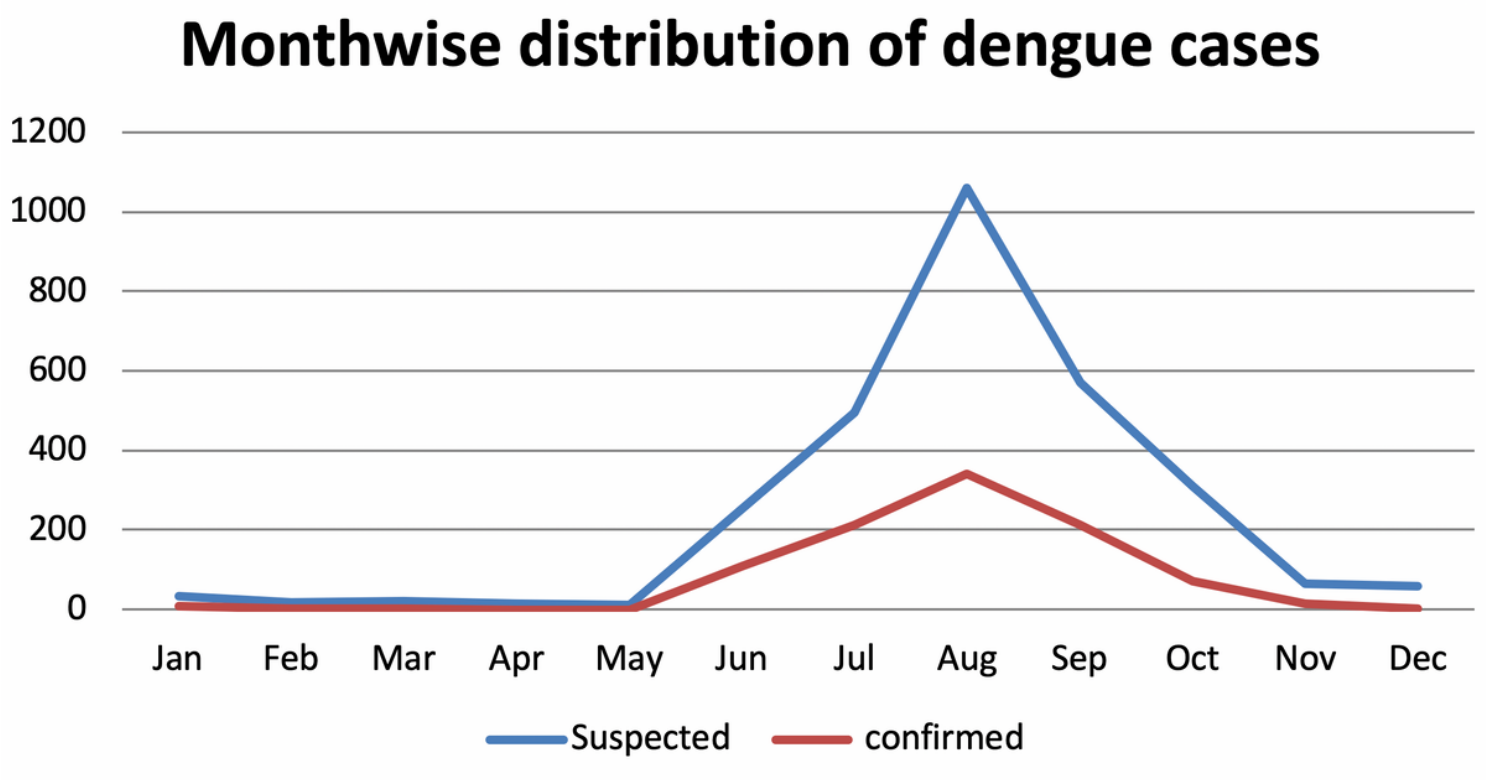

Figure 1

Month wise distribution of suspected and dengue positive cases

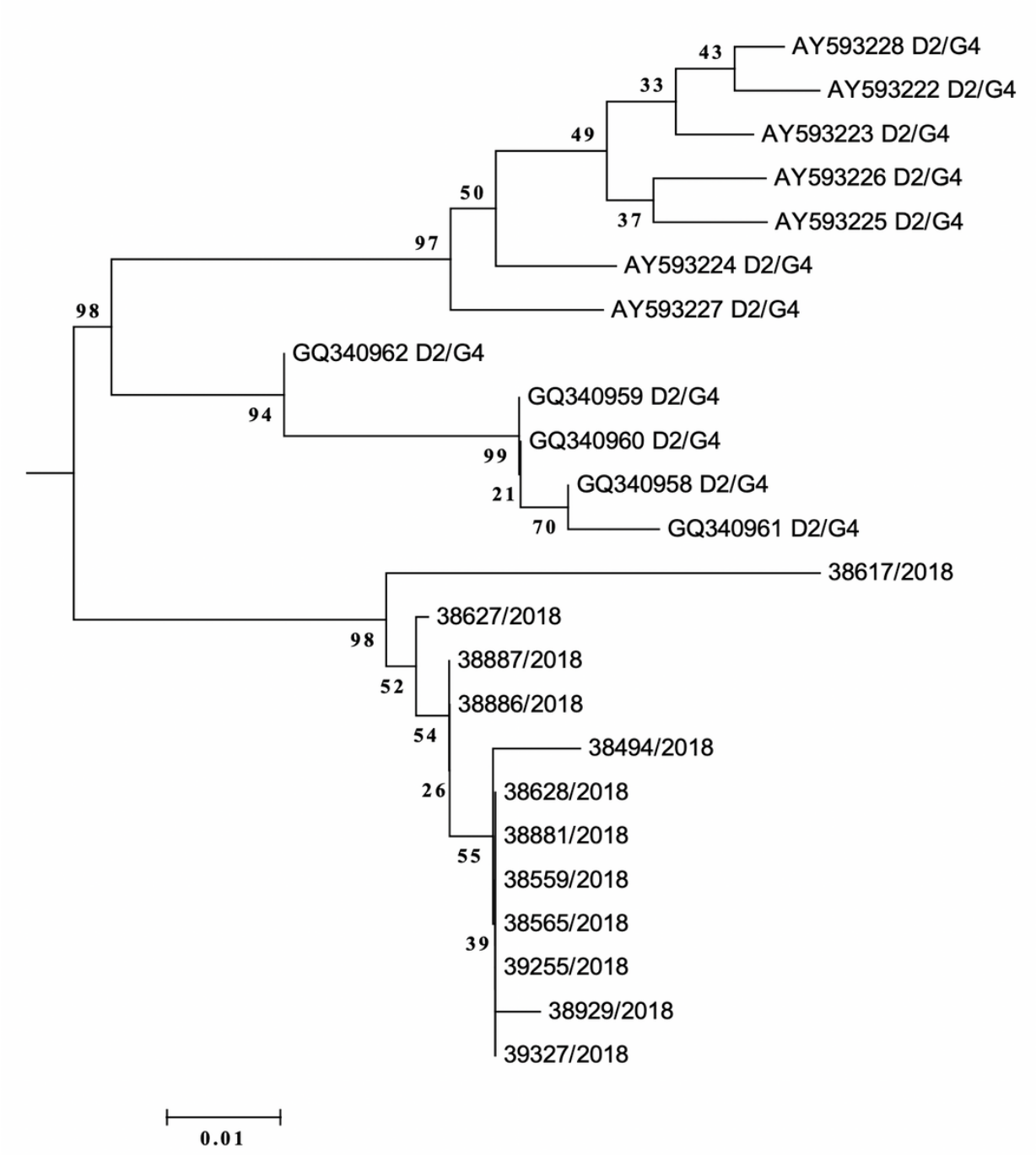

Figure 2

Phylogenetic tree of identified four dengue serotypes. Phylogenetic tree computed among four serotypes using Kimura 2 parameter matrix and neighbour joining (NJ) 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