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1 author:

M Rajesh Kumar Rao
ICMR-National Institute of Malaria Research

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Research paper

Episodes of the epidemiological factors correlated with prevailing viral infections with dengue virus and molecular characterization of serotype-specific dengue virus circulation in eastern India

M. Rajesh Kumar Rao[a,*], Rabindra N. Padhy[b], Manoj Kumar Das[c]

[a] Department of Biotechnology, Sai Nath University, Ranchi, Jharkhand, India
[b] Central Research Laboratory, Institute of Medical Sciences & Sum Hospital, Siksha 'O' Anusandhan University, K-8, Kalinga Nagar, Bhubaneswar 751003, Odisha, India
[c] National Institute of Malaria Research, Field Unit, Itki, Ranchi, Jharkhand, India

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ABSTRACT

Background: Dengue is one of the most important and widespread viral infection comprises 4 related serotypes (DEN-1, 2, 3, and 4). Infection with one serotype does not protect against the others, and sequential infections put people at greater risk for dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). This study determines the epidemiology of prevailing viral infections with dengue and molecular characterization of serotype-specific DENV circulation in Odisha of eastern India.
Methods: During the year 2013, 1980 blood samples with suspected dengue cases were obtained between days 1–10 of illness and analyzed by NS1 Ag-RDT, NS1 Ag-ELISA, and RT-PCR. The differential detection of dengue infections and DENV serotyping were carried out by IgM/IgG Ab-ELISA and RT-PCR, respectively.
Results: Of the 1980 samples, 733 (37.0%) were positive for dengue RNA by RT-PCR. The confirmed cases of dengue were more in males (73.6%) in comparing to females (26.4%). The age group of 15–44 years (527 cases, 71.9%) were more susceptible to dengue infections. 656 (89.5%) cases had infected with monotypic infection by different DENV serotype and 77 (10.5%) cases had multitypic infections by multiple serotypes of DENV. Of the total multitypic infections, there were 74 (10.1%) cases had infected with DENV-2 and DENV-3 serotypes at a time; and only 3 (0.4%) cases had the concurrent infections of all three serotypes that were, DENV-1, DENV-2, and DENV-3. Of the 28 DHF cases, there were 17 (2.3%) cases had infected with multitypic infections and 11 (1.5%) cases had infected with monotypic infection.
Conclusion: Dengue infections have prevailed from the month of July and grasped it's the peak in September. Rain, temperature and relative humidity have favored the dengue infections. Young adults and males are more susceptible to dengue infections. Serotypes DEN-2 followed by DEN-3 was dominant among the confirmed dengue cases. Co-circulation of multitypic infections with multiple DENV serotypes and the emergence of DHF cases suggested that eastern Indian state Odisha was becoming a hyper-endemic province for dengue; therefore, continuous surveillance is suggested for understanding the epidemiology of the diseases and monitoring the changes in the characteristics of circulating DENV strains.

1. Introduction

Dengue viral infections are one of the most important arboviral diseases within the family *Flaviviridae* and comprise four serotypes, designated as DENV-1, DENV-2, DENV-3, and DENV-4 (OhAinle et al., 2011). Infection with any of these serotypes causes a mild and self-limiting febrile illness (classical dengue fever (DF)); a few cases are severely developing to life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).

During the past few years, dengue has become one of the leading causes of morbidity and mortality in tropical and subtropical regions throughout the world posing a major public health problem (Kyle and Harris, 2008). Overall, two-fifths of the world populations are living in regions with the risk of dengue infections (WHO, 2017; Bhatt et al., 2013; Guzman et al., 2010). Dengue infects about 284–528 million individuals and handles 500,000 hospitalizations with 24,000 deaths worldwide every year (WHO, 2017). Of these, 10,000 infant deaths caused because of DHF and DSS (Halstead, 1988). Southeast Asia and Western Pacific are representing about 75% of the global dengue burden (World Health Organization, 2009).

* Corresponding author.
E-mail address: raomrajeshkumar@gmail.com (M.R.K. Rao).

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In India, dengue virus (DENV) was first isolated in 1945 and several outbreaks have been reported thereafter (Balaya et al., 1969; Rodrigues et al., 1972). Dengue is endemic in Odisha state. The first case of dengue was documented in the state in 1998, and several epidemics due to dengue were precipitated after 2005. In the year 2011, an outbreak of dengue occurred in the Angul district of Odisha in August and September. During this period, 1846 DF cases had detected in Odisha. Of these, 33 death cases were occurring with the epicenter at Talcher coalmine area in the Angul district (Anon., 2014). DF has described in the Angul district because of the vector species *Aedes* were obtained throughout the year in these provinces (Rao and Padhy, 2014).

Dengue viral infections are usually symptomatic. The epidemiology of dengue infections is an intricate phenomenon. It depends upon the correlation between the epidemiological factors, including the host, agent, and ecosystem. The intricacy of the relationships between these factors determines the level of dengue endemicity in an area (World Health Organization and National Vector Borne Disease Control Programme, 2015).

The exact causes of dengue severity are yet on doubtful for some instances even if there is an interaction takes place between the host and agent. Infecting cases plays a significant role in introducing the dengue virus by their movement towards newer areas. Because of limited studies were done in various regions of the Indian subcontinent, there is little scientific information available on circulating DENV serotypes or their molecular and epidemiological characterization (Mishra et al., 2015; Zangmo et al., 2015). Till date, systematic studies on the epidemiological risk factors of dengue viral infections, molecular detection of DENV, and serotype-specific DENV circulation in the eastern Indian state Odisha had not taken on.

Therefore, this paper presents an update report on the epidemiology of dengue infections, clinical features, and an episode of prevailing infections with DENV serotypes in eastern India.

2. Materials and methods

2.1. Study site

The district of Angul found in the central province of the Odisha state in eastern India lies between 20° 31′ N and 21° 40′ N latitude and 84° 15′ E and 85° 23′ E longitude. The geographical area of the Odisha state is 155,707 km^2. In the 2011 census of India, Odisha state had a population of 41,947,358 of which males 21,201,678 and females 20,745,680. With a brief winter, the climate of the district is subtropical with the temperature ranging from 6 to 47 °C (see Fig. 1).

2.2. Study period

To accomplish the aims of the research study was held out from January 2013 to December 2013.

2.3. Epidemiological factors correlated with dengue viral infections

The risk factors of dengue viral infections depend upon the correlation between the epidemiological factors, comprising the host (Human), agent (Virus), and ecosystem (Abiotic factors) (see Fig. 2).

2.4. Study population

The study population comprises of < 1 year, 1–14 years, 15–24 years, 25–44 years, 45–60 years and > 60 years of age groups are defined as infants, children, young adults, adults, middle-aged persons and senior citizens, respectively.

2.5. Clinical criteria for dengue viral infections

Dengue viral infections may be asymptomatic or symptomatic and clinical manifestations vary from undifferentiated fever, i.e. dengue fever (DF) to dengue hemorrhagic fever (DHF) with plasma leakage and that may lead to hypovolaemic shock (dengue shock syndrome, DSS) (World Health Organization and National Vector Borne Disease Control Programme, 2015; Dutta et al., 2011).

2.5.1. Dengue fever (DF)

A suspected dengue case comprised an acute febrile illness of 2–7 days duration of two or more of the following non-specific constitutional symptoms and signs: high fever, headache, retro-orbital pain, nausea/vomiting, myalgia (muscle pain), generalized skin rashes, arthralgia, and diarrhea in febrile phase; fatigue, pleural effusion, hypotension, ascites, and gastrointestinal bleeding in the critical phase; and itching, slow heart rate, seizures, and altered level of consciousness in recovery phase.

2.5.2. Dengue hemorrhagic fever (DHF)

DHF is characterized by a sudden rise of fever (38.3–39.4 °C), with a transient increase in vascular permeability resulting in plasma leakage, bleeding thrombocytopenia (Platelet counts ≤ 100,000/mm^3) and hemoconcentration or other signs of a headache, retro-orbital pain, conjunctival congestion, and facial flushing with fever sustaining for 2–15 days or melena (Equivalent to WHO classification of DHF grades I and II.).

2.5.3. Dengue shock syndrome (DSS)

DSS is characterized by a weak rapid pulse of narrowing of the pulse pressure (≤ 20% mm Hg (2.7 kPa), regardless of pressure levels, e.g. 100/90 mm Hg (13.3/12.0 kPa)), hypotension, cold clammy skin, and restlessness (Equivalent to WHO classification of DHF grades III and IV.).

2.6. Dengue virus

Dengue virus, the etiological agent of dengue, is separating out four different serotypes, designated as DENV-1, DENV-2, DENV-3, and DENV-4.

2.6.1. Monotypic infection of DENV serotype

Monotypic infection is defined as a single infection in an individual by any serotypes of dengue virus.

2.6.2. Multitypic infections of DENV serotypes

Multitypic infections are defined as concurrent infections in an individual with multiple serotypes of dengue virus.

2.7. Specimens

A total number of 1980 acute-phase blood samples collected from suspected cases of dengue infections under observation of non-specific constitutional symptoms (Zangmo et al., 2015; Dutta et al., 2011), coming to the various health departments in the Angul district, Odisha, was analyzed in the laboratory for diagnosing dengue infections using NS1 antigen RDT kit, enzyme-linked immunosorbent assay (ELISA) based on the NS1 antigen and molecular-based on reverse transcriptase-polymerase chain reaction (RT-PCR). The differential detection of dengue infections and DENV serotyping were carried out by ELISA-based IgM/IgG antibody and RT-PCR, respectively.

2.7.1. Detection of dengue infections using dengue NS1 Ag-RDT and NS1 Ag-capture enzyme-linked immunosorbent assay (MAC-ELISA) methods

Dengue infections are characterized by elevations in specific NS1 Ag levels 1 to 9 days after the onset of symptoms; this persists up to 15 days. Dengue NS1 Ag card is a rapid solid phase immunochromatographic test. All acute serum/plasma specimens were screened for the presence dengue infections using NS1 Ag-RDT and NS1

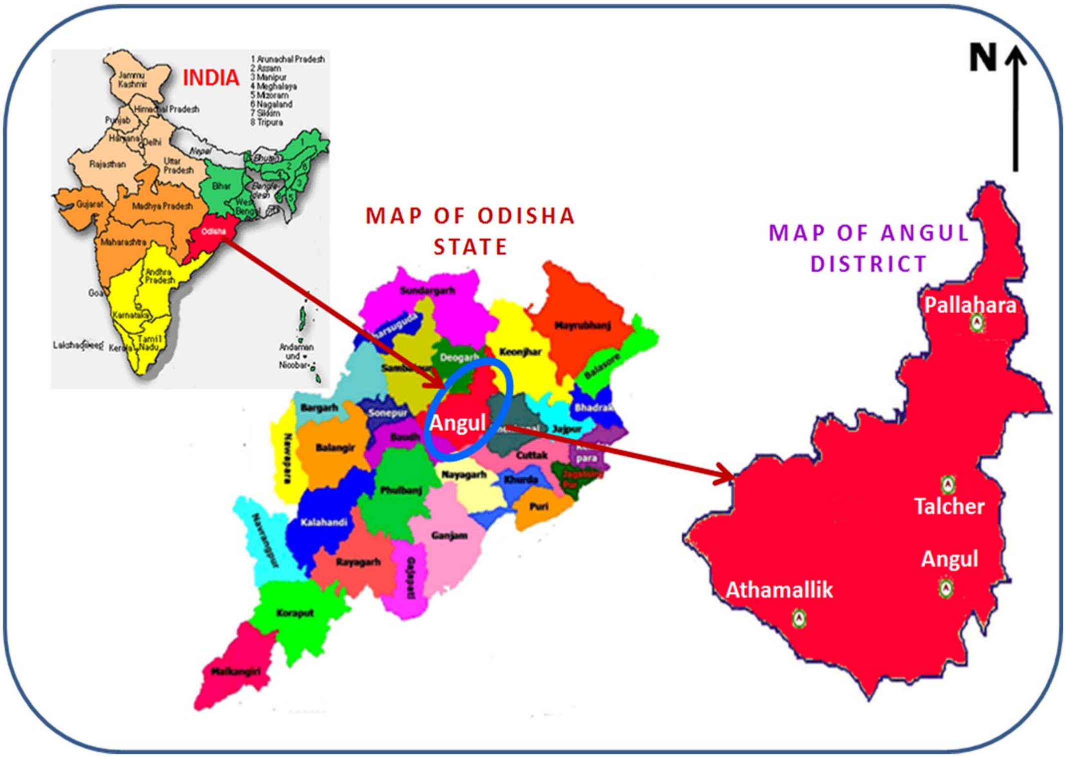

Fig. 1. Map showing the location of the study sites of Odisha in eastern India.

Ag-ELISA following the manufactures' protocol (Anon., 2013b; Anon., 2013c).

2.7.2. Detection of primary and secondary dengue infections using IgM/IgG antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) methods

Primary dengue infection is characterized by elevations in specific IgM antibody levels 3 to 7 days after the onset of symptoms; this persists with 30 to 60 days. IgG levels also become elevated after 10 to 14 days and remain detectable for life. All acute serum/plasma specimens were screened for the differential dengue infections using IgM/IgG Ab-ELISA. Tests were performed according to the manufacturer's instructions (Anon., 2013a).

2.7.3. Detection of DENV serotype-specific using reverse transcriptase polymerase chain reaction (RT-PCR) methods

Dengue virus RNA was isolated from the serum samples using the QIA amp viral RNA mini kit (Qiagen, Germany) as per manufacturer's protocol (Anon., n.d.-c). Extracted RNA was stored at − 70 °C or used for RT-PCR. The RT-PCR assay can distinguish the 4 serotypes of

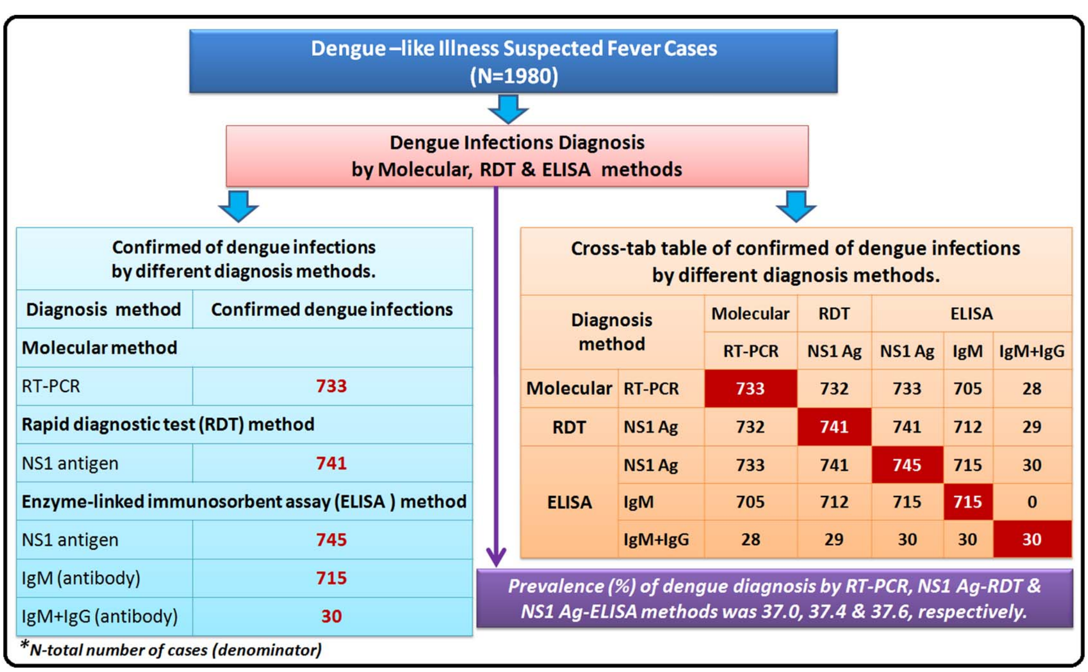

Fig. 2. Confirmed dengue infections using different laboratory diagnostic methods in Odisha of eastern India during January to December 2013.

Table 1
Primers for detection of DENV serotypes.

Primers	Sequences (5′-3′)
TS1	CGTCTCAGTGATCCGGGGG
TS2	CGCCACAAGGGCCATGAACAG
TS3	TAACATCATCATGAGACAGAGC
TS4	CTCTGTTGTCTTAAACAAGAGA

dengue viruses up to the tenth day after the onset of the symptoms using the whole blood, serum or plasma specimen by the size of the products as described by Lanciotti et al. (Lanciotti et al., 1992). This includes a step of RT-PCR using a conserved primer pair, D1 (forward) and D2 (reverse) and a step of second-round PCR using the primer D1 and dengue virus type-specific primers listed in Table 1. The PCR products were electrophoresed through 1.5% agarose gel, stained with ethidium bromide and examined under ultraviolet light using a digital gel documentation system.

2.8. Seasonal distributions

According to the intensity of rainfall, the weather data was divided into three periods, such as pre-monsoon period: February–May, monsoon period: June–September and post-monsoon period: October–January.

2.9. Analysis of data

MS Excel version 2007 was used for graphics and statistical analysis. Prevalence of dengue infections were dependent variables, whiles the demographic and climatic factors were independent variables. The independent z-test was used to compare means with various features. Frequencies/percentage of clinical symptoms was compared using Chi-square (χ^2) Goodness-of-fit tests. Probability (p-value) analysis was performed with the statistical package for the social sciences. A probability value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Demography distributions of suspected dengue cases

Table 2 presents the demographic distributions of dengue-like illness suspected cases (N = 1980). Of these, there were 1335 (67.4%) males and 645 (32.6%) females with the ratio of male to female = 2.1:1. In infants up to 1 year age, there were no suspected cases of dengue infections; however, maximum suspected cases of dengue infections were found in the age group of 25–34 years (538 cases, 27.2%) followed by those 15–24 years (456 cases, 23.0%); and the fewest suspected cases of dengue infections were found in the age group of 1–4 years (3 cases, 0.28%) followed by those 5–9 years (17 cases, 0.9%).

3.2. Distribution of the prevalence of dengue confirmed cases according to sex and different age groups

A total number of 1980 acute-phase blood samples of dengue-like illness suspected cases was examined and the confirmed cases of dengue infections, diagnosis by RT-PCR, NS1 Ag-RDT and NS1 Ag-ELISA methods were 733 (37.0%), 741 (37.4%) and 745 (37.6%), respectively. Of the 733 samples confirmed by RT-PCR, 733 were positive by NS1 Ag-ELISA, 732 were positive by NS1 Ag-RDT, 705 were positive for primary dengue infection by IgM Ab-ELISA and 28 were positive for secondary dengue infection by IgM + IgG Ab-ELISA only (see Fig. 3).

Of these 733 dengue infections confirmed cases, the maximum cases were occurring in the age groups of young adult and adult's (527 cases, 71.9%) followed by those middle-aged persons (139 cases, 19.0%) and the fewest cases were occurring in the age group of children (30 cases, 4.1%) followed by those senior citizens (37 cases, 5.0%). The confirmed cases of dengue infections were more in males (540 cases, 73.6%) in comparing to females (193 cases, 26.4%). The highest dengue case positivity rate (CPR) was noted in the age group of 15–24 years (37.9%) followed by those 25–34 years (37.4%) and lowest CPR was noted in the age group of 1–4 years (33.3%) followed by those 5–9 years (35.3%). In among all age groups, there was no death case occurring due to dengue infection during the study (see Table 2).

In 733 dengue infections confirmed cases, 705 (96.2%) cases of DF and 28 (3.8%) cases of DHF were observed; however, there were no DSS cases of dengue infections were observed during the study. Of the 28 DHF cases, 21 were male and 7 were female, and the age group of 15–44 years (71.4%) was the most vulnerable to dengue (see Table 3).

3.3. Clinical features

Table 4 summarizes the clinical features of the 1980 cases. Fever, headache, myalgia, athralzia, and nausea comprised the most common clinical symptoms occurring in all cases. The highest percent of differences in clinical symptoms such as retro-ocular pain (92.4, 64.4), hypotension (23.0, 14.6), itching (16.2, 12.4), pleural effusion (11.4, 12.8), rash (9.5, 14.5), diarrhea (4.0, 1.8) and slow heart rate (3.8, 2.6) between suspected (N = 1247) and confirmed dengue infections cases (N = 733) were observed, respectively. Gastrointestinal bleeding was not found in any cases during this study.

Table 2
Distribution of the prevalence of dengue suspected cases versus dengue confirmed cases according to sex and different age groups in Odisha of eastern India during January to December 2013.

Age group (years)	Male				Female				Total				Case positivity rate (CPR) (%)[a]
	Suspected cases		Confirmed cases		Suspected cases		Confirmed cases		Suspected cases		Confirmed cases		
	No	%	No	%	No	%	No	%	No	%	No	%	
< 1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0.0
1–4	0	0.0	0	0.0	3	0.2	1	0.1	3	0.2	1	0.1	33.3
5–9	7	0.4	3	0.4	10	0.5	3	0.4	17	0.9	6	0.8	35.3
10–14	39	2.0	16	2.2	23	1.2	7	1.0	62	3.1	23	3.1	37.1
15–24	340	17.2	138	18.8	116	5.9	35	4.8	456	23.0	173	23.6	37.9
25–34	393	19.8	158	21.6	145	7.3	43	5.9	538	27.2	201	27.4	37.4
35–44	252	12.7	102	13.9	171	8.6	51	7.0	423	21.4	153	20.9	36.2
45–60	240	12.1	97	13.2	142	7.2	42	5.7	382	19.3	139	19.0	36.4
> 60	64	3.2	26	3.5	35	1.8	11	1.5	99	5.0	37	5.0	37.4
Total	1335	67.4	540	73.6	645	32.6	193	26.4	1980	100.0	733	100.0	37.0

[a] Prevalence (%) of dengue: 37.6; Prevalence (%) of dengue virus serotypes: 37.0.

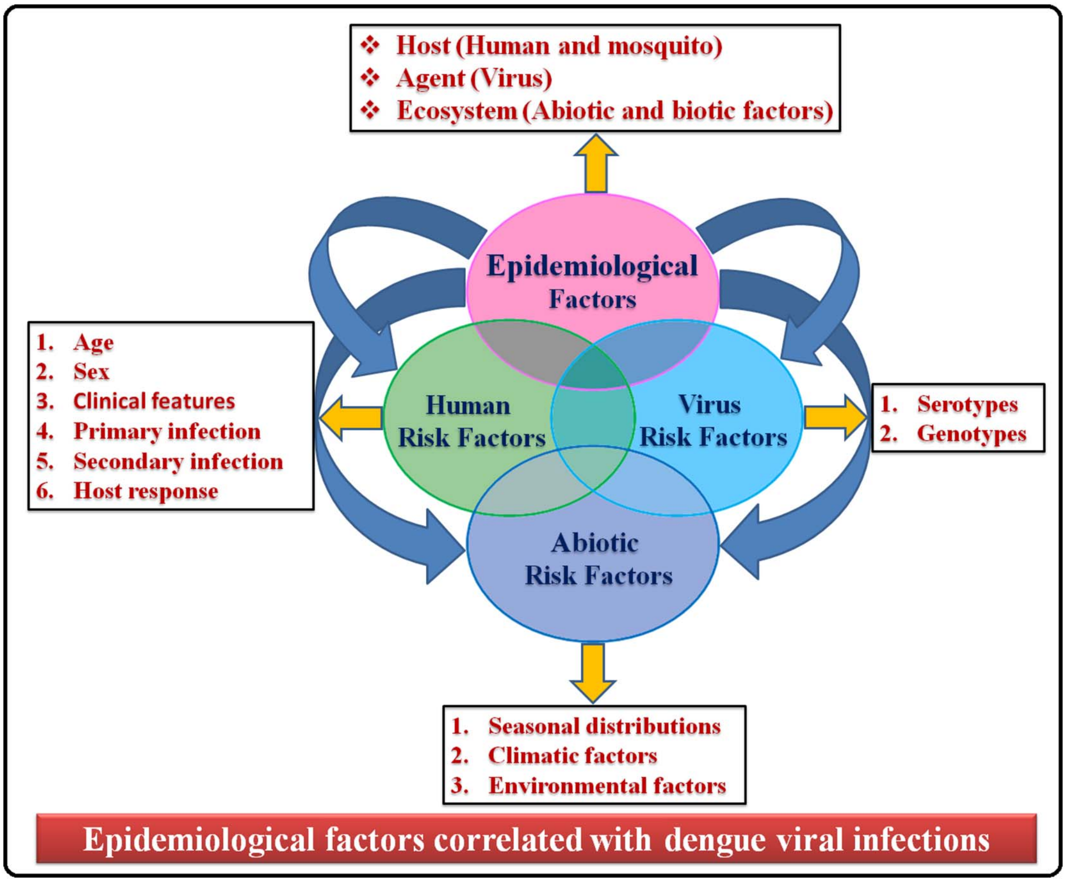

Fig. 3. Epidemiological factors correlated with dengue viral infections.

3.4. Distribution of the incidences of dengue infections correlated with seasonal and climatic factors

Fig. 4 presents the incidences of dengue infections correlated with seasonal distributions. The result revealed that dengue-like illness suspected cases during the pre-monsoon period were found as negative for dengue infections. During the post-monsoon period, the confirmed cases of DENV infection were 163 (22.2%), 17 (2.3%) and 2 (0.3%) in the months of October, November, and December, respectively. The maximum positive cases of dengue infections occurred during the monsoon period. The maximum number of cases were noted in September (42.4%), followed by those in August (29.1%) and July (3.7%).

Table 3
Distribution of the prevalence of the disease manifested as dengue fever (DF) and dengue hemorrhagic fever (DHF) according to sex and age groups in Odisha of eastern India during January to December 2013.

Age group (years)	Disease manifested of dengue viral infections						Total dengue cases (DF + DHF)	Percentage (%)
	DF			DHF				
	Male	Female	Total	Male	Female	Total		
< 1	0	0	0	0	0	0	0	0
1–4	0 (0.0)	1 (0.1)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	1	0.1
5–9	3 (0.4)	3 (0.4)	6 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	6	0.8
10–14	14 (1.9)	8 (1.1)	22 (3.0)	1 (0.1)	0 (0.0)	1 (0.1)	23	3.1
15–24	132 (18.0)	35 (4.8)	167 (22.8)	5 (0.7)	1 (0.1)	6 (0.8)	173	23.6
25–34	153 (20.9)	40 (5.5)	193 (26.3)	6 (0.8)	2 (0.3)	8 (1.1)	201	27.4
35–44	98 (13.4)	49 (6.7)	147 (20.1)	4 (0.5)	2 (0.3)	6 (0.8)	153	20.9
45–60	94 (12.8)	39 (5.3)	133 (18.1)	4 (0.5)	2 (0.3)	6 (0.8)	139	19
> 60	25 (3.4)	11 (1.5)	36 (4.9)	1 (0.1)	0 (0.0)	1 (0.1)	37	5.1
Total	519 (70.8)	186 (25.4)	705 (96.2)	21 (2.9)	7 (1.0)	28 (3.8)	733	100.0

Table 4
Clinical features recorded in suspected and laboratory confirmed dengue cases in Odisha of eastern India during January to December 2013.

Clinical symptoms	Clinical symptoms (%)		p-Value
	Suspected dengue cases[a] (N = 1247)	Isolated dengue infections[b] (N = 733)	
Febrile phase			
Fever	100.0	100.0	–
Headache	95.5	95.5	–
Vomiting	53.4	51.4	0.84
Myalgia (muscle pain)	100.0	100.0	–
Retro-ocular pain	92.4	64.4	0.02
Athralzia	94.6	95.6	1.0
Fatigue	18.4	20.4	0.74
Nausea	85.2	86.2	1.0
Rash	9.5	14.5	0.3
Diarrhea	4.0	1.8	0.36
Critical phase			
Hypotension	23.0	14.6	0.17
Pleural effusion	11.4	12.8	0.77
Gastrointestinal bleeding	0.0	0.0	–
Recovery phase			
Altered level of consciousness	2.8	1.6	0.56
Itching	16.2	12.4	0.47
Slow heart rate	3.8	2.6	0.63

[a] Does not include laboratory confirmed dengue cases.
[b] Positive (reactive) result of anti-dengue RT-PCR for dengue infections.

The difference between numbers of dengue infections positive cases as compared to dengue infections negative ones in monsoon period was higher than the remaining period with 55% of total annual cases reported. The climatic evaluation of the district showed that, the humid months (July to November 2013), with humidity ranging from 64% to 90%, high rainfall (cumulative rainfall = 184.5 mm) and maximum temperature (32–38 °C), had a higher prevalence of dengue CPR (99.7%) was noted and this tapered off in December to 0.3% (see Fig. 5).

3.5. Distribution of the prevalence of dengue cases infects with monotypic and multitypic infections by DENV serotypes according to sex and different age groups

Distribution of DENV serotyping was presented in Tables 5 and 6. Of these 733 (37.0%) positive cases of dengue infections, there were 656 (89.5%) cases had infected with monotypic infection by different DENV serotype, and 77 (10.5%) cases had infect with multitypic infections by multiple serotypes of DENV. Among these 656 monotypic infection cases, 63 (8.6%) was typed as DENV-1, 411 (56.1%) typed as DENV-2, and 182 (24.8%) typed as DENV-3. Of the monotypic infection cases, 484 (73.8%) were males and 172 (26.2%) were females. The maximum number of cases were occurring in the age group of 25–34 years (180, 27.4%) followed by those 15–24 years (155, 23.6%). Of the total multitypic infections, there were 74 (10.1%) cases had infected with DENV-2 and DENV-3 serotypes at the same time; and only 3 (0.4%) cases had the concurrent infection of all three serotypes that was, DENV-1, DENV-2, and DENV-3. Of the 77 cases of multitypic infection, 56 (72.7%) were males and 21 (27.3%) were females. The maximum number of cases were occurring in the age group of 25–34 years (21, 27.3%) followed by those 15–24 years (18, 23.4%). However, there were no positive cases of DENV-4 serotype infection were observed over one year.

Dengue hemorrhagic fever (DHF) was observed in 28 cases. Out of which, 11 cases had infected with monotypic infection of different serotypes, i.e. DENV-2 and DEN-3; and 17 cases had infected with multitypic infection by multiple serotypes of DENV. Of the total multitypic infections, there were 14 (10.1%) cases had infected with DENV-2 and DENV-3 serotypes at the same time; and only 3 (0.4%) cases had the concurrent infection of all three serotypes that was, DENV-1, DENV-2, and DENV-3. In infants up to 1 year of age and from children 1–9 years of age, there were no positive cases of DHF infection (see Table 7).

The serotype-specific dengue virus specimens were identified by the detection of DNA bands showing on 1.5% agarose gel electrophoresis stained with ethidium bromide (see Fig. 6).

4. Discussion

Dengue viral infections rank as the most important rapidly emerged mosquito-borne viral disease in recent years and are endemic in India

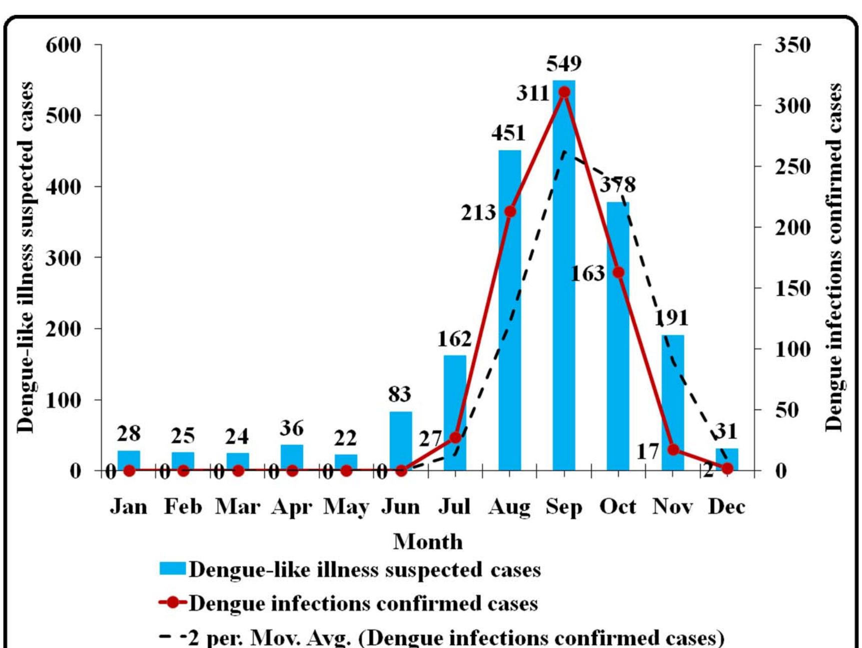

Fig. 4. Distribution of dengue-like illness suspected cases versus dengue infections confirmed cases correlated with seasonal occurrences in Odisha of eastern India during January to December 2013.

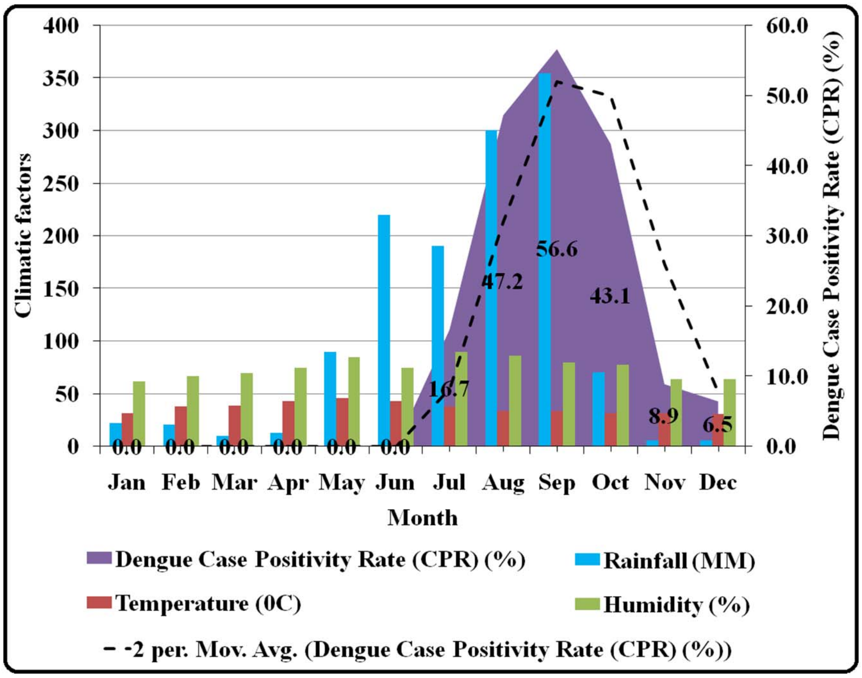

Fig. 5. Incidences of dengue infections correlated with seasonal distributions and climatic factors in Odisha of eastern India during January to December 2013.

Table 5
Distribution of the prevalence of dengue cases infects with monotypic infection by different DENV serotype according to sex and different age groups in Odisha of eastern India during January to December 2013.

Age group (years)	Dengue virus serotyping by RT-PCR	Dengue virus serotype monotypic infection								
		DEN1			DEN2			DEN3		
		M	F	T	M	F	T	M	F	T
< 1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
1–4	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
5–9	6 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.3)	2 (0.3)	4 (0.5)	1 (0.1)	1 (0.1)	2 (0.3)
10–14	23 (3.1)	1 (0.1)	1 (0.1)	2 (0.3)	8 (1.1)	4 (0.5)	12 (1.6)	4 (0.5)	2 (0.3)	6 (0.8)
15–24	173 (23.6)	12 (1.6)	3 (0.4)	15 (2.0)	77 (10.5)	20 (2.7)	97 (13.2)	34 (4.6)	9 (1.2)	43 (5.9)
25–34	201 (27.4)	14 (1.9)	4 (0.5)	18 (2.5)	90 (12.3)	22 (3.0)	112 (15.3)	39 (5.3)	11 (1.5)	50 (6.8)
35–44	153 (20.9)	9 (1.2)	4 (0.5)	13 (1.8)	58 (7.9)	28 (3.8)	86 (11.7)	25 (3.4)	13 (1.8)	38 (5.2)
45–60	139 (19.0)	8 (1.1)	4 (0.5)	12 (1.6)	55 (7.5)	23 (3.1)	78 (10.6)	24 (3.3)	10 (1.4)	34 (4.6)
> 60	37 (5.0)	2 (0.3)	1 (0.1)	3 (0.4)	15 (2.0)	6 (0.8)	21 (2.9)	6 (0.8)	3 (0.4)	9 (1.2)
Total	733 (100.0)	46 (6.3)	17 (2.3)	63 (8.6)	305 (41.6)	106 (14.5)	411 (56.1)	133 (18.1)	49 (6.7)	182 (24.8)

(Angel and Joshi, 2009). Dengue virus has now well established that different genotypes belonging to four DENV serotypes were circulating, either or in combinations (Gupta et al., 2008; Chaturvedi and Nagar, 2008). These serotypes and genotypes are replacing each other (Chakravarti et al., 2010; Kumar et al., 2010). Monitors at DENV activity were required for public health importance, as the dengue fever and DHF/DSS were increasing worldwide and were spreading in the places, where it was not reported. Considering the difference in sensitivity of NS1 Ag-ELISA and RT-PCR methods at various stages of illness (Peeling et al., 2010), using both methods was useful in detecting cases positive by just one method. The trend of using a molecular-based diagnostic tool such as RT-PCR-based tests has become the new approach to confirm of dengue infections and its characteristics. It has been reported that RT-PCR is positive in serum samples collected during the febrile stage of primary dengue infection (Guzman and Kouri, 1996; Yamada et al., 1999).

Our study showed that higher frequencies of clinical features designated as athralzia, nausea, fatigue, rash and pleural effusion (specific to the febrile and critical phase of dengue infections) were observed in dengue positive cases as compared to suspected dengue cases. Higher percentages of dengue infections were confirmed by NS1 Ag-ELISA (37.6%) as compared to the NS1 Ag-RDT (37.4%) and RT-PCR (37.0%). It is unlikely that this is due to higher detection sensitivity of the NS1 Ag-ELISA. This lower rate of the prevalence of dengue infections is likely due to the public awareness, skills and confidence of their responsibilities in dengue surveillance, treatment, and control after facing

Table 6
Distribution of the prevalence of dengue cases infects with multitypic infections by multiple serotypes of DENV according to sex and different age groups in Odisha of eastern India during January to December 2013.

Age group (years)	Dengue virus serotyping by RT-PCR	Dengue virus serotypes multitypic infections					
		DEN1 + DEN2			DEN1 + DEN2 + DEN3		
		M	F	T	M	F	T
< 1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
1–4	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
5–9	6 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
10–14	23 (3.1)	2 (0.3)	1 (0.1)	3 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)
15–24	173 (23.6)	14 (1.9)	4 (0.5)	18 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)
25–34	201 (27.4)	16 (2.2)	4 (0.5)	20 (2.7)	0 (0.0)	1 (0.1)	1 (0.1)
35–44	153 (20.9)	10 (1.4)	5 (0.7)	15 (2.0)	0 (0.0)	1 (0.1)	1 (0.1)
45–60	139 (19.0)	10 (1.4)	4 (0.5)	14 (1.9)	1 (0.1)	0 (0.0)	1 (0.1)
> 60	37 (5.0)	3 (0.4)	1 (0.1)	4 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
Total	733 (100.0)	55 (7.5)	19 (2.6)	74 (10.1)	1 (0.1)	2 (0.3)	3 (0.4)

the hazards due to an outbreak of dengue infection occurred in the Angul district of Odisha during the month of August and September 2011. In this study, infants were not found to suffer from dengue infections because they are taken good care by covering the body of clothes and bed nets to prevent mosquitoes bite, and also due to the maternal gained antibodies (Infection-fighting proteins called antibodies pass from the woman's body to the developing fetus through the placenta.) (Simister and Story, 1997). However, more prevalence was observed among the young-adult and adult age groups (15–45 years); which due to the immunity to one serotype does not afford protection from the infection by a heterotopous serotype (World Health Organization and National Vector Borne Disease Control Programme, 2015), and also they are working personnel's working outside of the premises during the daytime where the vector of *Aedes* species can bite

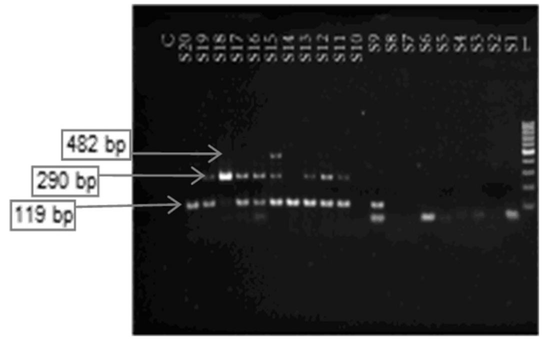

Fig. 6. Serotype-specific dengue virus samples, showing band in 1.5% agarose gel electrophoresis stained with ethidium bromide. Lane L: 100 bp DNA ladder, Lane S1–S8 and S10: negative sample, Lane S9, S14 and S20: sample positive for single infection of DEN-2 (119 bp), DEN-3 (290 bp), and DEN-1 (482 bp); Lane S11–S13 and S16–S19: sample with dual infection of DEN-2 (119 bp) and DEN-3 (290 bp); Lane S15: sample with concurrent infection of DEN-1 (482 bp), DEN-2 (119 bp), and DEN-3 (290 bp); and Lane C: negative control.

because these species are the daytime feeders and preferable in outdoors (Rao and Padhy, 2014). The confirmed cases of dengue infections were more in males in comparing to females. The prevalence of dengue infections was also significantly different (z-value = 1.7250, $p < 0.05$) between the males and females among different age groups.

The first isolation of DENV serotypes (DENV-1 and DENV-4) was reported from India in 1964 (Myers et al., 1964) and serotype 3 in 1968 (Myers et al., 1970). The earlier reports on monotypic infection of DENV serotypes revealed that DENV-3 was the predominant serotype in Delhi and many parts of northern India during 2003–2006 (Gupta and Ballani, 2014; Dash et al., 2015). After onwards, in Delhi DENV-2 and DENV-3 were replaced by DENV-1 (Chakravarti et al., 2010). Since 2006, DENV-1 prevalence has increased in India (Dash et al., 2015; Kukreti et al., 2009). Whereas, in our study, we report a high percentage (56.1%) of DENV-2 was the most common etiologic agent, followed by DENV-3 (24.8%) and DENV-1 (8.6%) among the DENV monotypic infection by single different serotypes were found to co-circulating in this province. Detection of DENV-2 serotype infection in our study corroborates active DENV circulation of this province, which is similar to the results of the study, was carried out in central India during 1992 (Mahadev et al., 1997). However, the reports on the DENV serotypes circulation of Andhra Pradesh, south India during the

Table 7
Distribution of the prevalence of dengue hemorrhagic fever (DHF) cases infects with monotypic and multitypic infections by serotypes according to sex and different age groups in Odisha of eastern India during January to December 2013.

Age group (years)[a]	Dengue hemorrhagic fever (DHF)												
	DENV serotyping by RT-PCR	DENV serotype monotypic infection						DENV serotypes multitypic infections					
		DEN2			DEN3			DEN1 + DEN2			DEN1 + DEN2 + DEN3		
		M	F	T	M	F	T	M	F	T	M	F	T
10–14	1 (3.6)	1 (3.6)	0 (0.0)	1 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
15–24	6 (21.4)	3 (10.7)	1 (3.6)	4 (14.3)	1 (3.6)	0 (0.0)	1 (3.6)	1 (3.6)	0 (0.0)	1 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)
25–34	8 (28.6)	2 (7.1)	0 (0.0)	2 (7.1)	1 (3.6)	0 (0.0)	1 (3.6)	3 (10.7)	1 (3.6)	4 (14.3)	0 (0.0)	1 (3.6)	1 (3.6)
35–44	6 (21.4)	1 (3.6)	0 (0.0)	1 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	3 (10.7)	1 (3.6)	4 (14.3)	0 (0.0)	1 (3.6)	1 (3.6)
45–60	6 (21.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)	1 (3.6)	2 (7.1)	2 (7.1)	4 (14.3)	1 (3.6)	0 (0.0)	1 (3.6)
> 60	1 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)	1 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)
Total	28 (100.0)	7 (25.0)	1 (3.6)	8 (28.6)	3 (10.7)	0 (0.0)	3 (10.7)	10 (35.7)	4 (14.3)	14 (50.0)	1 (3.6)	3 (10.7)	3 (10.7)

[a] Infants up to 1 year of age and from children 1–9 years of age, there were no positive cases of DHF infection.

outbreak of 2007 and Maharashtra, western India during 2009 has revealed significantly different that the emergence of DENV-4 serotype was dominant one (Dash et al., 2011; Cecilia et al., 2011).

The first case of multitypic infections with two DENV serotypes was reported from Puerto Rico in 1982 (Gubler et al., 1985), since then different countries have reported occurring to multitypic infections in areas where multiple DENV serotypes co-circulates (Kanesa-Thasan et al., 1998; Fang et al., 1997; Loroño-Pino et al., 1999; Wang et al., 2003). Although concurrent infection with more than one serotype of DENV in the same individual was uncommon, a high percentage of multitypic infections with multiple DENV serotypes was detected during an outbreak in Delhi, India, in 2006 (Bharaj et al., 2008). In this study, we reported 10.5% of multitypic infections with multiple DENV serotypes. This is the first and foremost study from this province to report multitypic infections with multiple serotypes of DENV. Which is similar to the reports on previous studies was carried out in Taiwan (9.5%), Indonesia (11.0%), and Mexico (11.0%) (Fang et al., 1997; Loroño-Pino et al., 1999). The findings of our study have been postulated that multitypic infections may influence the clinical expression of the disease. This has been considered as a single major factor of emerging as DHF (Loroño-Pino et al., 1999).

In India, first DHF case was reported from Delhi in 1988 (Kabra et al., 1992) and thereafter, several dengue outbreaks of the increase in the number of DHF/DSS cases were reported from Delhi and its adjoining areas. During the major dengue outbreak of 1996, revealed that DHF/DSS was seen in 59.0% and DF in 40.0% cases of dengue infections (Dar et al., 2003). In 2006, 70.0% cases of single serotype infection and 30.0% cases with multitypic serotype infections cases had suffered from DHF (Bharaj et al., 2008). In our study, we reported that DHF was diagnosed as 3.8% and DF in 96.2% cases of dengue infections. Young adults and males were more vulnerable to DHF. DHF was present in 60.7% (17/28) cases of multiple DENV serotypic infections and 39.3% (11/28) cases of dengue monotypic infection. Combine of DENV-2 and DENV-3 serotypes were identified a high percentage (82.4%) of occurring multitypic serotype infections. Only a male patient of 53 years old and two female patients of 26 and 38 years age had infected with DENV-1, DENV-2, and DENV-3 serotypes at the same time and suffered from DHF. Thus a higher percentage of cases of multitypic infections had suffered from DHF although the numbers of cases are small and therefore studies with sizeable samples are needed to prove this association.

In our study, we have observed that the temperature remains high during the pre-monsoon period and decreases towards the end of monsoon period, most of all it remains constant during the later months of rainy season. Among the three seasonal periods, there is a significant positive relationship between the divergence in temperature and humidity, z-value = -7.064, $p < 0.05$. The largest proportion of DENV infections was described during the humid months of July and grasped its peak in the September with high rainfall, which was the post-monsoon period. It is due to the continuous rain pouring for a long period that brings down the temperature during the period from June to November, which managed an increase in the relative humidity and decreases the evaporation rate thus maintaining secondary reservoirs containing rain waters favored to cause to reproduce the vector mosquitoes. These findings of our study agree with the previous studies, from Myanmar by Naing et al. during 1996–2001 reported that the maximum cases of dengue during the monsoon period (Naing et al., 2002), from Brazil by Rebelo et al. reported that dengue cases were higher during the rainy season which showing the influence of rain in forming prime breeding sites for *Aedes aegypti* thus spread of dengue infections (Rebelo et al., 1999), and from Venezuela by Barrera et al. reported that peaks of dengue cases were observed to be near concurrent with rain peaks and also DF has a positive correlation with the relative humidity and negative relation with evaporation rate (Barrera et al., 2002).

5. Conclusion

This prospective study highlighted that over one year, 733 (37.0%) cases were positive for dengue RNA by RT-PCR. The confirmed cases of dengue infections were more in males in comparing to females. The age groups of young adults and adults (15–45 years) are most vulnerable to dengue; however, infants were not suffering from dengue infections. DENV-2 and DENV-3 have dominated in these provinces, even though three (DENV-1, DENV-2, and DENV-3) serotypes were found to be co-circulating. Dengue infections were prevailed from the month of July and grasped it's the peak in September, which was the post-monsoon period. Rain, temperature and relative humidity as the major and important climatic factors are favored breeding of the vector mosquitoes and spread of the dengue infections. Co-circulation of multitypic infections with multiple DENV serotypes and the emergence of DHF cases suggested that Odisha was becoming a hyper-endemic province for dengue in eastern India.

Therefore, it will be worthwhile to carry out more studies to understand the age groups as well the sex-related differences in dengue severity and the characteristics of dengue among them. Furthermore, studies are required to establish the relationship between the climatic changes and dengue infection and analysis of the patterns, causes, and effects of health and disease conditions in defined populations, which would help in planning the shapes policy decisions and evidence-based practice by identifying risk factors of disease and targets for preventive health care.

Ethics

An ethical clearance for this study was not required.

Authorship statement

This work was carried out under the supervision of R.N.P. and M.K.D. M.R.K.R. designed the study, carried out field and laboratory work, performed the statistical analysis and wrote the manuscript. R.N.P. corrected the final manuscript. All of the authors read and approved the final manuscript.

Conflict of interest

The authors have declared that no competing interests exist.

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