Original Article

Demographic and Clinical Profile of Patients Infected with Dengue Virus Serotypes 1, 2, and 3 in North Karnataka

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Abstract

Introduction: Dengue fever is a mosquito-borne disease caused by flavivirus and has clinical presentation varying from being asymptomatic to severe complications (dengue shock syndrome and dengue hemorrhagic fever) depending on the serotype of the virus involved. Cross-protective immunity between the serotypes is lacking, hence the severity of the disease is more if multiple infections occur with two different serotypes. Hence, data on the demographic-specific prevalence of virus serotypes are vital to optimal clinical measures. **Aim:** The present study aimed to identify the dengue virus serotypes prevalent in the North Karnataka region of India in correlation to the clinical presentation of the disease. **Materials and Methods:** A prospective study was carried out in a Teaching hospital of North Karnataka, India, from June 2012 to March 2016. One thousand serum samples were tested for NS 1 antigen aIgM and IgG antibodies by enzyme-linked immunosorbent assay (ELISA) method. Samples positive for NS-1 was subjected to reverse transcription polymerase chain reaction (RT-PCR) for the detection of serotypes. **Results:** Of the 1000 serum sample test 462 serum samples were positive for dengue virus antigen or antibodies. Two hundred and forty-five patients (53.03%) were male and 217 patients (46.96%) were female. Age group of16-–30 years was more affected followed by 31–45 years, over 45 years, and 0–15 years of age group. Maximum number of cases were observed in Bidar city followed by Humnabad, Aurad, Bhalki, and Basavakalyan regions. Malaise was a predominant symptom in dengue virus serotype-3 (DENV-3) ($P < 0.05$), while headache ($P < 0.001$), and retro-orbital pain (<0.05) were predominant symptoms in DENV-2. GI symptoms (nausea, abdominal pain, and diarrhea) were significantly common in DENV-2 ($P < 0.001$). Hepatomegaly was frequently observed in DENV-2 (17.02%), ($P < 0.05$). A total of 462 samples were positive for either NS-1, IgM, or IgG or in combination. Viral RNA was extracted from 119 samples positive for NS-1 antigen by ELISA. Of the 119 samples tested for serotyping by RT-PCR, 38 belonged to dengue serotype-1 (DENV-1), 46 were of dengue serotype 2 (DENV-2) and 35 belonged to dengue serotype 3 (DENV-3). A change in the earlier serotype 1 and 2 from 2011 to 2013 to the present serotype DENV-2 and DENV-3 was observed and constant presence of DENV-2 in circulation was recorded. **Conclusion:** Dengue virus serotype 1, 2, and 3 were prevalent in our study population, and severe clinical manifestations were observed in patients suffering from dengue virus serotype 2 and 3.

Keywords: Dengue, North Karnataka, serotype

Introduction

Dengue fever is one of the mosquitos borne diseases caused by flavivirus. Dengue infection is reported in 50–100 million individuals from developing world and is responsible for over 500,000 hospitalizations.[1] The severity of the disease varies from acute infection to fatal consequences such as dengue shock syndrome and dengue hemorrhagic fever (DHF).[2] Dengue virus has four different serotypes, DENV-1, DENV-2, DENV-3, and DENV-4, which are further subclassified based on their individual genotype.[3]

In India, a major outbreak of dengue serotype 2 was reported in the year 1996.[4] This was later displaced by dengue serotype 3, although many outbreaks with mixed serotypes are also reported subsequently. The first isolated dengue viruses belonged to serotype 3 and 4 in the year 1964 and 1965, respectively. Dengue virus type 3 has been very rarely reported in North Karnataka.[4-6] There are various studies from the Indian subcontinent investigating DHF in various parts of the country. However, there are no studies investigating the overall prevalence of the dengue serotypes

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circulating in the endemic zone, apart from the epidemic outbreak regions.

The purpose of our study was to identify the circulating serotypes of dengue virus in North Karnataka from suspected cases of dengue presenting to our hospital during the years 2012–2016 and correlate it with the clinical presentation.

Materials and Methods

This is a prospective study, which was carried out in Bidar Institute of Medical Sciences Teaching Hospital of North Karnataka, India during the year 2012–2016. Ethical clearance was obtained from the Institutional Ethical Committee. Patients attending the hospital with a history of fever, headache, retro-orbital pain, nausea/vomiting, joint pain, malaise, and generalized skin rashes were initially considered for the diagnosis of dengue fever.[7-11] Patients presenting with two or more of these symptoms were included and were classified according to the WHO criteria[12] (dengue with or without warning signs). Patients of both gender and all age groups were included in the study.

A volume of 2–5 ml peripheral venous blood was collected in plain vial and blood was allowed to clot at room temperature and then centrifuged. Serum was separated for detection of NS-1antigen, IgM, and IgG antibodies by enzyme-linked immunosorbent assay (ELISA). In case of delay, test serum samples were preserved at −70°C. Positive samples were stored at −80°C until they were processed for serotyping by reverse transcription polymerase chain reaction (RT-PCR).[13]

Blood samples were collected in ethylenediaminetetraacetic acid vial from the patients for complete blood count (i.e., Hb estimation [Hb], total leukocyte count, differential count, hematocrit, and platelet count) using an automated cell counter.

All the 1000 serum samples were tested for the presence of NS-1 antigen (Pan-bio) and IgM and IgG antibodies by capture ELISA[12,13] by using the kit prepared by the National Institute of Virology, Pune, India. Following the prescribed protocol, optical density was measured at 450 nm using ELISA reader.

RNA extraction: Samples which were positive for NS-1 antigen by ELISA were only subjected for extraction of viral RNA by using the QiagenQIAmp RNA Mini Kit according to the manufacturer's protocol.

For the conventional PCR using serotype-specific E-NS1 region primers, viral RNA was reverse transcribed to cDNA before enzymatic DNA amplification by use of MoMLV reverse transcriptase. For RT of viral RNA to cDNA before Taq polymerase amplification, corresponding reverse primer of the particular set was used.

Primer and probe details

The initial viral quantitation of the samples was done using real-time PCR to establish that there was enough viral load for carrying out amplification and sequencing reactions. The primers (Eurofins genomics) were designed to target the 3 prime untranslated region on the dengue viral genome. The primers were combined in the required ratio with the corresponding Taqman probe (Eurofins Genomics) to make a common screening primer-probe mixture (ppm). The primer probe mix also contained primers and probe for a housekeeping gene (internal control region). The internal control probe signal was read in the VIC dye channel whereas the viral C-DNA amplification signal was read in the FAM channel on the real-time machine.

Table 1: List of primes and programs for E-NS-1 region amplification

Serotype	Primers	PCR program		Product size
DEN 1	D1-1229F	95°C- 5 min	X 1	482 BP
		95°C - 30 s	X 35	
	D1-1710R	57°C - 30 s		
		72°C - 1 min		
		72°C - 5 min	X 1	
DEN 2	ENVF-PO1	95°C - 2 min	X1	445 BP
		95°C - 30 s	X 30	
	D2R-PO1	52°C - 30 s		
		72°C - 2 min		
		72°C - 7 min	X1	
DEN 3	D3-1307F	95°C - 5 min	X 1	881 BP
		95°C -30 s	X35	
	D3-1867R	57°C - 30 s		
		72°C - 1 min		
		72°C - 5 min	X 1	
DEN 4	D4-1954R	95°C - 5 min	X 1	195 BP
		95°C - 30 s	X 35	
	D4-1760F	57°C -30 s		
		72°C -1 min		
		72°C - 5 min	X 1	

PCR: Polymerase chain reaction

After the viral load for all the serum samples was carried out, serotyping by serotype-specific primer-probe mix (Eurofins Genomics) was done to categorize the samples into their particular serotypes.

Primers were designed in-house at Huwel Lifesciences Pvt. Ltd [Table 1].[14]

Statistical analysis

Statistical analysis was done by using SPSS version 22 software (IBM corporation., Armonk, NY, USA). Chi-square test was applied. $P < 0.05$ was considered statistically significant.

Results

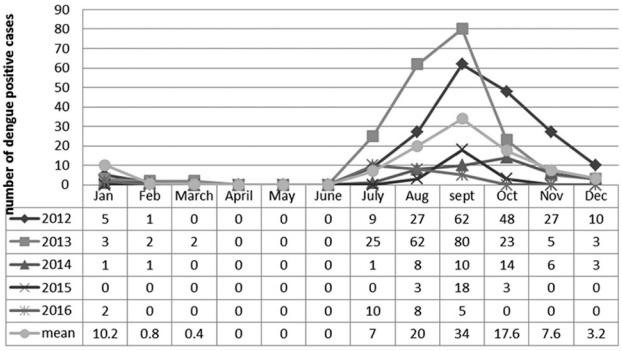
Of the 1000 clinically suspected patients, only 462 serum samples were positive for dengue antigen or antibodies, hence achieving an incidence rate of 46.2% in our patient population evaluated. Age group wise more number of positive case were observed in the age group 16–30 years, i.e., 238 patients (51.1%) followed by 31–45 years (107 patients; 23.16%), 46 years and above (61 patients; 13.20%), and

0–15 years (56 patients; 12.1%). Male patients (245 patients; 53.03%) were relatively more affected then female patients (217 patients; 46.96%) [Table 2]. Sporadic cases occurred throughout the year, more number of dengue cases were observed in monsoon and postmonsoon months, i.e., in July and attained maximum number of cases in the month of September, with a mean number of seven cases in July, 20 cases in August, 34 cases in September, 17 cases in October, 8 cases in November, and 3 cases in December [Table 3].

Table 2: Age and sex wise distribution of seropositive samples for dengue antigen and antibodies

Age (years)	Male (%)	Female (%)	Total (%)
0-15	31 (6.70)	25 (5.41)	56 (12.1)
16-30	121 (26.19)	117 (25.32)	238 (51.1)
31-45	65 (14.06)	42 (9.09)	107 (23.16)
45 and above	28 (6.06)	33 (7.14)	61 (13.20)
Total	245 (53.03)	217 (46.96)	462 (100)

Table 3: Month wise distribution of dengue positive cases



	Jan	Feb	March	April	May	June	July	Aug	sept	Oct	Nov	Dec
2012	5	1	0	0	0	0	9	27	62	48	27	10
2013	3	2	2	0	0	0	25	62	80	23	5	3
2014	1	1	0	0	0	0	1	8	10	14	6	3
2015	0	0	0	0	0	0	0	3	18	3	0	0
2016	2	0	0	0	0	0	10	8	5	0	0	0
mean	10.2	0.8	0.4	0	0	0	7	20	34	17.6	7.6	3.2

More cases of dengue DENV-1 (7) serotype were observed in Basavakalyan. All the three serotypes were isolated from Bidar and Humnabad, while serotypes 1 and 2 were isolated from Basavakalyan, Bhalki, and Aurad regions.

Of the 462 positive samples, 183 (39.61%) were only NS-1 positive, 120 (25.97%) were IgM positive and 247 (53.46%) were both NS-1 and IgMpositve by ELISA method. The positivity of NS-1 was 241 (97.57%) in acute phase (1–7 days), and IgM positivity (174 cases; 94.56%) was more in convalescence (6–14 days) phase.

Clinical manifestations of patients were compared with the dengue serotypes identified from 119 samples, which was statistically analyzed by Chi-square test. Malaise was a predominant symptom in DENV-3 ($P<0.05$), while headache ($P<0.001$) and retro-orbital pain (<0.05) were predominant symptoms in DENV-2. GI symptoms (nausea, abdominal pain, and diarrhea) were also significantly observed in DENV-2 ($P<0.001$). Hepatomegaly was commonly observed in DENV-2 (17.02%), ($P<0.05$). Respiratory symptoms (cough, dyspnea, rhinorrhea, and congestion) were more commonly observed in DENV-3 infection. Musculoskeletal symptoms (myalgia, joint pain, and bone pain) commonly observed ($P < 0.001$) in DENV-2 with 89.36%, 78.72%, and 53.19% incidence, respectively. Cutaneous signs (maculopapular rash) was frequently ($P < 0.05$) seen in DENV-2 (12.07%) [Table 4].

Hematological assessment revealed that the mean platelet count was less in serotype-2 (67150 c/mm^3), followed by serotype-3 (71800 c/mm^3) and 75200 in DENV-1 ($P < 0.01$). Hematocrit was 37.57 in DENV-2, 38.10 in DENV-3, and 39.01 in DENV-1 ($P < 0.05$) [Table 5].

PCR Result: NS-1 ELISA positive samples were subjected for dengue virus RNA isolation. We could identify dengue virus

Table 4: Serotypes isolated and its comparison with clinical manifestations

Signs and symptoms	Total cases (n=119)	DENV-1 (n=38; 31.93%), n (%)	DENV-2 (n=46; 38.65%), n (%)	DENV-3 (n=35; 29.41%), n (%)
Fever	119 (100)	38 (100)	46 (100)	35 (100)
Malaise	63 (52.94)	10 (26.31)	23 (50)	30 (85.71)
Headache	78 (65.54)	11 (28.94)	44 (95.65)	23 (65.71)
Retro-orbital pain	64 (53.78)	17 (44.73)	31 (65.95)	16 (45.71)
Nausea	44 (36.97)	7 (18.42)	37 (78.72)	0 (00)
Vomiting	4 (3.36)	2 (5.2)	60 (24.25)	0 (00)
Abdominal pain	42 (35.29)	8 (21.05)	32 (68.08)	2 (5.71)
Diarrhea	09 (7.56)	0 (00)	9 (19.14)	0 (00)
Splenomegaly	15 (12.60)	05 (13.15)	10 (21.27)	0 (00)
Hepatomegaly	12 (10.08)	03 (7.89)	8 (17.0)	20 (12.85)
Cough	8 (6.72)	1 (2.6)	30 (24.25)	5 (14.28)
Dyspnea	8 (6.72)	0 (00)	6 (12.76)	2 (5.71)
Rhinorrhea	6 (5.04)	1 (2.6)	30 (24.25)	3 (8.57)
Congestion	4 (3.36)	0 (00)	1 (2.1)	20 (38.57)
Myalgia	62 (52.10)	18 (47.36)	42 (89.36)	2 (5.71)
Joint pain	53 (44.53)	12 (31.57)	37 (78.72)	4 (11.42)
Bone pain	34 (28.57)	9 (23.63)	25 (53.19)	0 (00)
Maculopapular rash	6 (5.04)	0 (00)	6 (12.70)	0 (00)

RNA from 119 (24.68%) samples by Multiplex RT-PCR. Out of 119 samples positive by RT-PCR, 38 samples had monotypic infection with DENV-1 (31.93%), 46 samples (38.65%) had monotypic infection with DENV-2 and 35 samples (29.41%) had monotypic infection with DENV-3. No DENV-4 serotype was detected among the samples [Table 6].

Serotype 1 was identified from the samples collected in 2012, 2013, and 2014 while Serotype 2 was identified from the samples collected in 2012, 2013, 2014, and 2015. Serotype 3 was identified from samples collected in the year 2013, 2014, and 2015.

Out of the 38 Serotype 1, we could identify 12 (31.57%), 15 (39.47%), 11 (28.94%) in 2012, 2013 and 2014 respectively and out of 47 DENV-2 19 (40.42%), 12 (25.53%), 10 (21.27%), and 05 (12.7%) were identified in the year 2013, 2014, 2015, and 2016, respectively and DENV-3 was isolated 03 (8.57%), 12 (34.28%), 20 (57.14%) in the year 2014, 2015, and 2016, respectively [Table 7].

Discussion

Dengue is the major public health problem in endemic regions of India.[15] The first epidemic of clinical dengue-like illness was recorded in Chennai in the year 1780 but proven virological epidemic of Dengue fever in India occurred in Calcutta and Eastern coast of India in 1963–1964.[16] The largest outbreak occurred in Delhi and surrounding areas in the year 1996. This outbreak was due to dengue serotype 2, which was followed by epidemic in 1997 due to dengue serotype 1. Subsequent epidemics were reported to be due to co-circulation of both serotype-1 and serotype 3 in the year 2003.[17] In this study, we are reporting the co-circulation and replacement of serotype-1 and 2 by serotype 2 and 3 during the years 2012–2016.

Table 5: Correlation of dengue serotypes with hematological parameters

Hematological parameter	DENV-1	DENV-2	DENV-3	*P*
Hb (mmHg)	12.2	11.5	12.5	<0.05
TLC (cells/mm^3)	6121	6520	6441	0.6
Hematocrit	39.01	37.57	38.10	<0.05
Platelet count (cells/mm^3)	75,200	67,150	71,800	<0.01

TLC: Total leukocyte count, Hb: Hemoglobin

Table 6: Different serotypes isolated from tested samples

Total	DENV-1	DENV-2	DENV-3
119	38 (31.93%)	46 (38.65%)	35 (29.41%)

Table 7: Year-wise distribution of dengue serotypes isolated

Serotype	2012	2013	2014	2015	2016	Total (%)
DENV- 1	12	15	11	00	00	38 (31.93)
DENV- 2	00	19	12	10	5	46 (38.65)
DENV- 3	00	00	3	12	20	35 (29.42)
Total (%)	12 (10.08)	34 (28.5)	26 (21.8)	22 (18.4)	25 (21.0)	119

The relative predominance in the prevalence of disease among male patients observed in our study is in concurrence to previous reports.[18,19] Majority of cases were observed in the age group of 16–30 years, this is comparable to the studies previously reported from other regions.[18,19] This age group is highly exposed to the external environment and mosquito vector. Infection in young patients results in nonproductivity and high number of cases in young age group indicates the disease is endemic to the region. We observed dengue virus incidence rate of 46.2% in our study population. Dengue cases started during July and reached the peak in September, which is the post-monsoon period. It may be explained by the fact that stagnant fresh water from August to October (Rainy season) favored the breeding of vector mosquitoes. Hence, these months must be effectively targeted to initiate preventive measures to control the breeding of mosquitoes.

Although the symptoms correlated with serotypes observed in our study is consistent with previous reports;[19-21] however, our finding is in contrast to the studies reporting hemorrhagic manifestations associated with dengue serotype-4.[20] In our study, we could not isolate dengue serotype 4 from any of the blood samples. A previous study reported from India[20] evaluated 80 serum samples and isolated all four serotypes of DENV. This study reported a high prevalence of Anorexia in serotype 2 as compared to serotype 1 and 4. Hepatomegaly was a prominent feature in serotype-2 followed by 1, 3, and 4. In our study, hepatomegaly was seen more in dengue serotype 2 followed by dengue serotype 3 and 1. In our study, out of the total 119 serum samples positive, 38 suffered from DENV-1 monotype infection, 46 DENV-2 monotype infection and 35 DENV-3 monotype infection, only four dengue monotypic-infected patients (age group 16–30 years) had suffered from DHF which may be explained by the absence of immunity against all-serotype of dengue virus in these patients.

In the year 2012 and 2013, the main serotype prevalent was DENV-1 and DENV-2. However, in the following years 2013, 2014, and 2015 displacement of DENV-1 was observed. The study shows that the serotype predominance can shift from one to another. Our study is in agreement with previous reports demonstrating DENV-1 was replaced by DENV-4.[18,19] As the majority of the samples in our study were obtained from hospitalized patients, the results may not actually reflect the predominant serotype in the general population in a given year. However, as currently there are not any approved vaccines or therapeutics for dengue the treatment is mainly supportive. Hence the containment of the spread of the vector and the disease is important.[22,23] Data on serotype will help develop improved, proactive, laboratory-based surveillance systems that can predict an impending dengue outbreak which will help to timely initiate preventive and control measures.

Conclusion

We observed circulation of dengue serotype-2 and serotype-1 and also a rise in number of dengue serotype-3. Co-circulations of these mixed serotypes may lead to severe clinical manifestations. Hence, detection of dengue serotypes helps achieve proper clinical management and probably develop an effective vaccine.

Limitations

Stage of the disease is important for isolation of RNA from serum. RNA isolation is only possible in the initial phase of the disease when high amount of circulating viruses are present, hence we have chosen only NS-1 positive samples for isolation. As the antibody develop, RNA load is lowered in circulation, which may result in less isolation rate, which may be the reason for less isolation of serotypes from our study samples.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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