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Original Article

Microbiology Section

Identification of Serotypes of Dengue Virus Circulating in Delhi and National Capital Region in Post-monsoon Period of 2018

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ABSTRACT

Introduction: Dengue is a major global public health issue with around 390 million infections occurring per year. It is the leading arboviral infection in India and is endemic in all states and union territories. In recent years, all the four serotypes have been seen in circulation in Delhi, India but the predominant serotype keeps changing. Considering the differences in the clinical picture caused by the different serotypes, knowledge of the circulating serotypes can help improve preparedness to deal with future outbreaks effectively.

Aim: To identify the dominant serotype(s) of Dengue Virus (DENV) circulating in Delhi, India during the post-monsoon season in the year 2018.

Materials and Methods: Acute phase serum samples of clinically suspected dengue patients presenting to the hospital were tested for the presence of Nonstructural protein 1 (NS1) Antigen (Ag) of DENV by ELISA from August to December 2018. Sixty two non-repetitive serum samples testing positive for the NS1 Ag by ELISA were subjected to Reverse Transcription Polymerase Chain Reaction (RT-PCR) for identifying the serotype of DENV.

Results: Total NS1 Ag ELISA positive cases were 462. Of the 62 samples subjected to RT-PCR, dengue viral Ribonucleic Acid (RNA) was detected in 43 samples. The predominant serotype was DENV-3 (32/43; 74.4%) followed by DENV-4 (7/43; 16.3%), DENV-1 (4/43; 9.3%) and DENV-2 (3/43; 7%). Two samples showed dual infection with serotypes 1 and 3 whereas one sample showed dual infection with serotypes 3 and 4. Twenty one out of 43 (48.8%) patients were admitted to the hospital while the rest were managed on an outpatient basis. Most of the DENV-3 infections were mild, except six patients with warning signs and three with severe dengue. On the other hand, although only three cases with DENV-2 were identified, one presented with dengue with warning signs, another with no such sign and the third one had severe dengue. Of the seven patients with DENV-4, four were admitted with dengue with warning signs.

Conclusion: The present study found that during 2018, DENV-3 was the predominant circulating serotype in New Delhi, India and most of the DENV-3 infections were mild. Intensive surveillance of circulating serotypes during the early stages of outbreaks can help in predicting the probability of serious outcomes and in calibrating the health care delivery protocols for dengue patients.

Keywords: Dengue serotypes, Epidemiology, New delhi

INTRODUCTION

Dengue is a febrile illness caused by mosquito-borne Dengue virus, member of the family *Flaviviridae* genus Flavivirus. About half of the global population has been projected as at risk of dengue [1].

The WHO has identified dengue as one of the major global public health issues, with around 390 million dengue infections occurring per year (95% credible interval 284–528 million) of which an estimated 96 million (67-136 million) are clinically apparent dengue cases; predominantly in Asia, followed by Latin America and to a lesser extent Africa [1]. India documented a total of 1,01,192 cases of dengue in 2018, with 172 deaths. Of these, 7,136 cases and four deaths were reported from Delhi [2].

There are four distinct, but closely related, serotypes of the DENV viz., DENV-1, DENV-2, DENV-3 and DENV-4. Most of the infections are caused by these four serotypes. However, a fifth serotype has also been identified. The serotype DENV-5, reported in 2013, was detected during screening of viral samples taken from a 37 year old farmer admitted to a hospital in Sarawak state of Malaysia in 2007 [3,4].

It has been reported that primary infections by DENV-1 and DENV-3 tend to be more apparent and more severe as compared to other serotypes [5,6]. While antecedents and prime movers of dengue severity are not completely understood, it is hypothesised that interaction between human host and DENV certainly contributes in the pathogenesis of disease. Difference in severity may also be related to plasma levels of viral RNA. Since there appears some definite correlation between DENV serotypes and their disease severity; it becomes scientifically logical that the circulating serotypes are identified and clinicians sensitised about the possible picture of impending clinical outcomes during the dengue outbreaks.

In light of the fact that the circulating DENV serotypes influence the clinical outcome, the present study was undertaken to detect the dominant serotype(s) of DENV circulating in Delhi, India during the post-monsoon season in the year 2018.

MATERIALS AND METHODS:

The present prospective study was conducted at Department of Microbiology, ABVIMS and Dr. Ram Manohar Lohia Hospital, a tertiary care centre, in New Delhi, India from August to December, 2018. This hospital is an important sentinel surveillance site for dengue cases. Testing is undertaken as per guidelines issued by National Vector Borne Disease Control Programme (NVBDCP) [7]. As a routine, all patients reporting to the Out-Patient Department (OPD)/emergency with clinical symptoms and/or signs of dengue are screened by IgM antibody capture enzyme-linked immunosorbent assay (MAC ELISA) and NS1 Ag ELISA for dengue. In keeping with this protocol, acute phase serum samples of clinically suspected dengue patients were taken after obtaining informed consent and tested for the presence of NS1 Antigen of DENV by ELISA {DENV Detect NS1 ELISA kit (InBios, USA)} according to the manufacturer's instructions. Optical density was read at 450 nm. Immune Status Ratio (ISR) was calculated from the ratio of optical density obtained

from test sample and the mean optical density of the cut-off control. ISR >= 1 was considered positive for NS1 antigen. Left over specimens after clinical investigations (acute phase serum samples) of suspected dengue patients with fever of less than five days and prominent clinical symptoms such as bodyache, headache, myalgia and rash, that tested positive for NS1 Ag by ELISA were included in this study. Sixty two non-repetitive serum samples testing positive for the NS1 Ag by ELISA were subjected to RT-PCR for identifying the serotype of DENV. Viral RNA was extracted from serum using SpinStarTM Viral Nucleic Acid Kit 1.0 (ADT Biotech) according to the manufacturer's instructions. RT-PCR for differentiation between serotypes 1,2,3 and 4 of DENV was performed on the extracted RNA using RealStar® Dengue Type RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany) that employs primers targeting the envelope protein gene, on Qiagen Rotor-Gene Q 5plex thermocycler.

STATISTICAL ANALYSIS

Data was entered into Microsoft Excel 2016 and represented in the form of frequency and percentages.

RESULTS

Total Dengue MAC ELISA positive cases from August-December 2018 at Dr RML Hospital were 704. Total NS1 Ag ELISA positive cases during the same period at this hospital were 462. A total of 62 non-repetitive samples that tested positive for the NS1 Ag by ELISA were subjected to RT-PCR for detection and differentiation of DENV 1-4. 39/62 (62.9%) patients were male. 27/62 (41.9%) patients were under the age of 18.

Of these 62 patients, Dengue viral RNA was detected in 43 samples. The predominant serotype was DEN-3 (32/43; 74.4%) followed by DEN-4 (7/43; 16.3%), DEN-1 (4/43; 9.3%) and DEN-2 (3/43; 7%). Dual infections were seen in three samples. Two samples showed dual infection with serotypes 1 and 3 whereas one sample showed dual infection with serotypes 3 and 4 [Table/Fig-1].

	DENV-1	DENV-2	DENV-3	DENV-4
Total samples positive	**4/43 (9.3%)**	**3/43 (7%)**	**32/43 (74.4%)**	**7/43 (16.3%)**
Dual Infections	2 samples (serotypes 1 & 3)	None	-2 samples (serotypes 1 & 3) -1 sample (serotypes 3 & 4)	1 sample (serotypes 3 & 4)
Dengue without warning signs	3 cases [including 1 case with dual infection by DENV-1 & DENV-3]	1 case	22 cases [including 1 case with dual infection by DENV-1 & DENV-3 and 1 case with dual infection by DENV-3 & DENV-4]	3 cases [including 1 case with dual infection by DENV-3 & DENV-4]
Dengue with warning signs	1 case with dual infection by serotypes 1 & 3	1 case	7 cases [including 1 case with dual infection by serotypes 1 & 3]	4 cases
Severe dengue	None	1 case	3 cases	None

[Table/Fig-1]: Dengue case classification for the various serotypes identified in the study.

21/43 (48.8%) patients were admitted to the hospital while the rest were managed on an outpatient basis.

Fourteen of the 21 admitted patients were infected with DENV-3. Of these, three presented with severe dengue, six with dengue with warning signs and five with dengue without warning signs.

The five DENV-3 patients without warning signs had other indications for admission, as follows:

- One patient was a primigravida at 36 weeks + 1 day period of gestation with placenta previa. She had to undergo an emergency Lower Segment Caesarean Section (LSCS) due to foetal tachycardia.
- Two patients were suffering from enteric fever in addition to dengue.
- One patient had seizure disorder with quadriparesis under evaluation with left cerebellar involvement.
- One patient was a follow-up case of Moya Moya Disease with epilepsy, developmental delay, hemiparesis, Broca's aphasia and *Klebsiella* bronchopneumonia with dengue.

Of the six DENV-3 patients with warning signs:

- Two had abdominal pain.
- Three presented with persistent vomiting.
- One patient had persistent vomiting, abdominal pain, hepatomegaly and mild pleural effusion.

Of the three DENV-3 patients with severe dengue:

- One was in decompensated shock with bilateral pleural effusion, ascites and free fluid in the pelvic cavity and had to be admitted to the PICU.
- One had anuria, thrombocytopenia and poor oral intake.
- One had bilateral pleural effusion with mild ascites and mild pericardial effusion.

One patient with dual infection with serotypes 1 and 3 was admitted, presenting with dengue with warning signs (abdominal pain, thrombocytopenia and hepatomegaly).

Four of the 21 admitted patients had DENV-4. All four presented with dengue with warning signs.

- Two had persistent vomiting and abdominal pain.
- One had increased haematocrit with thrombocytopenia.
- One had abdominal pain, thrombocytopenia, mild ascites and pleural effusion.

Two of the admitted patients had DENV-2. One presented with dengue with warning signs (abdominal pain, hepatomegaly) and the other with severe dengue (hypovolemic shock).

All the admitted patients were managed appropriately and discharged. No mortality occurred amongst these patients.

DISCUSSION

Dengue is the fastest growing mosquito-borne disease across the world today. It is the leading arboviral infection in India and is endemic in all states and union territories [2]. India witnessed its first dengue epidemic during 1963-64 in Kolkata. Since then, till date, there have been numerous outbreaks all over India. A major outbreak occurred in Delhi and adjoining areas in 1996 [8]. This was also the first major Dengue Haemorrhagic Fever (DHF) outbreak in India and was caused by DENV-2 genotype IV [9-11]. The predominant DENV serotype seen in Delhi in 1997 was DENV-1 [12]. Thereafter, DENV-2 predominated from 1997 till 2003 [13].

In the year 2003, another outbreak occurred in Delhi and all four DENV serotypes were found to be co-circulating for the first time, making it a hyperendemic state [14,15]. However, DENV-3 was reported to predominate [16,17]. DENV-2 and DENV-4 were not identified in Delhi in 2005. The outbreaks occurring in 2006 and 2010 were predominated by DENV-3 and DENV-1 respectively [18-20]. A study conducted by National Centre for Disease Control (NCDC), New Delhi during the post-monsoon period of 2012, again showed a change in the dominant serotypes: from DENV-1 (implicated in the 2010 outbreak) to re-emergence of the previously seen DENV-2 (genotype IV) and DENV-3 (genotype III) in a co-dominant pattern [21]. The 2013 dengue outbreak in Delhi was predominantly caused by DENV-2; however, DENV-1 and DENV-3 were also reported in 19% and 8% of cases, respectively [22]. All four serotypes were identified in 2015 with DENV-2 being predominant [23]. However, a change in this epidemiological trend was seen in 2016 with only one serotype being identified, i.e., DENV-3 Genotype III [24].

These epidemiological trends of Dengue virus in Delhi have been summarised in [Table/Fig-2] [2,10,11,15,18-25].

Year	Circulating strains	Remarks
1996	DENV-2 [10]	DHF/DSS Outbreak [10]
	DENV-2 Genotype-IV predominant [11]	DEN-2 genotype V from 1967 replaced by genotype IV [11]
	No information available	Cases: 10,252 Deaths: 423 [25]]
2003	Simultaneous transmission of all 4 dengue virus types demonstrated for the first time in India with no particular type predominating [15]	No information available
	All 4 serotypes co-circulating for the first time; emergence of DENV-3 Genotype III [21]]	No information available
2006	Sustained evolution of a distinct Indian lineage of DENV-3 genotype III in Delhi [18]	Worrying finding as this genotype had been implicated in several outbreaks in South-East Asia and other parts of the world [18]
	All the four dengue virus serotypes found to be co-circulating with DENV-3 being the predominant serotype [19]]	DHF/DSS outbreak. Co-infection by more than one serotype in 19% of cases [19]
2010	DENV-1 Genotype III [20]	1st time dominant co-circulation of DENV and CHIKV in equal numbers. Cases of DENV, CHIKV co-infection [20]
	No information available	Cases: 6259 Deaths: 8 [25]
2011	No information available	Cases: 1131 Deaths: 8 [25]
2012	DENV-3 Genotype III (45%) DENV-2 Genotype IV (39%) DENV-1 Genotype III (16%) [21]	Dominance shift of DENV-1 (causative agent of 2010 outbreak), toward re-emergence and co-dominant circulation of erstwhile DENV-2 (genotype IV) and DENV-3 (genotype III). Imperative for dengue epidemiology as both DENV-2 and DENV-3 had been associated with major DHF/DF outbreaks previously [21]
	No information available	Cases: 2093 Deaths: 4 [25]
2013	DENV-2 predominant (86%) DENV-1 (19%) DENV-3 (8%) [22]	Co-infection with more than one DENV serotype detected in 14% samples [22]
	No information available	Cases: 5574 Deaths: 6 [25]
2014	No information available	Cases: 995 Deaths: 3 [25]
2015	All four serotypes identified. DENV-2: 66.66%, DENV-1: 22.22%, DENV-3: 16.7%, DENV-4: 16.7% [23].	Predominance of DENV-2 serotype. Co-infection with more than one serotype observed in 22.22% samples [23].
	No information available	Cases: 15,867 Deaths: 60 [2]
2016	DENV-3 Genotype III [24]	Circulation of one single serotype of Dengue Virus: a change in the epidemiological trend [24]
	No information available	Cases: 4431 Deaths: 10 [2]
2017	No information available	Cases: 9271 Deaths: 10 [2]
2018	No information available	Cases: 7136 Deaths: 4 [2]

[Table/Fig-2]: Circulating Dengue virus strains in Delhi over the years [2,10,11,15,18-25].
DF: Dengue fever; DHF: Dengue hemorrhagic fever; DSS: Dengue shock syndrome; CHIKV-Chikungunya virus

The present study found DENV-3 to be the predominant serotype circulating in Delhi in the post-monsoon season of 2018, accounting for 74.4% of the viraemic dengue infections that were diagnosed at Dr RML Hospital. The other three serotypes were also found to be co-circulating.

While access to care, appropriate interventions, host genetic factors, and previous exposure to DENV are all known to affect the outcome of the infection, it is not entirely understood why some individuals develop more severe disease. It has been hypothesised that the four dengue serotypes differ in disease severity and clinical manifestations [5]. Studies by Chen RF et al and Vaughn DW et al have found a significant correlation between DENV-2 and greater disease severity [26,27].

While DHF can occur during infection with any of the four dengue serotypes, several prospective studies have suggested that the risk is highest with DENV-2 [28-31]. In a study conducted in Bangkok over 11 years by Fried JR et al., DENV-2 appeared to be marginally associated with more severe dengue disease as evidenced by a significant association with DHF grade I when compared to DENV-1. Ascites and larger pleural effusions were significantly associated with DENV-2 [5].

Balmaseda A et al., on the other hand, found DENV-1 to be associated with an increased incidence of plasma leakage and thrombocytopenia compared to DENV-2 in their study of dengue infections in Nicaragua from 1999-2001 and during a 2003 outbreak. However, these findings should be interpreted with caution owing to the short observation period [32].

In the present study, of the 32 cases of DENV-3, only 3 (9.4%) had severe dengue. Six (18.8%) DENV-3 patients presented with warning signs. Five patients were admitted due to their co-morbidities. On the other hand, although only three cases with DENV-2 were identified, one presented with dengue with warning signs and another had severe dengue. This limited circulation but high severity corroborates earlier experience in Delhi during previous years [Table/Fig-2]. Of the seven patients with DENV-4, four were admitted with dengue with warning signs. One patient with dual infection with serotypes 1 and 3 was admitted, presenting with dengue with warning signs. One case of dual infection with serotypes 3 and 4 was identified. He presented with mild illness and was managed on outpatient basis.

It is known that after recovering from infection by one serotype of the dengue virus (primary infection), the individual is immune to re-infection by the same serotype for life. However, cross-immunity to the other serotypes after recovery is only partial and temporary. Subsequent infections by other serotypes (secondary infection) increase the risk of developing severe dengue [33].

Restricting the analysis to secondary disease, Fried JR et al., found DENV-2 and 3 to be twice as likely to result in DHF as compared to DENV-4. The study found that, among those who presented for medical attention, dengue cases caused by DENV-2 and DENV-4 were overwhelmingly secondary infections, and there were no cases of DHF caused by primary DENV-2 and DENV-4. The authors hence suggested that DENV-1 and DENV-3 are more pathogenic without immune priming from other serotypes and that DENV-4 causes milder disease in primary DENV infections. The authors also found that the same serotype caused different disease severity in different study years. This could add weight to the hypothesis that disease severity is influenced by the sequence in which different serotypes cause infection [5]. A limitation of the present study is that the authors cannot comment whether the infections were primary or secondary as Dengue IgG ELISA was not performed.

DENV-5 has not yet been reported from India. However, since the vector Aedes niveus, and the ideal sylvatic hosts, i.e. non human primates are available in our country, it may very well be possible that a hitherto undetected sylvatic transmission cycle may be present in the forests of India. Further studies are needed to look for the presence of DENV-5 in India.

Considering the differences in the clinical picture caused by the different serotypes, knowledge of the circulating serotypes will help improve our preparedness to deal with future outbreaks effectively. The clinical picture caused by a particular serotype needs to be monitored over the years, to look for any change in clinical

presentation. Hence, regular clinical correlation of dengue cases with serotyping is important. Change in predominance of serotypes in an area in different years leads to a larger number of cases, possibly due to a larger population susceptible to that particular serotype [34]. This also highlights the need for regular serotyping. Further studies may be conducted to identify the genotypes and carry out sequencing to look for mutations or variations in the virus.

Limitation(s)

In the present study, infections could not be categorised as primary or secondary as Dengue IgG ELISA was not done. Also, genotype determination and phylogenetic analysis could not be performed due to infrastructural and financial constraints.

CONCLUSION(S)

The present study found that during 2018, DENV-3 was the predominant circulating serotype in New Delhi, India. Most of the DENV-3 infections were mild, except six patients with warning signs and three with severe dengue.

It is felt that intensive surveillance of circulating serotypes during the early stages of outbreaks can act as an effective tool in predicting and envisioning the probability of serious outcomes. This may help calibrate the health care delivery protocols for dengue patients.

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