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Co-Circulation of Dengue Serotypes in a Tertiary Care Hospital in NCR Region

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A B S T R A C T

Dengue is the most important and rapidly spreading mosquito-borne viral disease in the world. It is caused by any of the 4 serologically related virus serotypes designated as: DEN-1, DEN-2, DEN-3 and DEN-4, each causing its characteristic symptoms which range from acute febrile illness to severe manifestations (DHF and DHS). The present study focuses on the circulating serotypes of Dengue in this region. A total of 246 clinically suspected patients of Dengue fulfilling the WHO case definition were undertaken in the study. Acute phase blood samples were collected, centrifuged and tested for NS1 antigen, IgM and IgG antibody and samples positive by one of the parameters: NS1 Ag and/or IgM Ab/IgG Ab were further subjected to Real time RT-PCR. Of the 246 clinically suspected cases of Dengue, 68 (27.6%) were positive for NS1 Ag and/or IgM Ab/IgG Ab and of these, 30 (44%) samples were positive by Real time RT-PCR. All the 4 Dengue serotypes were found to co-circulate in this region of which, 22 cases (73.33%) were positive for DEN-3, 4 cases (13.33.%) were positive for DEN-1, 3 cases (10%) were positive for DEN-4 and 1 case (3.33%) was positive for DEN-2 respectively. The study highlights the co-circulation of all the 4 dengue serotypes with DEN-3 being the predominant serotype. The present investigation will not only help us to determine the evolutionary pattern of emerging Dengue virus but also assist us in forecasting dengue outbreaks and improving control measures of vectors thus helping us to prevent future outbreaks.

Introduction

In recent decades, Dengue has emerged as a notable public health problem in terms of mortality and morbidity[1,2]. An estimated 390 million dengue infections occur per year, of which 96 million manifest clinically (with any severity of disease)[3]. Dengue illnesses are caused by any of the four serologically related viruses designated as: DEN-1, DEN-2, DEN-3 and DEN-4 which follow the Human Cycle[4]. A fifth serotype DENV-5 was discovered recently in October 2013 from Bangkok which follows the sylvatic cycle. Most parts of Indian subcontinent are endemic for dengue and all the 4 serotypes have been reported from India[5]. DENV-5 has not been reported from India yet. Serotype prevalence varies between seasons and places. Infection with any 1 serotype confers lifelong immunity to

the infecting serotype but only 2 to 3 months immunity to other serotypes. Sequential infection with different serotypes is believed to induce more serious pathologies such as DHF and DSS. In recent years, co-circulation of multiple serotypes has been reported from different regions of India[6]. Co-infection with more than 1 serotype with increased disease severity was also observed in high percentage[7,8,9]. In the year 2017, Odisha[10] and Hyderabad[11] reported co-circulation of all 4 DENV serotypes in a single outbreak.

Dengue serotyping is not performed routinely; therefore, data on circulation of different serotypes in various parts of the country are limited. Thus, periodic monitoring of circulating DEN viral serotypes is essential for epidemiological purpose, patient management as each dengue serotype is associated with different symptoms and severity. Hence the present study was conducted to look into the circulating serotypes of dengue in this region.

Materials and Methods

This prospective study was conducted in the Department of Microbiology, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh, India. Institute Ethics Committee approval was obtained for the study. Patients of all age groups and both sexes having body temperature of >38.5° C for >24 hour and <10 days of illness who were clinically diagnosed as having Dengue fever fulfilling the WHO case definition from various Outpatient departments, Emergency services and IPD were included in the study during August 2017-August 2018. Febrile patients with duration of illness >10 days and cases with evidence of bacterial or other viral illness based on laboratory testing were excluded from the study. Demographic data, details of clinical history and clinical presentations were collected and recorded on a pre-structured datasheet.

Sample collection

Before taking the sample, patient was informed about the procedure and consent for the same was taken. A standard protocol was followed for venipuncture and collection of blood sample[12,13]. 5 ml of venous blood was collected under full aseptic conditions in a sterile plain vial.

NS1 antigen, IgM antibody, IgG antibody testing

All the blood samples were centrifuged at 3000 rpm for 10 minutes. Serum obtained was tested for NS1, IgM and IgG testing. Sera showing haemolysis, icterus, lipaemia or microbial growth were excluded as they may cause false positive/negative interpretation. Dengue Ag+Ab Duo Rapid Test Kit manufactured By SD Biosensor Healthcare Pvt. Ltd. which is an in-vitro ICT for the detection of Dengue NS1 antigen and IgM/IgG antibodies against Dengue virus in human serum was used.

Dengue serotyping

All seropositive samples obtained after NS1, IgM and IgG testing were used for RNA extraction using Geno Sen's® Viral RNA Extraction Mini Kit and amplification by Real time RT-PCR using Geno-Sen's® Dengue Typing 1/2/3/4 Real Time PCR Kit for Rotor Gene™ 6000 manufactured by Corbett Research.

Results and Discussion

Of the 68 seropositive samples, 36 (53%) samples were NS1 only positive. 7 (10.2%) samples were positive for both NS1 and IgM. 5 (7.3%) samples were positive for both NS1 and IgG and 2 (3%) samples were positive for all the three parameters i.e. NS1, IgM and IgG. 18 (26.4%) samples were only IgM

positive. None of the samples were IgG positive only or IgM and IgG both positive (Table 1). 30 (44%) samples were positive by Real time RT-PCR and 38 (56%) samples were negative of the total 68 seropositive samples. Of the 30 PCR positive samples, most prevalent serotype was DEN-3 in 22 patients (73.33%) followed by DEN-1 in 4 patients (13.33%), DEN-4 in 3 patients (10%) and DEN-2 in 1 patient (3.33%) respectively (Figure 1). Of the 68 seropositive cases, a maximum of 36 (53%) cases were positive for NS1 Ag and 7 (10.2%) cases were positive for NS1 Ag+IgM Ab. 5 cases (71.4%) out of these 7 NS1 Ag+IgM Ab cases were positive by Real time RT-PCR showing maximum percentage of Real time RT-PCR positivity among all serological parameters (Table 2).

In recent years, co-circulation of multiple serotypes has been reported from different parts of India[14]. Co-infection with more than one serotype has also been reported from places where multiple serotypes co-circulate[15,16] further leading to complexity in the serotypic distribution of dengue. Our study documents the co-circulation of all four serotypes with absence of concurrent infection. Study conducted by Gupta E *et al.*, (2006)[17] in the neighbouring State of Delhi reported all the four dengue serotypes to be co-circulating in the year 2003, followed by complete predominance of DEN-3 in the year 2005. However, it was observed that no sample was harbouring more than one serotype indicating absence of concurrent infection. However, a recent study by Reddy *et al.*, (2017)[18] reported the co-circulation of all four serotypes with samples harbouring more than one serotype of dengue indicating 100% concurrent infection. Another recent study by Racherla *et al.*, (2018)[19] also reported co-circulation of all the four dengue serotypes.

The first serotype of dengue infection in India was DENV-1. DENV-2 emerged as the predominant serotype from the early 1970s to 2000, during which time it was responsible for the majority of epidemics of dengue. One of the largest dengue epidemic in North India occurred in Delhi and adjoining areas in the year 1996 which was mainly due to DEN-2[20,21]. In the year 1997, DEN-1 was seen to circulate in Delhi[22]. In the year 2003 another outbreak occurred in Delhi in which all the four serotypes were found to co-circulate[23,24] thus making it a hyperendemic region. However, DEN-3 was reported to predominate in certain parts of North India in 2003[25].

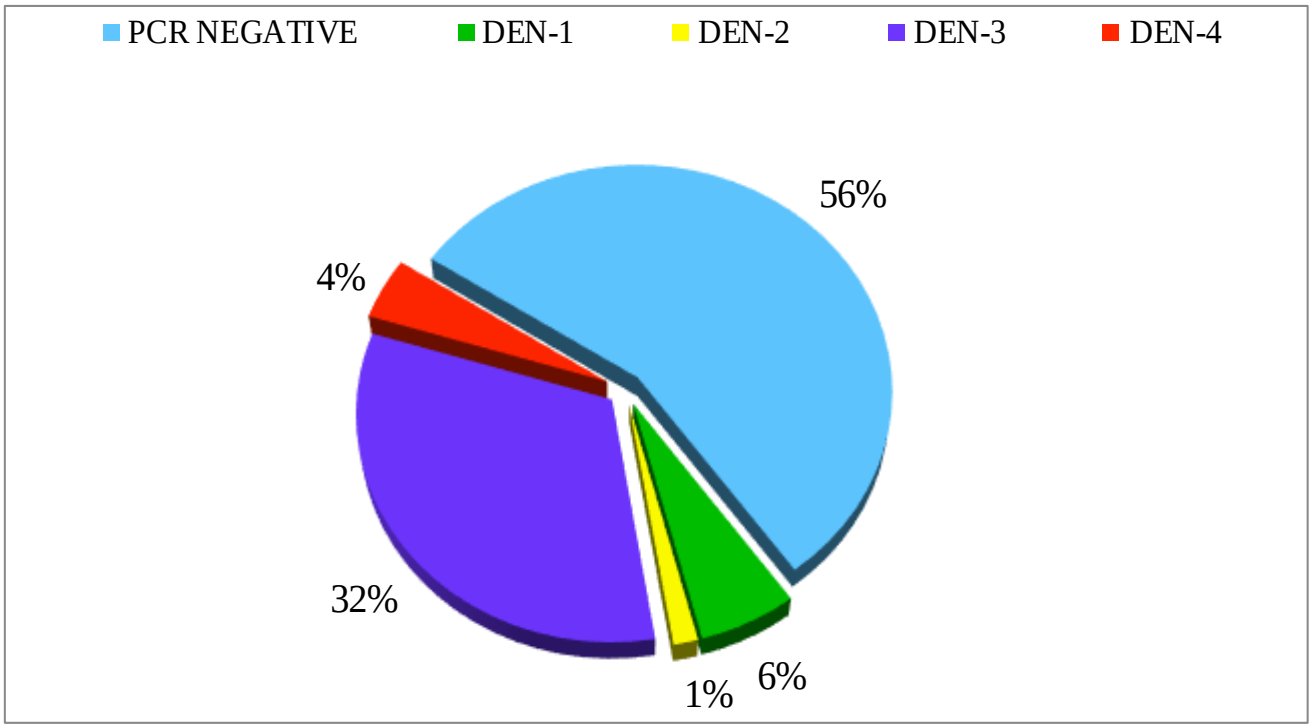
Table.1 Dengue seropositive samples

Test (n=246)	Number of Positive Samples	Percentage(%)
Only NS1 Positive	36	**53**
NS1+IgM Positive	7	10.2
NS1+IgG Positive	5	7.3
NS1+IgM+IgG Positive	2	3
Only IgM Positive	18	26.4
Total	68	27.6

Table.2 Correlation between serological parameters & real time RT-PCR

Serological Parameter	Cases Positive by Serological Tests (n=68)	Cases Positive by Real Time RT-PCR (n=30)	DEN-1	DEN-2	DEN-3	DEN-4
NS1	36	20	4	1	12	3
NS1+IgM	7	**5**	-	-	5	-
NS1+IgG	5	3	-	-	3	-
NS1+IgM+IgG	2	1	-	-	1	-
IgM	18	1	-	-	1	-
Total	68	30	4	1	22	3

Fig.1 PCR positivity and serotypes isolated



DEN-3 was also the most prevalent serotype in our study which has also been reported to be the prevalent serotype in different studies from various places all over India like the study in Lucknow (North India) by Prakash *et al.*, (2015)[26] and Kerala (South India) by Kumar *et al.*, (2013)[27]. A study in Delhi by Gupta *et al.*, (2006)[28] showed complete predominance of DEN-3 in in the year 2005. Bharaj *et al.*, (2008)[29], Dash *et al.*, (2006)[30] also reported DEN-3 to be the most prevalent serotype in their studies in the neighbouring state of Delhi. A study by Khan *et al.*, (2014)[31] from the North Eastern most state of Arunachal Pradesh also stated DEN-3 to be the most prevalent serotype in that year.

Muruganandam *et al.,* (2014)[32] in Port Blair reported DEN-3 to be the prevalent serotype. This data clearly suggests that DEN-3 has been frequently isolated as the most prevalent serotype not only from Northern or Southern India but from all the different regions of the country. A single case of DEN-2 was detected as the least common serotype (3.33%) in our study. In contrast, however in a study by Mishra *et al.,* (2014)[33] from the year 2009-2012 in Uttar Pradesh, DEN-2 was predominantly detected in 56.6% patients. Thus this study highlights the importance of continuous molecular monitoring of DENV, as the circulating serotype usually gets replaced with a new serotype which may bear the potential of causing severe outbreak.

In conclusion, this study infers that there is a circulation of multiple serotypes which suggests that this could be a hyperendemic 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. The return of DEN-3 in our study coincides with the recrudescence of dengue activity in India in the recent years, supporting the idea that increase in dengue activity may be connected with changes in predominant serotypes. Thus the present investigation will assist in designing control strategies for the epidemics. Further this molecular study will also help us to determine the evolutionary pattern of the emerging Dengue virus.

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