J Vector Borne Dis 49, June 2012, pp. 82–85

Circulation of Dengue virus-1 (DENV-1) serotype in Delhi, during 2010–11 after Dengue virus-3 (DENV-3) predominance: A single centre hospital-based study

Ekta Gupta[1], Sweta Mohan[1], Meenu Bajpai[2], Aashish Choudhary[1] & Gaurav Singh[1]

[1]*Department of Virology;* [2]*Department of Transfusion Medicine, Institute of Liver and Biliary Sciences, New Delhi, India*

ABSTRACT

Background: Delhi, a city in north India, has so far witnessed several reported outbreaks of dengue. Dengue in Delhi from being epidemic is slowly changing towards being endemic and hyper-endemic. Circulating type of the virus is also changing over the years. In the absence of an effective vaccine, dengue prevention to a major extent relies on virological surveillance, and development of effective, locally adapted control programmes. In the present study, we tried to identify the between-year non-epidemic serotype of dengue virus circulating in Delhi, during 2010–11.

Methods: Acute-phase samples were collected from the patients attending the Institute of Liver & Biliary Sciences, New Delhi, India. Dengue diagnosis was done using WHO case definitions. All the samples were subjected to Dengue NS1 Ag ELISA and modified nested RT-PCR.

Results: A total of 75 acute-phase samples were received, of which 19 (25.3%) were positive for dengue NS1 antigen. Dengue RT-PCR was positive in 14.6% (11/75) samples. All the RT-PCR isolates were of DENV-1 serotype. No case of concomitant infection with more than one serotype was observed. Median age of involvement was 23 yr (range10–86). Maximum number of cases were seen in the age group of 21–30 yr. Male to female ratio was 1.2 : 1. Maximum number of suspected dengue cases (n=79) was seen during September and October.

Conclusions: DENV-1 was circulating in Delhi in the year 2010–11 in non-epidemic period following reported predominance of DENV-3 and co-circulation of all dengue serotypes in the epidemic years 2003, 2006 and 2007.

Key words Dengue; endemic; epidemic; non-epidemic

INTRODUCTION

Dengue viruses belong to the genus *Flavivirus* within the *Flaviviridae* family. Dengue is the most important arboviral infection with four serotypes (DENV 1–4) that are capable of producing disease ranging from self-limiting dengue fever (DF) to severe life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)[1]. Each serotype has unique characteristics and can present with severe manifestations, in a particular population depending upon its interaction with the host response[2]. Epidemiology of dengue is ever changing in India. All the four serotypes have been reported from India. Delhi, a city in north India, has so far witnessed several reported outbreaks of dengue[3]. In the year 1996, DENV-2 was responsible for the massive epidemic followed by total absence for almost a decade[4]. Following this, DENV-1 was reported in the post-epidemic phase[5]. Co-circulation of all the four dengue serotypes with predominance of DENV-3 was seen in outbreaks reported in 2003, 2005 and 2006[6–8]. In the years 2004 and 2008, DENV-1 was isolated in the post-epidemic phase[9].

In the absence of an effective vaccine, dengue prevention to a major extent relies on virological surveillance, and development of effective, locally adapted control programmes. Hence, continuous monitoring of circulating type of dengue virus is important in a given population to develop such efficient local control programmes. Most studies highlight the circulating serotypes during epidemics, however, between epidemics, there can be significant variation for which public health systems are normally unprepared. In the present study, we tried to identify the between-year non-epidemic serotype of dengue virus circulating in Delhi in the year 2010–11.

MATERIAL & METHODS

Acute-phase samples (within 5 days of fever) were collected from clinically suspected patients of dengue fever presenting to the outpatient departments, emergency services and indoor services of our Institute from January 2010 to May 2011. The samples were collected aseptically by venipuncture and centrifuged at 2000 rpm for 10 min. Plasma samples were stored at –80°C till further test-

ing. Clinical criteria for diagnosing dengue was based on WHO case definitions[10]. All the samples were subjected to Dengue NS1 Ag ELISA (PANBIO, Brisbane, Australia) and modified nested single tube reverse transcriptase polymerase chain reaction (RT-PCR).

Viral RNA extraction

Dengue viral RNA was extracted from the patients' plasma, using QIAamp Viral RNA Mini Kit (Qiagen, Germany) as per the manufacturer's protocol. The extracted RNA was eluted in 60 µl of elution buffer.

cDNA synthesis and amplification

The extracted viral RNA was reverse transcribed to cDNA using transcriptor first strand cDNA synthesis kit (Roche, Germany), and then amplified in the external round of RT-PCR using dengue virus consensus primers (D1: 5' TCAATATGCTGAAACGCGCGAGAAACC G 3'; D2: 5' TTGCACCAACAGTCAATGTCT TCA GGT TC 3') in 25 µl reaction volume. The DNA product obtained was of 511 bp.

Semi-nested PCR (Internal round)

Subsequent amplification of cDNA was carried out with the dengue virus consensus forward primer (D1) and four dengue serotype-specific reverse primers (TS 1: 5' CGTCTCAGTGATCCGGGGA 3'; TS 2: 5' CGCCACAAGGGCCATGAACAG 3'; TS 3: 5' TAACATCATCATGAGACAGAGC 3'; TS 4: 5'CTCTGTTGTCTTAAACAAGAGA 3') as described by Lanciotti *et al*[11], but all the 4 serotype-specific primers were added in a single reaction mixture. A 1 : 20 dilution of the external PCR product was used in the nested PCR reaction. Dengue virus serotypes were identified by the size of the resulting DNA bands (DENV 1–482 bp, DENV 2–119 bp, DENV 3–290 bp, DENV 4–392 bp).

RESULTS

A total of 75 acute-phase samples were received, of which 19 (25.3%) were positive for dengue NS1 antigen. Dengue RT-PCR was positive in 14.6% (11/75) samples. All the RT-PCR isolates were of DENV-1 serotype. No case of concomitant infection with more than one serotype was observed (Fig. 1). In cases where NS1 antigen was positive but RT-PCR was negative, duration of fever was 5 days, as by this time viremia declines and only NS1 antigen persists till IgM antibodies are formed.

The median age of 11 confirmed dengue cases was 23 yr (range :10–86). Maximum number of cases was seen in the age group 21–30 yr (Fig. 2). Male to female ratio was 1.2 : 1. Maximum number of suspected dengue cases (n=79) were seen in the months of September and October (Fig. 3). All the confirmed cases were followed up and in all complete clinical recovery was seen.

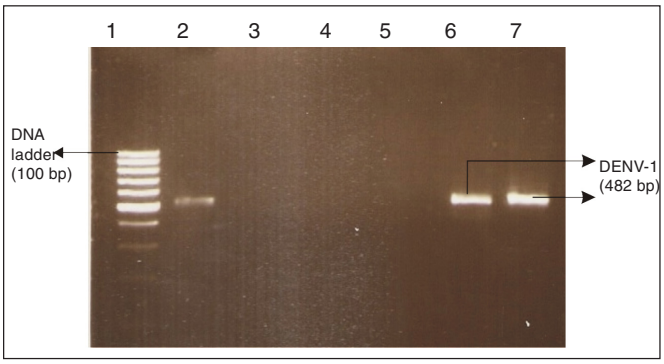

Fig. 1: A gel documentation image of nested RT-PCR. Lane 1: Molecular weight markers (100 bp); Lanes 2, 6, 7: Positive samples; Lanes 3, 4: Negative samples; and Lane 5: Negative control (water).

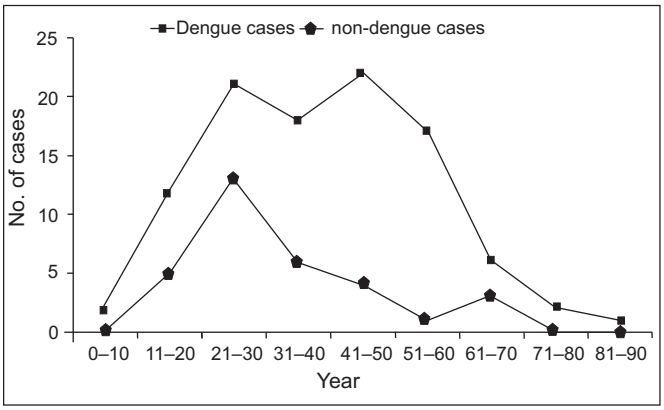

Fig. 2: Age-wise distribution of dengue and non-dengue cases. Maximum number of dengue cases seen in the age group of 21–30 yr.

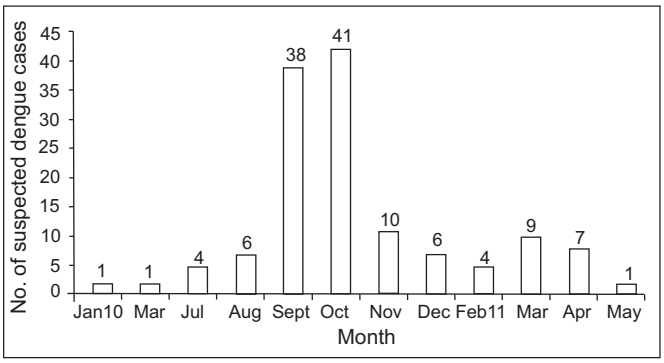

Fig. 3: Month-wise distribution of cases. Maximum number of dengue cases seen during the months of September and October.

DISCUSSION

Dengue virus infection is a major, growing public health problem with an estimated 2.5 billion people at risk

of infection. Dengue viruses cause a range of well-described clinical illnesses. Infection ranges from an asymptomatic infection to a self-limiting febrile illness, DF, to severe dengue, a clinical syndrome that typically presents with capillary permeability and can lead to DSS and DHF.

Dengue epidemics can have a significant economic and health toll in any country. Globally dengue transmission has expanded in recent years and all the four dengue virus serotypes (DENV 1–4) are now circulating in Asia, Africa and the Americas, a dramatically different scenario from that which prevailed 20 or 30 years ago[12]. Development of effective surveillance and disease prevention programmes for any disease depend a lot on the accounting for variation in the epidemiology.

In north India, epidemiology of dengue is changing fast and from epidemic pattern it has now become endemic in most of the cities. Incidence of fatal DHF and DSS cases, which are medical emergencies, are also on the rise[13]. History of dengue epidemics in Delhi and circulating serotype is interesting[14]. Both circulating as well as predominant strain of the virus kept changing as depicted in Table 1. In 1967, major outbreak was reported by DENV-2[15,16] after a gap of almost 3 decades major hemorrhagic outbreak by DENV-2 occurred. Since then, apart from brief occurrence of DENV-1 in 1997, DENV-2 dominated the circulation in Delhi. This was replaced by co-circulation of all types in 2003 followed by complete predominance of DENV-3. Co-circulation of all the serotypes and concurrent infection with multiple serotypes was reported, which clearly indicates endemicity of dengue in this region[3,17]. Increasing frequency of outbreaks with co-circulation of all serotypes also hint at hyperendemicity of dengue in Delhi. This may be attributed to several factors like rapidly growing population, increasing urbanization and lack of effective vaccine and vector control programmes. Continuous monitoring of dengue infection and its trend is essential in any endemic area to prevent further epidemic transmission of this virus and also it will help in the development of local control programmes[18]. However, most of the studies focus on epidemics and very few studies have been done to find out the circulating type of dengue virus in the non-epidemic period.

Table 1. Circulation of various dengue virus serotypes in Delhi

Year	DENV serotype	Epidemic/ non-epidemic	References
1967	2	Epidemic	Balaya *et al*[15], Singh *et al*[16]
1996	2	Epidemic	Dar *et al*[4]
1997	1	Non-epidemic	Vajpayee *et al*[5]
2003	1,2,3,4	Epidemic	Gupta *et al*[7]
2004	1	Non-epidemic	Gupta *et al*[3]
2005	3	Epidemic	Gupta *et al*[3]
2006	3	Epidemic	Bharaj *et al*[8]
2008	1	Non-epidemic	Chakravarti *et al*[9]
2010	1	Non-epidemic	Present study

In the present study, we found the circulation of DENV-1 type in Delhi during the non-epidemic period of 2010–11 after complete predominance of DENV-3 in earlier reported outbreaks[3,7,8]. Epidemic causing aggressive and more pathogenic strain is always replaced by milder strain of the virus during the non-epidemic period. Earlier similar pattern was observed following massive outbreak by DENV-2 in Delhi in the year 1996 following which DENV-1 strain predominated the circulation in the non-epidemic period[5]. Clinical significance of the findings are that most of the people living in Delhi are probably immune to DENV-3 and DENV-1 but chances of an outbreak by less prevalent serotypes are still there. Subsequent infection with different serotype of the virus leads to more severe clinical disease due to antibody enhancement phenomenon. Mosquito control programme needs to be more active to prevent any future outbreak.

Our study also shows that in the non-epidemic period usually one strain circulates and co-circulation of multiple types with concurrent infection with more than one serotype is not seen. Another finding in our study was the shifting trend of the age group being mostly affected. Dengue affects all the age groups but certain age groups may be more prone to being affected in different parts of the world. It may be regarded as a disease mainly affecting children (<10 yr of age) or it may be characterized as an adult-onset disease. In the present study and in other well-documented studies from north India, it has been found that the age-group 21–30 yr, is the most affected[6]. Whereas contemporary studies from south India found children to be more vulnerable than adults, to dengue infection[19]. With household mosquito elimination programmes on an overdrive, dengue-causing *Aedes* mosquitoes seemingly infect the young employed population which is mostly outdoors. Our finding further corroborates that dengue infection in Delhi more commonly affects the age group 21–30 yr. Maximum cases were seen during the months of September–October in concordance with various other studies and similar to that is seen during epidemic period. This is due to post-monsoon collection of water and increased availability of breeding sites for the *Aedes* mosquito.

CONCLUSION

The problem of dengue is huge in our country and needs proper implementation of control measures before

any further huge epidemic occurs. All of us have developed immunity to DENV-3 but DENV-1 serotype is replacing DENV-3 in Delhi which is new to the population and may take a form of epidemic strain. Adequate and timely preventive measures should be taken to prevent any future epidemic. Such issues may be of interest to epidemiologists and virologists for planning effective control of the virus or future vaccine development. Future endeavours should be made to develop improved laboratory-based surveillance systems that can forecast impending dengue epidemics.

REFERENCES

1. Gubler DJ. Dengue and dengue haemorrhagic fever. *Clin Microbiol Rev* 1998;*11:* 480–96.
2. Guzman MG, Kourf G. Dengue: An update. *The Lancet Infect Dis* 2002, *2:* 33–42.
3. Gupta E, Dar L, Kapoor G, Broor S. The changing epidemiology of dengue in Delhi, India. *Virol J* 2006; *3:* 1–5.
4. Dar L, Broor S, Sengupta S, Xess I, Seth P. The first major outbreak of dengue haemorrhagic fever in Delhi. *Emerg Infect Dis* 1999; *5:* 589–90.
5. Vajpayee M, Mohankumar K, Wali JP, Dar L, Seth P, Broor S. Dengue virus infection during post-epidemic period in Delhi, India. *Southeast Asian J Trop Med Public Health* 1999; *30:* 507–10.
6. Gupta E, Dar L, Narang P, Srivastava VK, Broor S. Serodiagnosis of dengue during an outbreak at a tertiary care hospital in Delhi. *Indian J Med Res* 2005; *121:* 36–8.
7. Dar L, Gupta E, Narang P, Broor S. Co-circulation of Dengue serotypes, Delhi, India. *Emerg Infect Dis* 2006; *12:* 352–3.
8. Bharaj P, Chahar HS, Pandey A, Diddi K, Dar L, Guleria R, Kabra SK, Broor S. Concurrent infections by all four dengue virus serotypes during an outbreak in 2006, Delhi, India. *Virol J* 2008; *9:* 5–10.
9. Chakravarti A, Kumar A, Matlani M. Displacement of dengue virus types 3 and 2 by type 1 in Delhi during 2008. *Indian J Med Microbiol* 2010; *28:* 412.
10. *Dengue hemorrhagic fever: diagnosis, treatment and control*. II edn. Geneva: World Health Organization 1997. Available from : *http://www.who.int/csr/resources/publications/dengue/012-23.pdf.* [Accessed on November 26, 2011].
11. Lanciotti RS, Calisher CH, Gubler DJ,Chang GJ, Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *J Clin Microbiol* 1992; *30:* 545–51.
12. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, *et al*. Dengue: a continuing global threat. *Nature Rev Microbiol* 2010; *8:* S7–S16.
13. Dash PK, Parida MM, Saxena P, Abhyankar A, Singh CP, Tewari KN, *et al*. Re-emergence of dengue virus type 3 (subtype III) in India: Implications for increased incidences of DHF, DSS. *Virol J* 2006; *3:* 1–10.
14. Chaturvedi UC, Nagar R. Dengue and dengue haemorrhagic fever: Indian perspective. *J Biosci* 2008; *33:* 429–41.
15. Balaya S, Paul SD, D'Lima LV, Pavri KM. Investigations on an outbreak of dengue in Delhi in 1967. *Indian J Med Res* 1969; *57:* 767–74.
16. Singh UB, Maitra A, Broor S, Rai A, Pasha ST, Seth P. Partial nucleotide sequencing and molecular evolution of epidemic causing Dengue 2 strains. *J Infect Dis* 1999; *180:* 959–65.
17. Gupta E, Dar L, Broor S. Concurrent infection by two dengue virus serotypes among dengue patients. *Indian J Med Microbiol* 2008; *26:* 402–3.
18. Gore MM. Need for constant monitoring of dengue infections. *Indian J Med Res* 2005; *121:* 9–12.
19. Gunasekaran P, Kaveri K, Mohana S, Arunagiri K, Babu BV, Priya PP, *et al*. Dengue disease status in Chennai (2006–08): A retrospective analysis. *Indian J Med Res* 2011; *133:* 322–5.

Correspondence to: Dr Ekta Gupta, Department of Virology, Institute of Liver and Biliary Sciences (ILBS), D-1, Vasant Kunj, New Delhi–110 070, India.
E-mail: ektagaurisha@gmail.com

Received: 24 January 2012 *Accepted in revised form:* 17 May 2012