DOI: 10.7860/JCDR/2019/37607.12462

Original Article

Microbiology Section

Dengue Fever Outbreak in Remote Tribal Village of Central India

DEVJYOTI MAJUMDAR[1], RANI SONI[2], DEBASHISH SAMAL[3], SATYARAM SATAPATHY[4], JAGDISH MAHANTA[5]

ABSTRACT

Introduction: Dengue fever is known to cause outbreaks in urban and semi urban dwellings and rarely in rural areas. The present study investigates an outbreak in remote tribal village of Chhattisgarh in 2015. Dengue virus is found in circulation of the infected patient in the first four days of fever and headache on day fifth after which dengue virus is not detectable in blood. Therefore, we analysed virus isolation and serotyping in serum of patients who presented with 1-4 days of fever. Since dengue IgM begin to appear after four days of symptoms, these samples were not included for RT-PCR.

Aim: Isolation and identification of dengue virus serotype from the patients in a dengue fever outbreak in remote tribal village of central India and also to find out the correlation of dengue symptoms with serotype of dengue virus.

Materials and Methods: This outbreak affected a population of 7038. Serum samples of clinically suspected patients were tested by dengue NS1 and dengue IgM capture ELISA. Dengue NS1 positive samples were serotyped by reverse transcriptase real time PCR.

Results: Out of 746 samples 167 were dengue NS1 positive and 171 were Dengue IgM positive. Twenty six samples were DENV 2 positive, 77 samples were DENV 3 positive and 36 samples were positive for both DENV 2 and DENV 3. There was no correlation between serotype of dengue and severity of symptoms.

Conclusion: Surveillance activities and serotyping by RT-PCR of dengue is necessary in the rainy season for prevention and control of dengue in future outbreaks.

Keywords: Flaviviridae, Haemorrhage, Serotype

INTRODUCTION

Dengue infection is caused by ssRNA positive sense virus of flaviravidae family. According to world health organization 390 million Dengue infections occur per year, of which 96 million manifest clinically as high fever, Dengue Haemorrhagic Fever (DHF) or Dengue shock syndrome [1]. Changing epidemiology shows that hyperendimicity with multiple serotypes infection is becoming more common [2]. Dengue is now prevalent around the globe and has spread into new areas, although it has been mostly reported from urban areas. As the vector *Aedes aegypti* is predominantly an urban vector, Dengue was more confined to urban areas. However, in recent years few outbreaks have been reported from rural areas [3-7]. One such outbreak of Dengue was in August 2015 in a remote tribal village of Konta, Bastar district in state of Chhattisgarh. Konta is a remote village with a population of about 7038 individuals directly inaccessible by road and mainly inhabited by tribal population of central India. (Latitude: 17° 47' 59.99" N Longitude: 81° 22' 59.99" E). The village is bordered by Andhra Pradesh and Orissa. It was conspicuous by absence of death and severe Dengue. DENV type 2 and DENV 3 predominantly were demonstrated from clinical cases using real time PCR. The aim of the present study was to isolate and identify dengue virus serotype from the patients living in remote tribal village of central India.

MATERIALS AND METHODS

The present study was a retrospective cohort study done from 13th August to 29th August 2015. The study was conducted at VRDL (Virology Research and Diagnostic Laboratory project established by department of health research, government of India), attached to the Department of Microbiology, Lt Baliram Kashyap Memorial Govt Medical College. Seven hundred forty six serum samples from symptomatic patients were received from Konta. Their symptoms include acute onset of high fever, headache and body ache. Case definition included these symptoms, with or without rash, gum bleeding, melena, positive tourniquet test and low blood pressure. Since Konta is endemic for malaria, it was excluded by rapid antigen test and microscopy in all cases. Serum samples of clinically suspected Dengue patients were transported to our laboratory in Jagdalpur in cold chain (ice packs). Serum samples of patients with <5 days symptoms (high fever, headache, body ache with or without rashes) were tested for NS1 Dengue antigen (PanBio) with ELISA and those of >4 days of symptoms with Dengue IgM ELISA (National institute of virology, Pune). NS1 positive samples were subjected to RT-PCR (Applied bioscience 7500 fast, USA). Quality assurance was done at National Institute of Virology, Pune. Samples which were not amplified were sent to NIV Pune for further virological study. The results were compiled in excel sheet and analysed by epiInfo CDC 7.2.

Inclusion criteria: Samples from patients with less than five days of fever history.

Exclusion criteria: Samples from patients with equal to or more than 5 days of fever. Fifty of the randomly selected Dengue NS1 negative and Dengue IgM capture ELISA negative were included as negative control in RT-PCR. No permission of the ethical committee was sought as it was a part of diagnostic service.

NS1 ELISA: Dengue NS1 was performed by PanBio dengue early ELISA. As per manufacturer's instructions, briefly 100 µL diluted test samples, controls and calibrators were pipetted into their respective micro wells then covered by Aluminum foil and incubated for 1 hour at 37°C. After incubation the wells were washed with diluted wash buffer, then each well was pipetted with 100 µL HRP conjugated Anti-NS1 MAb. The wells were incubated for 1 hour at 37°C. The wells were washed with dilute wash buffer. A 100 µL of TMB was added into each well, incubated for 10 minutes at room temperature.

After stopping the test with 100 µL of stop solution, colour was read by Erba automated Elisa reader, LisaScan™ within 30 minutes at a wavelength of 450 nm with a reference filter of 600-650 nm.

IgM ELISA: Dengue IgM Capture ELISA was done on samples of patients with history of fever for more than four days. For IgM capture ELISA, kit manufacturer's by National Institute of Virology, Pune was used. Briefly, 50 µL diluted test samples and controls were pipetted into their respective micro wells then covered by Aluminum foil and incubated for 1 hour at 37°C. After incubation the wells were washed with diluted wash buffer, then 50 µL Ag (Antigen) were added. The wells were incubated for 1 hour at 37°C. Then, 50 µL monoclonal Abs (Antibody) was added. The wells were incubated for 1 hour at 37°C. Then each well was pipetted with 50 µL HRP conjugate. The wells were incubated for 30 minutes at 37°C. The wells were washed with dilute wash buffer. A 100 µL of TMB was added into each well, incubated for 10 minutes at room temperature.

After stopping the test with 100 µL of stop solution, colour was read by Erba automated Elisa reader, Lisa Scan^{em} within 30 minutes at a wavelength of 450 nm with a reference filter of 600-650 nm.

RNA extraction: RNA extraction was done using Invitrogen Pure link viral RNA/DNA mini kit according to manufacturer instruction.

For analysing Dengue serotypes we extracted RNA from NS1 positive serum samples. Briefly, in an eppendorf tube 200 µL of lysis buffer taken, 200 µL of serum sample and 25 µL of proteinase K were added, vortexed for 15 seconds then incubated at 56°C for 15 minutes. Briefly, Centrifugation was done to remove any drops from inside the lid and 250 µL 96-100% ethanol was added to the lysate, vortexed for 15 seconds and incubation at room temperature for 5 minutes was done. Lysate was centrifuged at 6800x g for 1 minute in spin column. A 500 µL wash buffer was added with ethanol to spin the column. The spin column was centrifuged at 6800x g for 1 minute. The flow through was discarded and the process was repeated. It was centrifuged at maximum speed for 1 minute to dry the membrane completely. The dried column was transferred to a 1.5 mL recovery tube. A 50 µL of RNase free water was poured to the center of the column and the lid was closed and incubated for 1 minute then centrifuged at maximum speed for 1 minute. The spin column was discarded and the recovery tube contained extracted viral nucleic acids. Storage of the viral RNA was done at -20°C for desired downstream applications.

Real time polymerase chain reaction (RT-PCR): RT-PCR was done by using Applied Bioscience 7500 fast equipment. Reagents for preparation of master mix was provided by CDC Atlanta, USA. First master mix was prepared at 0°C on ice tray.

2.2 µLNuclease free water, 12.5 µL 2X premix, 0.5 µL Primer D1-F, 0.5 µL Primer D1-R, 0.25 µL Primer D2-F, 0.25 µL Primer D2-R, 0.5 µL Primer D3-F, 0.5 µL Primer D3-R, 0.25 µL Primer D4-F, 0.25 µL Primer D4-R, 0.45 µL Probes (DENV-1-4), 0.5 µL SuperScript TM III RT/Platinum®Taq Mix for each test. Twenty µL of master mix is taken in each micro well. There after 5 µL of extracted RNA is added in to the respective micro well. Thermocycling is done by setting the temperature and timing are set at 50°C for 30 minutes once stage one, and thereafter at 95°C for 2.0 minutes once stage two and then at 95°C for 15 seconds and, 60.0°C for 1 minutes stage three than set stage three for 45 cycles.

RESULTS

Between 13th August-29th August 2015, 746 serum samples from symptomatic patients were received from Konta, 432 samples from male and 314 samples from female patients. One hundred and sixty seven cases were positive for NS1 antigen and 171 were positive for Dengue IgM. Dengue NS1 positive samples were tested for viral RNA by RT-PCR, because viral RNA is found only in acute phase (Less than 5 days of symptoms) and dengue IgM positive samples were not tested because virus titer falls after the acute phase. Out of the 167 NS1 samples that were tested for serotype by RT PCR, 26 patients were DENV 2 positive, 77 patients were DENV 3 positive, and one patient was DENV 4 positive [Table/Fig-1]. Dual infection with DENV 1 and DENV 2 was seen in one patient, with DENV 2 and DENV 3 in 36 patients. In 26 samples DENV was not amplified [Table/Fig-2]. There was no severe manifestation such as bleeding from gum, melena or shock. There were only three patients who presented with mild rashes. There was no death. Fifty randomly selected Dengue NS1 negative and Dengue IgM negative samples were did not yield any amplification of Dengue virus. The age range of the patients who were positive either for Dengue NS1 or Dengue IgM was 1.5 years to 82 years (standard deviation 9.61 and 7.01 respectively). Overall attack rate was 4.80%.

Serotype	Primer	Quantity	Probe
DENV 1	D1 F	0.5 µL	0.45 µL
	D1-R	0.5 µL	
DENV 2	D2-F	0.25 µL	0.45 µL
	D2-R	0.25 µL	
DENV 3	D3-F	0.5 µL	0.45 µL
	D3-R	0.5 µL	
DENV 4	D4-F	0.25 µL	0.45 µL
	D4-R	0.25 µL	

[Table/Fig-1]: Primers and quantities for different serotypes of dengue in RT-PCR.

Serotypes	Result (%)
DENV 1+DENV 2	1/167=0.59%
DENV 2	26/167=15.56%
DENV 3	77/167=46.10%
DENV 4	1/167=0.59%
DENV 2+DENV 3	36/167=21.55%
Not amplified	26/167=15.56%

[Table/Fig-2]: Different serotype of Dengue amplified by RT-PCR of 167 Dengue NS1 positive patients.

DISCUSSION

Dengue and severe dengue (Dengue haemorrhagic fever and Dengue shock syndrome) are most important arboviral disease in Asia and other parts of the world and 2.5 billion people in more than 100 endemic countries are at risk of acquiring Dengue viral infection [8]. Dengue is spreading to new areas due to various factors such as increasing urbanization, international travels, global warming, etc. However, recently dengue is being reported in rural areas and sometimes in epidemic proportions. The mosquito vectors of dengue i.e., *Aedes aegypti* and *Aedes albopictus* are both responsible for this changing epidemiological scenario. In most epidemics it has been found that 1-5% cases of severe dengue are associated with deaths [9,10]. In the current study out of 746 samples collected, 408 samples were Dengue NS1 as well as Dengue IgM negative. Clinically suspected cases were confirmed in the laboratory by serological test i.e., Dengue NS1 ELISA in those samples from patients with history of 1-4 days of fever and by IgM ELISA in samples from patients with history of 5 or more days of fever. In none of the laboratory confirmed cases there were any haemorrhagic manifestation such as positive tourniquet, test bleeding gum, melena, shock or low platelet count. In remote locations of Chhattisgarh no dengue fever had been reported earlier to August 2015. We suspected dengue to be newly introduced in Konta. Hence, we decided to serotype the isolate so as to test the hypothesis of occurring severe dengue in suspected probable dengue outbreak with heterologous serotypes in subsequent years [11,12]. In this study we found dengue RNA in 141 of 167 dengue NS1 positive samples (84.43%). In 26 samples no RNA was detected. Out of the 141 samples where DENV RNAs

were detected in 36 (21.55%) both DENV 2 and DENV 3 dual infection was detected. In a similar study by Requena-Castro R et al., 21.5% samples were found to be duel infection with DENV 1 and DENV 2 [13]. In the study of Vinod Kumar CS et al., in August 2013 found that 18 of the 42 samples were due to concurrent infection with two DENV serotypes [Table/Fig-3] [14]. No virus was detected in 26 (15.5%) Dengue NS1 positive samples. In the current study although multiple serotypes of dengue were found in most samples there was no statistically significant difference in clinical manifestations in the outbreak. In the Konta outbreak interestingly, there was no severe dengue or death, probably because it was for the first time such an outbreak has occurred in that area. No dengue like case has been reported in the two subsequent years in the same area probably because effective mosquito control program initiated by the State Infectious Disease Control Program workers.

	Mixed DENV Serotypes	Percent	Total dengue positive samples	Method
Current study	DENV 2 and DENV 3	21.55%	141	NS1 ELISA, RT-PCR
Requena-Castro R [13]	DENV 1 and DENV 2	21.5%	71	NS1 ELISA RT-PCR,
VinodKumar CS [14]	DENV 2 and DENV 3	42%	42	RT-PCR

[Table/Fig-3]: Mixed DENV serotype infection in different studies [13,14].

LIMITATION

The main limitation of this study was that all the dengue NS1 positive samples could not be amplified by RT-PCR. Out of 167 NS1 positive samples only in 141 we could amplify the viral RNA and 26 were not amplified (15%). This was probably due to error in recording duration of fever, because if the duration of fever was more than four days virus cannot be detected in samples. It will be useful in control of dengue if sequencing facility and creation of a data base is available for future reference.

CONCLUSION

This study reveals that large scale outbreaks of dengue fever can occur even in non urban settings and in such outbreak there may be cases due to simultaneous infection by multiple dengue serotypes. Active surveillance and regular serotyping by public health workers of this region is very important, particularly in rainy seasons to prevent dengue and severe dengue in this area.

REFERENCES

[1] http://www.who.int/mediacentre/factsheets/fs117/en/
[2] Villabona-Arenas CJ, de Oliveira JL, Capra Cde S, Balarini K, Loureiro M, Fonseca CR. Detection of four dengue serotypes suggests rise in hyperendemicity in urban centers of Brazil. PLoS Negl Trop Dis. 2014;8(2):e2620.
[3] Kumar A, Sharma SK, Padbidri VS, Thakare JP, Jain DC, Datta KK. An outbreak of Dengue fever in rural areas of northern India. The Journal of Communicable Diseases. 2001;33(4):274-81.
[4] Mehendale SM, Risbud AR, Rao JA, Banerjee K. Outbreak of Dengue fever in rural areas of Parbhani district of Maharashtra (India). Indian J Med Res. 1991;93:6-11.
[5] Gupta P, Kumar P, Aggarwal OP. Knowledge, attitude and practices related to Dengue in rural and slum areas of Delhi after the Dengue epidemic of 1996. J Commun Dis. 1998;30(2):107-12.
[6] Tewari SC, Thenmozhi V, Katholi CR, Manavalan R, Munirathinam A, Gajanana A. Dengue vector prevalence and virus infection in a rural area in south India. Trop Med Int Health. 2004;9(4):499-507.
[7] Paramasivan R, Thenmozhi V, Hiriyan J, Dhananjeyan K, Tyagi B, Dash AP. Serological & entomological investigations of an outbreak of Dengue fever in certain rural areas of Kanyakumari district, Tamil Nadu. Indian J Med Res. 2006;123(5):697-701.
[8] Who fact sheet. Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic Fever. Publication details Editors: WHO Number of pages: 196 Publication date: 2011 Languages: English ISBN.
[9] Rajapakse S. Dengue shock. J Emerg Trauma Shock. 2011;4(1):120-27.
[10] Ong A, Sandar M, Chen MI, Sin LY. Fatal Dengue hemorrhagic fever in adults during a Dengue epidemic in Singapore. Int J Infect Dis. 2007;11(3):263-27.
[11] Halstead SB. Observations related to pathogenesis of Dengue hemorrhagic fever. VI. Hypotheses and discussion. Yale J Biol Med. 1970;42:350-62.
[12] Gubler DJ. Dengue and Dengue hemorrhagic fever. Clin Microbiol Rev. 1998;11(3):480-96.
[13] Requena-Castro R, Reyes-López MÁ, Rodríguez-Reyna RE, Palma-Nicolás P, Bocanegra-García V. Molecular detection of mixed infections with multiple Dengue virus serotypes in suspected Dengue samples in Tamaulipas, Mexico. Mem Inst Oswaldo Cruz. 2017;112(7):520-22.
[14] Vinodkumar CS, Kalapannavar NK, Basavarajappa KG, Sanjay D, Gowli C, Nadig NG,. Episode of coexisting infections with multiple Dengue virus serotypes in central Karnataka, India. J Infect Public Health. 2013;6(4):302-06.

PARTICULARS OF CONTRIBUTORS:
1. Professor, Department of Microbiology, Lt. BRKM GMC, Jagdalpur, Chhattisgarh, India.
2. Research Scientist, Department of Microbiology, Lt. BRKM GMC, Jagdalpur, Chhattisgarh, India.
3. Demonstrator, Department of Microbiology, Lt. BRKM GMC, Jagdalpur, Chhattisgrah, India.
4. Demonstrator, Department of Microbiology, Lt. BRKM GMC, Jagdalpur, Chhattisgarh, India.
5. Distinguished Scientist, RMRC Lahowal, Dibrugarh, Assam, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Devjyoti Majumdar,
Lt. BRKM GMC, Jagdalpur-494001, Chhattisgarh, India.
E-mail: dmsmims@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jun 22, 2018**
Date of Peer Review: **Aug 04, 2018**
Date of Acceptance: **Nov 09, 2018**
Date of Publishing: **Jan 01, 2019**