International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Molecular Serotyping of Dengue Viruses in a Tertiary Care Hospital of Western Odisha

Sudipta Kumar Ram

Abstract: ***Molecular serotyping of dengue viruses in a tertiary care hospital of western Odisha Introduction- Dengue is one of the most important mosquito- borne viral infections of public health concern. It belongs to the family flaviviridae and have five antigenically related serotypes designated as DEN-1, DEN-2, DEN-3, DEN-4 and DEN-5. All the five serotypes can cause clinical manifestation ranging from mild self limiting dengue to dengue heamorragic fever (DHF) to dengue shock syndrome (DSS). Material and methods- A total of 355 samples are received from clinically suspected dengue patients during July 2021 to Dec 2021.All the 355 sample were subjected to dengue specific NS1 antigen detection by ELISA(Erbalisa Dengue NS1 Ag).31 seropositive sample on NS1 AG subjected for multiplex real time PCR employed for diagnosis to identify the different serotypes. Result- Of the 355 samples,31 were positive for NS1 antigen ELISA .7 samples were found to be infected with DEN-2 serotype, DEN-1 serotype was detected in 4 samples. All the RT- PCR positive samples were also positive for dengue NS1 antigen. The age of the dengue case was in the range between 15-50 years of age and male outnumbered the female patient. Discussion- Dengue infection presents with nonspecific fever that mimics other viral illness. The availability of commercial ELISA assays to detect the DENV NS1 protein in acute plasma provide an additional diagnostic tool to existing approaches of multiplex real-time RT- PCR. It is important to establish the diagnosis of acute dengue virus infection during the first few days after clinical manifestation. Conclusion –The present study comprehensively established the utility of NS1, MULTIPLEX real-time PCR is rapid diagnostic test for dengue patients. DEN-2 was the predominant serotype in severe case. Continued surveillance of serotype should be carried out in endemic area.***

Keywords: Dengue, Dengue Heamorrhagic Fever, Dengue Shock Syndrome, NS1 AG, IGM MAC ELISA, DEN-1, DEN-2,DEN-3, DEN-4, Multiplex Real Time PCR

1. Introduction

Dengue (DEN) is the most important arboviral disease.[1] Every year, about 100 million infections are detected across the world, [1] of which 500,000 manifest in haemorrhagic cases and 22,000 deaths are attributed to DEN.[2] The disease is reported from more than 100 countries, and is prevalent in tropical and subtropical regions. Among World Health Organization (WHO) regions, South-East Asia, the Americas and Western Pacific regions are the most seriously affected,[3] and 75% of the population is at the risk of exposure, thus the situation in the South-East Asian region is alarming.

The infecting DENV serotype and genotype are shown to have an impact on the clinical picture and outcome.[2] DENV serotypes and genotypes are also related to their outbreak, causing potentials of varying severity.[9] Further, the introduction of new serotype or genotype may have both clinical and epidemiological implications.[2] Moreover, infection by multiple serotypes is shown to have a severe outcome.[10] It is also established that the transmission dynamics of arbovirus can change with even a single amino acid change. Thus, to understand epidemiological and clinical outcome/s, it is important to monitor circulating serotypes by conducting molecular studies; Reports from western Odisha confirm that all the four serotypes are in circulation causing sporadic cases, and DENV-1 and DENV-2 are shown to cause outbreaks with high positivity.Hence, we present this molecular analysis identifying serotypes of DENV circulating in western Odisha in the year 2021.

2. Materials and methods

Sambalpur is geographically situated in the western part of Odisha. The laboratory of the institute vimsar, burla for diagnosis of dengue in Western Odisha. Blood/serum samples of clinically suspected dengue cases are referred for diagnosis and serotyping of DENV. Study period was between July 2021 to Dec 2021

Testing of samples

Serum was separated from whole blood sample by centrifugation at 3000 revolutions per minute at 4°C. Samples collected in the acute phase of illness (0–5 days of illness, n = 355) were tested for the presence of DENV NS1 antigen by ELISA (erba elisa) as described by manufactures.

Dengue virus serotyping

Primer and probe design.

To be able to find serotype-specific conserved regions, all whole genome sequences of DENV1-4 available at the design stage (2014–10–31) were downloaded from NCBI and used for assay design. Multiple sequence alignments containing the genomic sequences of the individual serotypes and alignments containing all DENV genomic sequences were created using CLC Genomics Workbench 7.5 (www.clcbio.com). Primers and probes were constructed using in-house software. Melting temperatures (Tm) were verified using Primer Express® v3.0 (Applied Biosystems®). Theoretical specificity of the systems was investigated using BLAST against theNCBI nucleotide database with very loose match criteria (word-size = 7, E-cutoff = 1000, match/mismatch cost +1/-1, gap cost 5/2).

Assay	Primer/probe	Sequences (5'-3')	Base Ct
One-step Real time RT-PCR	DENV1_F	5' CCTTTCCACAGAGYAAYGCAG 3'	21
	DENV1_R	5' CCTGAGTTCCATGATCTYTCA 3'	21
	DENV1_P	6FAMTCCAAGATGAGGAAARAGACATTCCMGBNFQ	25
	DENV2_F	5'-TCTGCAAACAYTCCATGGT-3'	19
	DENV2_R	5'-CACAGGTCACRATRCCTCC-3'	19
	DENV2_P	VICCATCCATTTCCCCATCCMGBNFQ	17
	DENV3_F	5'-CTCAAGAGCATGGAATGTGT -3'	20
	DENV3_R	5'-GCCGACATTAGCCTRTGG-3'	18
	DENV3_P	NEDCCGTARTCTTCYACCTCCCMGBNFQ	19
	DENV4_F	5'-GCTGAYGTCAGGAAYGACAT-3'	20
	DENV4_R	5'-TCTTCTTTRTCCCATTTRTCTCC-3'	23
	DENV4_P	6FAMCATCCCCCACCGTATGATATCMGBNFQ	21

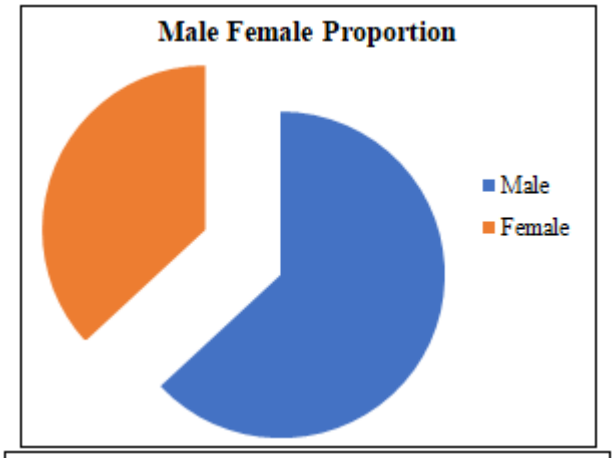
Real-time RT-PCR (TaqMan) assay

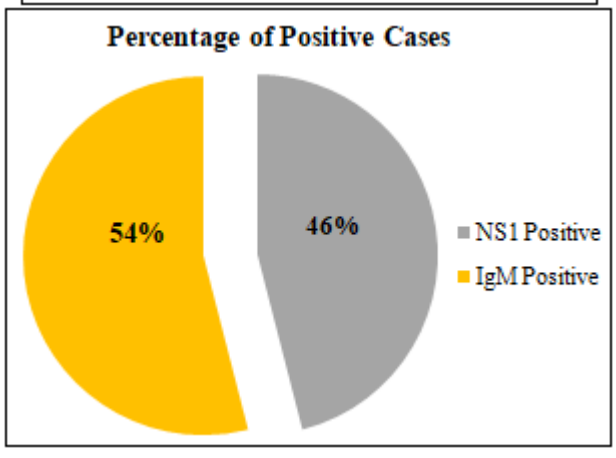
Serotype-specific DEN virus primers and fluorogenic probes were designed by using the PrimerExpress, version 2.0.0 (PE Applied Biosystems, Foster City, CA), and Beacon Designer(Premier Biosoft International, Palo Alto, CA) software packages (Table 1). Serotype specificities of primer sequences were evaluated by comparison of available published sequences of the complete genome of each DEN virus serotype from GenBank, which were aligned with Megalign software (DNAstar, Madison, WI). Comparisons of specific regions were also done with PrimerSelect software programs and by BLAST searches. Sequences of primers and probes designed to detect DEN-2 virus (strain PUO218) were reported previously. TheDEN-1 probe was labeled at the 5′ end with the 6-carboxyfluorescein (FAM) reporter dye and atthe 3′ end with Nonfluorescent quencher-minor groove binder (NFQ-MGB) fluorophore; theDEN-2 probe was labeled with VIC and NFQ-MGB; the DEN-3 probe was labeled with NED reporter dye and NFQ-MGB; and the DEN-4 probe was labeled with FAM reporter dye and NFQ-MGB. RNA from other flaviviruses or negative extractions of tissue culture media was included as a negative control in all real-time RT-PCR assays. The specificities of the primer-probe sets were determined as previously described. The DENV1, DENV2, DENV3, and DENV4 RT-PCR assays were carried out in 25 µL reaction mixtures containing 5 µL template RNA, TaqMan® Fast Virus 1-step mastermix (AppliedBiosystems®), UltraPure™ DNase/RNase-Free Distilled Water (Invitrogen™), 0.9 µM of each primer, and 0.2 µM probe (Table 1). The MGB-probes were labeled with FAM reporter dye anda non-fluorescent quencher. Primers and probes were purchased from Life Technologies. Amplification and detection were performed in ABI 7500 real-time PCR system (AppliedBiosystems®). Thermocycling parameters were as follows: reverse transcription at 50 °C for 30min, inactivation at 95 °C for 2 min, followed by 45 cycles of fluorescence detection at 95 °C for15 s, and annealing at 60 °C for 1 min. The baseline and threshold were set using the auto baseline and threshold feature in StepOne Software v2.2.2 (Applied Biosystems®). Samples were considered positive if target amplification was recorded within 40 cycles. The DENV-1-4 Real-Time RT-PCR Assay was performed in Multiplex reactions following the manufacturer's instructions (Centers for Disease Control and Prevention) in 25 µL volumes using the SuperScript® III Platinum® One-qRT-PCR Kit (Invitrogen™). Amplification and detection were performed in a 7500 Fast DX Real-time PCR instrument (Applied Biosystems®).

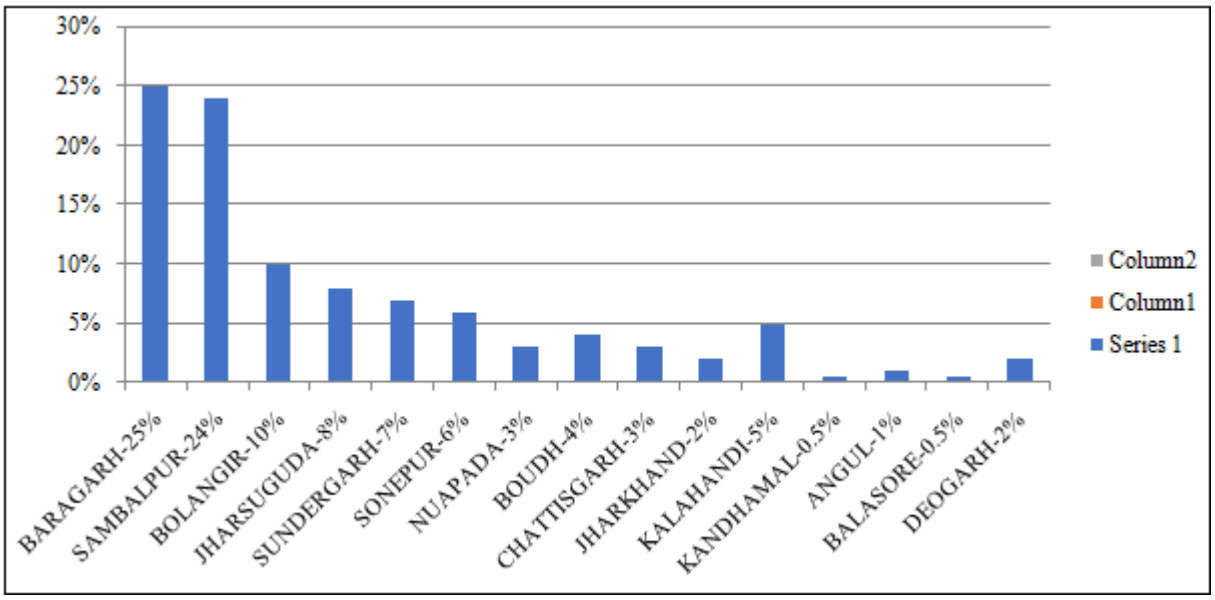
The data analyses were performed as described in the manufacturer's guidelines. In short, thethreshold was adjusted to fall within the PCR exponential phase in the linear view. The manufacturer's instructions specifies that a specimen is considered positive for either DENV1, 2,3, or 4 if the amplification curve crosses the threshold line within 37 cycles (Cq < 37).

3. Result

Of the 355 samples,31 were positive for NS1 antigen ELISA .7 samples were found to be infected with DEN-2 serotype, DEN-1 serotype was detected in 4 samples.All the RT- PCR positive samples were also positive for dengue NS1 antigen. The age of the dengue case was in the range between 15-50 years of age and male outnumbered the female patient.







4. Discussion

Dengue infection presents with nonspecific fever that mimics other viral illness.The availability of commercial ELISA assays to detect the DENV NS1 protien in acute plasma provide an additional diagnostic tool to existing approaches of multiplex real-time RT- PCR.It is important to establish the diagnosis of acute dengue virus infection during the first few days after clinical manifestation.

DENV-2 is regarded as the most virulent serotype of DENV,[3] and was the predominant serotype associated with the outbreaks of DEN fever and DEN haemorrhagic fever cases during 1970–2000; loss of RNA during transportation, reporting incorrect days of onset of illness by patients, sensitivity of NS1 ELISA, specificity of qRT-PCR and very low amount of viral RNA in the sample could be the reasons for failing of serotyping twenty samples.

5. Conclusion

The present study comprehensively established the utility of NS1, MULTIPLEX real-time PCR is rapid diagnostic test for dengue patients. DEN-2 was the predominant serotype in severe case. Continued surveillance of serotype should be carried out in endemic area.

References

[1] World Health Organization Regional Office South East Asia. Fact Sheet. Available from: http://www.searo.who.int/entity/vector_borne_tropical_diseases/data/data_factsheet/en/. [Last accessed on 2017 Aug 27].
[2] Centers for Disease Control and Prevention. Available from: https:// www.cdc.gov/dengue/epidemiology/. [Last accessed on 2017 Apr 12].
[3] Rico-Hesse R. Microevolution and virulence of dengue viruses. Adv Virus Res 2003;59:315-41.
[4] World Health Organization SEARO. Global Strategy for Dengue Prevention and Control. Available from: http://apps.who.int/iris/bitstream/10665/75303/1/9789241504034_eng.pdf. [Last accessed on 2017 Apr 12].
[5] Gupta N, Srivastava S, Jain A, Chaturvedi UC. Dengue in India. Indian J Med Res 2012;136:373-90.
[6] Shrivastava S, Tiraki D, Diwan A, Lalwani SK, Modak M, Mishra AC, et al. Co-circulation of all the four dengue virus serotypes and detection of a novel clade of DENV-4 (genotype I) virus in Pune, India during 2016 season. PLoS One 2018;13:e0192672.
[7] Chakravarti A, Arora R, Luxemburger C. Fifty years of dengue in India. Trans R Soc Trop Med Hyg 2012;106:273-82.