Short Communication

Circulating Genotypes of Dengue-1 Virus in South West India, 2014–2015

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SUMMARY: Dengue is the most prevalent arboviral disease affecting humans, with tropical and subtropical regions having the highest risk of dengue virus infections. The clinical symptoms often range from mild fever to fever with hemorrhagic manifestations and shock. Since 2010, India has been witnessing a marked increase in the number of dengue virus serotype 1 (DENV-1) infections, apart from those of serotypes 2 (DENV-2) and 3 (DENV-3). The present study was undertaken to understand the circulating genotypes of DENV-1 in South West India, by sequencing the envelope gene of DENV-1 samples representative of the 2014–2015 time period. The extracted RNAs from dengue NS1 antigen ELISA-positive samples, archived at the APEX Referral Laboratory for Arboviruses (National Vector Borne Disease Control Programme, Manipal Centre for Virus Research), were used for the study. The currently circulating genotypes of DENV-1 identified from the study area belong to genotypes V and I. Multicentric laboratory-based studies analyzing the envelope gene of all dengue serotypes are required from endemic countries like India.

Dengue is a single-stranded RNA virus belonging to the genus *Flavivirus* (family *Flaviviridae*). This mosquito-borne virus is transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes, with *Aedes aegypti* being the predominant vector. An infection with any dengue serotype provides immunity against that particular serotype, but not against different dengue serotypes (1). Asian genotypes of dengue virus serotype 2 (DENV-2) and serotype 3 (DENV-3) are frequently associated with severe disease manifestations, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (2).

Dengue infections are widely prevalent in India, where all 4 serotypes are found. Outbreaks have been reported from rural areas of Haryana, Tamil Nadu, and Maharashtra (3). Although outbreaks in India were mainly due to DENV-2 and DENV-3, dengue virus serotype 1 (DENV-1) was the predominant serotype reported in the 2010 Delhi outbreak. The circulating genotypes of DENV-1 have been less extensively studied worldwide (4), and there is a scarcity of available literature regarding the currently circulating DENV-1 strains from South West India. In this context, we decided to undertake a laboratory-based study to determine the circulating genotypes of DENV-1 strains.

The Institutional Ethical Committee (IEC) of Manipal University (Manipal, Karnataka, India) approved the protocol (IEC 308/2016), and the samples archived between January 2014 and December 2015 at the APEX

Referral Laboratory for Arboviruses (National Vector Borne Disease Control Programme, Manipal Center for Virus Research) were used in this retrospective cross-sectional study. The nucleic acid extraction of NS1 antigen ELISA-positive samples was performed using the QIAquick® RNA Extraction Kit (Qiagen, New Delhi, India) according to the manufacturer's instructions. Amplification of the envelope (E) gene was performed to obtain a product size of 1,761 bp by the reverse-transcription polymerase chain reaction (RT-PCR), using the forward primer D1-820-S and reverse primer D1-2600-AS (5). The extracted nucleic acid (5 µl RNA) was added to the 20-µl reaction mixture containing 10 µmol of each primer, 12.5 µl of buffer mix (Ambion® Life Technologies, Carlsbad, CA, USA), 1.0 µl of enzyme mix (Ambion® Life Technologies), and 5.5 µl of nuclease-free water. The amplification was carried out on the Veriti® Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA).

The initial reverse transcription was carried out at 50°C for 30 min, followed by denaturation at 95°C for 15 min. The cDNA was amplified by 40 cycles of denaturation (94°C, 30 s), primer annealing (57°C, 90 s), and primer extension (72°C, 30 s), with a final extension at 72°C for 5 min. The PCR products were analyzed by electrophoresis on a 1% (w/v) agarose gel. RT-PCR positive samples were purified using the QIAquick® PCR Purification Kit (Qiagen). The purified PCR products were directly sequenced using specific primers and the Big Dye Terminator Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions, in a 3500 XL genetic analyzer (Applied Biosystems).

A total of eight DENV-1 E gene sequences were analyzed in this study. Phylogenetic analysis of DENV-1 was further carried out using MEGA7 software (<http://www.megasoftware.net/>). The phylogenetic tree was constructed using the maximum-likelihood method, and the reliability of the tree was tested by the bootstrap method (1,000 replicates).

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The study included 200 NS1 antigen-positive samples, of which 64 were serotyped by the Lanciotti protocol (6) as follows: 37 as serotype 1, 20 as serotype 2, and 7 as serotype 3. None were of serotype 4. The nucleotide sequences and deduced amino acid sequences of the partial encoding E gene (480 bp) of 8 DENV-1 strains were compared with previously published E gene sequences in the NCBI nucleotide database. In the phylogenetic analysis, 7 of the DENV-1 strains showed similarities to the reference strain of genotype V, and one strain was genotype I. The genotype V strains in the present study showed 99% similarity with Delhi strains (DENV1/AIIMS_Delhi/1263/2010 and DENV1/AIIMS_Delhi/1132/2010) (Fig.1).

The genotype I strain from Mananthawady taluk in Wayanad district had 96% similarity with a Vietnam strain (DENV1/Vietnam/2005) and 95% similarity with a Myanmar strain (DENV1/MYANMAR/2002). The sequences for the E gene of the DENV-1 strains identified in this study were submitted to GenBank under the

accession numbers KY021893, KY021894, KY021895, KY021896, KY021897, KY021898, KY021899, and KY021900.

In our study, the E genes of eight DENV-1 samples representing the study period of 2014–2015 were sequenced and compared with the available sequences of 17 globally representative strains. Except for one strain from Wayanad district of Kerala, all the strains from the Dakshina Kannada, Shimoga, and Udupi districts of Karnataka and Wayanad district of Kerala were found to be genotype V.

India has been witnessing a rapid change in dengue epidemiology in the last 2 decades, with disease outbreaks occurring at regular intervals (3,4). There is a gradual geographic extension of the infection to rural and remote areas. South East Asia reports the greatest genetic diversity of dengue viruses, and study of the co-circulation of dengue serotypes in the population has epidemiologic significance (7). Although the detection of dengue virus virulence is difficult owing to the

Fig.1. The tree is based on a 480bp (nt 965bp-1480bp) fragment encoding the partial envelope gene. Classification and naming of DENV-1 genotypes are based on the report by Domingo *et al.*, and Chakravarti *et al.*, (9, 3). Only bootstrap values $\geq 75\%$ are shown on the major nodes.

absence of animal models, the association between the specific genotypes and the clinical presentations can be made by phylogenetic studies of dengue virus samples (8).

Domingo et al. reported 5 distinct DENV-1 genotypes based on the E gene sequence (9). Genotype V was stated as the one with a high epidemiologic impact due to its potential to cause DHF (8). Genotypes V and III were the main ones reported from India, and genotype I was detected in Delhi in 1997 and 1998 on the basis of the capsid premembrane protein junction (*cprM*) gene. To date, genotypes II and IV have not been found in India, whereas genotypes III and V have been in circulation in the country for the last 50 years (10). Genotype V (America-Africa) of DENV-1 was reported by Patil et al. to be present in serum samples collected from different parts of India during epidemics spanning over 40 years (10). The representative samples from the 2001–2007 dengue outbreak in North India demonstrated a predominance of DENV-1 genotype III (11). Another study carried out in Kerala, South India reported DENV-1 genotype III strains from a non-seasonal dengue outbreak in 2009 (12). The circulating DENV-1 genotypes in Uttar Pradesh, North India during 2009–2012 were detected to be genotype III by sequencing the *cprM* gene (13).

DENV-1 has been circulating in India since the 1940s, with a change in the disease profile from mild to severe (10). This serotype was implicated in the New Delhi dengue fever outbreaks of 1997, 2006, 2008, and 2010 (4, 11). Previous studies attributed changes in the disease spectrum to variation in the circulating dengue virus as a result of the evolution or introduction of a new genotype of the virus, as had occurred in Cuba (2). Burke et al. suggested an increased risk of DHF and DSS from dengue infections with DENV-2 (14). However, all 4 dengue serotypes can cause manifestations of severe infections, such as DHF and DSS (15). DENV-1 genotype V has been in circulation in Asia, Oceania, and Africa for the last 75 years (16). The DENV-1 genotype V strains circulating in South West India were introduced from adjacent areas. However, the genotype I strain reported from the Wayanad study showed similarity with strains from South East Asia (Vietnam and Myanmar).

In summary, we have analyzed and sequenced the E glycoprotein gene of DENV-1 samples from South West India, and ascertained that genotype V and I strains of this serotype are currently in circulation in this studied region.

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Conflict of interest None to declare.

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