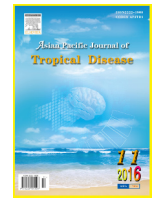




Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Dengue research

doi: 10.1016/S2222-1808(16)61144-1

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Genetic analysis of imported dengue virus strains by Iranian travelers

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ARTICLE INFO

Article history:

Received 1 Sep 2016

Received in revised form 18 Sep 2016

Accepted 20 Sep 2016

Available online 26 Sep 2016

Keywords:

Dengue virus

Phylogeny

Serotype

Genotype

Epidemiology

Iran

ABSTRACT

Dengue virus sequences used in this study were obtained from two Iranian patients who were both with a history of traveling to Malaysia. The maximum likelihood phylogenetic tree demonstrated that two sequences were grouped into dengue virus 1. Specifically, strains Iran-DF1 and Iran-DF2 clustered in genotype I and III, respectively.

1. Introduction

Dengue virus (DENV), an arthropod-borne RNA virus of the

Flaviviridae family, has 4 serotypes that cause dengue fever (DF) or dengue hemorrhagic fever (DHF) in humans[1]. The genome length of DENV is approximately 11 kb and encodes three structural proteins including capsid, premembrane/membrane and envelope, and seven nonstructural proteins (NS1, NS-2A, NS-2B, NS-3, NS-4A, NS-4B and NS-5)[1,2]. DENV consists of four closely related but genetically distinct virus serotypes (DENV-1 to 4). Phylogenetic studies of DENV have revealed genetic diversity within each serotype. DENV-1 has been sub-divided into five genotypes (I–V) based on phylogenetic analysis of the envelope gene, while other

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The journal implements double-blind peer review practiced by specially invited international editorial board members.

phylogenetic studies on capsid, premembrane and membrane genes of DENV-1 have revealed three distinct genotypes (genotypes I–III)[3,4]. DF is characterized by general signs and symptoms that could occur with many other illnesses including general fever, nausea, vomiting, abdominal pain, vomiting and severe headache. This virus is principally transmitted by *Aedes aegypti* mosquitoes[5].

The first report of DF in Iran was in 2008. The patient had previously travelled to Malaysia (Kuala Lumpur) with a history of entering a forest[6]. Since 2008, additional human cases with DF symptoms were diagnosed in Iran. Commonly, those patients had travelled to Malaysia. Due to the continued monitoring of DF, 24 cases have now been confirmed in Iran. The majority of these patients are travelers with a travel history to Malaysia, India and Thailand[7]. However, DENV RNA was not detected in these imported DF cases. This study characterized imported DENV strains in Iran by using a phylogenetic approach. These data will lead to a better understanding of DENV epidemiology in Iran and may help to discriminate imported DENV strains from locally circulating DENV strains.

2. Material and methods

Two previously healthy women in her 40s and 50s were treated in a hospital in Iran in 2009 and 2011 after returning from Malaysia. For the previous days, they had been suffering from fever (up to 40 °C) and myalgia. Serum samples collected during the first week of illness gave positive results in DENV immunoglobulin M ELISA, as well as for DENV RNA with a DENV-specific RT-PCR, demonstrating an acute DENV infection[1]. The amplified fragments from the RT-PCR were purified for Sanger sequenced. The sequences described in this study have been deposited in the GenBank database under accession numbers KM669157 (strain: Iran-DF1) and KP144198 (strain: Iran-DF2).

In addition to the two DENV sequences obtained from Iranian patients, several sequences representing all serotypes (DENV-1 to 4) and three genotypes within serotype 1 based on capsid, premembrane and membrane genes (available from GenBank at www.ncbi.nih.gov) were incorporated into the alignments for phylogenetic analyses. The sequence alignment was undertaken using ClustalW and phylogenetic trees were generated by the maximum likelihood method with kimura 2-parameter distance using molecular evolutionary genetics analysis software. Afterwards, analysis of split decomposition was performed

by the Splits Tree 4.0 software to assess the presence of a “phylogenetic network” at the genotype level for DENV-1[8].

3. Results and discussion

The phylogenetic tree based on maximum likelihood showed that two sequences obtained from two Iranian patients were grouped within the DENV-1 lineage. The Iran-DF1 (KM669157) sequence showed 72% closeness to the 428 bp region, which corresponds to nt 198–628 in the late region of the capsid gene and the early region of the membrane gene of the strain isolated from China (JQ048541). Iran-DF2 (KP144198) sequence showed 52% closeness to the 428 bp region of the strain isolated from India (JF815210) (Figure 1).

The phylogenetic network revealed clustering of isolates in three distinct genotypes (I, II and III). Iran-DF1 clustered in genotype I with the other viral isolates from Malaysia, Cambodia, Singapore, Thailand and China. Genotype III showed three distinct sub-lineages. All Myanmar isolates were grouped together with significant identity, whilst all isolates from South America formed a separate sub-lineage. Iran-DF2 formed the third sub-lineage within genotype III with other isolates from Southeast Asia (Figure 2).

The estimate of dengue patients among the Iranian population is of concern and is currently being more regularly monitored by the Iranian health authorities. We performed the first phylogenetic analysis of imported DENV strains in Iran in order to trace the potential origin of these strains.

Our data are in accordance with previous reports that DENV-1 has been sub-divided into three genotypes (I, II and III) based on phylogenetic analysis of the capsid, premembrane and membrane genes[3,4]. Recent data present that DENV-1 genotype I and III strains were imported to Iran by travelers returning from Malaysia. DENV-1 strains are responsible for the autochthonous DF cases in mainland Europe and thus may be able to cause autochthonous DF cases in Iran. The main DENV vectors *Aedes aegypti* and *Aedes albopictus* are present in Iran[9]. Thus, this might increase the risk of the introduction and circulation of DENV in Iran. We reported the genetic diversity of DENV strains among Iranian DF patients who acquired the infection following the travel to DENV-endemic regions in Southeast Asia. The Iranian public health authorities should implement preventive measures in order to reduce the risk of autochthonous DF cases in Iran caused by imported DENV initially seeded into the local mosquito population by Iranian travelers.

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