PHYLOGENETIC ANALYSIS OF ZIKA AND DENGUE VIRUS

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ABSTRACT

Zika and Dengue virus are two viruses from of the family *Flaviviridae*, mosquito-borne *Flavivirus,* known as zoonosis disease because the viruses can transmitted to human by mosquito vector mainly *Aedes aegypti*. The outbreaks of Zika virus (ZIKV) was first confirmed case in the Brazil in May 2015. It is currently one of the most important emerging viruses in the world and has outbreaks and epidemics with severe clinical manifestations. Like Zika, Dengue virus has rapidly spread in all areas of WHO in recent years and the source of outbreaks in areas of recent ZIKV introduction. This study aimed to determine the evolutionary relationship among Zika and Dengue virus. The 220 complete genome (11kb) were downloaded from GenBank database. Phylogenetic tree examined by constructed by Neighbor-Joining (NJ) method in the KIMURA-2 parameter model and valued by bootstrap with 1000 replicates. The result showed that Zika virus has relationship between two strains of DENV-4 where they were clustered together in phylogenetic tree.

INTRODUCTION

Zika virus (ZIKV) was first identified in the Zika forest in Uganda in 1947 from the serum of species rhesus monkey (*Macacamulatta*) (Zanluca & Duarte dos Santos, 2016). According to World Health Organization (WHO), ZIKV now reached a dangerous level and were reported in areas of Africa, Southeast Asia and the Pacific Islands. Zika infection is commonly reported with characteristics of acute febrile illness that similar with dengue fever. The common symptoms associated with fever, joint pain, rashes, fatigue, and conjunctivitis (Endy *et al*., 2002). Zika virus infection can caused brain deformation known as microcephaly and it was reported in pregnant women, commonly Brazilian, being tested positive with Zika virus (Chang *et al*., 2016). The Brazil Ministry of Health has reported an increased number of cases who have been infected with Zika infection who also have Guillain-Barre syndrome (GBS). GBS is a condition where the body's immune system attacks part of the peripheral nervous system (Willison *et al*., 2016).

Like Zika, Dengue virus (DENV) infection is one of the significant pathogens infecting people around the world, causing an expected 50-100 million cases resulted about 10,000 cases deaths every year (Stanaway *et al*., 2016) and more than 2.5 billion people being at risk of infection (Guha-Sapir & Schimmer, 2005).

Infection with any of the DENV serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) might be asymptomatic in the majority cases or may bring about a wide range of clinical side effects (Harris *et al*., 2000), ranging from a mild flu-like syndrome to the most severe forms of the disease. In Asia, the risk of developing severe disease is more noteworthy in DENV infected children (15 years) than adults (Guzmán *et al*., 2002).

In clinically obvious, dengue infection symptoms create after an incubation period of 4-7 days with a sudden onset of fevers regularly joined by headache with severe retro-orbital pain, myalgia, arthralgia, gastrointestinal discomfort and usually rush (Endy *et al*., 2002). Dengue poses a risk to those who travel to endemic regions and progressively being accounted in travellers returning from trips to endemic countries (Allwinn *et al*., 2008).

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MATERIALS AND METHODS

This research does not use any wet lab works that is used to calculate the genetic distance and determine the relatedness among the viruses of the family *Flaviviridae* by using computational method. The complete genome of Zika, Dengue and Hepatitis C virus were used as a part of this study and it had been already available in GenBank database.

Viral sequence dataset

A total of 220 complete genome of two viruses of the family *Flaviviridae* namely Zika and Dengue virus with the length 11 kb were downloaded from GenBank database at National Center for Biotechnology Information (NCBI). Four reference sequences of dengue virus from subtype 1-4 (accession number: NC_001477, NC_001474, NC_001475, NC_002640) were used as "gold standard" in this analysis. Biological data acquired for each sequence included species name, accession number, country and date of collection. None of the data was straightforwardly derived from human or animals samples.

Sequence alignment

The complete genome sequences with all available full-length of the virus sequences from GenBank were subjected to multiple sequence alignment (MSA) analysis using ClustalW that is implemented in the BioEdit software followed by manual sequence editing for significant changes. Sequences consensuses were trimmed until 10 kb to create a sequence block.

Phylogenetic analysis

Phylogenetic trees were generated by using Neighbor-Joining (NJ) method. Neighbor-Joining tree were generated and analyzed with 1000 replicates, transversion and transitionally analyze using pair-wise deletion for bootstrap testing using MEGA 6.0 software in the KIMURA-2 parameter model.

RESULTS AND DISCUSSION

The four serotype of DENV and Zika virus were clearly arranged in well-defined clades with Neighbor-Joining method. Fig. 1 showed the evolutionary relationship between the viruses of family *Flaviviridae*. Moreover, for phylogenetic analysis of both Zika and two strains of DENV-4 from Venezuela (accession number: GQ868644) and USA (accession number: FJ024424) were root away from their respective DEV-4 cluster and were slightly

Fig. 1. Phylogenetic tree showing the relationship between important *Flaviviruses* causing human infection. The evolutionary history was inferred using the bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using Kimura-2 parameter method and are in the units of the number of base substitutions per site. The analysis involved 220 nucleotide sequences. Evolutionary analyses were conducted in MEGA 6.

Table 1. List of viruses used for the analysis

Virus	No. of Sequences	No. of Countries	Date of Collection
DENV1	50	15	1963-2014
DENV2	51	9	1964-2011
DENV3	51	11	1998-2013
DENV4	51	13	1998-2013
ZIKV	17	4	1976-2015

rooted nearer to Zika. It is assumed that these two strains of DENV-4 could possibly play role in the emergence of Zika virus.

It is reasonable to suspect that cross-border transmission contributes to the spread of Zika virus. DENV-4 is newly emergence of dengue strain where two strains of DENV-4 from Venezuela and USA country were rooted to all DENV strain. On a global scale, DENV-1 (red lines in Fig. 1) has showed homogenous across the globe.

In this study, the available genomic data of ZIKV and DENV in the GenBank database were analyzed to provide quick search about the relationship of the viruses. There is no specific drug and vaccines to treat the infection, appropriate control measurements and public health intervention should be applied to control the mosquitoes which are known to vector of the viruses.

As exhibited in recent epidemics of emerging viral infections, characterization of the viral may encourage the distinguishing proof the important virulence factor and diagnostic, treatment and drug and vaccines target.

CONCLUSION

In summary, the phylogenetic study revealed the relationship between viruses of the family *Flaviviridae* especially Zika and Dengue virus. The findings in the current study may provide useful information for Zika and Dengue prevention and control.

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