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Original Article

Biotechnology Section

Genetic Variability of NS5 Gene Sequences amongst Zika Virus Isolates of India, China and Japan

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ABSTRACT

Introduction: Zika Virus (ZIKV) infection is transmitted by Aedes mosquitoes, predominantly in tropical and subtropical regions of the world, including India, and can lead to birth defects in humans. Due to genetic similarities among the *Flavivirus* genus, it may lead to cross-reactivity, especially in diagnostic tests. The ZIKV's non structural protein 5 (NS5) is a potential vaccine development and diagnosis target. Due to limited studies on NS5 genetic diversity, it needs to be explored.

Aim: To investigate the genetic variation in the NS5 region of the ZIKV and phylogenetic analysis among Asian isolates.

Materials and Methods: This study is descriptive based on genomics and was conducted from January to June 2024 atViral Research and Diagnostic Laboratory (VRDL), Department of Microbiology, Shyam Shah Medical College, Rewa, Madhya Pradesh, India. In this study, bioinformatics approach for the analysis of ZIKV RNA-dependent Ribonucleic Acid (RNA) polymerase (NS5) gene sequences retrieved from the National Centre of Biotechnology Information (NCBI) database. Phylogenetic analysis of the ZIKV RNA-dependent RNA polymerase NS5 gene sequence was performed using MEGA 11. Briefly, NS5 sequences were aligned and compared with ZIKV reference sequences retrieved from GenBank. The phylogenetic tree was constructed using a neighbour-joining method and the Tamura-Nei model, after 1,000 bootstrapped replicates.

Results: The NS5 genetic diversity among Indian isolates ranged from 0% to 9.93%. ZIKV isolates from India, China, and Japan showed similarity to KX369547.1_Germany, KJ776791.2_France, and KU940224.1_US sequences. Overall, the highest genetic divergence was observed in MK696551.1_Beijing_China, while the lowest genetic divergence was observed in OK054351.1_Maharashtra_India. Phylogenetic analysis suggests that the recent ZIKV outbreak in India originated from the Asian lineage and may have spread to other parts of the world, including China and Japan.

Conclusion: Zika NS5 gene diversity of Asian lineage is the highest among Indian ZIKV isolates, which may affect the efficiency of NS5 gene-based molecular diagnostics. Therefore, an extensive study on NS5 genetic variation and new diagnostic targets for the ZIKV is required from India.

Keywords: Asian lineage, Coding, Dengue, *Flavivirus*, Phylogeny

INTRODUCTION

Zika virus (ZIKV) is an arbovirus (a virus transmitted by arthropods) that belongs to the genus *Flavivirus* in the family *Flaviviridae*, which includes dengue virus, yellow fever, and West Nile virus. It was first observed in rhesus monkeys in the Zika forest near Kampala, Uganda, in 1947 [1]. ZIKV has spread across half of the North African continent and into countries including Vietnam, Malaysia, Indonesia, the Philippines, India, Thailand, and Pakistan [2,3]. A ZIKV outbreak was reported in French Polynesia in 2013 [4], and cases were subsequently reported in India from 2017 onwards. A total of 153 Zika cases were reported in Rajasthan between September and October 2018 [5]. In Madhya Pradesh, a total of 130 confirmed cases of ZIKV infection/disease were diagnosed in October and November 2018 [5]. This represents the spread of ZIKV to India.

ZIKV in humans is thought to be transmitted by Aedes mosquitoes (*A. africanus*, *A. luteocephalus*, and *A. aegypti*). ZIKV RNA is usually detected in the blood within the first 10 days after infection (i.e., within the first 3-5 days after the onset of symptoms), with the highest viral load occurring at the onset of symptoms [6]. The ZIKV genome encodes a polyprotein (approximately 3,423 amino acids in length), which contains 3 structural proteins (capsid C, membrane M, and envelope E) and 7 nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [7]. NS1 region is widely used in Dengue and other flaviviruses diagnosis, the sequence of this region shares similarity along with NS1 region of ZIKV [8]. The NS5 region is majorly targeted for the diagnosis of ZIKV. The NS5 protein has two domains: an RNA-dependent RNA polymerase (RdRp) domain at the C-terminus and a Methyltransferase (MTase) domain at the N-terminus [9]. The NS5 RdRp domain promotes bacterial RNA synthesis through a novel mechanism [10]. The NS5 coding region has been used as a potential biomarker for diagnosis [11].

Phylogenetic analysis is important to understand the evolution and transmission of ZIKV. The occurrence of specific changes in each part of phylogeny branches may be important for disease control, especially when this branch is associated with disease cases. Only a few complete ZIKV genomes are available and therefore the number of studies investigating the genetics of ZIKV relatively small [12]. The pairwise alignment is used as a preliminary tool to examine the ZIKV genome-wide genetic diversity and individual protein data. At the molecular level, genetic diversity can be measured by identifying mutations. The highest nucleotide variability has been found in protein C, M, peptide 2K, and NS5 [13].

The increased cases and outbreaks in the Asia region are major concerns. In addition, the NS5 region is recommended for the diagnosis of ZIKV. Therefore, it is needed to keep investigating the NS5 genetic variability of ZIKV isolates from India, China, and Japan. Therefore, the study is investigating the NS5 gene sequence variability among circulating ZIKV isolates from India, China, and Japan.

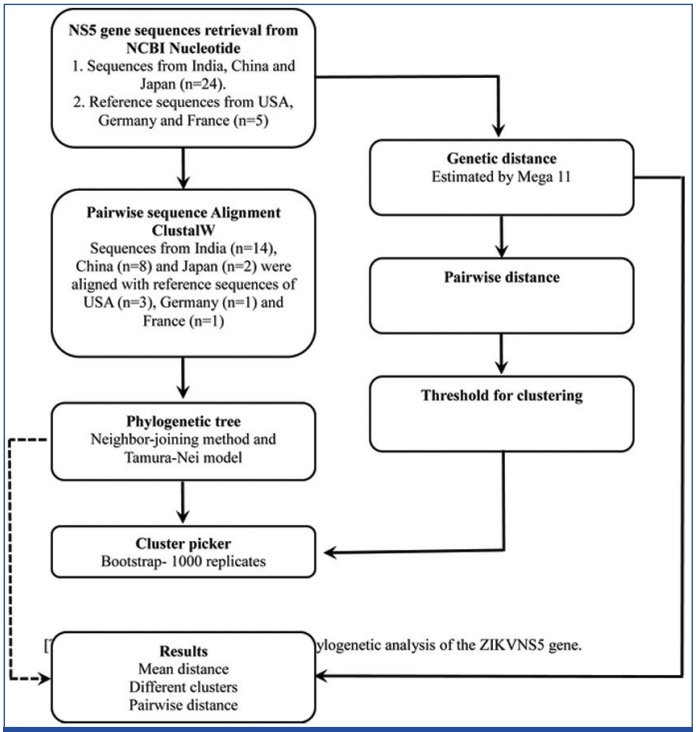
MATERIALS AND METHODS

The present study is descriptive based on genomics and was conducted from January to June 2024 at VRDL, Department of Microbiology, Shyam Shah Medical College, Rewa, Madhya Pradesh, India. The Institutional Ethical Committee approved the study (No. 31131). A bioinformatics approach was used and attentive precautions were taken for sequence retrieval, arrangements and categorisation.

Study Procedure

NS5 gene sequence retrieval: NS5 gene sequences of ZIKV isolates from India, China, and Japan, along with reference sequences, were retrieved from the NCBI Nucleotide database in a FASTA format (http://www.ncbi.nlm.nih.gov/genbank/).

Phylogenetic analysis by maximum likelihood method: Phylogenetic analysis of the ZIKV RNA-dependent RNA polymerase (NS5) gene sequence was performed by MEGA 11. The Zika sequences were obtained from NCBI database and were aligned with ZIKV reference sequences retrieved from GenBank. The phylogenetic tree was constructed by using a neighbour-joining method and Tamura-Nei model after 1000 bootstrapped replicates [14]. The details of the screening procedure are shown in [Table/Fig-1].



[Table/Fig-1]: Flowchart describing the phylogenetic analysis of the ZIKV NS5 gene.

STATISTICAL ANALYSIS

Tamura-Nei-based descriptive statistics were applied. Briefly, mean/pairwise distance was calculated to show nucleotide sequence-based evolutionary/genetic divergence. Data were expressed in terms of percentage divergence to understand the extent of genetic variability among different ZIKV isolates.

RESULTS

In present study, NS5 gene sequence-based genetic variability of ZIKV isolates was estimated by phylogenetic analysis. ZIKV sequences from the USA (AY632535.2, KX377335.1, and KU940224.1), Germany (KX369547.1), and French (KJ776791.2) were taken as reference sequences. ZIKV sequences from India (n=14), China (n=8), and Japan (n=2) were phylogenetically analysed. To ascertain the genetic changes in a recent outbreak of ZIKV, sequences from the Maharashtra outbreak in 2018 (OM666893.1, OM666892.1, and OM666891.1) and Rajasthan outbreak in 2021 (MK238037.1 and MK238035.1) were also included as Indian isolates. The overall mean distance was 0.03. The highest genetic divergence was observed in MK696551.1_Beijing_China; however genetic divergence was lowest in OK054351.1_Maharashtra_India in comparison to reference sequences.

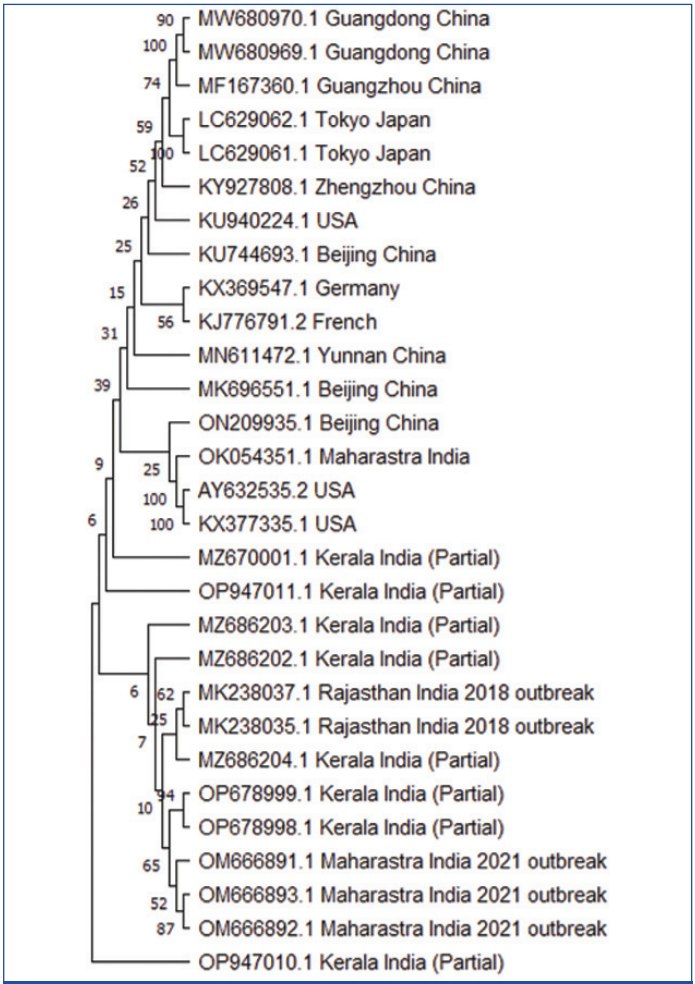
Within Chinese isolates, maximum genetic divergence was observed in MK696551.1_Beijing_China, while the minimum genetic divergence was observed in KY927808.1_Zhengzhou_China. Among Indian isolates, maximum genetic divergence was observed in OP678999.1_Kerala_India (Partial) and OP678998.1_Kerala_India (Partial), with the minimum genetic divergence observed in OK054351.1_Maharashtra_India. Both isolates of Japan were found to be genetically similar. The genetic variability among Chinese sequences ranged from 0% to 3.12%. In comparison, the genetic variability among the Indian sequences ranged from 0% to 9.93%. Genetic variability between ZIKV sequences has been shown in [Table/Fig-2].

Based on the phylogenetic analysis, NS5 gene sequences of ZIKV isolates from India, China, and Japan had shown more similarity with KX369547.1 (Germany), KJ776791.2_French, and KU940224.1_USA sequences [Table/Fig-3].

Zika Virus (ZIKV) isolates (India, China and Japan)	Zika virus (ZIKV) reference sequences				
	AY632535.2 USA	KX377335.1 USA	KU940224.1 USA	KX369547.1 Germany	KJ776791.2 French
MF167360.1_Guangzhou_China	14.75	14.60	0.60	0.45	0.45
KU744693.1_Beijing_China	14.98	14.83	0.91	0.68	0.68
ON209935.1_Beijing_China	14.84	14.69	1.21	0.84	0.84
KY927808.1_Zhengzhou_China	14.56	14.41	0.41	0.26	0.26
MK696551.1_Beijing_China	15.02	14.87	1.09	0.72	0.72
MW680970.1_Guangdong_China	14.79	14.64	0.64	0.49	0.49
MW680969.1_Guangdong_China	14.83	14.68	0.68	0.53	0.53
MN611472.1_Yunnan_China	14.86	14.71	1.02	0.64	0.64
MK238037.1_Rajasthan_India_2018_outbreak	13.81	13.66	1.40	1.03	1.03
MK238035.1_Rajasthan_India_2018_outbreak	13.76	13.61	1.35	0.98	0.98
OM666893.1_Maharastra_India_2021_outbreak	13.78	13.63	1.67	1.29	1.29
OM666892.1_Maharastra_India_2021_outbreak	13.78	13.63	1.67	1.29	1.29
OM666891.1_Maharastra_India_2021_outbreak	13.70	13.55	1.59	1.22	1.22
OK054351.1_Maharastra_India	12.82	12.67	8.60	8.22	8.22
MZ686204.1_Kerala_India_(Partial)	13.68	13.53	1.28	0.90	0.90
MZ686203.1_Kerala_India_(Partial)	13.54	13.39	1.14	0.76	0.76
MZ686202.1_Kerala_India_(Partial)	13.46	13.31	1.22	0.84	0.84
MZ670001.1_Kerala_India_(Partial)	14.09	13.94	1.68	1.31	1.31
OP947011.1_Kerala_India_(Partial)	13.46	13.31	1.22	0.84	0.84
OP947010.1_Kerala_India_(Partial)	13.54	13.39	1.14	0.76	0.76
OP678999.1_Kerala_India_(Partial)	14.10	13.94	1.99	1.61	1.61

OP678998.1_Kerala_India_(Partial)	14.10	13.94	1.99	1.61	1.61
LC629062.1_Tokyo_Japan	14.60	14.45	0.45	0.30	0.30
LC629061.1_Tokyo_Japan	14.60	14.45	0.45	0.30	0.30

[Table/Fig-2]: Estimates of NS5 gene sequence-based genetic divergence (%) between ZIKV isolates and reference sequences.



[Table/Fig-3]: Phylogenetic tree showing the genetic variability among ZIKV isolates, revealed by maximum likelihood method and Tamura-Nei model based on NS5 gene sequence.

DISCUSSION

The ZIKV is an Aedes mosquito-borne *flavivirus* that rapidly spreads throughout the tropical and subtropical regions of the world, including India. ZIKV infection has often been neglected for diagnosis in Asia, Africa, and America, likely due to mild or no clinical symptoms [15,16]. The outbreaks of ZIKV have occurred in Southeast Asian countries such as Thailand, Cambodia, Indonesia, the Philippines, India, Singapore, Japan, and Vietnam [17-26]. It indicates that over time, the Asian lineage of ZIKV has been wide spread all over the world, including India, China, Japan, Germany, France and some parts of the USA. Phylogenetic analysis showed that this phenomenon originated from the Asian lineage, probably from Southeast Asian countries [27]. Similar results were observed in the ZIKV outbreaks in the Pacific Islands and French Polynesia in 2013-2014 [27-29]. The outbreaks during different times points and places require constant molecular surveillance and accurate diagnosis of ZIKV. However, the cross-reactivity with other flaviviruses may lead to an overestimate the cases of ZIKV. In addition, the genetic variability analysis showed diverse mutations within the patients in cases of persistent infection, providing insight into the transmission dynamics of ZIKV. The various regions of its genome are targeted to enhance sensitivity and specificity, but the NS5 region is widely used for diagnostics. The NS5 region has shown a lower mutation as compared to the NS1 ZIKV gene [30].

In the present study, results showed that genetic variability within Indian ZIKV isolates is much higher than in Chinese isolates. This variability in the NS5 gene sequences can reduce the efficacy of NS5 gene-based ZIKV molecular diagnosis. Although other diagnostic targets, like NS1 and E gene, have been investigated by researchers, they are not as well established as NS5. Few studies have found that both NS1 and E gene targets were suitable for testing from serum, plasma, and urine samples [31,32]. There are several Zika vaccine candidates in clinical trials, among them one Deoxyribonucleic Acid (DNA) and two messenger Ribonucleic Acid (m)RNA -based vaccine candidates in phase two clinical trials [33]. The circulating strain of the ZIKV and genetic variability play crucial factors in vaccine efficacy. Hence, genome sequencing from different regions of the world is required to monitor diversity among Zika strains. Although, the study focused on genetic variation of the NS5 region, further research may be extended to include other gene targets of ZIKV.

CONCLUSION(S)

The present study observed that NS5 gene-based genetic variability within Indian ZIKV isolates is much higher than that in Chinese ZIKV isolates. Phylogenetic analysis has shown that the Asian lineage of ZIKV is responsible for recent outbreaks in India, and it has spread worldwide, including to China, Japan, and Germany, among other places. Genetic variability in the Zika NS5 region of the gene can affect the efficacy of both diagnosis and vaccines.

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