Readers Insight
Publisher of open access books and journals

PJMLS
Print ISSN: 2707-4471. Online ISSN: 2707-448X

Research Article	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/pjmls.v4i4.2205	Copyright © All rights are reserved by Corresponding Author
Vol. 4 No. 4, 2021: pp. 227-234	
www.readersinsight.net/pjmls	
Submission: November 23, 2021	**Revised:** December 23, 2021 **Accepted:** December 27, 2021

MOLECULAR EVOLUTION OF ZIKA VIRUS BY PHYLOGENETIC ANALYSIS OF INDIVIDUAL STRUCTURAL CAPSID (C) GENE AND WHOLE GENOME SEQUENCES WORLDWIDE

Muhammad Amir[1, 2], Muhammad Ali Khan[1], Saima Iram[3], Samiullah Khan[2], Muhammad Asif[2, 4*]

[1]**Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB),** University of Balochistan, Quetta, Pakistan

[2]**Department of Biotechnology,** Balochistan University of Information Technology, Engineering and Management Sciences (BUITEMS), Quetta, Pakistan

[3]**Department of Pathology,** Bolan Medical College, Quetta, Pakistan

[4]**Office of Research Innovation and Commercialization (ORIC),** Balochistan University of Information Technology, Engineering and Management Sciences (BUITEMS), Quetta, Pakistan

***Corresponding Author:** Dr. Muhammad Asif. E-mail:asifjallali@yahoo.com

Abstract

Background: *Zika virus was first discovered in Uganda in 1947 considered to be an emerging, arbovirus, mosquito-borne, Flavivirus, which is transmitted through Aedes mosquito possesses global health concerns. Distribution of zika virus all through much of Africa as well as Asia. In this study, using phylogenetic trees based analysis; we reported first full-length genome and partial capsid (C) gene of zika virus distributed over the globe.*

Materials and Methods: *Herein, 22 full-length genome including a Spondweni virus and partial capsid (C) gene sequences of geographically diverse zika virus strains were retrieved from GenBank database. Phylogenetic investigation and evolutionary distances from nucleotide and amino acid sequences were calculated by using maximum likelihood (ML) methods choosing best fitted model in MEGA X software.*

Results: *Phylogenetic and geographical distinct strains followed maximum-likelihood approach based full-length genome analysis showed that all strains fell into two main lineages which confined the genetically closeness. While, phylogenetic analysis of capsid (C) gene nucleotide and amino acid sequences revealed extensive differences in the topologies, followed by full genome sequences either in term of bootstrap support or distribution of the strains into various groups. These results propose significant distinction in the evolutionary trail of the studied genome and gene.*

Conclusions: *This data highlight the significance of studying the phylogenetic constructed viral evolution conferring to global perspective. This line of attack will expand our understanding the spread of zika virus to prevent their emergence as well as improve health surveillance.*

Keywords: *Capsid gene, Full-length genome, Phylogenetic, Zika virus*

INTRODUCTION

Zika virus (ZIKV) is an emerging pathogen and major health threat to humans worldwide. ZIKV is an arbovirus was initially confined in 1947 from the blood of sentry rhesus monkey squatting in the Zika forest of Uganda. At similar location a second isolation tailed from *Ae. africanus* mosquito (1, 2). The virus spread by way of *Aedes* mosquito species such as *Ae. aegypti* and *Ae. albopictus* (3). The specie *Aedes aegypti* is extensively circulated in the regions of tropical and sub-tropical domain (4, 5).

The principal epidemic of zika virus (ZIKV) infection was noticed up to 73 percent in the residents of Yap Island in 2007 (6). The zika virus (ZIKV) epidemic seemed in French Polynesia where about 28,000 peoples infested in the year of 2013 (7, 8)

Zika virus (ZIKV) which is associated with the *Flavivirus* genus, and the *Flaviviridae* family has a single-stranded, positive-sense RNA with genome size around 11 kb (9, 10). Zika virus (ZIKV) genome similar to

other *flaviviruses*, that comprises single ORF (open reading frame) which is flanked by 5′- and 3′- UTRs (untranscribed region). This ORF that encodes ten mature proteins, comprising three structural proteins; (capsid (C), pre-membrane/membrane (prM/M), and envelope (E) and seven non-structural (NS) proteins; (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) polyproteins (11).

Further, other mosquito-borne viruses, genus flaviviruses also contains viruses important to human health are West Nile Virus (WNV), Dengue virus (DENV), Yellow Fever Virus (YFV), St. Louis encephalitis virus, and Japanese Encephalitis Virus (JEV) (5). Clinically infections of zika virus (ZIKV) diagnosed in human are frequently asymptomatic comparatively associated with headache, myalgia, retro-orbital pain, acute febrile illnesses with fever, conjunctivitis, arthralgia, rash, abdominal pain, anorexia. Majority of these clinical indications meticulously look a lot like that instigated by dengue virus (DENV) as well as chikungunya virus (CHIKV) (1, 12).

Moreover, the ZIKV disease mostly transmitted through mosquito bite, but non-vector possibility through man to women to man transmission including breast milk, semen, urine, amniotic fluid and saliva has been reported (11, 13, 14).

Due to lack of adequate research for assessing either whole genome or individual genes are suitable for molecular and evolutionary analysis (6, 15, 16). Previously a comparative phylogenetic investigation based on whole polyprotein and partial non-structural (NS) coding region NS2B have been used and suggested that for evolutionary and molecular characterization of virus the method is more effective (17)

Therefore, in the present study, we conducted an evolutionary depiction of globally sequestered ZIKV isolates using the whole genome and partial capsid (C) gene sequences analysis. We describe the likely origin, evolution as well as geographic distribution of zika virus that explains the recent changes of zika epidemiology worldwide.

MATERIALS AND METHODS

SOURCE OF ZIKA VIRUS (ZIKV) SEQUENCES

The complete 21 strains of zika virus genome as well as one (SPOV) virus were downloaded (Table I) from NCBI GenBank database (18) as they were characteristic of various geographical areas. Previously, zika virus (ZIKV) epidemics in these regions had been documented. For comprehensive illustration and vibrant understanding of the spatiotemporal progression we concealed distinguishing sequences of zika virus (ZIKV) from various topographical extents. Complete zika virus (ZIKV) genome sequences of each isolate including capsid (C) gene were manually split into piece.

PHYLOGENETIC ANALYSIS VIA MAXIMIUM LIKELIHOOD METHOD

The different strains of complete genome and Capsid (C) nucleotide gene as well as amino acid of zika virus (ZIKV) sequences were aligned by using the Clustal *W* tool (19). Phylogenetic reconstructions were created by means of maximum-likelihood (ML) approach in MEGAX (20). The trees were constructed and based first on best suitable model like Bayesian Information Criterion (BIC), Akaike Information Criterion (AICs) and Log of the Likelihood (lnl) scores proposed the model that most appropriate. The consistency of phylogenetic trees was performed by a bootstrap resampling analysis using 1000 replications.

EVOLUTIONARY DISTANCE BETWEEN ZIKV STRAINS

Further analysis to figure out the overall evolutionary distances among the ZIKV isolates subsequently using the whole genome and capsid (C) gene sequences were aligned and analyzed. This analysis was carried out by testing 1000 bootstrap resampling in MEGA X program.

RESULTS

The 21 whole genome zika virus (ZIKV) strains size between 10141 to 10807 bp nucleotide from diverse geographical localities and time points of isolation (Table I).

Table I. Location and names of Zika virus (ZIKV) isolates collected globally

Country	Name of Isolate	Genome (bp)	Year	Nucleotide Accession	Protein Accession	Reference
Brazil	SSABR1	10648	2016	KU707826	AMH87239	Direct Submission
Cambodia	KHM/2010/FSS13025	10807	2016	KU955593	AMR39834	Direct Submission
Canada	Vero E6	10141	2013	KF993678	AHL37808	Direct Submission
Central African Republic	ARB7701	10755	2013	KF268950	AHF49785	Direct Submission
China	VE_Ganxian	10676	2016	KU744693	AMK79469	Direct Submission
Colombia	FLR	10800	2016	KU820897	AMM39804	Direct Submission
French Polynesia	H/PF/2013	10617	2014	KJ776791	AHZ13508	Direct Submission
Guatemala	103344	10272	2016	KU501216	AMC13912	Direct Submission
Haiti	Haiti/1225/2014	10807	2016	KU509998	AMB37295	Direct Submission
Italy	Brazil/2016/INMI1	10643	2016	KU991811	AMS00611	Direct Submission
Martinique	MRS_OPY_Martinique_PaRi_2015	10617	2016	KU647676	AMC33116	Direct Submission
Mexico	MEX/InDRE/Lm/2016	10617	2016	KU922923	AMQ34003	Direct Submission
Micronesia		10272	2008	EU545988	ACD75819	Direct Submission
Nigeria	IbH_30656	10251	2010	HQ234500	AEN75265	Direct Submission
Philippines	PHL/2012/CPC-0740	10807	2016	KU681082	AMD61711	Direct Submission
Puerto Rico	PRVABC59	10675	2016	KU501215	AMC13911	Direct Submission
Senegal	SEN/1984/41671-DAK	10806	2016	KU955595	AMR39836	Direct Submission
South Africa	SM-6 V-1	10290	2016	NC_029055	YP_009222008	Direct Submission
Suriname	Z1106033	10374	2015	KU312312	ALX35659	Direct Submission
Thailand	THA/2014/SV0127- 14	10807	2016	KU681081	AMD61710	Direct Submission
Uganda	MR766-NIID	10807	2014	LC002520	BAP47441	Direct Submission
USA	FB-GWUH-2016	10798	2016	KU870645	AMQ48986	Direct Submission

WHOLE GENOME NUCLEOTIDE BASED PHYLOGENETIC REPRESENTATION

Our maximum likelihood based method analysis indicates the reconstruction that best describe the evolutionary trail from the sequence data. The model was utilized on the way to renovate the evolutionary antiquity of the sequences, which depend between the sequence closeness in the form of phylogenies with specific nodes which illustrate most existing shared ancestor to be found at the tips of the tree.

Our full length nucleotide genome based phylogenetic examination, performed in MEGAX showed two main lineages (1 and 2) and an out-group which represent a country South Africa (Accession No: NC_029055). The lineage 1 was further divided into two sub-lineages, which were designated lineage 1A and lineage 1B (Fig. 1).

Phylogenetic study revealed both lineages (1 and 2) with bootstrap value of 100 presented at the branch node. The five isolates which was found in a discrete subgroup and clustered in lineage 1A are Puerto Rico (Accession No: KU501215), Suriname (Accession No: KU312312), Brazil (Accession No: KU707826), USA (Accession No: KU870645), Guatemala (Accession No: KU501216), but meticulously correlated to lineage 1B that comprises four zika virus (ZIKV) isolates from and Italy (Accession No: KU991811).

The second core lineage 2 confined four zika virus (ZIKV) isolates Central African Republic (Accession No: KF268950), Uganda (Accession No: LC002520), Nigeria (Accession No: HQ234500) and Senegal (Accession No: KU955595).

Our full genome nucleotide investigation proposed that the lineage 1, which were further distributed into two distinctive subgroup named lineage (1A and 1B) were genetically closer, whereas the isolate Italy (Accession No: KU991811) was found away in the cluster (Fig. 1).

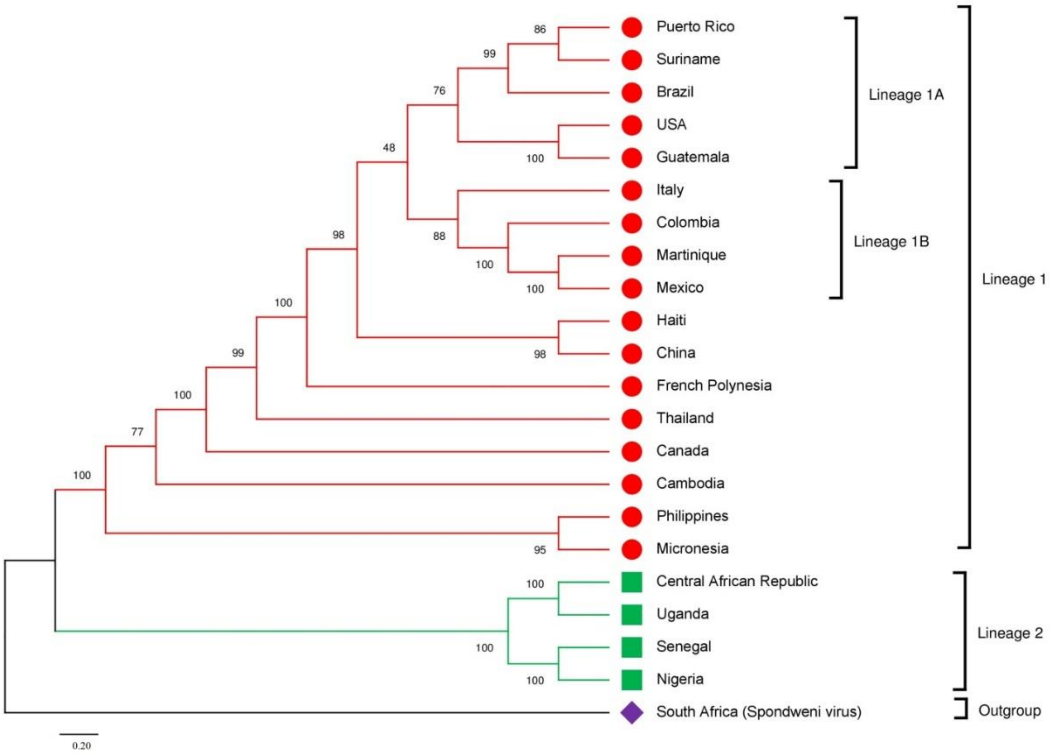

Fig. 1. Phylogenetic analysis of the 22 whole-genome ZIKV nucleotide strains, rooted by SPOV. The tree was inferred by the maximum likelihood method based on the GTR model. Evolutionary analysis was directed in MEGAX tool. In the final dataset there were overall 10787 positions after complete deletion of gaps and missing data.

AMINO ACID SEQUENCES BASED PHYLOGENETIC REPRESENTATION

Phylogenetic examination of 21 ZIKV isolates derived from whole amino acid genome was inferred by maximum likelihood method showed four distinct clusters in lineage 1 (Fig 2). In this analysis, the cluster 1A with more than 90 bootstrap supports and the isolates fell are Mexico (Accession No: KU922923), Martinique (Accession No: U647676), and Colombia (Accession No: KU820897). Consequently, there was a significant change among the whole genome nucleotide (Fig 1) as well as amino acid generated trees (Fig. 2).

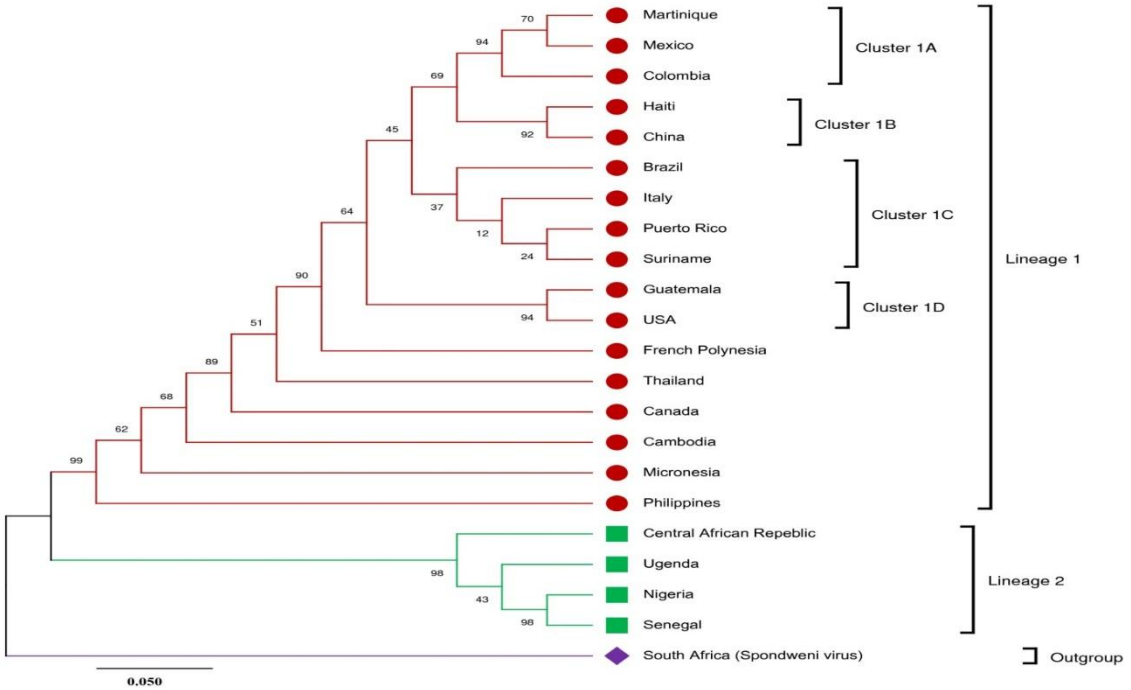

Fig. 2. Phylogenetic analysis of the 22 whole-genome ZIKV amino acid strains, rooted by SPOV. The tree was inferred by the maximum likelihood method. Evolutionary analysis was directed in MEGAX tool. In the final dataset there were overall 3423 positions after complete deletion of gaps and missing data.

PHYLOGENETIC ANALYSIS BASED ON CAPSID (C) GENE

The evolutionary analysis was inferred by ML method based on capsid (C) gene indicated that the topologies were dissimilar comparatively to complete nucleotide and amino acid genome phylogenies, both strains distribution in various clusters or bootstrap sustenance. With the inconsistency such as translocation of certain isolates between the sub-clusters within lineage 1 (Fig 3 and 4) resulted, while analysis performed on C gene NT and AA sequences. While, analyzing the amino acid based tree (Fig 4) the isolate Haiti was found at a distinct position in cluster 1A. Similarly, the isolates (Philippines, Cambodia and Micronesia) formed a distinct sub-cluster.

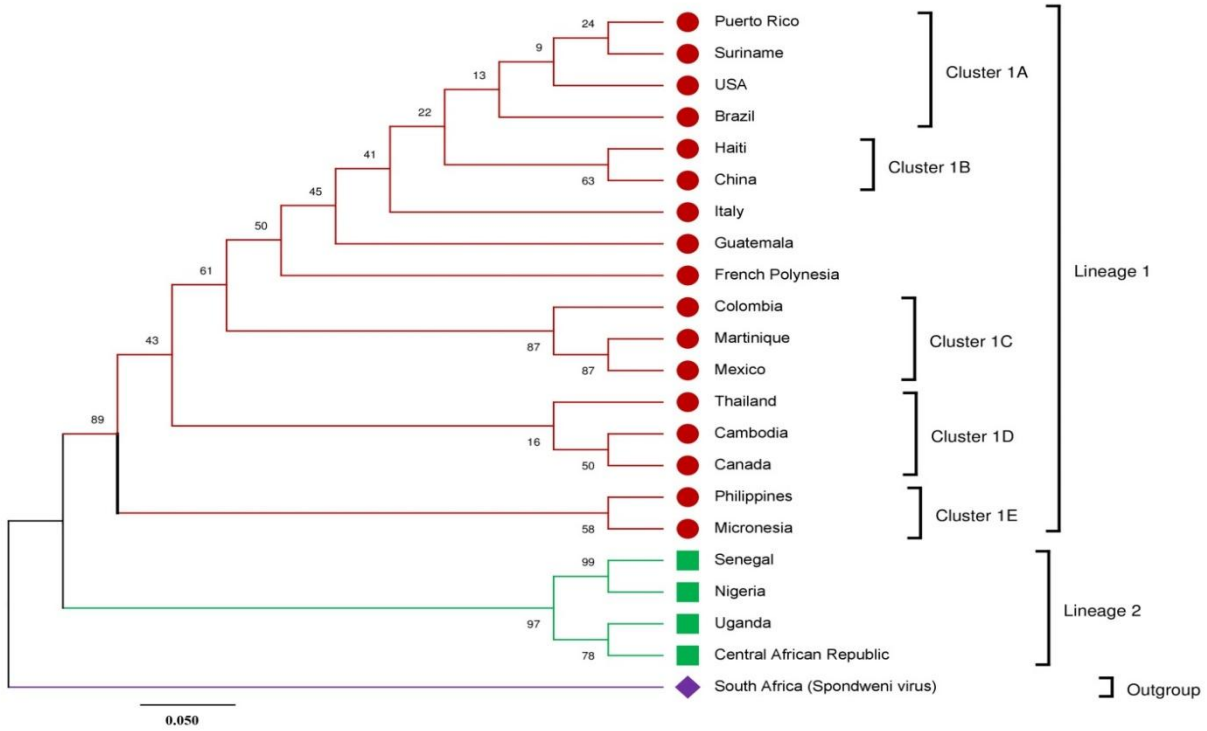

Fig. 3. Phylogenetic analysis of the 22 individual Capsid ZIKV gene nucleotide strains, rooted by SPOV. The tree was inferred by the maximum likelihood method based on Kimura 2-parameter model. Evolutionary analysis was directed in MEGAX tool. In the final dataset there were overall 651 positions after complete deletion of gaps and missing data.

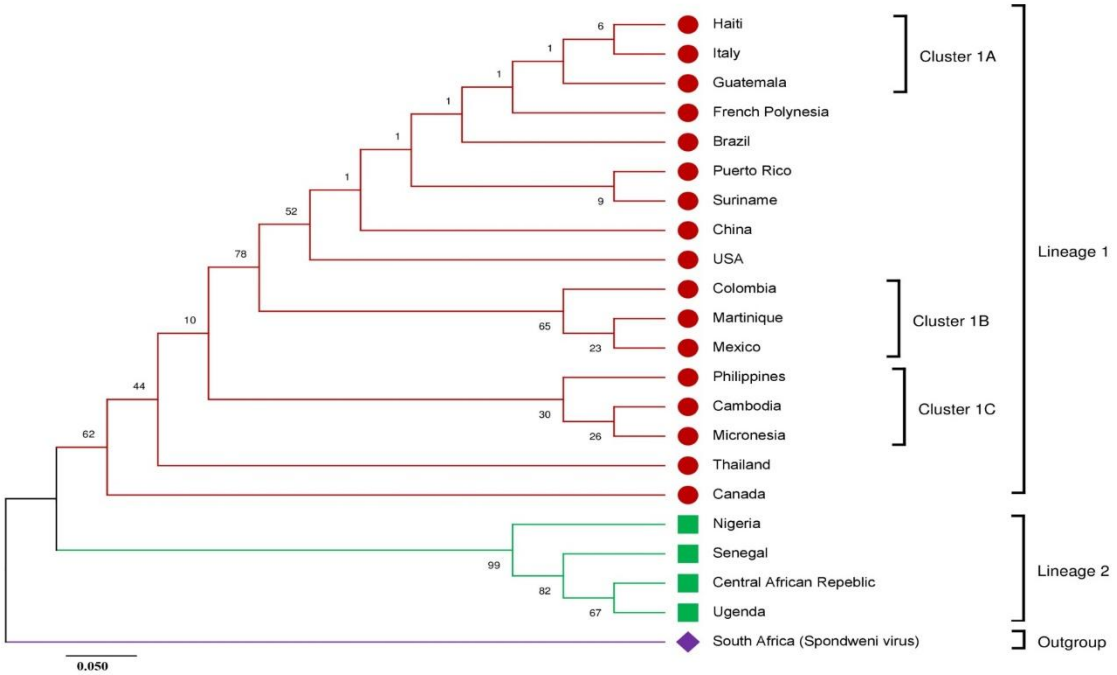

Fig. 4. Phylogenetic analysis of the 22 individual Capsid ZIKV amino acid strains, rooted by SPOV. The tree was inferred by the maximum likelihood method based on JTT matrix model. Evolutionary analysis was directed in MEGAX tool. In the final dataset there were overall 217 positions after complete deletion of gaps and missing data.

DISCUSSION

A number of genus *flaviviruses*, including DENV, Yellow Fever Virus (YFV), Japanese Encephalitis Virus (JEV) as well as West Nile Virus (WNV), annually instigating infections globally. Infections specific with arbovirus are contingent on a change of non-environmental influences, which disseminate the viruses to places where mosquito vectors previously exist ascribed to human mobility from travel, trade and migration (21, 22).

For a broad depiction of mosquito-transmitted *flaviviruses*, it is necessary to investigate its genomic behaviors in the same way mode of transmission. Previously, number of full genome and partial gene sequences of zika virus (ZIKV) have been used for phylogenetic study (23-26). Most of the studies used envelop (E) and non-structural 5 (NS5) polyprotein sequences of zika virus (ZIKV) (23, 27-31). For example during the 2014 ZIK-virus outbreak in Haiti a phylogenetic study based on whole genome was conducted and revealed that the sequences of Haiti from South American cluster within Asian lineage (15).

Formerly, investigators were more focused on individual genes for the genotyping of the ZIKV; in Brazil based on E and NS5 genes phylogenetic study revealed that the sequences from the suspected were clustered with the strains belong to the regions where ZIKV outbreaks previously occurred such as South and North-America, Pacific as well as Southeast Asia (23).

However, for the identification of genetic variation, and ZIKV genotyping investigators are using nucleotide as well as amino acid sequences. A genomic variation based on complete genome ZIKV strains were recognized and observed 34 mutations in amino acid and greater than 400 mutations in the nucleotide sequences (32).

In this study, 21 full genomes equally (nucleotide and amino acid) of ZIKV isolates described from geographically discrete regions were investigated, the strains distributed into two main lineages (1 and 2), which confirmed the previous findings by using the sequences of whole genome (10).
On the other hand by analyzing the whole genome polyprotein sequences an additional significant fact figured out that we perceive many ZIKV isolates exchange clusters subsequently study of amino acid sequences.

In the current study, phylogenetic trees produced by means of Maximum Likelihood technique from whole genomes exhibited two major lineages (1, and 2) and an out group (Fig. 1).
While a considerable change observed in the topologies of the partial nucleotide gene and amino acid sequences (Figure 3 & 4) were examined. Similarly, analysis through Capsid protein isolates as of regions corresponding Puerto Rico, Suriname, Haiti, and Italy focused on a solitary node however, formerly observed at different positions when study was achieved via Capsid nucleotide gene (Fig. 3, 4).

Furthermore, analysis of phylogenetic tree based on whole genome nucleotide the lineage 1 which has been divided into two sub lineages (1A and 1B) had a distinctive tree pattern comparatively amino acid (Fig. 3, 4). Study revealed the fundamental variance of the lineage 1, proposing a contemporary shared ancestor. Within the sub lineage 1A, strains with Puerto Rico, Suriname, Brazil, USA, and Guatemala. In the same way strains which had shared ancestor are Mexico, Martinique, Colombia, and Italy.

In the early stage of the investigation, we hypothesized that the nucleotide as well as amino acid based phylogenies depicted the consistent outcomes. On the other hand there was a vast contradiction in our analysis. This was may be the consequence of the codons biasness. Variations at nucleotide level are at certain time not revealed at the amino acid level. Therefore, in the current investigation phylogenies created from nucleotide sequences dissimilar comparatively to amino acid constructed tree such as certain strains changed clusters in amino acid phylogenetic representation (Fig. 1, 2).

These results proposed that a considerable change in the viral evolution between whole (ZIKV) and individual Capsid structural region. The topological network from whole-genomes comes to be considerably diverse to the topology from the individual gene. Furthermore, investigation of sequences based on

complete genome of nucleotide as well as amino acid suggested that the coding "Capsid" region is also guided the evolutionary pathway of ZIKV genome.

CONCLUSION

The current investigation of phylogenetic trees based on full genome and partial gene permitted us to estimate the evolutionary antiquity and the passage configurations that were connected with the introduction of zika virus (ZIKV). The comparative analysis indicates the closely but not the entirely evolutionary tendencies among the full genome and partial gene. The full genome and partial gene nucleotide and amino acid sequences of ZIKV strains aimed at this study will serve as baseline for the future epidemiology and evolutionary map globally.

Conflicts of interest:

The authors declare no conflict of interest.

References:

1. Tognarelli J, Ulloa S, Villagra E, Lagos J, Aguayo C, Fasce R, et al. A report on the outbreak of Zika virus on Easter Island, South Pacific, 2014. Archives of virology. 2016;161(3):665-8.
2. Dick G, Kitchen S, Haddow A. Zika virus (I). Isolations and serological specificity. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1952;46(5):509-20.
3. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerging infectious diseases. 2008;14(8):1232.
4. Musso D, Nilles E, Cao-Lormeau VM. Rapid spread of emerging Z ika virus in the P acific area. Clinical Microbiology and Infection. 2014;20(10):O595-O6.
5. Haddow AD, Schuh AJ, Yasuda CY, Kasper MR, Heang V, Huy R, et al. Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. PLoS neglected tropical diseases. 2012;6(2):e1477.
6. Guerbois M, Fernandez-Salas I, Azar SR, Danis-Lozano R, Alpuche-Aranda CM, Leal G, et al. Outbreak of Zika virus infection, Chiapas State, Mexico, 2015, and first confirmed transmission by Aedes aegypti mosquitoes in the Americas. The Journal of infectious diseases. 2016;214(9):1349-56.
7. Lazear HM, Diamond MS. Zika virus: new clinical syndromes and its emergence in the western hemisphere. Journal of virology. 2016;90(10):4864-75.
8. Weaver SC, Costa F, Garcia-Blanco MA, Ko AI, Ribeiro GS, Saade G, et al. Zika virus: History, emergence, biology, and prospects for control. Antiviral research. 2016;130:69-80.
9. Calvez E, O'connor O, Pol M, Rousset D, Faye O, Richard V, et al. Differential transmission of Asian and African Zika virus lineages by Aedes aegypti from New Caledonia. Emerging microbes & infections. 2018;7(1):159.
10. Gu SH, Song DH, Lee D, Jang J, Kim MY, Jung J, et al. Whole-genome sequence analysis of Zika virus, amplified from urine of traveler from the Philippines. Virus Genes. 2017;53(6):918-21.
11. Tripathi S, Balasubramaniam VR, Brown JA, Mena I, Grant A, Bardina SV, et al. A novel Zika virus mouse model reveals strain specific differences in virus pathogenesis and host inflammatory immune responses. PLoS pathogens. 2017;13(3):e1006258.
12. Shrivastava S, Puri V, Dilley KA, Ngouajio E, Shifflett J, Oldfield LM, et al. Whole genome sequencing, variant analysis, phylogenetics, and deep sequencing of Zika virus strains. Scientific reports. 2018;8(1):15843.
13. Kauffman EB, Kramer LD. Zika virus mosquito vectors: competence, biology, and vector control. The Journal of infectious diseases. 2017;216(suppl_10):S976-S90.
14. Poland GA, Kennedy RB, Ovsyannikova IG, Palacios R, Ho PL, Kalil J. Development of vaccines against Zika virus. The Lancet infectious diseases. 2018;18(7):e211-e9.
15. Lednicky J, Beau De Rochars VM, El Badry M, Loeb J, Telisma T, Chavannes S, et al. Zika virus outbreak in Haiti in 2014: molecular and clinical data. PLoS neglected tropical diseases. 2016;10(4):e0004687.
16. Giovanetti M, Milano T, Alcantara LC, Carcangiu L, Cella E, Lai A, et al. Zika Virus spreading in South America: Evolutionary analysis of emerging neutralizing resistant Phe279Ser strains. Asian Pacific journal of tropical medicine. 2016;9(5):445-52.

17. Zhu Z, Chan JF-W, Tee K-M, Choi GK-Y, Lau SK-P, Woo PC-Y, et al. Comparative genomic analysis of pre-epidemic and epidemic Zika virus strains for virological factors potentially associated with the rapidly expanding epidemic. Emerging microbes & infections. 2016;5(1):1-12.
18. Hatcher EL, Zhdanov SA, Bao Y, Blinkova O, Nawrocki EP, Ostapchuck Y, et al. Virus Variation Resource–improved response to emergent viral outbreaks. Nucleic acids research. 2016;45(D1):D482-D90.
19. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic acids research. 1994;22(22):4673-80.
20. Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. Molecular biology and evolution. 2021;38(7):3022-7.
21. Wilder-Smith A. Murray and Quam. Epidemiology of Dengue: Past, Present and Future Prospects Clin Epidemiol. 2013;299.
22. Weaver SC, Reisen WK. Present and future arboviral threats. Antiviral research. 2010;85(2):328-45.
23. Calvet G, Aguiar RS, Melo AS, Sampaio SA, De Filippis I, Fabri A, et al. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. The Lancet infectious diseases. 2016;16(6):653-60.
24. Camacho E, Paternina-Gomez M, Blanco PJ, Osorio JE, Aliota MT. Detection of autochthonous Zika virus transmission in Sincelejo, Colombia. Emerging infectious diseases. 2016;22(5):927.
25. Weber DS, Alroy KA, Scheiner SM. Phylogenetic insight into Zika and emerging viruses for a perspective on potential hosts. Ecohealth. 2017;14(2):214-8.
26. Shrivastava S, Puri V, Dilley KA, Ngouajio E, Shifflett J, Oldfield LM, et al. Whole genome sequencing, variant analysis, phylogenetics, and deep sequencing of Zika virus strains. Scientific reports. 2018;8(1):1-11.
27. Zanluca C, Melo VCAd, Mosimann ALP, Santos GIVd, Santos CNDd, Luz K. First report of autochthonous transmission of Zika virus in Brazil. Memórias do Instituto Oswaldo Cruz. 2015;110(4):569-72.
28. Buathong R, Hermann L, Thaisomboonsuk B, Rutvisuttinunt W, Klungthong C, Chinnawirotpisan P, et al. Detection of Zika virus infection in Thailand, 2012–2014. The American journal of tropical medicine and hygiene. 2015;93(2):380-3.
29. Alera MT, Hermann L, Tac-An IA, Klungthong C, Rutvisuttinunt W, Manasatienkij W, et al. Zika virus infection, Philippines, 2012. Emerging infectious diseases. 2015;21(4):722.
30. Hayes EB. Zika virus outside Africa. Emerging infectious diseases. 2009;15(9):1347.
31. Villamil-Gómez WE, Rodríguez-Morales AJ, Uribe-García AM, González-Arismendy E, Castellanos JE, Calvo EP, et al. Zika, dengue, and chikungunya co-infection in a pregnant woman from Colombia. International Journal of Infectious Diseases. 2016;51:135-8.
32. Wang A, Thurmond S, Islas L, Hui K, Hai R. Zika virus genome biology and molecular pathogenesis. Emerging microbes & infections. 2017;6(1):1-6.