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SHORT COMMUNICATION

Chikungunya outbreak in Karachi Pakistan 2016-2017: An analysis of viral isolates

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Abstract

In December 2016 physicians in Karachi, Pakistan,witnessed an increase in patients presenting with febrile illness and severe polyarthralgia. Subsequently, chikungunya virus (CHIKV)) was isolated from three patients. This virus was sequenced and compared with other isolates of CHIKV obtained in India and Pakistan during recent outbreaks. Phylogenetic analysis indicated that the Karachi isolates were most similar to the East Central South African CHIKV lineage and showed sequence homology to isolates obtained in other parts of Pakistan and India. More importantly, two of the CHIKV isolates had a nucleotide substitution in the E1 gene corresponding to an amino acid change at chain F portion of the E1 protein.

Keywords: Chikungunya virus, Arbovirus, Pakistan, Karachi-Sindh, Nucleotide sequence.

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Introduction

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that produces severe polyarthralgia, joint pains, and rash.[1] Over the past decade, CHIKV has emerged in many new regions such as Europe, the Western Hemisphere, and the Pacific Islands.[2] In December 2016, physicians in Karachi, Pakistan started seeing a sudden increase in patients presenting with febrile illness with severe arthralgia. Some reports mentioned an outbreak of CHIKV in Pakistan including Karachi with 818 suspected cases (between December 19, 2016 and February 22, 2017) and 107 confirmed cases (as of July 2017).[3-5]

As part of a cross-sectional, observational study performed at the Aga Khan University Hospital (AKUH) to identify which arboviruses were the cause of acute undifferentiated febrile illness in Pakistan, patients were enrolled to assist physicians in diagnosing the cause of these symptoms.[3,4] CHIKV was subsequently isolated from three patients presenting with headache, arthralgia, myalgia, and vomiting. Prior to this outbreak, there were no data for sequences of CHIKV from Pakistan in GenBank. Recently, seven full CHIKV genome sequences have been submitted from the 2016 CHIKV outbreak in Pakistan. For this report we sequenced the envelope (E1) protein and performed a phylogenetic analysis utilising sequence data from isolates obtained during the 2016 Pakistan and 2015-2016 India outbreaks from GenBank. This finding complements previous CHIKV sequences published in the GenBank, since these samples were collected by a different hospital. In addition, examining the evolution of CHIKV can highlight routes of virus dissemination which will help local authorities implement better surveillance which may decrease the disease burden of CHIKV in Pakistan.

Patients and Methods

A cross-sectional study enrolling patients meeting the CDC (Centres for Disease Control)[6] clinical description of arboviral disease was conducted from April 2015 to July 2017.[4,6] Patients included males and females between 10 to 86 years of age on the day of enrollment meeting the enrollment criteria.[3]

This study was performed to identify which arboviruses (DENV, WNV, Japanese Encephalitis virus (JEV), and CHIKV) were the cause of acute undifferentiated febrile illness in the Sindh region of Pakistan.[4]

Clinical findings at the time of presentation for confirmed CHIKV exposed patients included headache, fever, rash, myalgia, arthralgia, eye pain, gastrointestinal symptoms, and symptoms of involvement of the central nervous system (CNS) including vertigo, altered mental state, seizures, ADEM, and encephalitis.[3]

A total of 1,000 patients (250/year) were targeted for enrollment under informed consent procedures that were reviewed and approved by the Ethics Review Committee, Aga Khan University (#3183-PAT-ERC-14) and the Institutional Review Board, University of Florida (#201500908).[4] All enrolled subjects gave written informed consent in accordance with the Declaration of

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Table-1: List of published sequences used in the phylogenetic analysis.

Accession number	Strain/ Sample ID	Origin	Year of isolation/ submission
AF490259	Ross	Tanzania	1953
AY549581	DRC1725	Congo	2000
DQ443544	LR2006OPY1	Reunion	2006
AY726732	37997	Senegal	1983
AF192900	SV045196	Thailand	1996
AF192902	P0731460	India	1973
AY424803	653496	India	2004
EF012359	D570	Mauritius	2006
EF193853	D586	Mauritius	2006
EF193854	D933	Seychelles	2006
EF193855	D1355	Mauritius	2006
EF193856	D1563	India	2006
EF193857	D2898	India	2006
EF193858	D3045	India	2006
EF193859	D3546	India	2006
EF533650	D5565	India	2006
MF774619	10/2016	Pakistan	2016
MF774618	09/2016	Pakistan	2016
MF774617	07/2016	Pakistan	2016
MF774616	06/2016	Pakistan	2016
MF774615	05/2016	Pakistan	2016
MF774614	04/2016	Pakistan	2016
MF774613	01/2016	Pakistan	2016
KX619424	JHCK96	India	2015
KX619425	JHCK87	India	2015
KX619426	JHCK128	India	2015
KY751908	IN16C1	India (Australia)	2016
KY057363	119067	India	2016
KT336777	RGCB711/09	India	2009
KJ679577	STMWG01	India	2011
KF818472	IND-12-WBST1	India	2012
MG516709	Karachi-1	Pakistan	2016
MG516710	Karachi-2	Pakistan	2016
MG516711	Karachi-3	Pakistan	2016

Helsinki.

Testing for Flaviviruses: Patients were screened for CHIKV IgM using a commercial ELISA (InBiosCHIKjjDetect™, InBios, Seattle, United States). The samples of all CHIKV IgM positive cases were screened for CHIKV via real-time PCR (rtPCR) of the NS2A gene.[7] Patients positive for CHIKV IgM were also tested for DENV NS1, WNV IgM and JEV IgM to rule out potential co-infections using a commercial kit (Panbio® Dengue Early Rapid,Alere, Waltham, Massachusetts, United States).

PCR for sequencing: Three rt PCR positive serum samples (Karachi-1, Karachi-2, and Karachi-3) were chosen for DNA sequencing of the envelope (E) 1 gene of the CHIKV.[8] Polymerase chain reaction (PCR) consisted of 500 nM forward and reverse primers, 25 µL of Q5® High-Fidelity 2X Master Mix (New England Bioscience, Ipswich, MA, United States), 2 µL of DNA template, and nuclease-free water to make 50 µL of reaction. The cycling conditions were 98°C for 30 seconds, followed by 45 cycles of 98°C for 10 seconds, 67°C for 30 seconds, 72°C for 30 seconds, and the final annealing was one cycle of 72°C for 2 minutes.[8] Once a single DNA band was confirmed in 2% agarose gel, a gel recovery kit (Zymoclean™ Gel DNA Recovery Kit, Zymo Research, Irvine, California, United States) was used to purify DNA from the gel according to the manufacturer's instructions. Purified DNA was sequenced using the Sanger sequencing method by a commercial company.

Sequence analysis: Nucleotide sequences were assembled using Sequencher 4.8 (Gene Codes, Ann Arbor, Michigan, United States). Phylogenetic and molecular evolutionary analyses were conducted using Mega 7.0.[9] Sequences were aligned using the MUSCLE algorithm followed by manual editing. Sequences were aligned and compared with sequences obtained from recent CHIKV outbreaks in India and Pakistan from GenBank.[8] A phylogenetic tree was constructed using Maximum-likelihood method using the best fitting substitution model (K2 + G) in Mega 7.0.[9]

Results

Sequence analysis indicated that the CHIKV isolated from these three patients in Karachi were of the East Central South African (ECSA) lineage (Figure-1). This corresponds to other sequences of CHIKV isolated during the 2016 Pakistan and 2016 India outbreaks.

Based on part of the E1 region sequenced (567 bp), sequences Karachi-1 (Genebank: MG516709) and Karachi-2 (Genebank: MG516710) had one nucleotide difference (99.8% similarity) compared with other CHIKV isolates from Pakistan, with the exception of CHIKV strain 01/2016 (Genebank: MF7746113) which had two nucleotides differences (99.6% similarity). This mutation occurred at nucleotide position 10,856 (Genebank: NC004162) and was a transition from cytosine into thymine (Figure-1). The nucleotide change resulted in an amino acid substitution of isoleucine for threonine (Figure-1). Based on three-dimensional modelling of the mature envelope glycoprotein complex (spontaneous cleavage) performed using a publicly available molecular visualisation programme (NGL in Protein Data Bank (PDB) ID:3N41) (www.rcsb.org), the mutation occured on the F-chain at the 288 residue (Figure-2).[10-13]

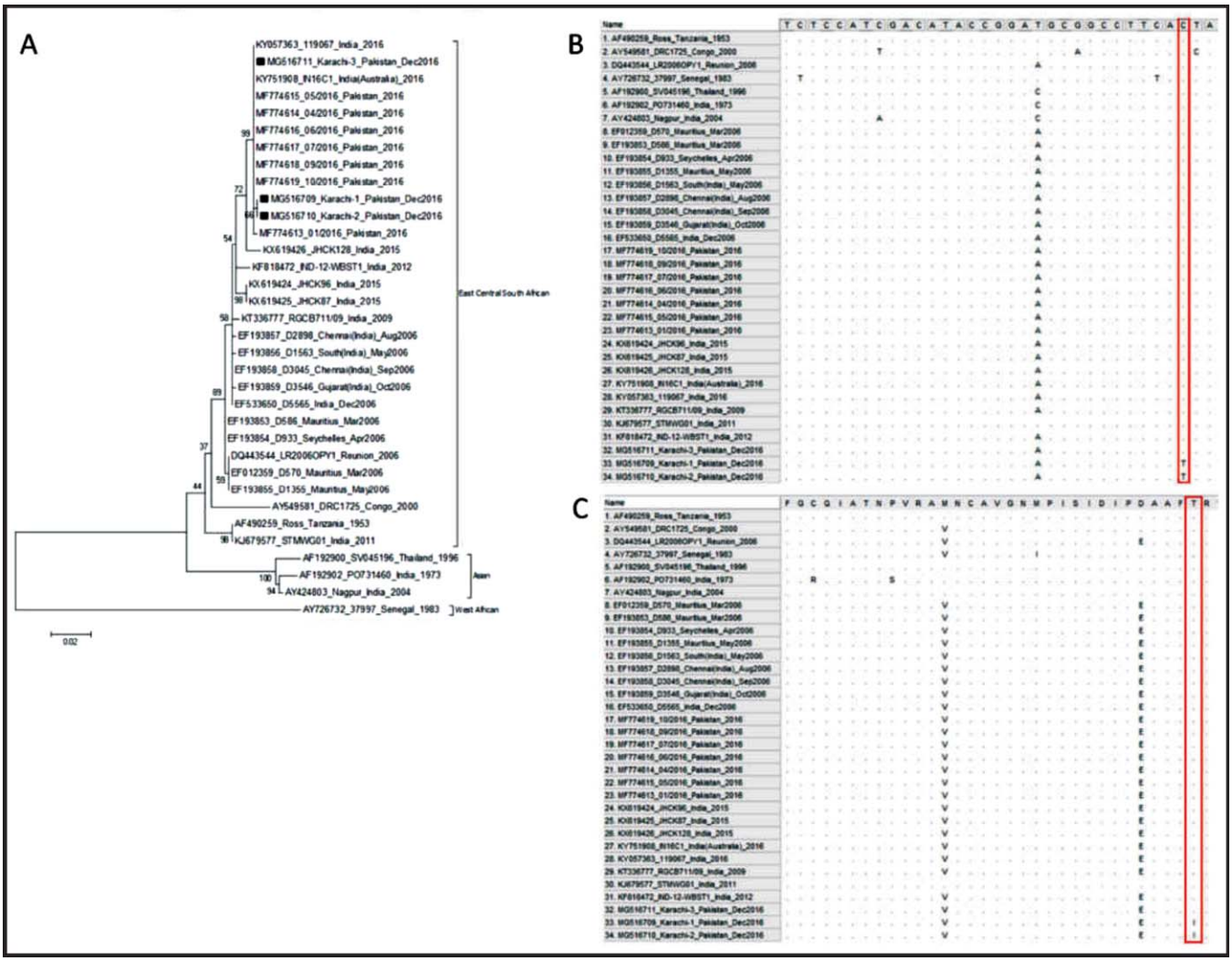

Figure-1: A Maximum Likelihood phylogenetic tree of CHIKV samples used in this paper, nucleotide and amino acid change positions. A) The phylogenetic tree shows three distinct lineages of CHIKV. Three CHIKV sequences from this paper are marked with rectangular boxes in front of the sequence name. The sequences included in this tree are named as follows: GenBank Accession number_ Sequence name_Origin_Month and/or Year. One thousand bootstrap replicates were used to assess statistical robustness and reliability of the branching order within the tree. The numbers on the branches represent bootstrap values. B) The position of the nucleotide change for Karachi-1 and Karachi-2 is marked by a red square. The picture is taken from the nucleotide sequences analysis using Mega version 7 software. C) The position of the amino acid change for Karachi-1 and Karachi-2 is marked by a red square. The amino acid change is a non-synonymous mutation. The picture is taken from the amino acid sequences analysis using Mega version 7 software.

Discussion

Analysis of CHIKV isolated from three patients during the 2016 Karachi outbreak indicated that they were descendants of the ECSA lineage of CHIKV. This is reasonable considering that CHIKV isolated from other areas of Pakistan during the 2016 outbreak were also from the same lineage. The three Karachi sequences were also similar to the CHIKV sequences from India in 2016, IN16C1 (Genebank KY751908) and 119067 (Genebank: KY057363). The 2016 CHIKV outbreak in Pakistan occurred a few months after the CHIKV outbreak in India.[14] It is possible that the 2016 Pakistan outbreak was introduced in the country from India since Pakistan and India share a border which hundreds of people cross each day while travelling between the two countries. Phylogenetic analysis conducted by other researchers using samples collected from the same region indicated that the 2016 CHIKV outbreak in Pakistan most likely came from India,[15] since health screening is not performed at border-crossing for either country.[14]

Based on the E1 region sequenced, two patients had one nucleotide difference (99.8 % similarity) from a third patient (Karachi-3, Accession MG516711) and other recent isolates from Pakistan (CHIKV Pakistan: 10/2016 (MF774619), 09/2016 (MF774618), 07/2016 (MF774617), 06/2016 (MF774616), 05/2016 (MF774615), and 04/2016

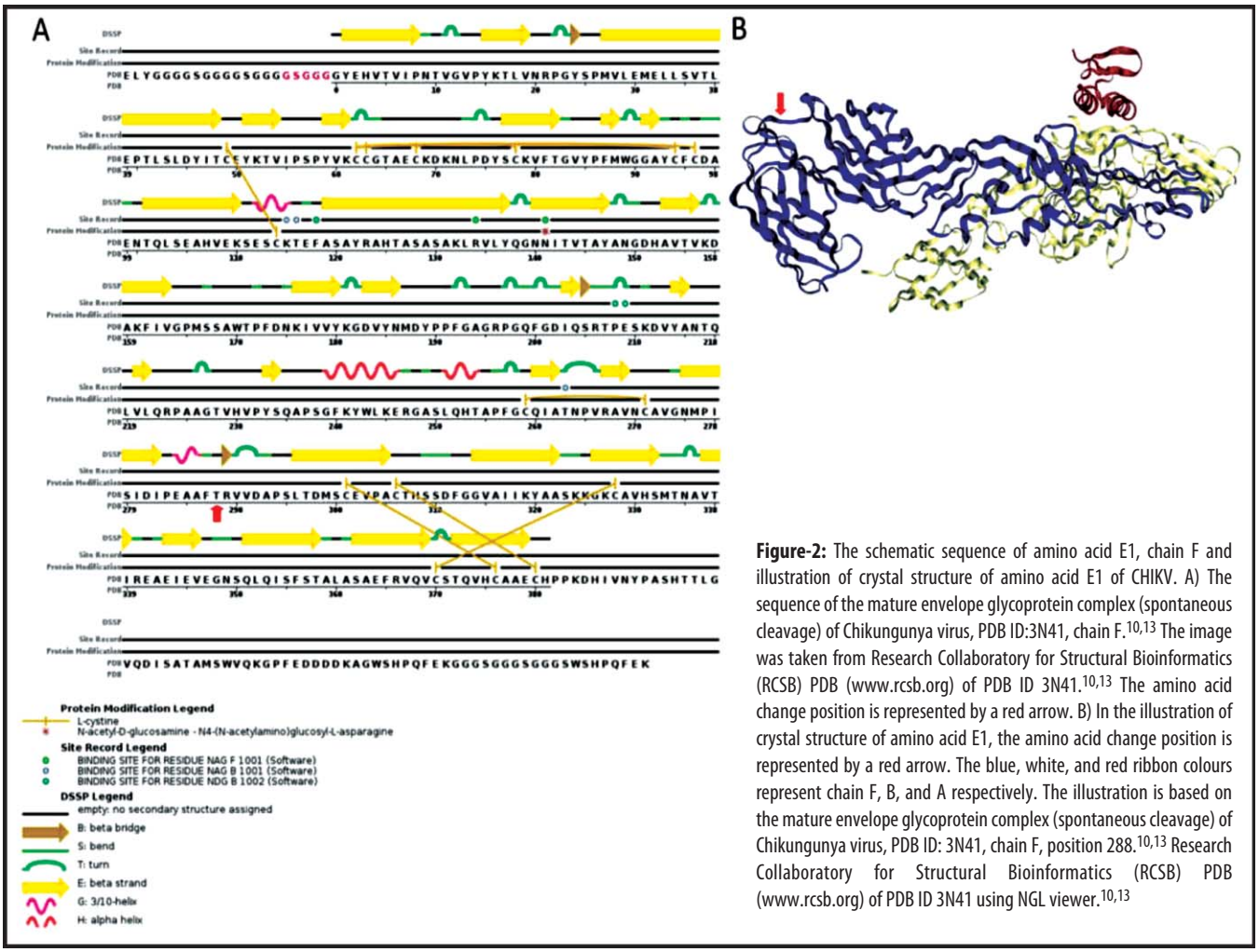

Figure-2: The schematic sequence of amino acid E1, chain F and illustration of crystal structure of amino acid E1 of CHIKV. A) The sequence of the mature envelope glycoprotein complex (spontaneous cleavage) of Chikungunya virus, PDB ID:3N41, chain F.[10,13] The image was taken from Research Collaboratory for Structural Bioinformatics (RCSB) PDB (www.rcsb.org) of PDB ID 3N41.[10,13] The amino acid change position is represented by a red arrow. B) In the illustration of crystal structure of amino acid E1, the amino acid change position is represented by a red arrow. The blue, white, and red ribbon colours represent chain F, B, and A respectively. The illustration is based on the mature envelope glycoprotein complex (spontaneous cleavage) of Chikungunya virus, PDB ID: 3N41, chain F, position 288.[10,13] Research Collaboratory for Structural Bioinformatics (RCSB) PDB (www.rcsb.org) of PDB ID 3N41 using NGL viewer.[10,13]

(MF774614)). The mutation occurs at residue 288 which corresponds to the F chain of the E1 protein (Figure-2). This portion of the protein is involved with dimerisation of the envelope and also functions as a serine type endopeptidase.[10,13] The substitution of isoleucine for threonine may have caused conformational changes in the E1 protein. Threonine is polar, hydrophilic, uncharged amino acid that typically participates in H-bonding, whereas isoleucine is a non-polar, hydrophobic, uncharged, and is not very reactive.[16]

People infected with CHIKV often have clinical symptoms such as sudden onset of high fever, headache, vomiting, rash, and arthralgia.[17] The three patients described herein presented with fever, arthralgia and nausea/vomiting. It is of interest to note that the two patients presenting with respiratory symptoms possessed the amino acid substitution. While we cannot relate this symptom with the mutation, it has been hypothesised that certain mutations can confer viral preference for certain tissues.[18] Additional isolation and in-vivo characterisation for increased virulence is important. Full genome sequencing, more sequencings of additional samples, and more sample data with collection time in combination with location would be useful for phylodynamic and pathogenesis analysis. Comparison of sequences between virus isolated from the respiratory system and virus isolated from blood/serum would be of interest for analysis of intra-host genetic diversity of CHIKV in humans.

Conclusion

The study showed that the CHIKV isolates collected from Karachi were from the ECSA lineage. It was also concluded that the CHIKV outbreak in Pakistan 2016 most likely came from India.

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Conflict of Interest: None to declare

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