HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

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

Short communication doi: 10.1016/S2222-1808(16)61087-3

Continued persistence of ECSA genotype with replacement of K211E in *E1* gene of Chikungunya virus in Delhi from 2010 to 2014

Priyanka Singh[1], Pankaj Sharma[2], Sachin Kumar[1], Mala Chhabra[2], Moshahid Alam Rizvi[3], Veena Mittal[2], Dipesh Bhattacharya[2], Srinivas Venkatesh[1,2], Arvind Rai[1,*]

[1]*Division of Biotechnology, National Centre for Disease Control, 22 Sham Nath Marg, Delhi, 110054, India*

[2]*Division of Zoonosis, National Centre for Disease Control, 22 Sham Nath Marg, Delhi, 110054, India*

[3]*Department of Biosciences, Jamia Millia Islamia University, New Delhi, 110025, India*

ARTICLE INFO

Article history:
Received 31 Mar 2016
Received in revised form 3 May 2016
Accepted 25 May 2016
Available online 21 Jun 2016

Keywords:
Chikungunya virus
E1 gene
Mutation
Genotype
India

ABSTRACT

Chikungunya is a viral disease caused by arthropod-borne Chikungunya virus (CHIKV) and transmitted by *Aedes* mosquitoes. In 2006, CHIKV re-emerged in Indian Ocean islands, Southeast Asia and India. Delhi witnessed the continued occurrence of CHIKV after its first outbreak in 2010. The constant monitoring of the circulating strains of CHIKV is important for designing and executing control strategies. Therefore, the present study was undertaken to unveil the genomic changes of CHIKV in Delhi during the period 2010–2014. RT-PCR of *nsP1* and *E1* gene region of CHIKV was performed for diagnosis and mutational study, respectively. Positive CHIKV samples were processed for the nucleotide sequence of *E1* gene region. Nucleotide alignments of study sequences revealed both synonymous and non-synonymous mutations, although amino acid alignments of all study sequences had single amino acid replacement lysine (K) by glutamic acid (E) at position 211 in *E1* gene. A phylogenetic analysis revealed that all the study sequences clustered in East, Central and South African genotype of CHIKV. The same strain of East, Central and South African genotype is circulating in this region during the period 2010–2014.

1. Introduction

Chikungunya, an acute mosquito-borne disease, is caused by Chikungunya virus (CHIKV) belonging to family Togaviridae, genus *Alphavirus*. The genome of CHIKV is linear, single-stranded and positive-sense ribonucleic acid of approximately 12 kb. CHIKV was first isolated from Tanganyika (Tanzania) in 1953 from the serum of a febrile human patient. The transmission of CHIKV in Africa is maintained in a sylvatic cycle involving wild primates and many species of *Aedes* mosquitoes. *Aedes aegypti* and *Aedes albopictus* are prevalent in India but the former is the main vector in Delhi[1].

CHIKV epidemic of unprecedented magnitude re-emerged in 2005 after 32 years in the islands of Indian Ocean and Southeast Asia including India. The prevalent genotype is East, Central and South African (ECSA) after its reemergence[2-5]. Globally, estimated one million symptomatic cases with 0.1% fatality per year have been reported after this reemergence. Factors such as the lack of herd immunity, vector control, globalization and trade are responsible for the spread of this disease. CHIKV has also acquired biologically important mutations during its evolution, which increases its geographic reach[6]. In 2006, the largest and most severe chikungunya epidemic affected more than 1.3 million populations in 15

*Corresponding author: Dr. Arvind Rai, Division of Biotechnology, National Centre for Disease Control, 22 Sham Nath Marg, Delhi, 110054, India.
Tel: +91-11-23912960
Fax: +91-11-23922677
E-mail: arvindrai.nicd@gmail.com
The journal implements double-blind peer review practiced by specially invited international editorial board members.

states and union territories of India[7]. Delhi experienced the presence of CHIKV in 2006 and remained in circulation up to 2009[8]. In Delhi, the first outbreak of CHIKV along with dengue virus was reported in 2010[9]. Besides, its continued presence was observed in the following years[10]. The present study was undertaken to study the genomic changes of CHIKV in Delhi, the national capital region, during the period 2010–2014.

2. Materials and methods

Acute-phase blood samples referred to the National Centre for Disease Control for diagnosis from different areas of Delhi during 2010–2014 were included in the study. Patients having constitutional symptoms such as headache, joint pain, rash and fever of less than 5 days duration were considered. Samples were studied as a part of the outbreak investigation and post surveillance.

A total of 104 acute-phase serum samples were taken for the study during the period 2010–2014, in which 38 serum samples belonged to 2010, 22 serum samples belonged to 2011, 12 serum samples belonged to 2012, 21 serum samples belonged to 2013 and 11 serum samples belonged to 2014. Viral RNA was isolated from the serum samples using QIAamp Viral RNA Mini kit (Qiagen, Germany) according to the manufacturer's instructions. Initially, the presence of CHIKV was confirmed by performing RT-PCR by amplifying 354 bp fragment from *nsp1* gene using published primers[11]. The samples which were positive for CHIKV infection were further for 1 013 bp fragment of *E1* gene using pre-published primers[12]. RT-PCR was performed using one step RT-PCR kit (Qiagen, Germany). The automated nucleotide sequence of *E1* gene region was performed using BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, USA) on ABI 3130XL automated capillary DNA sequencer (Applied Biosystems, USA). The nucleotide sequences obtained from the study samples were aligned with the reference sequences from diverse geographical areas, which were available at National Center for Biotechnology Information database and S-27 African prototype, using ClustalW multiple alignment software. The sequences were submitted to GenBank for obtaining accession numbers. The multiple alignment of amino acid sequences was carried out to compare studied sequences with reference sequences of the same genotype and other genotype sequences to find out mutational changes. A phylogenetic tree was generated using the neighbour-joining method with bootstrap analysis of 1 000 replicates using the software MEGA, version 6.1.

3. Results

In the present study, out of those 104 clinical samples, 32 samples were found positive for CHIKV infection by RT-PCR of diagnostic *nsP1* gene region. A 1 013 bp *E1* gene fragment was amplified and sequenced for all 32 positive samples. Sixteen from 2010, eight from 2011, two from 2012, four from 2013 and two from 2014 were found positive for CHIKV infection. Out of those 32 samples, 21 were male and 11 were female. Among them, 8, 18 and 6 samples belonged to 60 years, 18–59 years and < 18 years, respectively. The most common clinical symptoms were fever, arthralgia, myalgia and rashes. The derived sequences were submitted to GenBank and the accession numbers were obtained (KU955432-KU955462). The nucleotide alignment of study sequences with respect to the S-27 African prototype having accession number AF369024 revealed substitution without insertion or deletion that leads to synonymous and non-synonymous mutations. Amino acid alignment of the *E1* gene region revealed that all study samples belonged to 2010–2014 showed the replacement of an amino acid at position 211 (K211E) of the *E1* gene region.

A phylogenetic tree was constructed using the study sequences and global sequences including India retrieved from National Center for Biotechnology Information, which depicted that all CHIKVs were grouped into three genotypes, namely ECSA, Asian and West African genotypes. The phylogenetic tree revealed that all the studied CHIKV sequences from the year 2010, 2011, 2012, 2013 and 2014 having accession numbers KU955432-47, KU955448-55, KU955456-57, KU955458-61, KU955462-63, respectively, were present in the ECSA genotype. The study sequences shared the same genotype in the phylogenetic tree with strains reported from different parts of India (HM159385, HM159390, EF555200, HM159384, EF027137, EF210157, EF555198, EF027138, GQ996376, GQ996377, JN711127, JN711130, EU287996, EU287996, EU287999) belonging to different years. The strains reported from Singapore (FJ445510), Sri Lanka (FJ445427, FJ445426, FJ513632), Japan (AB455494), Italy (EU244823), Malaysia (FN295485), Australia (GQ168719), Reunion Island (AM258992), Mauritius (EU564334), Tanzania (AF192905), South Africa (AF192904), Cameroon (EF051584), Uganda (AF192907) and Republic of Congo (AY549583) also belonged to the ECSA genotype.

However, the earlier reported CHIKV sequences from India (AF192902, AY424803, EF027140), Thailand (AF192896, EF452493, AF192898), Taiwan (EU192143), Philippines (AF192895), Malaysia (FN295483) and Nigeria (AF192899) formed a separate cluster of the Asian genotype, while the West African genotype was represented by the sequences of Senegal

(AF192891, AF192892, AY726732) and Nigeria (AF192893).

4. Discussion

CHIKV infection has emerged and re-emerged in tropical and subtropical regions of the world as a result of changes in human activities and ecology. The reemergence of chikungunya in Indian Ocean island nations and Southeast Asia region especially, India has been coupled with mortality and greater morbidity. In North India, CHIKV emerged for the first in 2006 and remained in circulation at different rates in this region. This study was conducted to monitor the genomic changes, phylogenetic analysis and clustering of genotypes of the circulating strains. The ECSA genotype of CHIKV was circulating in the Delhi region during 2010–2014. The same genotype was reported in Indian Ocean islands, Southeast Asia, India and Delhi since its reemergence in 2006. A nonsynomous mutation K211E has been detected in all CHIKV samples during 2010–2014. Earlier, this mutations was reported in New Delhi samples of the year 2010[13]. This mutation has been reported previously in an isolate from Kerala and one imported case from Rajasthan[14,15]. Few Sri Lankan isolates have asparagine at this position in ECSA genotype[16]. Therefore, it can also be concluded that this amino acid position in the *E1* gene could be variable. However, further studies are required to detect their role in virulence and pathogenesis of the virus, but till then close monitoring of circulating strains are important for designing and executing control strategies.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors gratefully acknowledge the financial support from the National Centre for Disease Control during the course of this study.

References

[1] Chhabra M, Mittal V, Bhattacharya D, Rana UVS, Lal S. Chikungunya fever: a re-emerging viral infection. *Indian J Med Microbiol* 2008; **26**(1): 5-12.

[2] Yergolkar PN, Tandale BV, Arankalle VA, Sathe PS, Sudeep A, Gandhe SS, et al. Chikungunya outbreaks caused by African genotype, India. *Emerg Infect Dis* 2006; **12**(10): 1580-3.

[3] Bessaud M, Peyrefitte CN, Pastorino BA, Tock F, Merle O, Colpart JJ, et al. Chikungunya virus strains, Reunion Island outbreak. *Emerg Infect Dis* 2006; **12**(10): 1604-6.

[4] Kariuki Njenga M, Nderitu L, Ledermann JP, Ndirangu A, Logue CH, Kelly CH, et al. Tracking epidemic Chikungunya virus into the Indian Ocean from East Africa. *J Gen Virol* 2008; **89**(Pt 11): 2754-60.

[5] Yusof MP, Kuen LS, Adnan N, Razak NIA, Zamri A, Hulaimi KI, et al. Epidemiology and molecular characterization of Chikungunya virus involved in the 2008 to 2009 outbreak in Malaysia. *J Gen Mol Virol* 2011; **3**(2): 35-42.

[6] Singh P, Chhabra M, Mittal V, Sharma P, Rizvi MA, Chauhan LS, et al. Current research and clinical trials for a vaccine against Chikungunya virus. *Vaccine Dev Ther* 2013; **3**: 35-46.

[7] Dash PK, Parida MM, Santhosh SR, Verma SK, Tripathi NK, Ambuj NK, et al. East Central South African genotype as the causative agent in the reemergence of Chikungunya outbreak in India. *Vector Borne Zoonotic Dis* 2007; **7**(4): 519-27.

[8] Singh P, Mittal V, Rizvi MA, Bhattacharya D, Chhabra M, Rawat DS, et al. Northward movement of East Central South African genotype of Chikungunya virus causing an epidemic between 2006–2010 in India. *J Infect Dev Ctries* 2012; **6**(7): 563-71.

[9] Singh P, Mittal V, Rizvi MM, Chhabra M, Sharma P, Rawat DS, et al. The first dominant co-circulation of both dengue and Chikungunya viruses during the post-monsoon period of 2010 in Delhi, India. *Epidemiol Infect* 2012; **140**(7): 1337-42.

[10] Government of India. Clinically Supected Chikungunya Fever Cases Since 2010. New Delhi: Government of India; 2016. [Online] Available from: http://www.nvbdcp.gov.in/chik-cd.html [Accessed on 15th February, 2016]

[11] Hasebe F, Parquet MC, Pandey BD, Mathenge EGM, Morita K, Balasubramaniam V, et al. Combined detection and genotyping of Chikungunya virus by a specific reverse transcription polymerase chain reaction. *J Med Virol* 2002; **67**(3): 370-4.

[12] Schuffenecker I, Iteman I, Michault A, Murri S, Frangeul L, Vaney MC, et al. Genome microevolution of Chikungunya viruses causing the Indian Ocean outbreak. *PLos Med* 2006; **3**(7): e263.

[13] Shrinet J, Jain S, Sharma A, Singh SS, Mathur K, Rana V, et al. Genetic characterization of Chikungunya virus from New Delhi reveal emergence of a new molecular signature in Indian isolates. *Virol J* 2012; **9**: 100.

[14] Kumar NP, Mitha MM, Krishnamoorthy N, Kamraj T, Joseph R, Jambulingam P. Genotyping of virus involved in the 2006 Chikungunya outbreak in South India (Kerala and Puducherry). *Curr Sci* 2007; **93**(10): 1412-6.

[15] Grandadam M, Caro V, Plumet S, Thiberge JM, Souarès Y, Failloux AB, et al. Chikungunya virus, Southeastern France. *Emerg Infect Dis* 2011; **17**(5): 910-3.

[16] Hapuarachchi HC, Bandara KB, Sumanadasa SD, Hapugoda MD, Lai YL, Lee KS, et al. Re-emergence of Chikungunya virus in South-east Asia: virological evidence from Sri Lanka and Singapore. *J Gen Virol* 2010; **91**(Pt 4): 1067-76.