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

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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
states and union territories of India\cite{7}. Delhi experienced the presence of CHIKV in 2006 and remained in circulation up to 2009\cite{8}. In Delhi, the first outbreak of CHIKV along with dengue virus was reported in 2010\cite{9}. Besides, its continued presence was observed in the following years\cite{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 \textit{nsp1} gene using published primers\cite{11}. The samples which were positive for CHIKV infection were further for 1 013 bp fragment of \textit{E1} gene using pre-published primers\cite{12}. RT-PCR was performed using one step RT-PCR kit (Qiagen, Germany). The automated nucleotide sequence of \textit{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 \textit{nsp1} gene region. A 1 013 bp \textit{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 \textit{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 \textit{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, EF207138, 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, AF192904, 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.
was reported in New Delhi samples of the year 2010 [13]. This isolates have asparagine at this position in ECSA genotype [16].

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 nonsynonymous 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.

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References


