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Molecular epidemiology of Chikungunya virus: Mutation in E1 gene region

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Chikungunya virus is a mosquito-transmitted RNA virus and emerging as a pathogen that has a major public health impact because of the high morbidity including high fever, headache, rash, nausea, vomiting, myalgia, arthralgia with or without neurological manifestation or fulminant hepatitis. One hundred fifty-one patient samples were analyzed during the years 2006–2008, and compared conventional tests and CCRT-PCR (cell culture RT PCR). The conventional tests included ELISA, inoculation into C6/36 cell line and CPE were examined by PCR after RNA extraction. A total of 20/151 (13.2%), 8/151 (5.29%) and 7/151 (4.6%) samples were found to be positive by ELISA, cell culture and PCR, respectively. While 7/20 (35%) of the samples were positive by CCRT_PCR when ELISA 20 positive samples were detected. A total of 5/7 positive strains were sequenced in the E1 gene region. Remarkable changes (M269V, D284E, P294L, S295F, A316V, V322A, and C328W) were observed in the membrane fusion glycoprotein E1. These unique molecular features of the isolates with the continuing epidemic demonstrated high evolutionary potential and thereby indicating higher virulence.

1. Introduction

Chikungunya virus (CHIKV) is a member of the genus *Alphavirus* and belongs to family *Togaviridae*. Chikungunya is a Makonde word meaning 'The one which bends up' referring to the posture of the affected patient due to excruciating pain in the joints (Robinson, 1955). The *Alphaviruses* consist of 30 species of arthropod-borne viruses, which are sub-grouped into seven sero-complexes based on serological data (Porterfield, 1980; Strauss and Strauss, 1994; Van Regenmortel et al., 2000). CHIKV was first isolated from the serum of a febrile patient during a dengue epidemic which occurred in the Newala district, Tanzania, in 1953 (Ross, 1956). In India, the first outbreak of CHIK was recorded in 1963 in Kolkata (Calcutta), followed by epidemics in Tamil Nadu, Andhra Pradesh and Maharashtra. The last outbreak was recorded in Barsi in 1973 before disappearing from the Indian subcontinent. Chikungunya reemerged after several years in Indian states in 2005. By the end of 2006, the virus spread to 15 other States including Kerala during April–July 2007, an outbreak of febrile arthritis started in the Kottayam and Pathanamthitta districts of south Kerala affecting more than 25,000 persons (Santhosh et al., 2008). The *Alphaviruses* are enveloped virus and their genome consists of single stranded, positive sense RNA with approximately 12000 bp nucleotides. Phylogenetic analysis based on E1 gene sequences of CHIKV isolate as three genotypes from worldwide: Asian, East/Central/South African (ECSA) and West African (Powers et al., 2000; Schuffenecker et al., 2006; Powers and Logue, 2007). CHIKV is an important human pathogen which causes a febrile illness characterized by sudden onset of fever, headache, rashes, fatigue, nausea, vomiting, myalgia and severe arthralgia (Thaikruea et al., 1997; Diallo et al., 1999; Powers et al., 2000). The arthralgia may persist in a small proportion of cases for months after other symptoms subsided. These clinical symptoms mimic dengue fever and therefore, many cases of Chikungunya are misdiagnosed as dengue infection (Carey, 1971; Johnston and Peters, 1996; Santhosh et al., 2008). At present, there is no vaccine or antiviral treatment against Chikungunya virus. Its association with a fatal hemorrhagic condition was reported in India (Sarkar et al., 1964). Recently, several isolates from the epidemics in Reunion (2005–2006) and India (2006) have been sequenced (Schuffenecker et al., 2006; Arankalle et al., 2007). The outbreaks in the Ocean regions, which were unprecedented in magnitude, were due to a mutation in the E1 protein of CHIKV (A226V), which helped in the adaptation of the virus to *Aedes albopictus* (Mishra and Ratho, 2006; Schuffenecker et al., 2006). *A. albopictus* is widespread in the India, with an especially high density of mosquitoes in the South India. Humans are the major reservoir of CHIKV for mosquitoes; which transmit the disease by biting an infected person to others. Thus, the onset and extent of the CHIKV epidemic in India are a prospective threat. Therefore, it is important to improve the surveillance system to avert an imported outbreak CHIKV.

In the present study, the nucleotide sequences of viruses isolated from five patients originating from the North India was determined. In addition, partial E1 sequences were determined by

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cell culture RT-PCR (cell culture supernatant) from a total of 20 positive patients. The further determination of the genome structure as well as the unique molecular features of the Indian Ocean isolates, which may distinguish them from other reported CHIKV sequences. In addition, the phylogenetic origin, the diversity, and minor evolution of the CHIKV strains responsible for the Indian outbreak were investigated. The nucleotide and amino acid sequences of CHIKV were compared with other virus strains of Chikungunya.

2. Materials and methods

2.1. Sample collection

A total of 151 serum samples were collected from suspected febrile illness cases from the Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India. In addition, some referred blood/serum samples were also collected from the adjoining district of the hospital in and around Lucknow, UP, India.

All serum samples were tested by ELISA and positive samples were reconfirmed by cell culture and were tested by RT-PCR.

2.2. IgM capture ELISA

ChikV specific IgM antibody (ELISA) detection kit was supplied by the National Institute of Virology (NIV), Pune, India (Yergolkar et al., 2006). 96-Well plates were coated with anti-human IgM (μ chain specific) serum sample in 1:1000 dilution and the well was charged with 100 μl patient serum which were diluted in 1:100. After incubation of 2 h, the wells were washed and Chikungunya antigen was added which binds to capture IgM if the IgM and antigen are homologous. Unbound antigen is removed during a washing step. In the subsequent step Biotinylated Anti Chikungunya monoclonal antibody is added followed by avoiding-HRP. Subsequently, substrate/chromogen is added and watched for the development of color. The reaction is stopped by 1 N H_2SO_4. The intensity of color/optical density is monitored at 450 nm. Optical density values are directly proportional to the amount of CHIKV specific IgM antibodies present in samples. Identification of CHIKV infection should not be based on the result of the ELISA but in conjunction with clinical findings. Epidemiological data and travel history to epidemic area should also be considered. IgM appears in circulation 3–5 post onset days and is a marker of recent infection; therefore the date of sample collection after onset of disease also influences the interpretation of ELISA results. The collection of patient samples within 3–10 days after the onset of disease. All the samples (positive and indeterminate) were tested further with the sensitive cell cultured RT-PCR (CCRT-PCR) method to improve the sensitivity compared with the conventional method.

2.3. Virus culture

Serum specimens from febrile patients were diluted (1:1000) and inoculated into a mono layer of C6/36 cell line (obtained from NIV, Pune, India) cultured with an *A. albopictus* clone and incubated at 28 °C in Eagle's salt essential media, 2% fetal bovine serum, 2% tryptose phosphate broth (TPB) with sodium pyruvate. After 4–5 days inoculated cultures were frozen at −20 °C and thawed at room temperature later a blind passage (one) was made in fresh mono layers of C636 cell line, subsequent cytopathic effects (CPE) were observed and positive samples were kept at −80 °C.

2.4. RNA extraction

The genomic viral RNA was extracted from serum/plasma and cell cultured isolates by QIAamp viral RNA mini kit (QIAGEN, Germany) according to manufacturer's instructions. The RNA was eluted from the QIAspin columns in a final volume of 50 μl of elution buffer and was kept at −80 °C.

2.5. RT-PCR

The partial E1 gene encoding region from CHIKV was amplified by RT-PCR with High Fidelity PrimeScript™ RT-PCR Kit TaKaRa Bio Inc. using primer F 5′-CTGCTCGCTTCGCTACTTGGA-3′ and R 5′-CGGCACCTGTCCTACGAGTTG-3′ according to manufacture's protocol. DNA was eluted from gel piece using QIAGEN gel extraction columns (according to the QIAGEN, Chatsworth, CA, USA protocol). Five selected PCR product of CHIKV isolates were analyzed and purified according to the manufacturer's instructions.

2.6. Sequencing analysis

Sequencing reaction mix consists of 4 μl big dye terminator ready reaction mix, 2 μl of each primer (10 pmol/λ), 3 μl Milli-Q water and 1 μl of template (100 ng/μl). PCR conditions followed by (25 cycles) initial denaturation 96 °C for 1 min, denaturation 96 °C for 10 s, hybridization 50 °C for 5 s and elongation 60 °C for 4 min. ABI 3130 genetic analyzer and chemistry big dye terminator version 3.1 cycle sequencing kit were used for sequencing. Polymer and capillary array used the POP_7 polymer 50 cm capillary array and BDTv3-KB-Denovo_v 5.2 protocol were analyzed, whereas data were analyzed by software Seq Scape_v 5.2 and reaction were tested in the Applied biosystem micro Amp optical 96-well reaction plate.

2.7. Construction of phylogenetic tree

Nucleotide BLASTn analysis (http://www.ncbi.nlm.nih.gov/BLAST) was used to identify related genes of the viruses, and the reference sequences were obtained from GenBank. The ClustalX version 2.0.12 (Thompson et al., 1994) was used to perform multiple nucleotide and amino acid sequence alignment of E1 gene and tree was constructed (Njplot Version 2.3), the neighbor-joining method according to the distances between all pairs of the sequences in a multiple alignment. The confidence of sequence clustering was evaluated by bootstrapping (1000 replicates) in Fig. 2.

2.8. Comparative analysis of amino acid sequences in E1 gene

Amino acid sequences of the Indian isolate virus (SGPGI/2007/01, SGPGI/2007/02, SGPGI/2007/03, SGPGI/2007/04 and SGPGI/2007/05 from Uttar Pradesh, India) proteins was retrieved and compared with all the five isolate virus proteins using the software BioEdit sequence alignment editor version 7.0.5.2.

3. Statistical analysis

Twenty patients in the analysis were those who were confirmed clinically with CHIKV during the period 2006–2008. The frequency distribution of the cases, characteristics and their signs and symptoms were calculated. Attack rates (both overall and stratified by age and sex) were calculated for the six districts which were affected. Confidence interval 95% was also estimated. The paired sample *t*-test was performed with all groups and was calculated separately. The origin and spread of CHIKV cases in the six districts affected initially were plotted. For each case, the address of the individual and the year of the onset of symptoms were analyzed.

4. Results

One hundred and fifty-one case of CHIKV infection were received during 2006–2008, from the State of Uttar Pradesh, India.

Table 1
Summary sheet of positive cases during infection in the state of Uttar Pradesh (India).

S. No.	Symptoms of Chikungunya positive cases	No. of cases (out of 20 positive)
1	Fever[a]	20 (100%)
2	Arthralgia/joint pain[b]	15 (75%)
3	Acute febrile illness	4 (20%)
4	Headache and bodyache	3 (15%)
5	Rash	2 (10%)
6	Arthritis	2 (10%)
7	Arthritis with pedal edema	1 (5%)
8	Vomiting	1 (5%)

Age group	No. of cases (out of 20 positive)
0–30	3 (15%)
31–40	6 (30%)
41–50	7 (35%)
51–60	4 (20%)

Sex	No of cases (out of 20 positive)
Male	13 (65%)
Female	7 (35%)

Recognized place of infection	No of cases (out of 20 positive)
Lucknow	10 (50%)
Kanpur	4 (20%)
Jhanshi	3 (15%)
Mathura	1 (5%)
Allahabad	1 (5%)
Urai	1 (5%)

[a] Mandatory in the case definition.
[b] Not mandatory if diagnosis is laboratory confirmed.

The majority of cases occurred in the Lucknow, Kanpur, Jhansi, Mathura, Allahabad, and Urai, respectively. The distribution of cases by age, sex, symptoms, and place where the infection occurred are shown in Table 1. Most patients reported that they lived or had visited one of the six districts. The first three identified case were male persons living in the Jhansi district of Uttar Pradesh, India. However, any one relative of his/her had arrived from another State of the Country (India) in the Uttar Pradesh during 2006 (an area affected by the CHIKV epidemic). A serum sample that had been collected at an early stage from these patients who was assumed to be the index case, showed high antibody titers against CHIKV (>1:1280). These patients were excluded from further data analyses. The spatial-temporal spread of CHIKV in the mainly affected area and the rest of the area are shown in Fig. 1. After the principal cases, which occurred in the Jhansi, the infection extended both by contiguity, as spreading out of the primary group and by spring from place to place in all districts and, irregular cases and clusters occurring outside the district.

The positive cases were mostly Lucknow (50%), Kanpur (20%), Jhansi (15%), and 5% each from Mathura, Allahabad, and Urai districts (Table 1). A significant association was found between age (0–60) and sex (male/female) of the studied cases 95% CI, 33.97–43.53 ($p < 0.01$), Table 2 which indicates the high risk rate in male (65%) compared to female (35%). Another relationship between age vs. disease also shows higher risk since both gender and disease depends upon the age group which is highly significant ($p < 0.01$). Association of Chikungunya positive in relation to the place and sex was not found to be significant in the present study. The relationship between age group vs. place, shows a high significance ($p < 0.01$) and also an important association with disease occurrence according to sex (male/female), $p = 0.03$ shows that the male group is more infected in contrast to female patients. While there is no association found between places vs. disease. A significant association of CHIKV was found in age vs. sex, disease vs. age, age vs. places, sex vs. disease, and marginal association between places vs. sex, respectively. The frequency of clinical symptoms is shown in Table 1. Patients presented with high fever and most of them had multiple joint pains (75%). About 20% cases of the acute febrile illness, headache, general aches (15%) and some cases of arthritis with edema of legs, vomiting, arthritis and skin rashes, respectively.

All the clinically suspected cases of CHIKV were tested over 3 years (2006–2008), The ELISA result shows 13 (8.6%) cases were positive along with 7 (4.63%) cases equivocally positive and the remaining 131 were negative, The cell culture positivity was 8 (5.29%) while RT PCR positive was 7 (4.63%) directly from clinical samples (Table 3). The flow diagram of all suspected cases with different methods is shown in Fig. 4. Five positive cases were sequenced partially (EU727245–EU727249) for the E1 gene region. Variations in the E1 gene region were found and the isolated viruses were phylogenetically different from the prototype strain.

4.1. Genetic analysis

The partial genomic sequences of five imported CHIKVs (SGPGI/2007/01, SGPGI/2007/02, SGPGI/2007/03, SGPGI/2007/04, and SGPGI/2007/05) from Uttar Pradesh, India were determined (EU727245–EU727249). These isolates were obtained directly from the sera of patients during the acute phase; and cultured in C636 cell line. Using these partial (cds) sequences (EU727247 and EU727248 are nonfunctional polyproteins due to mutation gene; contains the envelope protein E1, partial sequence), to detect genetic differences between these CHIKVs and those isolated during a large outbreak in the Indian region and from other countries. Phylogenetic analysis demonstrated that the five viral sequences similar to the homogeneous complete genome of the Indian sequences (Fig. 2). The sequence of the present isolates was compared to 33 published CHIKV genome sequences from GenBank. These changes produced specific amino acid alterations in SGPGI/2007/01 (E1 S295F and V322A), SGPGI/2007/02 (E1 P294L and V322A), SGPGI/2007/03 (E1 A316V, and V322A), SGPGI/2007/04 (E1 A316V, A322V and C328W), and SGPGI/2007/05 (E1 S295F), these novel

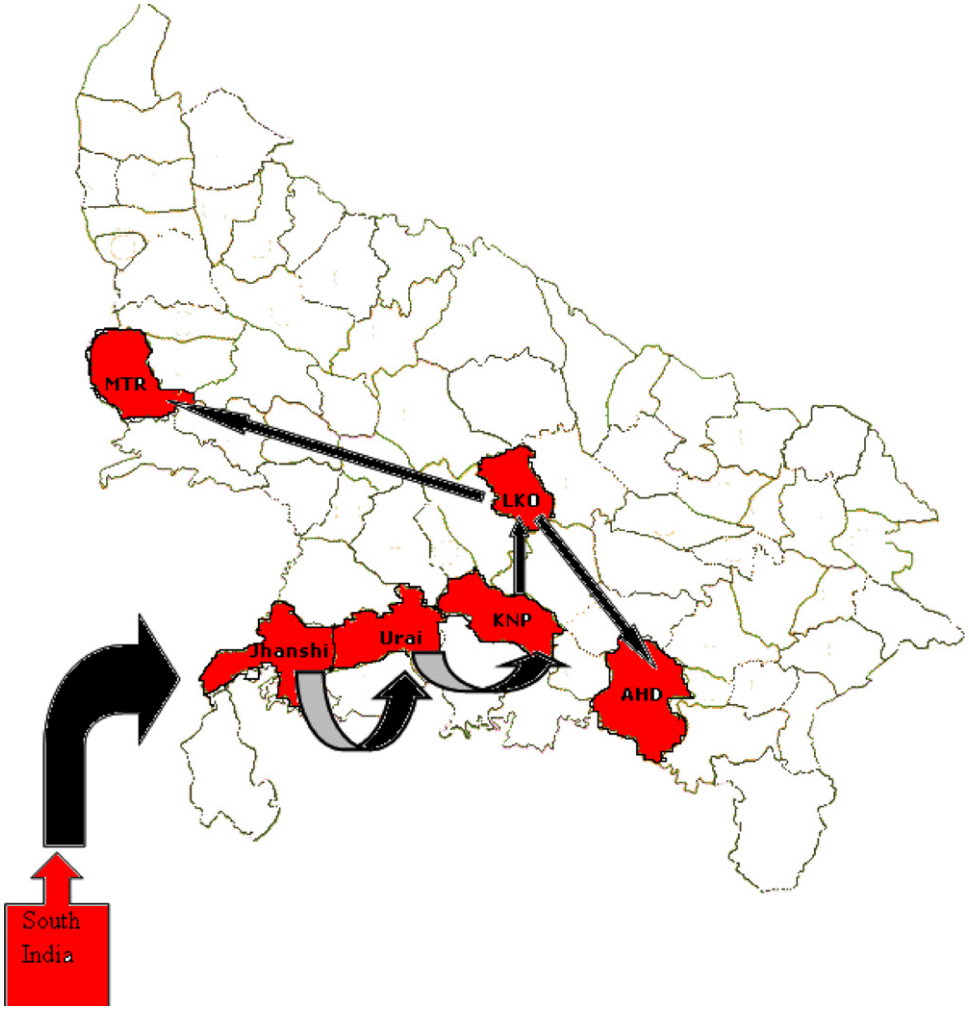

Fig. 1. Infected area of Chikungunya virus in the state of Uttar Pradesh Map, India (MTR: Mathura; LKO: Lucknow; KNP: Kanpur; AHD: Allahabad).

changes indicate the evolutionary potential of the virus. Furthermore, remarkable changes in the amino acid positions along with the S27 and the Ross strain at the position of E1 region V269M and E284D was also observed whereas no changes were found at E1 A226V in all five isolates (Fig. 3).

5. Discussion

The largest epidemic of CHIKV in the North India is now declining, while outbreaks with the North Indian isolate were reported in many other countries, and opportunities for the introduction of CHIKV to India were not limited. In this study, five cases of CHIKV were isolated from the Uttar Pradesh, India during 2006–2008. Similar statistical findings were reported in La Réunion (Staikowsky et al., 2008) whereas a lower proportion of cases with joint pains (78%) were found in the Malaysia in 1998 (Lam et al., 2001). About half of the patients presented with a skin rash, similar to previous findings (Pialoux et al., 2007).

The five cases were detected among the travelers from the South to North India because of possible transmission of the virus by the travelers, 5–7 days after the onset of the disease. None of the local close relatives were found to be infected. As the epidemic is more common in the Southwest of India (Lescar et al., 2001); it is considered as the origin of the infection, rather than the North India. A CHIKV outbreak of unprecedented magnitude swept the Indian Ocean territories principally the Reunion Island, Comoros, Mauritius, Seychelles, and Southwest India in 2005–2006 (Laras et al., 2005; Muyembe-Tamfum et al., 2003; Pastorino et al., 2004; Ravi, 2006). The precise reasons for the re-emergence of CHIKV in the Indian as well as other in the

Table 2
Statistical analysis of positive cases including all aspects in state of Uttar Pradesh (India) using the paired sample test.

Symptoms between groups	Mean	Standard deviation	95% of CI		*t* value	*p* value
			Lower	Upper		
Age–Sex	38.75	10.223	33.97	43.53	16.951	<0.001
Disease–Age	37.05	10.821	31.99	42.11	15.312	<0.001
Age–Place	38.00	10.518	33.08	42.92	16.157	<0.001
Place–Disease	−0.95	3.103	−2.40	0.50	−1.369	0.187
Places–Sex	0.75	1.650	−0.02	1.52	2.032	0.056
Sex–Disease	1.70	2.227	0.66	2.74	3.414	0.003

Table 3
Comparatively analysis of positive CHIKV cases by ELISA, PCR and cell culture.

No. of samples	ChikV Elisa (IgM)	PCR	Cell culture	Isolates sequenced
1	P	N	N	
2	E	P	P	SGPGI/2007/01
3	E	P	P	SGPGI/2007/02
4	P	N	N	
5	P	N	N	
6	P	N	N	
7	E	N	P	†
8	E	P	P	SGPGI/2007/03
9	P	P	N	
10	E	N	P	†
11	P	P	N	
12	P	N	N	
13	E	P	P	SGPGI/2007/04
14	P	N	N	
15	P	N	N	
16	E	P	P	SGPGI/2007/05
17	P	N	P	†
18	P	N	N	
19	P	N	N	
20	P	N	N	

P: positive; E: equivocal; N: negative; †: not performed.

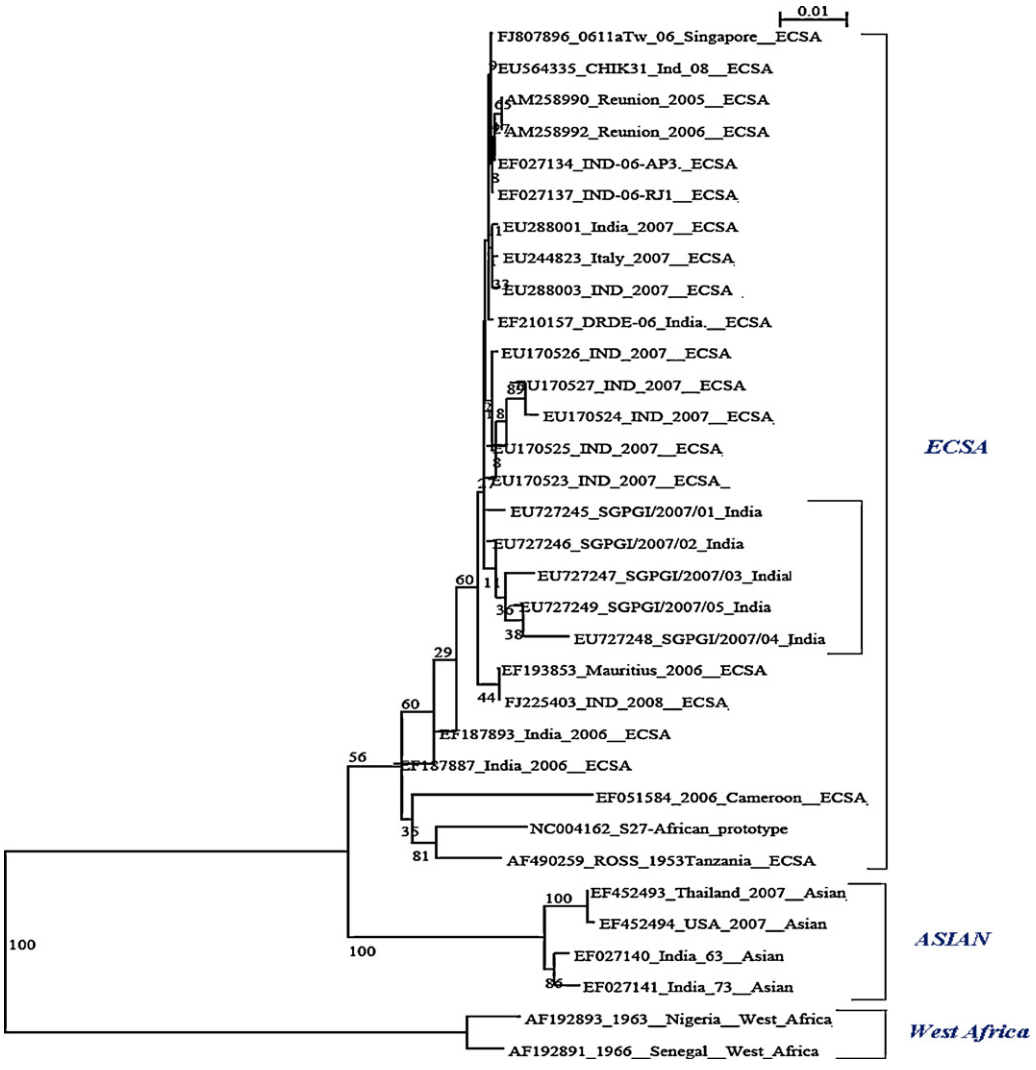

Fig. 2. Phylogenetic tree of Chikungunya viruses generated by neighbour-joining method based on the nucleotide sequence of Partial E1 gene of 33 isolates.

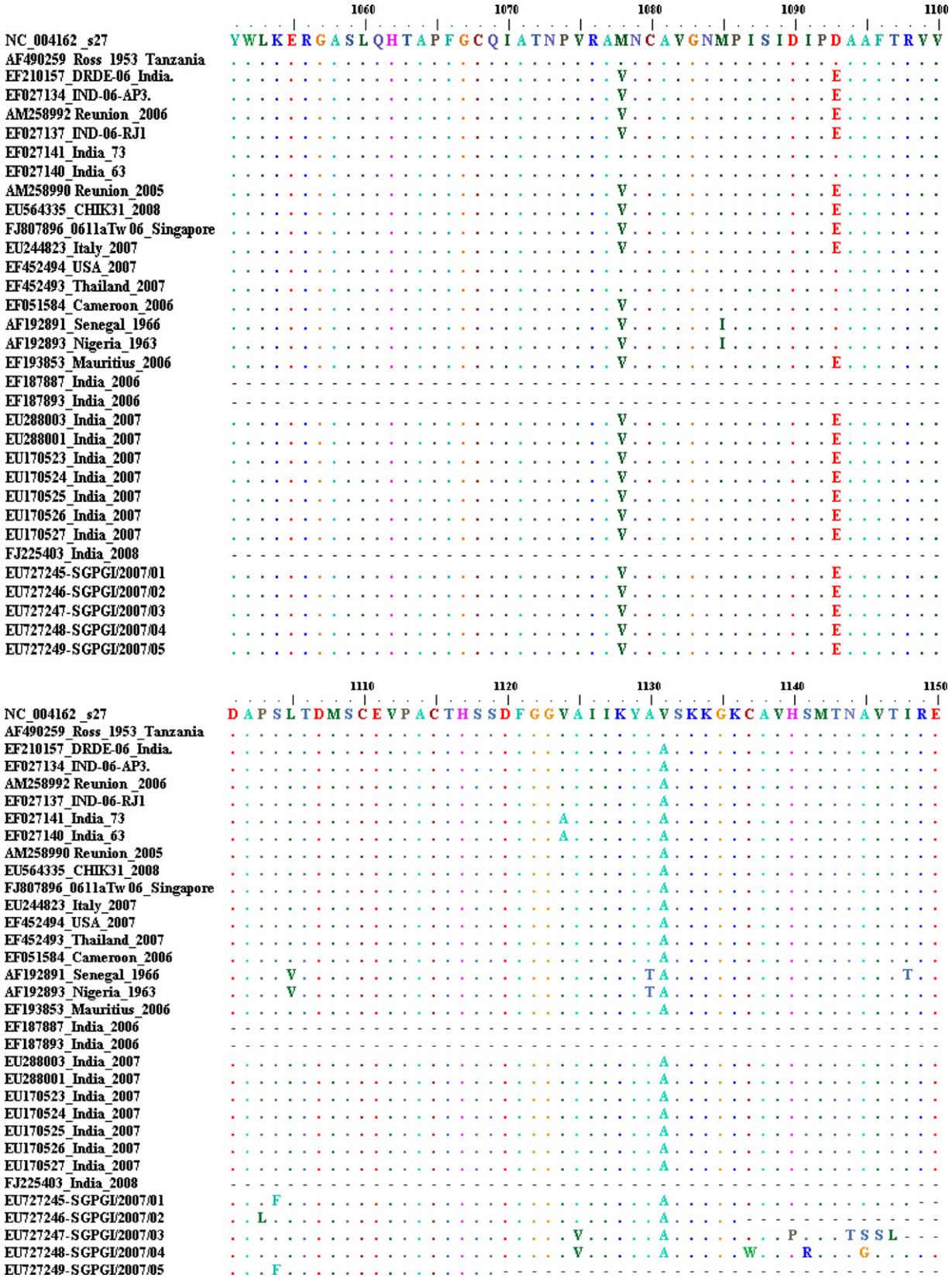

Fig. 3. Comparison of amino acid substitutions identified in Uttar Pradesh isolates with that of S-27 and other closely related genotypes of CHIKV.

southern Indian Ocean are an enigma. However, plausible explanations include increased tourism, the introduction of the virus into a native population and viral mutation (Schuffenecker et al., 2006).

The isolation of CHIKV in C636 cell line is a simple and easy on the cell line because the rate of growth is rapid with very high titer virus (Sourisseau et al., 2007). RNA viruses are usually extremely genetically varied, and their genomes contain signs of disparity and

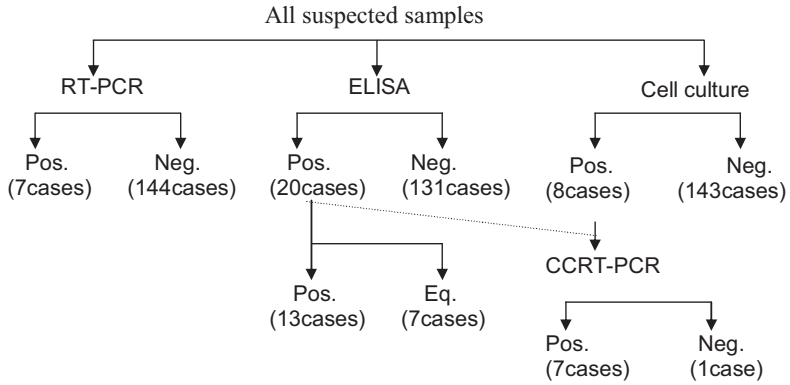

Fig. 4. Flow diagram of CHIKV suspected cases shows that ELISA 131 negative cases were also negative in PCR and cell culture method.

mobility. Genetic analyses of viral genomic sequences conducted over short times during an epidemic can be used to distinguish between dissimilar strains of a virus. This evolutionary situation is most likely based on the five sequences obtained, although the purpose of quasi-species heterogeneity is desired to obtain a more specific picture of viral evolution during the epidemic. Therefore, the evolutionary route of the viral infection cannot be predicted solely from the characteristics of the fittest sequence. Such data are helpful in understanding the evolutionary potential of a virus and mechanisms underlying the development of a disease epidemic; moreover, they may be used in the progress of new strategies for viral disease anticipation and control.

In CHIKVs, structural proteins E2 and E1 occurs as a closely associated heterodyne on the surface of the virion, with E2 projecting outward and over E1, covering the E1 fusion loops (Lescar et al., 2001; Pletnev et al., 2001). With this findings changes in E1 A226V were absent in the original isolates although these observations show point mutations in E1 at a different position. Recently, a study on mosquitoes (*A. albopictus*) using both wild (E1: 226A) and mutant (E1: A226V) viruses revealed that the mutant strain is better adapted and possesses the higher epidemic potential with increased transmissibility. The E1: A226V mutation in CHIKV results in increased fitness in *A. albopictus* mosquitoes with respect to midgut infectivity, dissemination to the salivary glands and transmission of vertebrate species (Tsetsarkin et al., 2007). In this study the microevolution of CHIKV in polypeptide position 1125 and protein position 316 of the Indian isolates (SGPGI/2007/03–SGPGI/2007/04) denotes the evolution to the mosquito vector in which C is replaced by T and due to this Alanine was replaced by Valine. Similarly, polypeptide position at 1104 (SGPGI/2007/01) protein position S295F and (SGPGI/2007/05) of other isolates genomic position at 10877 in the E1 gene also indicate the changes between Serine to Phenylalanine after replacing C to T. However in E1 gene protein of SGPGI/2007/02 isolate at the position 294 and polypeptide at 1103 in genomic position 10874; C is replaced by T nucleotide that further causes the replacement of Proline to Leucine. Similarly SGPGI/2007/04 isolate replaced T to G nucleotide (genomic position 10977) at the polypeptide position 1137 Cysteine to Tryptophane (C → W) at the protein position C328W, another substitution at polypeptide position 1131 and protein position at V322A were observed (Table 4). These results may indicate the higher prevalence of *A. albopictus* in the Kerala, compared to *Aedes aegypti* in other parts of the India which may be one of an important factor for the unusual spread of the epidemic. Novel mutations in the viral nucleic acids and proteins may represent adaptive mutations for the human or mosquito hosts. The unique observation of the E1 region in all five isolates was Glutamine at the amino acid position 284. This is a highly conserved position, which displays an Asp in the majority of Chikungunya viruses. This change is situated at the interface between E1 at the surface of the virion, participate in contacts that make up the icosahedral E1 scaffold (Schuffenecker et al., 2006). This microevolution reveals changes in the genomic diversity and it will be also an important to increase surveillance in other southern states of India with higher prevalence of *A. albopictus* where a scope for evolution of mutant strains (Santhosh et al., 2008). Recently it was reported that the E1 region at polypeptide position 1125 and protein A316V (*Ala to Val*) microevolution was observed in China to complete genome of strain FD080178 (Zheng et al., 2010). The amino acid differences detected among the CHIKVs might be related to their biological or pathogenic characteristics. Changes at polypeptide position 1078 in E1 protein M269V, suggesting the importance of mutations to increased transmissibility of the isolated sequences of the virus (Arankalle et al., 2007).

In order to determine the progenitor phylogroup from which the Indian Ocean outbreak isolates emerged, the E1 coding sequence from the five partial nucleotide genomes compared with 28 other available published CHIKV sequences. Phylogenetic analysis clearly demonstrated that the current Indian Ocean isolates represent a homogeneous clade within a broad group comprising isolates from the East, Central, and South Africa (Fig. 2). There was no ECSA group member showing a significantly closer relationship with the Indian Ocean isolates. Asian isolates were more distantly related to the Indian Ocean isolates and constituted the sister group of group ECSA, whereas West-African isolates were more divergent. The identity of EU727249 sequences is 99% with EU727247–EU727248 and EU727245 while EU727246 match with EU727248 (99%) and same identity of other complete genome of CHIKV EF027137, EF210157 and EF027134 respectively were matched. The sequence of EU727245 and EU727247 shows 99% homology with the Indian strain of IND-06-RJ1, DRDE-06, IND-06-AP3 and CHIK31 (EF027137, EF210157, EF027134 and EU564335), while it also shows 99% homology with complete genome of Singapore strain 0611aTw (FJ807896). However both sequences also show 99% homology, whereas EU727248 sequence shows 98% homology with five complete genomes of India and Singapore strain. The involvement of A316V mutant virus was attributed to the continued circulation of the 2008 China strain in the current outbreak due its geographical proximity coupled with higher prevalence of *A. albopictus* vector, supporting the higher epidemic potential of A316V mutant virus. This data reveal molecular analysis of Chikungunya virus circulating around the world which remains unclear and need more emphasis and understanding of its epidemiology. This is the first report regarding the protein analysis, appearance of this mutation in the Uttar Pradesh, India. The present study demonstrates that the highly pathogenic CHIKV variants within the E1 gene region is still the dominating viruses in the India. The genetic diversity of CHIKV strain existed in India. These results might be useful for

Table 4
Polymorphism in nucleotide position identified between Indian isolates versus reference strain.

Protein	Structural proteins							
	E1	E1	E1	E1	E1	E1	E1	E1
Polypeptide position	1035	1078	1093	1103	1104	1125	1131	1137
Protein position	226	269	284	294	295	316	322	328
S27 strain	A	M	D	P	S	A	V	C
Ross strain	A	M	D	P	S	A	V	C
SGPGI/2007/01	A	V	E	P	F	A	A	C
SGPGI/2007/02	A	V	E	L	S	A	A	–
SGPGI/2007/03	A	V	E	P	S	V	A	C
SGPGI/2007/04	A	V	E	P	S	V	A	W
SGPGI/2007/05	A	V	E	P	F	–	–	–

the origin and genetic diversity of CHIKV Indian isolates and the development of a vaccine in the future. In addition, the similar evidences provide the CHIKVs in India as well as other countries, and its changes described the recent evolution in the virus to cause an infection. Thus, public health laboratory surveillance should monitor its spread and track possible evolution of the virus during the epidemic.

Conflict of interest

None.

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