Chikungunya virus infection - a resurgent scourge

Chikungunya virus (CHIKV) is an arbovirus that belongs to the genus *Alphavirus* (family *Togaviridae*). The major vectors are *Aedes aegypti* and *Ae. albopictus* mosquitoes. Chikungunya was first described in Tanzania, Africa in 1952. The viral genome consists of a single-stranded, positive-sense RNA molecule of approximately 11.8 kb. Genetic analysis of CHIKV sequences show evidence that the virus originated in Africa and subsequently was introduced into Asia. Chikungunya virus was documented in the early 1960s in different parts of South and South eastern Asia. It had caused major outbreaks in India, Sri Lanka, Burma, and Thailand. The virus was isolated in India at Calcutta (Kolkata) in 1963. A well documented outbreak of CHIKV infection occurred in India in 1971, and since then the virus activity was noticeably absent for several decades in India. Small outbreaks and sporadic cases continued in Burma, Thailand, and the Philippines in the 1980s and the virus spread into Indonesia for the first time from 1982 to 1985, with outbreaks in several islands. The virus was never idle and caused major outbreaks in Thailand in 1995 and Malaysia in 1998-1999.

Re-emergence of chikungunya (CHIK) disease, caused by CHIKV was recorded in India during 2005-2006 after a gap of 32 years, causing 1.3 million cases in 13 States. Several islands of the Indian Ocean reported similar outbreaks in the same period. These outbreaks were attributed to the African genotype of CHIK virus. Large scale outbreaks of fever in several parts of southern India and areas such as the French Reunion Islands, Mauritius and Seychelles had all recorded the disease activity. This particular epidemic seems to have started in the Reunion Island. The majority of the cases were from Andhra Pradesh (AP), followed by Karnataka. Several hundred clinical cases were identified in Maharashtra and Orissa. There are two distinct lineages delineated, one from western Africa and the second from southern Africa, east Africa and Asia. Phylogenetic analysis at the nucleotide level revealed the present Indian isolates of the 2005-2006 epidemic to be related to the central African isolates from Reunion islands with high degree of homology.

More recently, the virus was active in southern Europe having been taken by tourists from India. About 160 people were infected in Italy’s northern Ravenna region with one fatality as indicated by the European Centre for Disease Prevention and Control (www.ecdc.europa.eu).

The major symptoms described for the viral infection have been short episode of 2-5 days fever, 2-3 days of maculo-papular rash on the trunk and limbs. Myalgia and arthralgia were typically seen. Arthralgia was seen in 80 per cent of affected individuals involving the small joints of the hands and feet is now recognized to have the sequelae of joint inflammation and causing prolonged discomfort. Symptoms could also include headache, suffusion of the conjunctiva and slight photophobia. Laboratory testing usually does not reveal a lowered platelet count. The virus has usually not been associated with fatal outcome in infected individuals. In the recent India epidemic the clinical features observed were high 2 day fever, crippling joint pain, intense headache, insomnia and extreme degree of prostration which was seen for about 5-7 days. However, patients have complained of joint pain for much longer time periods depending on the age of the patients, with younger patients recovering within 5-15 days, middle aged recovering in 1-2.5 months and a longer recovery period for old people. It has been observed that the severity of the disease as well as its duration was less in younger patients and pregnant women. No untoward effect on pregnancy was noticed following the infection. The National Institute of Virology (NIV), Pune, identified the 2005-2006 epidemics by IgM
antibody testing in acute and early convalescent phase serum samples showing a 40 per cent rate of detection. Serum samples tested from Vellore, Tamil Nadu, at the NIV also showed about 50 per cent IgM detection rate in clinically typical cases (unpublished data). It should be noted that the virus is transmitted by the same vector Aedes aegypti mosquito that spreads dengue fever. The virus was also active in India in the period when dengue virus is usually active. The diagnosis of Chikungunya was made showing absence of dengue virus infection in individuals fitting the case definition of the former. The isolation of the virus and molecular detection of genomic RNA and its sequencing have all clearly established the 2005-2006 epidemics of chikungunya in several parts of the country.

Viral diagnostics include isolation in culture, serological tests using haemagglutination inhibition or ELISA, and also the polymerase chain reaction (PCR) tests can be used to confirm the infection; these tests are not available in India widely. Virus isolation fromuffy coat cells or serum samples collected from 2-5 ml of heparinized whole blood obtained during the first week of illness is the most definitive test. A number of cell lines like BHK-21, HeLa and Vero cells show cytopathic effects (CPE) of the CHIKV to be confirmed by neutralization test. This work must be done in biosafety level-3 (BSL-3) laboratories to reduce the risk of viral transmission. Suckling mice- or cell culture-based isolations are cumbersome and the special skills required are not available except in some laboratories such as in NIV. An ELISA test to detect IgM is available at the NIV. Infected individuals become IgM positive by 7 days of illness and the antibody may last 6 months. In the literature there are reports of the availability of molecular diagnosis. A reverse transcriptase (RT)-PCR technique for CHIKV using nested primer pairs amplifying specific components of three structural gene regions, capsid (C), envelope E-2 and part of envelope E1 is described in the literature.

A real time RT-PCR assay has been evaluated on clinical serum samples with a synthetic RNA transcript as a positive control. The real-time RT-PCR was 10-fold more sensitive than a conventional block-based RT-PCR and could detect as low as 20 copies of RNA transcript. There is also the development of a one-step SYBR Green I-based real-time RT-PCR assay reported for detection and quantification of CHIKV in acute-phase patient serum samples by targeting the E1 structural gene. A linear relationship was obtained between the virus concentration and cycle of threshold (Ct) value over a range of 10^6 to 0.1 plaque forming unit/ml (PFU/ml). The feasibility of this reported assay system for clinical diagnosis was validated on suspected acute-phase serum samples of the recent CHIKV 2006 epidemic in southern India in 2006. The quantification of the viral load in the acute-phase serum samples was also determined employing the standard curve, which varies from 0.1 to 10^7 PFU/ml. These findings demonstrated that the reported assay has the potential usefulness for clinical diagnosis due to simultaneous detection and quantification of chikungunya virus in acute-phase patient serum samples.

Nucleotide sequencing of part of the E1 gene of CHIKV was used to investigate the relatedness of the samples. Analysis of sequences from patients that had traveled to India, Mauritius or the Seychelles showed high similarity with published sequences from the Indian Ocean island of Reunion. The relatedness of the Indian isolates (IND-06) with Reunion Island isolates (RU) was examined with the full-genome sequences of five CHIK virus isolates drawn from different States in India. An isolate from mosquitoes in the year 2000, identified as being of the African genotype, and two older strains isolated in 1963 and 1973 (of the Asian genotype), were sequenced by the NIV team. It was shown that the IND-06 isolates shared 99.9 per cent nucleotide identity with RU isolates, incriminating the same strain in these outbreaks. The IND-06 isolates shared 98.2 per cent identity with the archived Yawat-2000 isolate available at the NIV. There are two known crucial substitutions reported for RU isolates in the E1 region. The M269V change was noted in the Yawat-2000 and IND-06 isolates. The D284E was documented in the IND-06 isolates alone. The A226V shift hypothesized to be associated with the adaptation to the mosquito vector shown for the strain of CHIKV involved in the epidemic in Reunion Island was absent in all of the Indian isolates. Substitutions unique for the IND-06 isolates, two (T128K and T376M) in the Nsp1 region and one (P23S) in the capsid protein, have been described by the NIV group.

The stability of the virus was inferred by the fact that the two Asian strains showed 99.4 per cent nucleotide identity to each other and the investigators found no evidence of recombination of the Asian and African genotypes. The results indicated that the virus polymorphism was probably the reason behind the explosive nature of the CHIK outbreak. A study published in this issue compared the partial E1 gene sequences implicated in the epidemic in Rayalaseema region of Andhra Pradesh with other geographical isolates showing a homology of about 95 per cent to the Central African isolates.
Researchers thus speculated that mutation of the virus and host factors like absence of herd immunity along with environmental factors like lack of vector control and globalization of trade and travel played a role in the resurgence of this infection.\textsuperscript{10}

Mosquito control is the appropriate strategy to control an epidemic or even pre-empt future ones. No vaccine or antiviral drugs are available for chikungunya. Chloroquine is considered a possible treatment for the symptoms associated with chikungunya and also as an antiviral agent to combat the Chikungunya virus. Chloroquine phosphate (250 mg/day) has given promising results in persistent arthritis not responsive to aspirin or other nonsteroidal anti-inflammatory drugs.\textsuperscript{12} The French health officials also supported this approach.\textsuperscript{13} The Centers for Disease Control (CDC), Atlanta advises against using aspirin but ibuprofen, naproxen and other non-steroidal anti-inflammatory drugs are recommended for arthritic pain and fever.\textsuperscript{14} Practitioners of homoeopathy in India apparently have successfully treated chikungunya. Of course the treatment is for the amelioration of symptoms. This is usually not specific for the diagnosis of chikungunya.\textsuperscript{15}

The chikungunya virus has now been clearly entrenched in India and has shown its ability to cause explosive outbreaks posing a major public health problem with major economic impact.\textsuperscript{10} This establishes the need to improve disease surveillance and the requirement for strengthening laboratory services in different parts of the country to detect such epidemics in their early phase. Only this will help institute appropriate control measures. A detailed mapping of vector borne diseases has to be part of country-wide integrated disease surveillance programme (IDSP). India also needs manpower training in disease surveillance (epidemiologists), laboratory personnel for manning diagnostic facilities and vaccine manufacturing. Scientists should be encouraged to study the pathogenesis of the arthropathy seen in infected individuals. Studies in animal models on related flaviviruses have shown the virus RNA in the synovial fluid, virus replication in the endosteme and periosteum of the affected joints and antibody indicating an immune complex aetiology of the arthritides through complement activation.\textsuperscript{16}

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References


