Re-emergence of Chikungunya virus infection in Eastern India

Siddhartha Sengupta¹ · Saikat Mukherjee¹ · Surja Kumar Haldar² · Nemaı Bhattacharya² · Anusri Tripathi¹

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Abstract

Chikungunya fever is a major public health issue in India. Re-emergence of chikungunya virus (CHIKV) in West Bengal was detected after 32 years in 2006. After 2010, this infection was in apparent decline, but in 2016 a massive outbreak affected the country. Present study was carried out to understand CHIKV infection dynamics during recent outbreaks in West Bengal, Eastern India and its implication on disease manifestations. Blood was collected from 641 symptomatic patients. Patients' sera were serologically diagnosed to detect presence of anti-chikungunya-IgM antibodies. Viral RNA was extracted; presence of CHIKV genome and its respective viral load was determined by real time quantitative reverse transcription-PCR (real-time qRT-PCR). Statistical analysis was performed using EPI INFO software. CHIKV infection was detected in 24.64% of symptomatic patients. Middle-aged patients (31–40 years) were predominantly affected; clinically, both arthralgia and joint-swelling were significantly prevalent among CHIKV-infected patients. Myalgia, joint-swelling, and arthralgic manifestation were found in significantly higher frequency among patients with high chikungunya viral load (> 10,000 copies/ml). Thus, this study clearly indicated the re-emergence of CHIKV in Eastern India. Significant presence of myalgia, joint swelling, and arthralgia among chikungunya patients with high viral load implied association of disease severity with viral load; requiring vigilance for proper management of infected patients as this disease is highly morbid in nature. However, in addition to chikungunya virus, other viral, bacterial, and protozoal infections also occur during post-monsoon season in India, having overlapping symptoms. Hence, continuous monitoring of these infections is required for better clinical management of patients.

Keywords Chikungunya · Viral load · qRT-PCR · IgM ELISA · Myalgia · Arthralgia

Introduction

Chikungunya is an arthropod-borne neglected tropical disease caused by chikungunya virus (CHIKV), which is a single-stranded positive sense RNA virus, belonging to *Alphavirus*

genus of *Togaviridae* family [1, 2]. While, *Aedes albopictus* serves as major vector in Indian Ocean islands, *Aedes aegypti* is the principal vector for CHIKV in mainland India [3, 4]. In Africa, CHIKV is maintained in sylvatic cycle involving non-human primates and a number of forest-dwelling mosquitoes (*Aedes furciferfartaylori*, *Aedes luteocephalus*, and *Aedes africanus*) [5]. Since the first detection of chikungunya infection during late 2013 in Saint Martin island, CHIKV has alarmingly infected a million people in Caribbean, Latin America, Mexico and parts of South America viz. Brazil, Bolivia, Colombia, Ecuador, Paraguay, and Venezuela having both imported from Asia-Africa and autochthonous cases [6]. Currently, chikungunya is a re-emerging vector-borne disease of high public health significance in South-East Asia Region viz., India, Indonesia, Maldives, Myanmar, Sri Lanka, and Thailand [7]. In India, CHIKV was first isolated in 1963 at Kolkata (Calcutta) and continued imparting several epidemics at different regions of India, viz. Chennai, Pondicherry, Vellore, Nagpur, and Mumbai between 1964 and 1975 [8, 9]. In the following years, CHIKV seemed to have

Siddhartha Sengupta and Saikat Mukherjee contributed equally to this work.

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✉ Anusri Tripathi
anusri.stm@gmail.com

- ¹ Department of Biochemistry and Medical Biotechnology, Calcutta School of Tropical Medicine, 108, C. R. Avenue, Kolkata, West Bengal 700073, India
- ² Department of Microbiology, Virus Unit, Calcutta School of Tropical Medicine, 108, C. R. Avenue, Kolkata, West Bengal 700073, India

disappeared from India, eventually making a sudden re-emergence in 2005 after a gap of 32 years causing an explosive outbreak [10, 11]. Kerala was the worst affected state in 2007 with approximately 3.6 million fever cases [12]. Subsequently, recurring mass infection and disease spread was reported from 13 different states of India, resulting in 1.4–6.5 million estimated cases across the country [13]. In 2006, West Bengal had witnessed a CHIKV outbreak [14]. Though 2010 onwards chikungunya fever has been in the decline, until date, there has been several sporadic cases of CHIKV infection reported from different parts of India necessitating implementation of surveillance programs in clinical settings [15–17]. CHIKV is responsible for an acute infection, characterized by abrupt onset of high fever, joint pain, myalgia, headache, and rash [18, 19]. Joint pain, persisting for months, or even years, is a typical clinical symptom of CHIKV infection [20]. In some patients, maculopapular rashes are found mainly on trunks or exterior surfaces of limbs, and rashes are usually accompanied by secondary rise in temperature [21].

The present study has been aimed to detect CHIKV among symptomatic patients from Eastern India and to review current status of chikungunya infection in West Bengal during 2013–2016. The study was also aimed to correlate the appearance of characteristic clinical symptoms with CHIKV infection.

Materials and methods

Inclusion criteria

As per World Health Organization (WHO) criteria, an acute febrile illness with history of headache, body ache, myalgia, arthralgia, and rash, with or without hemorrhagic manifestation from all age groups and both sexes was selected.

Exclusion criteria

Patients with hematological malignancies, bleeding disorders, chronic liver disease, diabetes mellitus, renal diseases, shock, meningoencephalitis, and neuropathy were excluded from the study.

Specimen collection

After obtaining the institutional ethical committee approval (CREC-STM/53 dated 26.09.2013), 5 ml of blood were collected from each of the 641 symptomatic febrile patients visiting Calcutta School of Tropical Medicine, Kolkata, West Bengal, India, from September 2014 to October 2016. At the time of blood collection, all patients exhibited fever. Sera were separated from the collected blood samples through density

gradient centrifugation and stored at -80°C and -20°C freezer for molecular and serological tests, respectively.

Molecular techniques

Viral RNA isolation and detection of CHIK virus using real-time qRT-PCR

Viral RNA was extracted from 140 μL of patient serum by using QIAamp Viral RNA Mini Kit according to the manufacturer's protocol (Qiagen, Courtaboeuf, France) [22]. Presence of CHIKV genome and their respective viral loads (copies/ml) was determined through real-time qRT-PCR in a total volume of 20 μL using 8 μL viral RNA, One Step qRT-PCR Master Mix and components of CHIKV Genesig standard kit (PrimerDesign Ltd., UK), according to the manufacturer's protocol. Real-time qRT-PCR was performed in 7500 fast real-time PCR system (Applied Biosystems, Waltham, MA, USA). Cloned CHIKV non-structural protein 2 gene, provided in the kit, was used as positive control [16]. Steps for one step qRT-PCR were reverse transcription (55°C for 10 min), activation (95°C for 8 min), amplification (50 cycles at 95°C for 10 s, 60°C for 60 s and 72°C for 12 s), and cooling (40°C for 30 s). A standard curve of cycle threshold (C_T) values was constructed using six 10-fold dilution series of a positive control template. Copy number of CHIKV in patient serum was calculated from the corresponding C_T values. Each sample was loaded in triplicate.

Determination of chikungunya viral load

Chikungunya viral load was determined using real-time qRT-PCR in total volume of 20 μL , using 5 μL of patient-extracted viral RNA, one step qRT-PCR master mix, and other components of CHIKV Genesig kit (Primer Design Ltd., UK). Real-time qRT-PCR was performed in 7500 fast real-time PCR system (Applied Biosystems, Waltham, MA, USA). A standard curve of cycle threshold (C_T) values was constructed using six 10-fold dilution series of the positive control template (provided in the kit). Copy number was calculated from the corresponding C_T values. Each sample was analyzed in triplicate, according to the manufacturer's protocol. CHIKV load of $\geq 10,000$ copies/ml and $< 10,000$ copies/ml were identified as high viral load (HVL) and low viral load (LVL), respectively [15].

Serological techniques

Detection of anti-CHIKV IgM antibody using ELISA

Sera samples of patients were subjected to serological diagnosis for presence of anti-chikungunya-IgM antibodies using NIV CHIKV MAC ELISA Kits (National Institute of

Fig. 1 Age-group wise distribution of CHIKV-infected patients. Infected age group (divided in ranges from 0–10 to above 60) has been plotted on X-axis and percentage of chikungunya-infected patients has been plotted on Y-axis

Virology, Pune, India), following the manufacturer's instructions. Briefly, 100 μ L of 100 times diluted serum samples, negative control, positive control, and calibrator were added to respective wells and incubated for 1 h at 37 °C. The wells were washed five times and were incubated with 100 μ L of 10 times diluted enzyme conjugate for 1 h at 37 °C. Following incubation, wells were washed five times and further incubated at room temperature with 100 μ L of TMB (3,3',5,5'-Tetramethylbenzidine). After 30 min of incubation, reaction was terminated by adding 50 μ L of stop solution and absorbance was measured at 450 nm wavelength with reference filter of 600–650 nm within 30 min in Infinite M200 (Tecan Group, Mannedorf, Switzerland). Results were interpreted as negative or positive according to assay manual.

Statistical analyses

A 2×2 contingency Chi square and odds ratio analysis were performed to find association between CHIKV infections or

Fig. 2 Cumulative distribution of CHIKV positivity across monsoon and post-monsoon seasons of 2014–2016. Monthly demography (plotted on X-axis) and total number of chikungunya-positive patients identified per month (plotted on Y-axis)

Fig. 3 Percentage of CHIKV positivity by real-time qRT-PCR and IgM ELISA according to the days of collection from symptomatic onset. Days of blood collection from symptomatic onset (plotted on X-axis) along with percentage of CHIKV positivity detected using real-time qRT-PCR (■) and anti-Chikungunya-IgM ELISA (□) (plotted on Y-axis)

CHIKV load with different clinical symptoms using CDC Epi-Info software (version 3, CDC, Atlanta, GA, USA). Probability values of $P < 0.05$ were considered as statistically significant. Odds ratio values > 1 indicated that a condition/event was more likely to occur in a particular disease group.

Results

During 2014–2016, incidence of chikungunya infection was investigated among the 641 symptomatic patients from various parts of West Bengal. Among them, 24.64% (158/641) were found to be chikungunya-infected through real-time qRT-PCR and anti-chikungunya-IgM ELISA (Table S1). Among these CHIKV infected patients, 90 were within acute stage (0–5 days of symptomatic onset). Male to female ratio of

Fig. 4 Comparison of clinical symptoms between **a** CHIKV infected and uninfected symptomatic patients, **b** HVL and LVL group of CHIKV infected patients. **a** Clinical symptoms exhibited by CHIKV-positive (■) and CHIKV-negative patients (■) (plotted on X-axis) and percentage prevalence exhibited by both groups (plotted on Y-axis). **b** Clinical

symptoms exhibited by CHIKV HVL (■) and CHIKV LVL patients (■) (plotted on X-axis) and percentage prevalence exhibited by both groups (plotted on Y-axis) asterisk (*) indicated χ^2 p value < 0.05 as statistically significant at 95% confidence interval

chikungunya patients was 1.36:1 and mean age group was 33.06 ± 14.7 years (range 7–72 years).

Frequency of CHIKV infection was highest among patients 21–30-year age group, and gradually decreased with increasing age (Fig. 1). Similar to other mosquito-borne infections, CHIKV infection peaked up after July, which corresponds to the onset of monsoon in India. Maximum percentage of CHIKV infection was reported during September ((51/158) = 32.27%) (Fig. 2). Depending upon day of sample collection after symptomatic onset, real-time qRT-PCR assay could detect highest percentage of CHIKV genome on the first day of infection, which gradually decreased with increasing number of days after symptomatic onset (Fig. 3). On the contrary,

percentage prevalence of anti-chikungunya-IgM followed the reverse trend. Anti-chikungunya-IgM ELISA could detect highest percentage of anti-chikungunya-IgM antibody in patients' sera on the sixth day of symptomatic onset (Fig. 3).

Clinically, arthralgia (OR = 2.14, 95% CI 1.46–3.14, p = 0.0001) and joint swelling (OR = 1.67, 95% CI 1.03–2.70, p = 0.03) were more frequent among chikungunya-positive patients than that of chikungunya-negative symptomatic patients (Fig. 4a). Approximately 23.8% PCR-positive chikungunya patients exhibited HVL of CHIKV, whereas, remaining 76.2% PCR-positive chikungunya patients showed LVL. Interestingly, compared with chikungunya LVL group, arthralgia, joint swelling, and myalgia were significantly

Fig. 5 District-wise prevalence of CHIKV infected patients in West Bengal, Eastern India. **a** Maps of India and West Bengal. **b** Districts of West Bengal has been plotted on X-axis and percentage of chikungunya-positive cases has been plotted on Y-axis

higher among chikungunya HVL patients (Fig. 4b). According to symptomatic history accounted by patients, pain started on the first or second day of fever. It then involved both small and big joints of both the extremities with no upper/lower limb predilection. Vomiting and abdominal pain were the other associated minor symptoms. Majority of the symptoms subsided within 3–5 days, whereas, joint pain persisted for more than 30 days. All the patients were treated symptomatically and no death was observed.

Burden of chikungunya infection was markedly noticed within Kolkata metropolitan, West Bengal, India and its adjoining districts, viz. South 24 Parganas, Howrah, Hooghly, and North 24 Parganas (Fig. 5).

Discussion

In this cross-sectional study, multi-diagnostic approach viz. real-time qRT-PCR and IgM ELISA were carried out for laboratory confirmation of chikungunya infection. Similar to an earlier study by Dutta et al. 2014, the present study also confirmed that real-time qRT-PCR is the most sensitive diagnostic tool for detecting chikungunya infection during early stage of infection as it confirmed the presence of CHIKV genome among acute stage patients' blood—indicating greater efficacy of molecular diagnosis compared with serology-based techniques for early detection [16]. Maximum number of CHIKV infection was reported during September, the first post-monsoon month in India. Furthermore, a higher rate of CHIKV infection was found among middle age groups [17, 23, 24]. In this study, arthralgic manifestation and joint swelling were significantly more prevalent among CHIKV infected patients compared with that of symptomatic uninfected patients which have been mentioned in several earlier studies [17, 23]. Chikungunya patients with HVL were found to demonstrate arthralgia, joint swelling, and myalgia at significantly greater frequency compared with patients with LVL. HVL has been characterized previously by higher production of pro-inflammatory cytokines, viz. IL15, which might be associated with development of joint inflammation [25]. Prevalence of joint swelling among chikungunya patients with HVL has been previously reported by Dutta et al. 2014 [16].

Since 2010, CHIKV infection had been declining in India until the current outbreak in West Bengal and Delhi depicted its reemerging path [26]. As both dengue and chikungunya are transmitted by the same vectors and having similar disease manifestation, CHIKV has been reported as a co-infection in almost 10% of dengue virus (DENV) cases in recent studies [27, 28]. In the present study, approximately 7% (45/641) of the chikungunya cases were also found to be dengue-infected using real-

time qRT-PCR, anti-dengue IgM, and dengue NS1 ELISA. However, the present study highlighted the re-emergence of chikungunya in Eastern India, West Bengal, especially surrounding Kolkata [15, 17].

In this study, chikungunya infection was detected among 24.64% of symptomatic patients. However, in addition to chikungunya, other viral, bacterial, and protozoal infections having similar symptoms might have occurred during post-monsoon season in India and hence might be considered for remaining other febrile patients. Hence, continuous monitoring of these infections is essential for better clinical management of patients.

Conclusion

This study confirmed the emergence and spread of CHIKV infection in West Bengal, India. Though in this study no mortality was reported among patients infected with CHIKV, a significant presence of joint swelling among chikungunya patients implied the severity of arthralgia, which required prioritized attention for proper management of these patients—as this disease is highly morbid in nature. However, in addition to chikungunya virus, other viral, bacterial, and protozoal infections also occur during post-monsoon season in India, which has overlapping symptoms. Thus, detailed analyses of outbreak will be important to understand the actual impact of the current outbreak. The health authorities and community should, therefore, keep a strict vigilance for early diagnosis of illness.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval and informed consent All procedures performed in this study involving human participants were in accordance with the ethical standards at the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments. The study was approved by the Clinical Research Ethics Committee of Calcutta School of Tropical Medicine (CREC-STM/53 dated 26 September .09.2013). Informed consent was obtained from all individual participants included in the study.

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