DOI: 10.7860/JCDR/2021/50545.15349

Original Article

Microbiology Section

Seroprevalence of Chikungunya (CHIK) Infection during Non Epidemic Periods in Chennai, Southern India

KALIYAMOORTHY KAYALVILI[1], MURUGAN DURAIVEL[2], ELANGOVAN NIVEDHITHA[3], SUBRAMANIAN ARUL SELVAN[4]

ABSTRACT

Introduction: Chikungunya (CHIK) infection has caused many outbreaks in India with more than 13 lac people affected by the disease. Epidemics of CHIK infection occur during post monsoon period when there is a high vector density. Evidences on the prevalence of CHIK infection during non epidemic periods are limited.

Aim: To determine the seroprevalence of CHIK infection during non epidemic periods among patients attending fever clinic in Chennai, Southern India.

Materials and Methods: This cross-sectional study was done in 180 suspected cases of CHIK infection between the months of September 2014 and February 2015. A 5 mL of blood samples were collected from the suspected cases and serum was separated to detect for the presence of CHIK-IgM antibody by using CHIK-IgM antibody capture Enzyme Linked Immunosorbent Assay (ELISA) kit. Chi-square test was done to find out the statistical significance with p-value <0.05 kept as statistically significant.

Results: The seroprevalence of CHIK infection during non epidemic periods was found to be 5.5% (10/180). Fever and joint pain were the major complaints present in all the study population. All the seronegative cases were tested for the presence of other infections and it was found that 13% were positive for typhoid, 9% were positive for leptospirosis, 4% for malaria and 2% for dengue infections.

Conclusion: There are no vaccines or specific medications available till date. Prevention is the only effective approach against the disease. Even though the prevalence of CHIK infection is low during non epidemic periods, strict vector control and elimination of mosquito breeding sites are very important in controlling the disease transmission.

Keywords: Antibodies, Enzyme linked immunosorbent assay, Viral infection

INTRODUCTION

The Chikungunya (CHIK) is a viral infection caused by CHIK virus which is an arbovirus that belongs to the genus alphavirus under the *Togaviridae* family. CHIK infection is transmitted to humans by the bite of mosquitoes namely *Aedes albopictus* and *Aedes aegypti*. The outbreak of CHIK infection has affected many countries since 2005 [1]. In India, during the year 2006, an outbreak occurred which was one of the biggest and most severe outbreaks caused by Chikungunya virus affected over 13 lac people [2]. The name "CHIK" is derived from the Makonde language, which is a language spoken by a population that lives in the Mozambique region meaning "that which bends up" [3]. The clinical manifestations of CHIK infection include sudden onset of fever, joint pain, joint swelling, myalgia, backache, headache and rashes. Epidemics occur in post monsoon period during when the vector density is very high [4-6]. Several methods are used in the diagnosis of CHIK virus infection such as: a) Virus isolation; b) Detection of CHIK virus antibodies in the serum by ELISA; c) Detection of RNA in the serum by Polymerase Chain Reaction (PCR) [7]. However, the most frequently used test to diagnose the CHIK virus infection is by detection of antibodies against CHIK virus (IgM and IgG) in the serum. IgM antibody to CHIK virus is used as a marker for acute infection and can be detected in the serum five days after the onset of symptoms and may be detectable up to five months, whereas, IgG antibody to CHIK virus is used to study the previous exposure to CHIK infection and it may persist in the serum for many years after infection [8].

There are only few studies pertaining to the prevalence of CHIK infection during non epidemic periods in India [9-12]. Hence, this study was designed with the aim to determine the seroprevalence of CHIK infection during non epidemic periods among patients attending fever clinic in a tertiary care centre in Chennai, Southern India.

MATERIALS AND METHODS

The present cross-sectional study conducted between September 2014 and February 2015 in a tertiary care centre in Chennai, Southern India, among 180 CHIK infection suspected patients aged between 8-70 years. A suspected patient is defined as patient with history of sudden onset of fever and joint pain (arthralgia) with one of the following symptoms: joint swelling, myalgia, backache, headache, rashes [13]. Before initiating the study, Institutional Ethics Committee clearance was obtained. Details about the study and study procedures were informed clearly to the patients in their native language and written informed consent was obtained from them.

Sample size calculation: Sample size was calculated by using the following formula: $n=z^2pq/d^2$. Assuming z=1.96 (for 95% confidence level), p=4% [12], q=94% (1-p) and d=3% (precision), the sample size required for the study was 166 but, authors included all the suspected patient samples collected during the study period.

Inclusion criteria

- Patients with history of fever and joint pain along with any one of the following complaints such as joint swelling, myalgia, backache, headache, rashes etc.,
- Both sexes were included in the study and the age limit was set at 8-70 years.
- Those who are willing to give written, informed consent.

Exclusion criteria

- Patients having history of fever without joint pain.
- Patients with non infectious causes of fever.
- Those who are having co-morbid conditions.
- Those who were aged <8 years and >70 years.
- Pregnant females.
- Immunocompromised individuals.

Study Procedure

All the study participants were evaluated by a structured questionnaire which contained information such as name, age, gender, socioeconomic status (using Modified Kuppuswamy Scale) and presence or absence of clinical manifestations like fever, joint pain, joint swelling, myalgia, backache, headache and rashes.

After collecting all the necessary information, then under aseptic precautions, 5 mL of blood samples were collected by venipuncture from the patients in a sterile vacutainer tubes. After blood collection, samples were transported to the Microbiology Department with duly filled requisition forms. Then serum was separated from the blood samples by centrifugation. Then, it was transferred to sterile aliquots, labeled with the particulars of the patient and stored in the deep freezer at -20°C. Further, the separated serum samples was used to detect for the presence of CHIK-IgM antibody using CHIK-IgM antibody capture ELISA kit supplied by National Institute of Virology, Pune, India. All the seronegative patients were followed up for the presence of other infections like Dengue (IgM ELISA), Leptospirosis (IgM ELISA), Malaria (Quantitative Buffy Coat) and Typhoid (WIDAL). All the serological tests were performed as per the manufacturer's instructions.

STATISTICAL ANALYSIS

The statistical analysis was done using statistical software GraphPad Prism version 9.1.2. Chi-square analysis was used to evaluate differences in seropositivity between age, gender, socioeconomic status and clinical manifestations of CHIK infection. The p-value <0.05 was considered as statistically significant.

RESULTS

A total of 180 serum samples were collected between the months of September 2014 and February 2015 from the CHIK infection suspected patients and were tested for the presence of CHIK-IgM antibody. Among the study population, 45% (82/180) were males and 55% (98/180) are females. A 50% (90/180) were in the age group of 41-60 years followed by 32.5% (59/180) in the age group of 21-40 years; 12% (21/180) in the age group >60 years and 5.5% (10/180) in the age group of <20 years [Table/Fig-1].

Characteristics	CHIK-IgM antibody		Total (N=180)	p-value
	Positive (n=10)	Negative (n=170)		
Age (years)				
<20	1 (10%)	9 (90%)	10 (5.5%)	0.23
21-40	3 (5%)	56 (95%)	59 (32.5%)	
41-60	3 (3%)	87 (97%)	90 (50%)	
>60	3 (14%)	18 (86%)	21 (12%)	
Sex				
Male	4 (5%)	78 (95%)	82 (45%)	0.71
Female	6 (6%)	92 (94%)	98 (55%)	
Socio-economic status				
Upper	1 (33%)	2 (77%)	3 (2%)	0.009
Upper middle	3 (5%)	62 (95%)	65 (36%)	
Lower middle	4 (4%)	99 (96%)	103 (57%)	
Upper lower	1 (50%)	1 (50%)	2 (1%)	
Lower	1 (14%)	6 (86%)	7 (4%)	

[Table/Fig-1]: Age and sex wise distribution of CHIK-IgM positive and negative cases.

The CHIK-IgM antibody was detected in 5.5% (10/180) of the study population. Among them, 40% (4/10) were males and 60% (6/10) were females. Out of the 10 positive cases, 10% (1/10) cases were in the age group of <20 years and 30% (3/10) cases were positive each in the age group between 21-40 years, 41-60 years and >60 years, respectively.

Although, the results portray that there were no statistically significant association between age, sex and CHIK-IgM seropositivity (p>0.05), it was found that the CHIK prevalence was higher in the age groups <20 years (1/10) and >60 years (3/21) with slightly increased female preponderance {6% (6/98)}.

With regard to month wise distribution of CHIK infection suspected and positive cases, the proportion (percentage) of positive cases among the CHIK infection suspected cases increases every month and it was maximum during the month of february 2015. The overall seroprevalence of CHIK infection was found to be 5.5% (10/180) [Table/Fig-2].

S. No.	Month	No. of CHIK infection suspected cases	No. of CHIK positive cases	Proportion (%) of positive cases among suspected cases
1.	September 2014	8	0	0
2.	October 2014	31	1	3
3.	November 2014	41	2	5
4.	December 2014	83	5	6
5.	January 2015	10	1	10
6.	February 2015	7	1	14
	Total	N=180	10 (5.5%)	

[Table/Fig-2]: Month wise distribution of CHIK infection suspected and positive cases.

The [Table/Fig-3] shows the clinical manifestations of CHIK-IgM positive and negative cases. Among the clinical manifestations recorded, fever and joint pain (arthralgia) were observed in all the study population (180/180) while other manifestations included myalgia (110/180), arthralgia with myalgia (52/180) and rashes (19/180).

Clinical manifestations	Yes/ No	CHIK-IgM positive	CHIK-IgM negative	No. of cases	p-value
Fever	Yes	10 (5.5%)	170	180	-
	No	0	0	0	
Joint pain (arthralgia)	Yes	10 (5.5%)	170	180	-
	No	0	0	0	
Myalgia	Yes	7 (6.4%)	103	110	0.55
	No	3	67	70	
Arthralgia+myalgia	Yes	7 (13.5%)	45	52	0.003*
	No	3	125	128	
Rashes	Yes	2 (10.5%)	17	19	0.32
	No	8	153	161	

[Table/Fig-3]: Clinical manifestations of CHIK-IgM negative and positive cases. Chi-square was done; *significant

Among the seropositive cases, fever and joint pain were present in all 10 cases. Myalgia and arthralgia with myalgia were seen in seven positive cases while rashes are seen in two cases.

Comparision of CHIK-IgM seropositivity with the clinical manifestations of CHIK revealed that there was a statistically significant association (p=0.003) exists in patients having arthralgia with myalgia. It was also observed that the prevalence of CHIK was much higher (13.5%) in those patients when compared to patients having other clinical manifestations such as fever, arthralgia, myalgia and rashes.

The [Table/Fig-4] shows the laboratory investigations done in 170 CHIK-IgM negative samples. All the seronegative samples were screened for the presence of other infections and it was found that 28% (48/170) were positive for other infections. A 13% (22/170) were positive for typhoid, 9% (16/170) were positive for leptospirosis, 4% (6/170) positive for malaria and 2% (4/170) for dengue infections.

S. No.	Diseases	No. of cases positive	Positivity rate (%)
1	Typhoid	22	13%
2	Leptospirosis	16	9%
3	Malaria	6	4%
4	Dengue	4	2%
	Total	48	28%

[Table/Fig-4]: Laboratory investigations in CHIK-IgM negative cases (N=170).

DISCUSSION

The CHIK virus, an arbovirus responsible for causing CHIK epidemics or outbreaks has affected many countries. Epidemics usually occur in the post monsoon period when the vector density is very high. The overall seroprevalence of CHIK infection during the non epidemic periods was 5.5% which was much less when compared to studies from southern India and from other parts of India. Srikanth P et al., in their study showed that out of the 118 serum samples which was collected from CHIK virus suspected patients, 14 (12%) samples were found to be positive for CHIK virus infection [10]. Study done by Kawle AP et al., in Maharastra shown that out of 482 study participants, 131 (27%) cases showed seropositivity for CHIK virus IgM antibody [12]. In another study done in Gujarat by Solanki MC et al., have shown that the 38 (27%) out of the 139 samples were tested positive for CHIK virus infection [11]. However, in one study from Tirunelveli, Tamil Nadu done by Sudeep AB et al., found out that, of the 217 serum samples tested for CHIK virus infection, 107 (49%) were tested positive [9]. Suryawanshi SD et al., in the year 2009 reported that 87 (52.4%) were IgM antibody positive out of 166 tested samples [13]. The higher prevalence rate in these studies may be because of the reason that the study might have been conducted during the epidemic or outbreak periods. However, there are few studies from southern India which showed a prevalence rate of 3-6% which was much similar to present study finding. In a study done by Shilpa C et al., on 509 serum samples, only 19 samples were tested positive for CHIK virus infection with the prevalence rate found to be 3.7% [14]. Padma V and Javid SM in the year 2014 did a cross-sectional study on 500 patients to find out the important causes of infectious fever and found out that CHIK virus infection was positive in 30 (96%) patients [15].

The detailed analysis on the various characteristics of the study population such as age, gender and clinical manifestations revealed that CHIK infection was having significant association in patients having arthralgia with myalgia.

Even though, there were no statistically significant association between age and CHIK-IgM seropositivity, it was found that the seroprevalence of CHIK was higher in the age groups <20 years and >60 years when compared to other groups. This was inconsistent with the study findings done by Solanki MC et al., Divya P and Krishna S and Modi KP et al., which showed that CHIK seroprevalence was higher in >15 age groups [11,16,17]. In a study done by Srikanth P et al., and Kawle AP et al., they showed that seroprevalence of CHIK infection was more common in the age group between 20-50 years [10,12]. Suryawanshi SD et al., showed that the prevalence of CHIK infection was more common in age group <30 years [13]. In another study done by Barve S et al., it was shown that patients in the age group 51-60 years were most commonly affected by CHIK infection [18].

Gender analysis showed that although there was no statistically significant association observed between gender and CHIK-IgM seropositivity, there was a slight female preponderance (60%). This correlates with the study findings of Solanki MC et al., which showed that 66% of the females and 34% of the males were affected by CHIK infection [11]. The findings from another study done by Kawle AP et al., suggested that seroprevalence of CHIK infection was higher in females (63%) [12]. Barve S et al., in their study also showed a higher seroprevalence rate of CHIKV in females (58%) [18].

Analysis of the socioeconomic status of the patient revealed that significant association exists between socioeconomic status and CHIK-IgM seropositivity (p=0.009). This was consistent with the study finding of Kumar CJ et al., which showed that lower socioeconomic status is the important factor responsible for the increase in the CHIK virus seroprevalence [19].

Analysis of the clinical manifestations of CHIK infection with IgM seropositivity showed that even though fever and joint pain were present in all the seropositive cases, there was no significant association observed. This coincides with the study findings of Srikanth P et al., Kawle AP et al., and Suryawanshi SD et al., which showed that fever and joint pain were found to have significant association with CHIK IgM seropositivity [10,12,13].

The present study helps us to find out the seroprevalence rate of CHIK infection in Southern India during non epidemic periods. The low seroprevalence rate of 5.5% coincides with the fact that CHIK cases starts to rise only during the post monsoon season (May-August) as vector density remains very high during this period. This clearly demonstrates that even though the prevalence is low, there is a considerable CHIK infection during non epidemic periods. Hence, continuous sero-surveillance has to be done in outbreak and non outbreak areas to get the baseline data and to know about the immune status of the person. By studying the seroprevalence rate of CHIK infection using cost effective diagnostic technique, authors could determine the exposure rate, percentage of people affected and that would be helpful in managing the disease outbreak situation.

Limitation(s)

The sample size was less because of the limited availability of CHIK-IgM antibody kits and as this study was conducted during non epidemic periods, there was a low seropositivity rate.

CONCLUSION(S)

From the above observation, it can be concluded that the seroprevalence of CHIK infection was 5.5% during non epidemic periods. There are no vaccines or specific medications available till date. Prevention and education is the only effective approach against the disease. People need to be educated more about the disease, their mode of transmission, treatment options available and adoption of control measures. Even though, the prevalence of CHIK infection is low during non epidemic periods, encouraging the public sector to strictly adhere to the vector control measures and elimination of mosquito breeding sites will help in controlling or preventing the transmission of disease. There was a sustained decrease in number of cases after the year 2010. Also, various geographical areas at different time period shows a variable range of seroprevalence rate which indicates that CHIK infection continues to pose a major public health problem. This recommends the need of appropriate strategies and early diagnosis with rapid testing at affordable cost to reduce the severity of disease burden.

Acknowledgement

Authors owe their heartfelt thanks to the patients for giving consent to take the samples. Authors express their deep gratitude for the technical staffs for their assistance in this work. Lastly, authors express their sincere gratitude to the almighty for helping us in completing this work.

REFERENCES

[1] Jain S, Kadri S, Venkatesh S, Lal S, Katyal R. Epidemiological investigation of an outbreak of chikungunya in hyderabad and nalgonda districts of Andhra Pradesh, India. Int J Health Sci (Qassim). 2007;1(2):303-08.
[2] Kaur P, Ponniah M, Murhekar MV, Ramachandran V, Ramachandran R, Raju HK, et al. Chikungunya outbreak, South India, 2006. Emerg Infect Dis. 2008;14(10):1623-25.

[3] Centers for Disease Control Prevention (CDC) (September). "Chikungunya fever diagnosed among international travelers". MMWR. Morbidity and Mortality Weekly Report. 2006;55(38):1040-42.
[4] Talawar AS, Pujar HS. An outbreak of chikungunya epidemic in South India, Karnataka. Int J Res Rev App Sci. 2010;5(3):229-34.
[5] Kalantri SP, Joshi R, Riley LW. Chikungunya epidemic: An Indian perspective. Natl Med J India. 2006;19(6):315-22.
[6] Ravi V. Re-emergence of chikungunya virus in India. Indian J Med Microbiol. 2006;24(2):83-84.
[7] Laboratory Diagnosis of Chikungunya Fever. World Health Organization. Archived from the original on 8 September 2012. Retrieved 20 May 2013.
[8] Johnson BW, Russell BJ, Goodman CH. Laboratory diagnosis of chikungunya virus infections and commercial sources for diagnostic assays. J Infect Dis. 2016;214(suppl 5):S471-74.
[9] Sudeep AB, Hundekar SL, Jacob PG, Balasubramanian R, Arankalle VA, Mishra AC. Investigation of a chikungunya-like illness in Tirunelveli district, Tamil Nadu, India 2009-2010. Trop Med Int Health. 2011;16(5):585-88.
[10] Srikanth P, Sarangan G, Mallilankaraman K, Nayar SA, Barani R, Mattew T, et al. Molecular characterization of chikungunya virus during an outbreak in South India. Indian J Med Microbiol. 2010;28(4):299-302.
[11] Solanki MC, Shingala HK, Sinha M. Sero-prevalence study of chikungunya cases in and around the area of Jamnagar, Gujarat (India). IP Int J Med Microbiol Trop Dis. 2017;3(4):171-75.
[12] Kawle AP, Nayak AR, Bhullar SS, Borkar SR, Patankar SD, Daginawala HF, et al. Seroprevalence and clinical manifestations of chikungunya virus infection in rural areas of Chandrapur, Maharashtra, India. J Vector Borne Dis. 2017;54(1):35-43.
[13] Suryawanshi SD, Dube AH, Khadse RK, Jalgaonkar SV, Sathe PS, Zawar SD, et al. Clinical profile of chikungunya fever in patients in a tertiary care centre in Maharashtra, India. Indian J Med Res. 2009;129(4):438-41.
[14] Shilpa C, Kavitha K, Sudheesh N, Sabeena S, Prasad V, Hindol M, et al. Estimating the seroprevalence of chikungunya virus exposure in Shimoga district, Karnataka state: A hospital-based study during 2014-2018. J Med Virol. 2020;92(1):119-23.
[15] Padma V, Javid SM. Infectious fever-cross-sectional study of 500 patients. Int J Pharm Bio Sci. 2014;5(4):479-85.
[16] Divya P, Krishna S. Seroprevalence of chikungunya virus infection in Ballari and nearby districts of Karnataka. IP Int J Med Microbiol Trop Dis. 2016;2(4):175-77.
[17] Modi KP, Patel DA, Vegad MM, Mistry AU, Padaria NJ, Rathod AB. Sero-prevalence of dengue and chikungunya, their co-infection and seasonal trends of these infections at a tertiary care hospital, Ahmedabad, Gujarat. Int J Microbiol Res. 2017;9(1):819-22.
[18] Barve S, Nanda S, Javadekar T. Chikungunya fever: The resurgence and epidemiological pattern in Western India. The Journal of Medical Research. 2013;3:159-61.
[19] Kumar CJ, Baboo CA, Krishnan BU, Kumar A, Joy S, Jose T, et al. The socioeconomic impact of the chikungunya viral epidemic in India. Open Med. 2007;1(3):e150-52.

PARTICULARS OF CONTRIBUTORS:
1. Consultant Microbiologist, Department of Microbiology, Orbito Asia Diagnostics, Chennai, Tamil Nadu, India.
2. Assistant Professor, Department of Pharmacology, Chettinad Hospital and Research Institute, Kelambakkam, Tamil Nadu, India.
3. Assistant Professor, Department of Microbiology, SRM Medical College Hospital and Research Institute, Kattankulathur, Tamil Nadu, India.
4. Assistant Professor, Department of Forensic Medicine, Government Mohan Kumaramangalam Medical College, Salem, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Murugan Duraivel,
Department of Pharmacology, Chettinad Hospital and Research Institute,
Kelambakkam, Chengalpattu-603103, Tamil Nadu, India.
E-mail: drvlmurugan@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jun 02, 2021
- Manual Googling: Aug 03, 2021
- iThenticate Software: Aug 04, 2021 (9%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **May 26, 2021**
Date of Peer Review: **Jun 26, 2021**
Date of Acceptance: **Aug 07, 2021**
Date of Publishing: **Sep 01, 2021**