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Original Research Paper

Microbiology

COMPARISON OF MOLECULAR AND SEROLOGICAL DIAGNOSIS FOR EARLY DETECTION OF CHIKUNGUNYA INFECTION: A HOSPITAL-BASED STUDY IN KOLKATA

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ABSTRACT

OBJECTIVE: The aim of the study was to compare molecular (Real Time RT-PCR) and serological (IgM ELISA test) diagnostic methods for the early detection of **chikungunya infection.**

MATERIALS AND METHOD: This study was conducted on a total of 100 patients (OPD &indoor) with chief complaints of fever, rash and arthralgia. Patient's serum was subjected to both the diagnostic methods. The exclusion criteria were hematological malignancies, bleeding disorder and certain chronic diseases.

RESULT: In case of fever of duration 1-3days ,Real time RT- PCR detected 9 cases and CHIK specific IgM ELISA detected none, whereas, the latter was a better diagnostic tool later on.

CONCLUSION: This study revealed that Real Time RT-PCR was more sensitive than CHIK specific IgM ELISA for early detection of Chikungunya fever cases.

KEYWORDS : Chikunguniya, RT-PCR, ELISA.

INTRODUCTION:

Chikungunya fever is an acute arthropod borne viral illness reported from many parts of Africa, South East Asia, Western Pacific and India. The resurgence of chikungunya in Indian ocean islands and India has drawn worldwide attention due to its explosive nature, high morbidity and complex clinic- pathological manifestations . [1]

The causative agent chikungunya virus (CHIKV) is a single strand positive sense RNA virus approximately 11.8 kb with 60-70 nm diameter capsid and phospholipid envelop [2], member of the genus Alpha virus of Togaviridae family and transmitted from primates to human [3,4]

The name derived from the Makonde word meaning that which bends up in reference to stooped posture developed as a result of arthritic symptoms of the disease. [4,5]

The sudden onset of the disease including crippling arthralgia and frequent arthritis that accompanying fever, chills, headache, nausea, vomiting, low back pain and rash are clinically distinctive. The disease is almost self-limiting and rarely fatal. [2]

In India, chikungunya virus was first reported in 1963 in Kolkata and subsequently,the virus was isolated from numerous well documented outbreaks from 1963-1973.Although no major outbreak was reported in India since the 1973,outbreak in Barsi , Maharashtra, yet, the virus was isolated in sporadic cases in human and mosquitoes during random surveillance and dengue virus outbreaks.[6,7]

The recent outbreak of chikungunya virus infection of an unprecedented magnitude in many parts of southern India is a point of major concern. It was the largest and most severe epidemic, affecting more than 1,000,000 persons in the Andhra Pradesh, Maharashtra, and Karnataka states of India, and spread to several new areas, causing huge public health and administrative [8].

Andhra Pradesh was the worst affected states ; about 80,000, cases were there, in Karnataka it was over 2000, from Orissa it was it was 4904.

After 1963, chikungunya virus made its entry in Kolkata and surroundings in 2009, 35 suspected chikungunya fever cases were reported and out of which 11 were confirmed for chikungunya.[9].

The re-emergence and persistence of chikungunya virus suggested the need for continuous monitoring and identification of new variants, to prevent the further spread of the virus to other parts of the country.

The proposed study was an effort towards early detection of chikungunya virus infection cases attending the outpatient department and amongst indoor patients of the Calcutta School of Tropical Medicine and various other hospital in Kolkata.

Global scenario

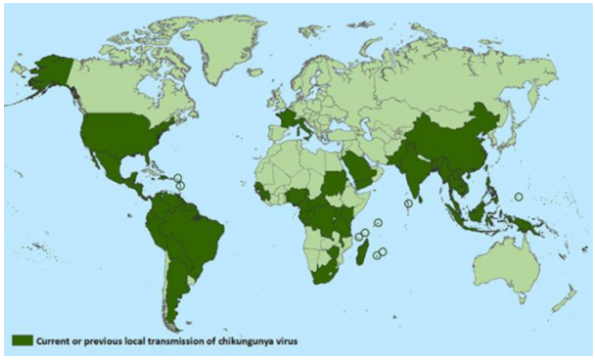


Fig.-1. Countries and territories where chikungunya cases have been reported* ***(as of April 22, 2016)***

*Does not include countries or territories where only imported cases have been documented.

MATERIALS AND METHODS

This cross-sectional study was conducted on 100 OPD and indoor patients admitted in different secondary and tertiary care hospitals in Kolkata and other districts of West Bengal with chief complaints of fever, rash and arthralgia over a period of one year (April2015-March2016). Clinically ,a case of chikungunya is a person of any age, at any time of the year with fever, petechial or maculopapular rash of trunkand occasionally on the limbs, arthralgia or arthritis and other nonspecific symptoms such as headache, nausea, vomiting and

conjunctivitis. These types of clinical cases were selected as the study population.

The exclusion criteria included haematological malignancies, bleeding disorder, chronic liver disease, diabetes mellitus and renal disease.

The study parameters incorporated :

A. Patient selection according to the inclusion criteria which consisted of fever, rash, arthralgia and joint swelling and collection of serum of those patients.
B. Detection of CHIKV specific antibody by IgM ELISA: This test was performed by using the serum collected from patients to detect evidence of recent infection of CHIKV. This test was done by using NIV CHIK IgM Capture ELISA Kit.
C. Detection of Chikungunya specific genome by Real Time RT PCR:

This test was performed to detect chikungunya virus genome in patient serum.

The QIA amp viral RNA extraction kit was employed for the Real Time RT-PCR.

All the serum samples were processed at the highly equipped virology unit of the Calcutta School of Tropical Medicine, Kolkata, India following stringent Bio-safety precautions.

RESULTS:

Hundred samples were collected according to the inclusion criteria and processed in the Virology unit of the Department of Microbiology and the Department of Bio-chemistry and Medical Bio-technology of the Calcutta School of Tropical Medicine, Kolkata. Of the 100 processed samples 14% were only Real Time RT-PCR positive, 15% were only CHIK specific IgM ELISA positive and 1% both Real Time RT-PCR and CHIK specific IgM ELISA positive.

Table-1:- Positive cases of chikungunya among the suspected cases by Real Time RT-PCR and CHIK specific IgM ELISA.

Suspected cases studied	100	
Only Real Time RT-PCR +ve	14	14%
Only CHIK IgM ELISA +ve	15	15%
Both Real Time RT-PCR and CHIK IgM ELISA +ve	1	1%
Total +ve cases	30	

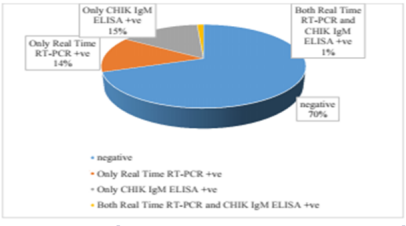
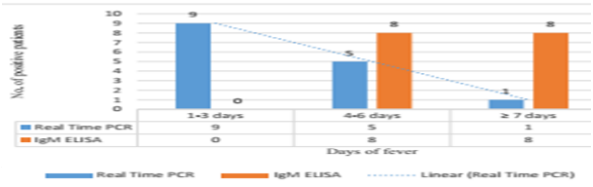


Table -2:- Comparison between CHIK IgM ELISA & Real Time PCR test result according to days of fever.

Days of Fever	Real Time RT-PCR	CHIK IgM ELISA
1-3 days	9 (30%)	0
4-6 days	5 (16.66%)	8 (26.66%)
≥7days	1 (3.33%)	8 (26.66%)



RT- PCR detected 9 cases and CHIK specific IgM ELISA detected none. In case of fever with duration between 4-6days, Real time RT-PCR detected 5 cases and CHIK specific IgM ELISA detected 8 cases. In fever of ≥7days duration, Real Time RT-PCR detected 1case and CHIK specific IgM ELISA detected 8 cases.

DISCUSSION:

The present epidemiological cross-sectional comparative study was conducted on the serum samples of suspected patients who attended the virology unit of the,Department of Microbiology of the Calcutta School of Tropical Medicine, Kolkatafrom various hospitals of Kolkata and other districts of West Bengal.

Out of 100 study samples ,chikungunya was found positive in15% by chik specificIgM ELISA and 14% by Real Time RT-PCR and 1% signaled positive by both the methods. However, RT-PCR was better for early diagnosis (9positivein fever of 1-3 days duration by RT-PCR against nil by chik specificIgM ELISA). Fever was the commonest symptoms (100%) followed by body aches 70% cases, joint pain 40% cases, rash 23.3% cases and joint swelling 13.3% cases. Among all positive cases 33.33% werefemale and 28.12% were male.

A study was conducted by Bandyopadhyay et al in West Bengal in 2007, it was found that the highest sero-positive (43%) was observed in age group of 31-50 years and 29% were above 50 years of age. Of all the patients,66 % of them were males. The predominant signs and symptoms were fever (100%), arthralgia (96%), and rash (94%) [10]

A study was conducted by Reddy V1, Ravi V, Desai A, Parida M, Powers AM,Johnsn BW.the utility of four laboratory diagnostic methods viz. IgM capture ELISA, an in house reverse transcription PCR for the diagnosis of Chikungunya fever, TaqMan real-time PCR, and a one step reverse transcription-loop mediated isothermal amplification assay (RT-LAMP). [11]Out of the 70 serum samples tested, 29 (41%) were positive for Chikungunya IgM antibody by ELISA and 50 (71%) samples were positive by one of the three molecular assays. CHIKV specific nucleic acid was detected in 33/70 (47%) by reverse transcription PCR, 46/70 (66%) by TaqMan real-time PCR, and 43/70 (62%) by RT-LAMP assay The molecular assays were more sensitive for diagnosis in the early stages of illness (2-5 days post onset) when antibodies were not detectable. In the later stages of illness, the IgM ELISA is a more sensitive diagnostic test.

Our study showed that in case of fever of 0-3days duration Real Time RT-PCR detected 9 cases (30%) out of 30 cases whereas CHIK specific IgM ELISA detected none.

In case of fever of 4-6 days duration Real Time RT-PCR detected 5 cases (16.66%) out of 30 cases whereas CHIK specific IgM ELISA detected 8 cases (26.66%)as positive.In case of fever of ≥7days duration CHIK specific RT-PCR detected 1 case (3.33%) out of 30 cases whereas CHIK specific IgM ELISA detected 8 cases (26.66%) as positive.

CONCLUSION:

In the present cross-sectional study of 100 cases, chikungunya fever was found positive in 30 cases. Highest positivity of chikungunya fever was found in the age group 21-40 years with female preponderance. TheStudy showed that in case of fever of 1-3 days duration, Real Time RT-PCRdetected 9cases and IgM ELISA detected none, in case of fever of 4-6 days Real Time RTPCR detected 5 cases and IgM ELISA detected 8 cases, in case of fever≥7days Real Time RT-PCR detected 1 case and IgM ELISA detected 8cases.So, our study revealed that Real Time RT-PCR was more sensitive test than CHIKspecific IgM ELISA for early detection of Chikungunya fever case.

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