Research Article

Chikungunya fever, a reemerging virus infection: Diagnosed by real-time polymerase chain reaction

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ABSTRACT

Background: Chikungunya virus (CHIKV), an emerging viral infection, has become a serious health problem in recent years. It has been frequently reported in the Indian subcontinent in the past few years. Reemergence of the other arboviral hemorrhagic fever has made diagnostic dilemma for clinicians. The prognosis of the disease is generally good; though some patients develop chronic arthritis and neurological involvement have been reported. **Objective:** To study the epidemiological and clinical profile of CHIKV infection. **Materials and Methods:** Study comprises total 1296 patients presented with clinical suspicion of Chikungunya from September to December 2016. Confirmation of cases was carried out by detection of viral RNA by real-time polymerase chain reaction (RT-PCR). **Result:** Of 1296 suspected cases, 845 (65.2%) cases were positive for CHIKV RNA, detected by RT-PCR assay. Majority of the confirmed cases were of age group 50–59 years. Male:female positivity ratio was 1.33:1. The most common clinical features were fever with joint pain and rash. Of 34 suspected cases with neurological involvement, 14 cases (41.17%) were confirmed positive by RT-PCR in cerebrospinal fluid specimen. **Conclusion:** Early diagnosis and monitoring of CHIKV infection is an important component of disease management. Viral RNA detection by RT-PCR gives a positive confirmatory result in the acute phase (first 7 days) of the disease. RT-PCR is rapid, specific, and sensitive method of choice for the early detection and confirmation of virus in clinical samples.

KEY WORDS: Chikungunya Virus Infection; Viral RNA detection; Real-time Polymerase Chain Reaction, Rapid, Specific, Sensitive

INTRODUCTION

Chikungunya is a mosquito-borne viral disease caused by the Chikungunya virus (CHIKV) (family: Togaviridae, genus: *Alphavirus*). It is transmitted to humans by virus-carrying Aedes mosquitoes (*Aedes aegypti* and *Aedes albopictus)*. Vertical transmission of the virus from mother to fetus has also been recorded.[1]

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In India, the virus was first isolated in 1963 in Kolkata.[2] The disease reemerged in India in October 2005 after remaining silent for nearly 32 years.[3]

The virus particles are enveloped and the genome consists of a single-stranded, positive-sense RNA molecule. Incubation period is 2–12 days. The symptoms of CHIKV infection include abrupt onset of fever, chills, headache, rash, and severe joint pain with or without swelling (usually the smaller joints) which gave rise to the name Chikungunya, which means "to walk bent over" in Makonde, an African language.

Most patients recover fully, but in some cases, joint pain may persist for several months or even years. Occasional cases neurological complications such as headache, reduced consciousness, acute encephalopathy,

and meningoencephalitis have been reported.[4] In older people, the disease can contribute to earlier death that may be due to the frequency of concomitant underlying diseases or decreased immunologic response.[5] Thus, rapid laboratory confirmation is crucial for adequate clinical therapeutic management and to initiate responses for control measures.

Laboratory confirmation is done by CHIKV IgM antibody, virus isolation, and viral RNA detection by polymerase chain reaction (PCR) technique in serum and cerebrospinal fluid (CSF) specimen, primarily. During the first 7 days of these illnesses, viral RNA can often be identified in serum. Virus-specific IgM antibodies may be detectable >4 days after onset of illness.[6]

Viral RNA detection by real-time PCR (RT-PCR) gives a positive confirmatory result in the acute phase (first 7 days) of the disease. RT-PCR is rapid, specific, and sensitive method of choice for the early detection and confirmation of infection.

MATERIALS AND METHODS

This study comprised all the suspected case of Chikungunya infection with an acute illness characterized by sudden onset of fever, joint pain, rash, etc., over a period of September 2016–December 2016. Confirmation of cases was carried out by the detection of viral RNA by RT-PCR.

Molecular Assay

Specimen collection

All 1296 samples, include 34 CSF and 1262 plasma/serum specimen, were tested over a period of 4 months (September–December 2016) for the presence of CHIK virus-specific RNA by RT-PCR. Positive and negative controls were included in each run of the assays, and all precautions to prevent cross-contamination were observed.

RNA extraction and RT-PCR

One-step RT-PCR was performed in accordance with the manufacturer's protocol, employing primer pairs targeting the neuroendocrine-specific protein gene. The assay principle is based on Taqman probes which allow higher specificity and sensitivity.

Statistical Analysis

The Chi-square test was used for analysis. $P < 0.05$ was considered to be statistically significant. Data were entered into an Excel file and analyzed using Epi Info 7.1.5.0.

RESULT

A total of 1296 samples from patients presented with clinical suspicion of Chikungunya from September 2016–December 2016 were received. RT-PCR for Chikungunya was done on all the 1262 serum and 34 CSF specimens, out of which 831 serum and 14 CSF were positive for Chikungunya. Hence, 845 (65.20%) were confirmed cases of Chikungunya infection.

Majority of Chikungunya cases were positive in the months of November (74.33%) (odds ratio [OR] 1.9, $P = 0.0001$) [Table 1].

Largest proportion of suspects was in age group of 50–59 years, with positivity of 75.79% (OR 1.8, [CI 1.3–2.5], $P = 0.0004$) [Table 2].

Among patients with confirmed Chikungunya infection, 492 (68.90%) were male. Male:female positivity ratio is 1.35:1 [Table 3].

The most common clinical features seen in CHIKV infected patients were fever with joint pain and rash (100%).

A total of 34 patients presented with clinical suspicion of viral encephalitis were tested for Chikungunya infection in CSF by RT-PCR, after excluding bacterial meningitis. Of these 34 patients, 14 cases were confirmed positive for Chikungunya infection.

DISCUSSION

Although cases of Chikungunya infection were reported throughout the year, a peak was observed in November 2016. Suddenly, the number of samples and positivity rate increased with an average of 74.3% during this month. As reported above, individuals aged 50–59 years accounted for the highest number of cases [75.79%].[7,8]

Table 1: Month wise distribution of clinically suspected and positive cases of Chikungunya

Month	Total suspected cases (*n*=1296)	Positive cases *n*=845 (%)	OR	*P*
September	63	29 (46)	0.4 (CI 0.2–0.7)	0.001
October	500	315 (63)	0.8 (CI 0.6–1)	0.2
November	487	362 (74.3)	1.9 (CI 1.5–2)	0.0001
December	246	139 (56.5)	0.6 (CI 0.4–0.8)	0.002

*Considered statistically significant when $P<0.05$. OR: Odds ratio, CI: Confidence interval

Table 2: Age wise distribution of clinically suspected and positive cases of Chikungunya

Age group (years)	Total cases *n*=1296	Positive cases *n*=845 (%)	OR	*P*
0-9	53	31 (58.49)	0.7 (CI 0.4–1.2)	0.36
10–19	105	52 (49.52)	0.4 (CI 0.3–0.7)	0.0006
20–29	170	84 (49.41)	0.4 (CI 0.3–0.6)	0.0001
30–39	174	108 (62.06)	0.8 (CI 0.6–1.1)	0.3
40–49	146	107 (73.28)	1.4 (CI 0.9–2.1)	0.07
50–59	219	166 (75.79)	1.8 (CI 1.3–2.5)	0.0004
60–69	213	154 (72.30)	1.4 (CI 1.0–2.0)	0.021
70–79	119	83 (69.74)	1.2 (CI 0.8–1.8)	0.32
80–89	97	60 (61.85)	0.8 (0.5–1.3)	0.5

*Considered statistically significant when $P<0.05$. OR: Odds ratio, CI: Confidence interval

Table 3: Distribution of the cases according to gender

Gender	Total suspects *n*=1296	Positive cases *n*=845	Percentage	Ratio
Male	758	482	57.26	1.33
Female	532	361	47.36	-

Of 14 confirmed cases with neurological involvement, 11 were above age of 60 years. A definitive diagnosis of Chikungunya infection can be made only with the aid of laboratory support since clinical presentation of infection resembles those of other arboviral hemorrhagic fever. Laboratory diagnosis is therefore critical to establish the differential diagnosis. Therefore, a molecular approach based on reverse transcription RT-PCR technologies is useful for early confirmatory diagnosis before the appearance of IgM antibody, irrespective of the presence of viable virus.

In the present study, the peak of the cases reported in the month of November, the classical season, due to high vector density in post-monsoon period.[7]

Similar studies conducted in New Delhi and Italy also reported the most affected age group as 40–59 years.[7,8]

Our study revealed males encountered more frequent infections than females.[7,8]Neurological involvement of elderly patients was also observed in study conducted at Nagpur.[9]

Strengths and Limitations

High prevalence (65.2%) may be attributed to the study population restricted to a small geographical area. Geographical conditions have not been accounted, which may have a significant impact on prevalence and morbidity.

CONCLUSION

It can be concluded that the prevalence of Chikungunya is high in the month of January, among the age group of 50–59 years. This study strongly supports CHIKV to be an important cause of central nervous system infection and demands for a more detailed understanding of its neurovirulence.

Early diagnosis and monitoring of CHIKV infection is an important component of disease management. Viral RNA detection by RT-PCR gives a positive confirmatory result in the acute phase (first 7 days) of the disease. RT-PCR is rapid, specific, and sensitive method of choice for the early detection and confirmation of virus in clinical specimen.

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