ROLE OF RAPID IMMUNOCHROMATOGRAPHIC TEST IN SERODIAGNOSIS OF CHIKUNGUNYA INFECTION IN A TERTIARY CARE CENTRE

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ABSTRACT

BACKGROUND

A large number of arthropod borne viruses (Arboviruses) have been diagnosed from humans in India in recent years. In India, there was a major outbreak of Chikungunya in 2006 wherein 1.39 million cases were diagnosed across 16 states. The main clinical features in this self-limiting disease include sudden onset fever, chills, rash and joints pain. A study was done to look for the seropositivity of Chikungunya IgM card test (Immunochromatographic assay) among suspected test samples.

MATERIALS AND METHODS

This is a hospital based study done in the Department of Microbiology at a tertiary care centre in Ludhiana from September to December 2016. A total of 481 blood samples from patients with suspected chikungunya were sent to the Microbiology Laboratory for detection of IgM Ab.

RESULTS

A total of 481 clinical samples were tested for Chikungunya IgM Ab during the study period. Majority of the samples (449) were from adults (93.3%), while only 32 samples (6.65%) were of paediatric age groups. Out of all samples, majority (420 out of 481, i.e. 87.3%) were from inpatient wards. A total of 87 patients were tested "Reactive" out of 481 (18.08%) and among them Male-to-Female ratio was 1.6:1.

CONCLUSION

Immunochromatographic test for IgM Ab against chikungunya virus has advantage of being rapid, easy, user friendly and does not require special equipment or training. It can be used as rapid tool for confirmation of diagnosis. However, its sensitivity and specificity needs to be determined and physicians should refrain from advising the test during first 3 - 4 days of illness.

KEYWORDS

Chikungunya, Arbovirus, Chikungunya IgM Ab, Immunochromatographic Assay.

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BACKGROUND

A large number of arthropod-borne viruses (arboviruses) have been diagnosed from humans in India in recent years. The family of arboviruses is heterogeneous and is divided into at least 7 major groups. Dengue and chikungunya are known to be commonly prevalent in India.

Chikungunya virus, a single stranded RNA virus, belongs to Group A (alphaviruses) arboviruses (togaviridae family) and causes a dengue-like illness. It is transmitted by bite of aedes mosquito (same mosquito that spreads dengue virus).[1] The name Chikungunya derives from a root verb in the Kimakonde language meaning "that which bends up," i.e. to become contorted. The name reflects the stooped appearance of sufferers with arthralgia.[2] The first epidemic of chikungunya was recorded in Tanzania in 1952 - 53.[3] Since then epidemics have occurred in several countries. Even in India, there was a major outbreak in 2006, wherein 1.39 million cases were diagnosed across 16 states.[4] During 2015, a total of 27,553 clinically suspected cases of Chikungunya were reported from 22 states and 3 UT's. While 14,656 clinically suspected cases were reported till mid-September in 2016 from 18 states and 2 UT's.[5]

The main clinical features include sudden onset fever, chills, rash and joints pain.[6] It is usually a self-limiting illness and majority of patients just need symptomatic treatment. However, joint pains can persist for many months and even years.[7] Leucopoenia with lymphocyte predominance is the usual observation and thrombocytopenia is also seen at times. But none of these haematological findings are pathognomonic. During acute phase both CRP and ESR are elevated.[5]

As the clinical manifestations of Chikungunya fever resemble those of dengue and other fevers caused by arthropod-borne viruses of the Alphavirus genus, laboratory diagnosis is critical to establish the cause of diagnosis and initiate specific response.

Diagnosis of chikungunya can be divided into virus culture, molecular and serological methods. Chikungunya virus isolation can be done by intracerebral inoculation of mice.[8] Specific detection of the virus can be performed using real time PCR/Nested PCR combination amplifying fragment of envelope E2 gene.[9] Recently, sensitive and specific one step TaqMan RT-PCR assay has been developed as a tool for diagnosis of chikungunya virus and rapid indicator by

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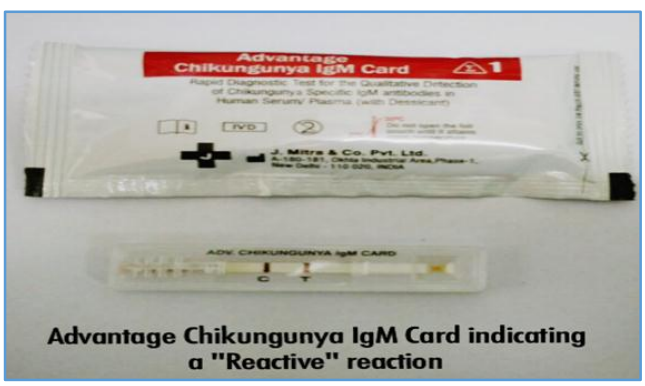
quantifying viral load in clinical samples and cell culture supernatant.[10]

Serological diagnosis can be made by demonstration of four-fold rise in antibody titre in acute and convalescent sera or by demonstrating IgM antibodies specific for chikungunya virus.[11] Virus specific IgM antibodies are readily detected by capture ELISA (Enzyme-linked immunosorbent assay) and ICT (Immunochromatographic tests) in patients. The gold standard of chikungunya diagnosis is culture and isolation, but this requires facilities and skills. Even the molecular methods like PCR need costly reagents and equipment. Serological diagnosis by detecting IgM/IgG antibodies is most widely used, as it is relatively cheaper and easier to perform. The disadvantage of antibody testing is the possibility of cross-reactivity with other alphaviruses. IgM antibody may persist for 3 - 6 months, so a single raised IgM may indicate a recent past infection rather than an acute infection. A study was done to look for the seropositivity of Chikungunya IgM card test (Immunochromatographic assay) among suspected test samples.

MATERIALS AND METHODS

This is a hospital based study done in the Department of Microbiology, at a tertiary care centre, Ludhiana from September to December 2016. A total of 481 blood samples from patients with suspected chikungunya were sent to the Microbiology Laboratory for detection of IgM Ab.

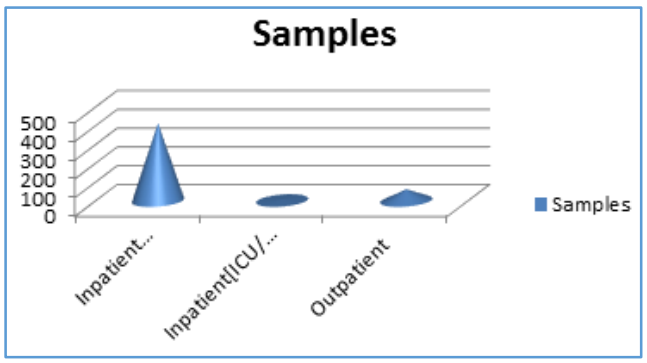
Test for chikungunya was performed in the Microbiology Lab using Advantage Chikungunya IgM Card (J. Mitra and Co Pvt. Ltd). The test is based on sandwich immunoassay principle and is a one-step Immunochromatographic assay. The test sample is introduced, which flows laterally through an absorbent pad where it mixes with the conjugate. If the sample contains Chikungunya specific IgM antibodies, it forms a complex with the conjugate. The complex then migrates through the nitrocellulose membrane by capillary action. When the complex meets the line of immobilised capture reagents (test line 'T'), it generates a pink purple line indicating that the sample is reactive. To serve as a procedural control, an additional control line 'C' has been immobilised at a distance from the test line on the strip. If the test is performed correctly, this results in the formation of a pinkish purple line at the control region upon contact with the conjugate. The results were read visually in 15 minutes. Detection of both control and test line was interpreted as 'reactive' and only control line was interpreted as 'Non-reactive' for presence of IgM Ab against Chikungunya [as per the manufacturer's protocol].



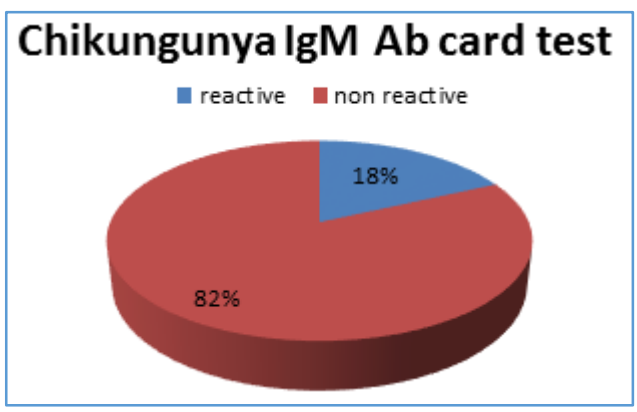
Advantage Chikungunya IgM Card indicating a "Reactive" reaction

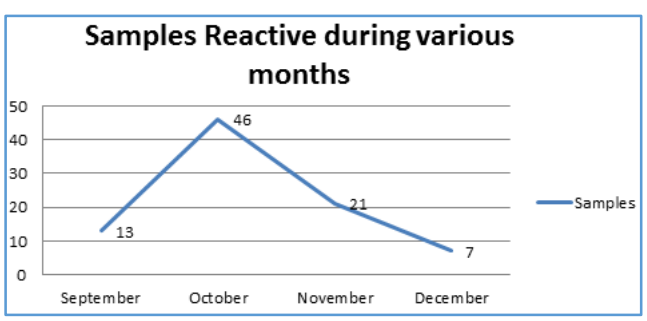
RESULTS

A total of 481 clinical samples were tested for Chikungunya IgM Ab during the study period. Majority of the samples (449) were from adults (93.3%), while only 32 samples (6.65%) were of paediatric age groups. Out of all samples majority (420 out of 481, i.e. 87.3%) were from Inpatient wards (including 7 patients from ICU/HDU's).



A total of 87 patients were tested "Reactive" out of 481 (18.08%) and among them Male-to-Female ratio was 1.6:1. Out of 7 ICU/HDU patients, 2 tested "Reactive" for Chikungunya. Both patients improved with treatment, none of them had any organ failure and were discharged. More than half of patients (46 patients, 52.8%) reported as "Reactive" were detected during the month of October.





DISCUSSION

In this study, rapid diagnostic test [immunochromatographic test] was used for diagnosis of chikungunya.

The clinical features of chikungunya infection are nonspecific and may resemble other viral illnesses, especially dengue fever. A total of 87 patients were found to be "Reactive" for Chikungunya out of 481 suspected samples. Our study also emphasises that suspected chikungunya patients actually may include a relatively large number of other febrile illnesses. In

our study, only 18% samples had IgM positive for chikungunya indicating that in remaining majority the reason for febrile illness would have been some other. Mulyatno et al also detected five isolates (31.3%) of Dengue fever in 16 clinically suspected chikungunya fever cases.[12] In a recently published study by S Chattopadhyay et al a total of 36.89% patients were seropositive for Chikungunya,[13] while a study from a tertiary care centre in Mumbai found seroprevalence of Chikungunya to be 12.5%.[14] Seropositivity in the given samples depends mainly on physician threshold for suspecting chikungunya, timing of sample withdrawal after disease onset and sensitivity of the test being used.

In our study, majority of reactive samples were from adults (94.3%) and only a handful of patients were in paediatric age group (5.7%). This finding could be attributed to the fact that adults work during daytime and are more prone to bite by aedes, whereas children in paediatric age group usually stay protected at home. Similar adult predominant infection has been shown in many studies. A study by Yergolkar P N et al showed that the disease preferably affects adults as 90% of the seropositive patients were > = 15 years of age.[15] Our results are similar to study by Patil et al, wherein 70.14% cases were found in adults.[16] Also in this study group, there was slight male predominance (1.2:1) similar to our result (1.6:1), although Chikungunya is known to affect both the genders. Majority of the samples were collected from inpatient wards (and very few from ICU/HDU) suggesting severe debilitating symptoms experienced by patients requiring admission, but at the same time not seriously ill.

In present study, a large proportion of Chikungunya cases were detected during the month of October indicating maximum Aedes mosquito transmission rate during this post monsoon period, as there occurs water accumulation. This artificial water collection is the source of breeding of the Aedes mosquito and the increased frequency of mosquito bites. The lack of awareness amongst the general public and the improper sanitation cannot be ignored and control measures are to be advocated during the same period. In another study, similar findings were observed where maximum Chikungunya cases occurred in the months of November and December.[17]
An early diagnosis of chikungunya is needed not just for management of chikungunya, but also to look for other causes of febrile illness in case the test is negative. Though IgM antibody based assay with only acute phase samples is not sufficient for diagnosis. Also the sensitivity of IgM antibody based assay is extremely low during acute phase.[18] In majority of chikungunya cases, IgM Ab only reach detectable levels between day 4 and day 7 of illness.[1,19] The IgM Ab remain in host for many months, so these Ab may not always correlate with an acute chikungunya infection.

Out of all samples, only 18% were “Reactive” for Chikungunya. The test was performed using the Immunochromatographic method. There would have been some false negative too, especially if samples were sent too early (before IgM levels start rising during illness). Also, the sensitivity/specificity of this test has not been exactly determined (J. Mitra kit). As most acutely infected patients seek medical attention within the first few days of illness, the ideal test should detect RNA or antigen.[20] Also we cannot deny for few false positives as similar arboviral illness, i.e. dengue occurs during same months and can cause cross-reactivity in few of the patients. In a study wherein a new immunochromatographic diagnostic test was used for detection of Chikungunya virus the sensitivity, specificity and real-time PCR (RT-PCR) agreement values of 89.4%, 94.4% and 91.1% were revealed respectively.[21]

CONCLUSION

Immunochromatographic test for IgM Ab against chikungunya virus has advantage of being Rapid, Easy and User Friendly and does not require specialist equipment or training. It can be used as rapid tool for confirmation of diagnosis. However, its sensitivity and specificity needs to be determined and physicians should refrain from advising the test during first 3 - 4 days of illness.

REFERENCES

[1] Pialoux G, Gaüzère BA, Jauréguiberry S, et al. Chikungunya, an epidemic arbovirosis. Lancet Infect Dis 2007;7(5):319-327.
[2] Guidelines for prevention and control of chikungunya fever. World Health Organization, regional office for south east Asia 2009. http://www.searo.who.int/linkfile/publication-SEA-CD-182.
[3] Robinson MC. An epidemic of virus disease in southern province, Tanganyika territory, in 1952–53. I. Clinical features. Trans R Soc Trop Med Hyg 1955;49(1):28-32.
[4] Ravi V. Re-emergence of chikungunya virus in India. Indian J Med Microbiol 2006;24(2):83-4.
[5] National guidelines for clinical management of chikungunya 2016. http://nvbdcp.gov.in/Doc/National-Guidelines-Clinical-Management-Chikungunya-2016.
[6] Hochedez P, Jaureguiberry S, Debruyne M, et al. Chikungunya infection in travelers. Emerg Infect Dis 2006;12(10):1565-7.
[7] Kennedy AC, Fleming J, Solomon L. Chikungunya viral arthropathy: a clinical description. J Rheumatol 1980;7(2):231-6.
[8] Myers RM, Carey DE, Reuben R, et al. The 1964 epidemic of dengue-like fever in south India: isolation of chikungunya virus from human sera and from mosquitoes. Indian J Med Res 1965;53(8):694-701.
[9] Pfeffer M, Linssen B, Parke MD, et al. Specific detection of chikungunya virus using a RT-PCR/nested PCR combination. J Vet Med B Infect Dis Vet Public Health 2002;49(1):49-54.
[10] Pastorino B, Bessaud M, Grandadam M, et al. Development of a TaqMan RT-PCR assay without RNA extraction step for the detection and quantification of African chikungunya viruses. J Virol Methods 2005;124(1-2):65-71.
[11] Thaikruea L, Charearnsook O, Reanphumkarnkit S, et al. Chikungunya in Thailand: a re-emerging disease? Southeast Asian J Trop Med Public Health 1997;28(2):359-64.
[12] Mulyatno KC, Susilowati H, Yamanaka A, et al. Primary isolation and phylogenetic studies of chikungunya virus from Surabaya, Indonesia. Jpn J Infect Dis 2012;65(1):92-4.
[13] Chattopadhyay S, Mukherjee R, Nandi A, et al. Chikungunya virus infection in West Bengal, India. Indian J Med Microbiol 2016;34(2):213-5.

[14] Galate LB, Agarwal SR, Shastri JS, et al. Chikungunya fever among patients with acute febrile illness attending a tertiary care hospital in Mumbai. J Lab Physicians 2016;8(2):85-9.
[15] Yergolkar PN, Tandale BV, Arankalle VA, et al. Chikungunya outbreaks caused by African genotype, India. Emerg Infect Dis 2006;12(10):1580-3.
[16] Patil SS, Patil SR, Durgawale PM, et al. A study of the outbreak of chikungunya fever. J Clin Diagn Res 2013;7(6):1059-62.
[17] Chundawat B, Madhulika M, Mehta KD, et al. Profile of the chikungunya infection: a neglected vector borne disease which is prevalent in the Rajkot district. Journal of Clinical and Diagnostic Research 2013;7(6):1008-11.
[18] Blacksell SD, Tanganuchitcharnchai A, Jarman RG, et al. Poor diagnostic accuracy of commercial antibody-based assays for the diagnosis of acute chikungunya infection. Clin Vaccine Immunol 2011;18(10):1773-5.
[19] Taubitz W, Cramer JP, Kapaun A, et al. Chikungunya fever in travelers: clinical presentation and course. Clin Infect Dis 2007;45(1):e1-e4.
[20] Dash M, Mohanty I, Padhi S. Laboratory diagnosis of chikungunya virus: do we really need it? Indian J Med Sci 2011;65(3):83-91.
[21] Okabayashi T, Sasaki T, Masrinoul P, et al. Detection of chikungunya virus antigen by a novel rapid immunochromatographic test. J Clin Microbiol 2015;53(2):382-8.