Evaluation of an Antibody-Based Test for the Diagnosis of Chikungunya Infection

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Abstract

Background: The diagnosis of chikungunya is confusing due to similar clinical presentations of different viral illnesses. Objective: The purpose of the present study was to evaluate the accuracy of an antibody-based test for the diagnosis of chikungunya infection. Methodology: This cross-sectional study was conducted at the Department of Internal Medicine, Bangabandhu Sheikh Mujib Medical University, Dhaka, from July to September 2017 when an outbreak of chikungunya occurred in Bangladesh. Chikungunya patients were evaluated by the IgM antibody test by immunochromatographic method (ICT) during both the early phase and the convalescent-phase. The sensitivity and specificity of the IgM antibody test were estimated considering the polymerase chain reaction (PCR) method as the gold standard. Result: The sensitivity and specificity of the IgM antibody test in the acute phase were 2.7 percent and 79.5 percent, respectively. In contrast, in the convalescence phase, sensitivity and specificity were 86.5 percent and 33.3 percent. Conclusion: Antibody-based testing was found not suitable for detecting chikungunya infection during the acute phase of the illness. [Journal of Current and Advance Medical Research, July 2020;7(2):68-72]

Keywords: Chikungunya; sensitivity; specificity; immunochromatographic method

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Introduction

Chikungunya is an arboviral infection that classically presents with high-grade fever, joint pain, and rash\(^1\). The vector for viral pathogen the Aedes mosquito (Aedes aegypti and Aedes albopictus) is abundant during the post-monsoon period due to an increase in breeding places facilitated by stagnant water\(^3\). The recent outbreak of chikungunya in Bangladesh during the monsoon period April-September 2017 was more widespread and debilitating than the previous incidences after the first detection of the virus in 2008\(^5\). The Institute of Epidemiology, Disease Control and Research (IEDCR), Bangladesh, confirmed 984 reverse transcription-polymerase chain reaction (RT-PCR) positive chikungunya cases during that period where the estimated clinically diagnosed cases were around 13000 cases\(^8\). Though diagnosed clinically, the chikungunya virus (CHIKV) infection is often confused with dengue and other viral illnesses due to similar clinical presentations. Moreover, arthralgia due to chikungunya often persists for long, which demands excluding the disease from other rheumatoid disorders. For these reasons, a confirmed diagnosis with lab support for chikungunya is often necessary\(^9\).

Polymerase chain reaction (PCR) is a reliable method for diagnosing CHIKV infection during acute phases. Among the different techniques, the conventional PCR has good sensitivity\(^12\). However, it is not affordable for most of the patients of developing countries due to its comparatively high price and lack of available testing facilities. The World Health Organization (WHO) has made a list of general characteristics that make a diagnostic test appropriate for low resource settings countries of the world. Accordingly, the ideal test should be affordable, sensitive, specific, user-friendly (simple to perform by persons with little training), rapid and robust use without special storage, equipment-free, and delivered to those who need it\(^14\). Antibody-based tests are much cheaper, quick, and available all over Bangladesh. Immunochromatographic test (ICT) for IgM detection is one of the most commonly used point-of-care (POC) tests for chikungunya in Bangladesh\(^15\). Despite its wide availability, the ICT IgM antibody test didn’t show much promise regarding sensitivity and specificity in the previous studies\(^16\). The validity of the ICT IgM antibody test has not been performed in our country before. Proper validation of this rapid POC test is necessary for an evidence-based national guideline formulation. A low cost, widely available POC test can save time and money during future outbreaks.

In this study, the ICT IgM antibody test was evaluated for sensitivity (SN), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) in comparison to gold standard PCR investigation.

Methodology

Study Population and Sample: This cross-sectional study was conducted by the Department of Internal Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, during the chikungunya outbreak from July to September 2017. The study enrolled a total of 102 probable cases of chikungunya fever attending the outpatient department (OPD) of Internal Medicine, BSMMU, during the study period. According to the national guideline on chikungunya management, patients with acute febrile illness and joint pain were diagnosed as probable cases of chikungunya\(^19\). Study participants were advised to attend a follow-up visit on the second week of their illness, and 76 participated in this regard.

Data Collection Method: A medical doctor collected data on demographic variables and the clinical history of all primary patients. Blood samples were collected two times, firstly within seven days of illness (acute phase sample) and secondly on the subsequent week of symptom appearance (convalescence sample). Professional phlebotomists collected 5 ml of blood from the antecubital vein of the respondents aseptically. They sent the blood samples to the Virology Department of the same university. Qualitative Polymerase Chain Reaction (PCR) and ICT-based IgM detection tests were done for the first sample, while the second samples were sent for IgM detection by the ICT method. ICT was done by the Standard Q Chikungunya IgM/IgG kit by SD Biosensor. Chikungunya RNA was extracted by the GeneRna RNA Isolation kit, and amplification was done by the GeneRna Biotechnology AG amplification kit. The amplified product was run by 2 percent agarose gel electrophoresis, and the band was visualized by transilluminator.

Statistical Analysis: During the acute and convalescence phase, the IgM antibody test results were analyzed for sensitivity, specificity, negative predictive values (NPV), and positive predictive values (PPV) with 95 percent confidence intervals (CI) in comparison with PCR finding.

Ethical Approval: The study was approved by the Institutional Review Board of BSMMU (Memo...
number: 9521). Informed written consent was taken from each participant.

**Result**

The mean age of the participants was 34 years, with a range from 8 to 70 years. Approximately two-thirds of the respondents were female. Almost half of the probable cases of chikungunya fever had PCR-confirmed positive infection during the acute-phase illness, where only 12 percent of patients had a positive IgM antibody. In the second week of the disease, 76 percent of 76 patients had a positive IgM antibody result. During the acute phase, the IgM test showed 2.7 percent sensitivity and 79.5 percent specificity (Table 1).

### Table 1: Diagnostic Validity of IgM Test During the Acute Phase For Detecting CHIKV Infection

| Diagnostic validity | Value  | 95% CI        |
|---------------------|--------|---------------|
| Sensitivity         | 2.7    | 0.1 to 14.2   |
| Specificity         | 79.5   | 63.5 to 90.7  |
| PPV                 | 11.1   | 0.3 to 48.2   |
| NPV                 | 46.3   | 34.0 to 58.9  |

PPV= Positive predictive value; NPV= Negative predictive value

However, in the convalescent stage, the sensitivity and specificity of the IgM test were 86.5 percent and 33.3 percent, respectively (Table 2).

### Table 2: Diagnostic Validity of IgM test during the Convalescent Phase for Detecting CHIKV Infection

| Diagnostic validity | Value | 95% CI        |
|---------------------|-------|---------------|
| Sensitivity         | 86.5  | 71.2 to 95.5  |
| Specificity         | 33.3  | 19.1 to 50.2  |
| PPV                 | 55.2  | 41.5 to 68.3  |
| NPV                 | 72.2  | 46.5 to 90.3  |

PPV= Positive predictive value; NPV= Negative predictive value

**Discussion**

In Bangladesh, a massive outbreak of chikungunya occurred in 2017. According to the Ministry of Health and Family Welfare of Bangladesh, 984 real-time PCR and more than 13176 clinically confirmed cases were reported. CHIKV infection is usually diagnosed clinically, but diagnosis can be confused with dengue or other arboviral diseases, which shows an almost similar clinical picture. Polyarthritis of chikungunya infection often persists for an extended period, which needs exclusion from other rheumatoid disorders, and confirmation of the diagnosis with laboratory support is sometimes required. However, this becomes more challenging in countries with limited resources where the facility to laboratory testing is minimal, particularly in rural areas. Also, sufficient time and resources needed for laboratory confirmation in an outbreak situation often will not be feasible. For a reliable diagnosis of chikungunya infection in the acute phase, the assay should detect chikungunya-specific analytes like RNA, antigen, or antibody during illness.

Various studies showed that CHIKV RNA peak rapidly in the first three days of illness and then decline. It may persist for up to 8 days after the onset of infection. Whereas anti-CHIKV IgM antibodies can be detected from 4th day onward, resulting in incorrect false-negative results when sera are tested in early acute-phase. These IgM antibodies may persist in the host for many months. In this study, most of the patients with chikungunya infection presented to the health facility at the 3rd to 4th day of illness, at which point antibody only starts to build up in the body. In early cases, we expect more PCR positivity than antibody positivity. This study found that PCR was positive in 49 percent of patients, and only 12 percent had a positive IgM antibody. As most of the patients attended the acute phase of illness on the 3rd to 4th day of their symptom appearance, PCR was also positive in the highest amount in these periods of illness. It has been documented that in the acute phase of the disease, the sensitivity of the anti-CHIKV IgM-based assay is very low. Blacksell and colleagues found only 1.9% to 3.9% sensitivity of the tests during this time. The sensitivity and specificity of the IgM test in the acute phase sample in this study were only 2.7% cases and 79.5% cases respectively which was similar to the findings from other studies. In the convalescent period, the sensitivity was raised to 86.5% with a specificity of 33.3%. With the progression of the day of illness, an antibody developed gradually, and the IgM antibody was detected at a higher rate. From this scenario, we can assume that the antibody-based test used in our study was not appropriate for detecting cases in the acute phase of the illness.

PCR assay is quite acceptable for diagnosing the acute phase of CHIKV infection up to 7 days of illness and is superior to antibody-based technologies. But PCR based molecular detection methods are not widely available, and they are costly. A single test costs about 50 USD, whereas the per capita income of Bangladesh is only 2065 USD. PCR tests also require high precision.
Antibody-Based Test for the Diagnosis of Chikungunya Infection

From this study, we found that antibody-based tests like ICT IgM detection had no role in diagnosing illness in the acute phase or first week of illness. The detection rate of the disease by antibody detection rises in the convalescent period with good sensitivity. As the antibody stays long for months, this test can be used in the sero-surveillance of the infection and differentiating rheumatological disorders present with sub-acute or chronic presentations.

ICT method for antibody detection can be used to detect CHIKV infection during the convalescent period in developing countries like Bangladesh. Polyarthralgia of chikungunya often persists for an extended period and needs exclusion from other rheumatoid disorders. During outbreaks, a low cost, widely available POC test like ICT, should be recommended to save time, money, and space; and national preparedness to control future propagation.

The study participants were selected purposively, and selection bias was a limitation. Larger sample size with the inclusion of a control group will provide more information in this regard.

Conclusion

Antibody-based testing was found not suitable for detecting chikungunya infection during the acute phase of the illness. The higher sensitivity of the test in the convalescence phase can help sero-surveillance and exclude other chronic joint pain diseases.

References

1. Patterson J, Sammon M, Garg M, Dengue, Zika and Chikungunya: Emerging arboviruses in the New World. Western Journal of Emergency Medicine 2016;17(6):671
2. Burt FJ, Chen W, Miner JJ, Lenschow DJ, Merits A, Schnettler E, et al. Chikungunya virus: An update on the biology and pathogenesis of this emerging pathogen. The Lancet Infectious Diseases. 2017;17(4):e107-17
3. Kothandam S, Arumugam S, Purushothaman I, Swaminathan R. Real time molecular detection of chikungunya and dengue virus in the female Aedes mosquitoes collected in Chennai, Tamilnadu, India. Tropical Biomedicine. 2017;34(1):166-73
4. Reiter P, Fontenille D, Paupy C. Aedes albopictus as an epidemic vector of chikungunya virus: Another emerging problem?. Lancet Infectious Diseases 2006;6(8):463-4
5. Kabir I, Dhimal M, Müller R, Banik S, Haque U. The 2017 Dhaka chikungunya outbreak. Lancet Infect Dis. 2017;17(11):1118.
6. Rahim MA, Uddin KN. Chikungunya: an emerging viral infection with varied clinical presentations in Bangladesh: Reports of seven cases. BMC Infectious Diseases. 2017;17(1):1-7.
7. Kalam MA. Chikungunya outbreak. The Independent, 17 July 2017. http://www.thainewsdirectory.com/post/104516. (Accessed 6 June, 2020)
8. Institute of Epidemiology, Disease Control and Research. Chikungunya News Letter. Sept 7, 2017. Website: http://www.iedcr.gov.bd/index. (Accessed 6 June, 2020)
9. Borgherini G, Poubpe P, Jossaume A, Gouix A, Cotte L, Michault A, et al. Persistent arthralgia associated with chikungunya virus: A study of 88 adult patients on reunion island. Clinical Infectious Diseases. 2008 Aug 15;47(4):469-75.
10. Laoprasopwattana K, Kaewjungwad L, Jarumonkul R, Geater A. Differential diagnosis of chikungunya, dengue viral infection and other acute febrile illnesses in children. The Pediatric Infectious Disease Journal. 2012;31(5):459-63
11. Chang AY, Encinales L, Porras A, Pacheco N, Reid SP, Martins KAO, et al. Frequency of chronic joint pain following chikungunya virus infection. Arthritis Rheumatol. 2018;70:578-584.
12. Mackay IM, Arden KE, Nitsche A. Real-time PCR in virology. Nucleic Acids Research. 2002;30(6):1292-305.
13. Esposito DL, Fonseca BA. Sensitivity and detection of chikungunya viral genetic material using several PCR-based approaches. Revista da Sociedade Brasileira de Medicina Tropical. 2017;50(4):465-9.
14. Urdea M, Penny LA, Olimsted SS, Giovanni MY, Kaspar P, Shepherd A, et al. Requirements for high impact diagnostics in the developing world. Nature. 2006;444(7305):739-41.
15. Yeasmin M, Yusuf MA, Rahman M. Diagnostic limitation of chikungunya fever and its future prospect: A review update. Bangladesh Journal of Infectious Diseases 2018;5(2):65-70
16. Prat CM, Flusin O, Panella A, Tenebray B, Lanciotti R, Leparc-Goffart I. Evaluation of commercially available serologic diagnostic tests for chikungunya virus. Emerging Infectious Diseases. 2014;20(12):2129-32
17. Blacksell SD, Tanganuchitharnchai A, Jarman RG, Gibbons RV, Paris DH, Bailey MS, et al. Poor diagnostic accuracy of commercial antibody-based assays for the diagnosis of acute chikungunya infection. Clinical and Vaccine Immunology. 2011;18(10):1773-5
18. Yap G, Pok KY, Lai YL, Hapsarachchi HC, Chow A, Leo YS, et al. Evaluation of chikungunya diagnostic assays: differences in sensitivity of serology assays in two independent outbreaks. PLoS Neglected Tropical Disease 2010;4(7):e753
19. Communicable Disease Control (CDC), Directorate General of Health Services (DGHS), Bangladesh. National Guideline on Clinical Management of Chikungunya Fever. 15 May, 2017
20. Goupi BA, Mores CN. A review of Chikungunya Virus-Induced Arthralgia: Clinical manifestations, therapeutics, and pathogenesis. Open Rheumatology Journal 2016;10:129
21. Chopra A, Anuradha V, Lagoo-Joshi V, Kunjir V, Salvi S, Saluja M. Chikungunya virus aches and pains: An emerging challenge. Arthritis Rheumatism 2008;58(9):2921-2
22. Petti CA, Polage CR, Quinn TC, Ronald AR, Sande MA. Laboratory medicine in Africa: a barrier to effective health care. Clinical Infectious Diseases. 2006;42(3):377-82
23. Appassakij H, Khuntikij P, Kemapunmanus M, Wutthanarungsan R, Silpapojakul K. Viremic profiles in asymptomatic and symptomatic chikungunya fever: a blood transfusion threat? Transfusion. 2013;53(10pt2):2567-74
24. Taubitz W, Cramer JP, Kapaun A, Pfeffer M, Drostten C, Dobler G, et al. Chikungunya fever in travelers: clinical presentation and course. Clinical Infectious Diseases. 2007;45(1):e1-4
25. Grivard P, Le Roux K, Laurent P, Fianu A, Perrau J, Gigan J, et al. Molecular and serological diagnosis of Chikungunya virus infection. Pathologie-biologie (Paris). 2007;55(10):490-4
26. Chua CL, Sam IC, Chiam CW, Chan YF. The neutralizing role of IgM during early chikungunya virus infection. PLoS One. 2017 Feb 9;12(2):e0171989
27. Rianthavorn P, Wuttiritthamakowit N, Prianantathavorn K, Limpaphayom N, Theamboonlers A, Poovorawan Y. Evaluation of a rapid assay for detection of IgM antibodies to chikungunya. Southeast Asian Journal of Tropical Medicine and Public Health. 2010;41(1):92
28. Johnson BW, Russell BJ, Goodman CH. Laboratory diagnosis of chikungunya virus infections and commercial sources for diagnostic assays. The Journal of Infectious Diseases. 2016;214(suppl_5):S471-4.
29. Economy of Bangladesh. https://en.wikipedia.org/wiki/Economy_of_Bangladesh (accessed 6 June, 2020)
30. Sissoko D, Ezzedine K, Moendandzé A, Giry C, Renault P, Malvy D. Field evaluation of clinical features during chikungunya outbreak in Mayotte, 2005–2006. Tropical Medicine & International Health. 2010;15(5):600-7