International Journal of Technical Innovation in Modern Engineering & Science (IJTIMES),(UGC APPROVED)
Impact Factor: 5.22 (SJIF-2017),e-ISSN:2455-2585
Research Symposium on
"Advancements in Engineering, Science, Management, and Technology"
Volume 5, Special Issue 04, April-2019.

Recent Trends in Chikungunya Virus diagnosis

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Abstract

Chikungunya is vector borne disease which is transmitted by female mosquito found in early morning and late afternoon. Chikungunya disease starts with onset of fever and severe joint pain. This paper presents the diagnostic techniques for CHIKV. Conventional as well as newer techniques are also studied and compared.Aim for the paper is to provide brief introduction to all the existing and experimental diagnostic techniques for Chikungunya.

Keywords— Chikungunya, diagnosis tools for Chikungunya, laboratory methods, RT-PCR, ELISA, Antigen-Antibody detection, Vector Borne Disease

I. INTRODUCTION

The word Chikungunya is from *Makonde* language, which is spoken on Monkonde Plateau in Southern Tanzania. First outbreak for the Virus is reported in 1952.Since then it has affected millions of people around the world mainly Africa, Asia and Indian subcontinent. It is a vector borne disease which belongs to *Togaviride* family and the transmitted by *Aedes Mosquitoes*. The term Chikungunya means "that which bends up", "to walk over" or "to become contorted", relating the bending of patients due to severe pain in wrists, hands, knees and ankles. Some Symptoms are high fever, nausea, muscle pain, Joint Pain (Lower Back, knees and wrists), Rash, Joint swelling, headache and fatigue. The virus is transmitted by the female mosquito found in day light Hours mainly in early morning and late afternoon[13].

II. VIRUS STRUCTURE

Chikungunya virus is a small (about 60–70 nm-diameter), spherical, enveloped, positive-strand RNA virus that is approximately 11 kb in length and codes for 9 proteins. The genome has two open reading frames (ORFs): the 5´ORF, translated from genomic RNA, encodes the nsP1, nsP2, nsP3, and nsP4 non-structural proteins, and the 3´ORF, genome is transcribed for subsequent translation into a polyprotein precursor containing the three structural proteins PE2 (the precursor of E3 and E2), E1, and the capsid protein .Figure 1 depicts CHICV genome structure.

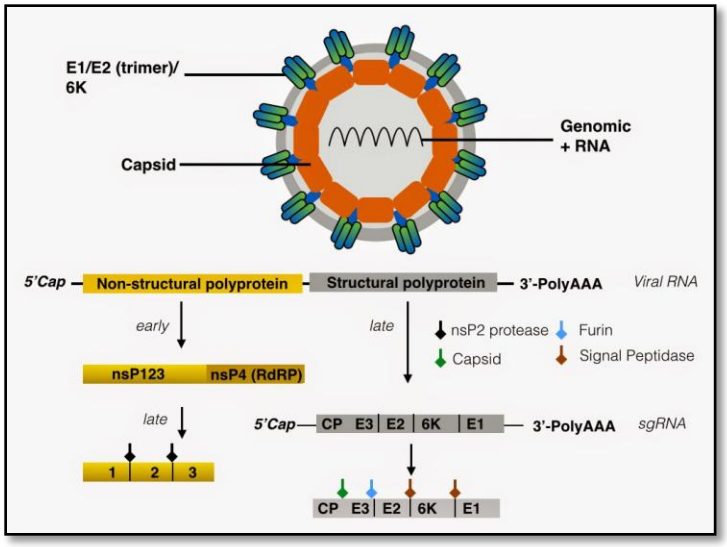

Figure 1. CHIKV virus genome structure. Reproduced from (26).

III. DIFFERENCES BETWEEN DENGUE AND CHIKUNGUNYA

Chikungunya and Dengue, are carried by the same mosquito type, but are caused by different viruses. While Dengue is caused by a *Flavirideae flavivirus* , Chikungunya is caused by a *Togaviridae alphavirus*. Swelling and intensity of joint pain is high in Chikungunya as compared to that in Dengue, whereas Dengue can cause breathing problems, bleeding in some cases.

IV. CHIKUNGUNYA DETECTION METHODS

Chikungunya starts showing symptoms like fever and joint pain within 2-3 days of mosquito bite. This can progress from mild to severe rapidly so early and correct diagnoses is necessary. Chikungunya symptoms are often confused with dengue, Zika and malaria so early diagnoses should also be able to distinguish the virus.

There are various methods for diagnosing Chikungunya. Some are used commercially and some are in developmental phase. We will discuss some of these in brief. Several experiments have been done to compare different diagnostic techniques for CHIK[6,14,25].Table I list comparison of convectional diagnosis techniques and Table II list the recently suggested diagnosis, technique for CHIKV.

It is important to keep in mind that the detection efficiency of these methods varies depending on both the presence of the viral particles in the bloodstream of a patient and on the time of sample collection (Figure 2).

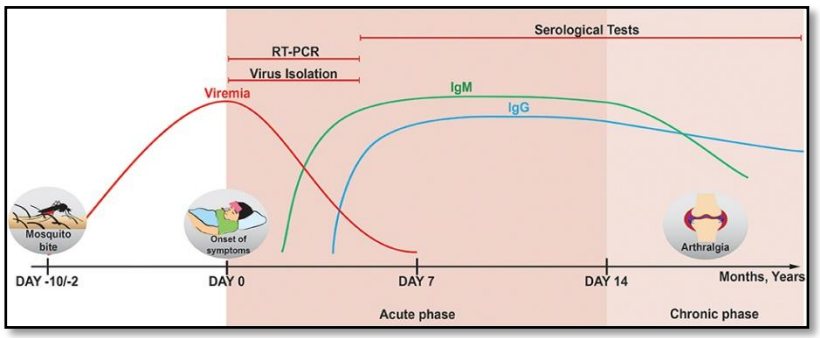

Figure 2. Time course of chikungunya virus (CHIKV) viremia and immune response along with detection methods. Reproduced from (27).

A. Virus Isolation

Virus isolation is the oldest method for detection of virus .Cell cultures, which are derived from dispersed cells taken from original tissue and disaggregated by, mechanical, enzymatic or chemical means, provided large numbers of cells suitable for virus isolation. Virus is isolated in insect and mammalian cell lines. Isolated virus can be confirmed by RT-PCR or IFA. Virus isolation is more convenient and less expensive however it takes almost a week to provide results.

B. RT-PCR

RT-PCR is expanded as Reverse Transcription Polymerase Chain Reaction. It targets the virus envelop and non structural genes. It is use to detect CHIKV and can also tell us the quantity of virus in given volume. Results of this test are available in two days. RT-PCR is preferred if the sample is taken 6 days after the illness onset. This technique combines reverse transcription of RNA into DNA and amplifies the specific DNA targets using Polymerase Chain Reaction (PCR).Using this technique specific amount of RNA can be measured. RT-PCR can be categorized into two categories namely conventional RT-PCR and real time RT-PCR (qPCR). Detailed studies for two techniques show that qPCR is 10 times more sensitive than conventional PCR, therefore it also the preferred technique for detection of CHIKV.

C. Serological Test

When a foreign particle attacks the body, antibodies are produced by the immune system. These foreign particles are called antigens. On recognition of antigens by the immune system, selective antibodies that are able to bind the specific antigen are produced. Serological tests can be done to calculate antibodies as well as antigens[15].

Serological test determines antibodies (mainly IgM and IgG) in our body. These tests are categorized as Enzyme Linked immunosorbent assay for IgM/IgG (ELISA) , indirect immunofluorescence assay (IFA) ,Plaque Reduction Neutralization test(PRNT) and antigen specific test.

Immunoglobulin M (IgM) is mainly found in blood and lymph fluid. IgM is the first antibody produced by the immune system to fight a new infection. It is detectable from 2-3 days from onset of illness and remains detectable for 3-4 months. Immunoglobulin G (IgG) antibody is found in all body fluids. It is detectable from 6-7 days from onset of illness and remains detectable for years. IgM/IgG ELISA is used because it is simple and sensitive. IgM/IgG is detectable long after illness. They can also produce false results due to cross reactivity with similar disease. It is because of these reasons they are not very reliable methods for diagnosis.

1) ELISA Test

In ELISA test procedure the blood sample is added with specific antigen for CHIKV. If the blood contains antibodies to antigen two will bind together. The contents is checked by adding an enzyme and observed how blood and antigen react. If virus is present then content will change the color.

2) IFA Test

IFA determines the specific antibodies against CHIKV. The biochips are coated with antigen and blood is added in it. If the blood contains antibodies to antigen two will bind together. In next step attached antibodies are strained with fluorescein labeled anti human antibodies and are made visible by florescence microscope.

3) PRNT Test

PRNT determines the presence of neutralizing antibodies specific for CHIKV. It calculates the percentage of reduction in virus activity by comparing and counting the number of plaques with standard amount of virus.

4) *ELISA Antigen Detection test*

Antigen detection test can detect directly whether the patient is affected by CHIKV or not. ELISA antigen technique provides high sensitivity and no case of cross reactivity with similar clinical diseases. In the detection procedure the antibodies are bound to a plastic surface, and then blood sample is added to it. If blood contains antibodies to antigen, two will bind together. The contents are checked by adding an enzyme and observe how blood and antigen react. If the virus is present then contents will change the color.

Experiments have been done using antigen testing for Chikungunya [7,8,18].Commercially these test are available for Dengue but not for Chikungunya.

D. *Differential Diagnosis*

It is the technique for distinguishing the particular virus from other virus that presents similar clinical conditions. In case of CHIKV, the viral diseases which confuse the diagnoses are malaria, Zika and Dengue. However it is also seen that both Dengue and CHIKV are present in patient body. A rapid, sensitive and specific method for the differential diagnosis of these CHIKV and DENV viruses simultaneously can also be done[2,10,17].

E. *Rapid Detection Kits*

There are several detection kits available in markets. Mostly are based on Immunoassay principle. It is one step procedure and does not require much skill or equipment for diagnosis. They are bio Hazard free and give results in simple yes or no. However, sometimes the cost exceeds the conventional detection techniques. They also have limited self life and do not provide elaborate report of the virus. Sensitivity is normally good, sometimes may give false results too.

Figure 3 Rapid Test Kits for Chikungunya

F. *Other Diagnosis Techniques*

Over the years researchers have provided excellent diagnoses techniques for CHIKV. They are not commercially available yet but the experimental results have shown promising future. Some such techniques are discussed in table II.

TABLE I
Comparison of Convectional Diagnosis Techniques

S. No	Diagnostic Tool	Method	Advantages	Disadvantages	Result	References
1	Virus Isolation	Virus is isolated in insect and mammalian cell lines. Isolated virus can be confirmed by RT-PCR or IFA	Requires facilities and skills	Requires large amount of blood for the sample.	Results are produced in almost a week	[3]
2	RT-PCR and qRT-PCR	Detection of Viral nucleic Acid	Highly sensitive and specific Target envelop and non structural gens Sensitivity based on viral particles per reaction volume Requires less amount of blood	Reagents and equipments are costly for widespread use.	2 days	[22]
3	Serological Test (ELISA,PRNT,IFA)	Detection of Host antibody Response	Widely acceptable Easy to perform Relatively cheaper Applicable to serum and cerebrospinal fluid	Cross reactivity with other alphavirus	1 day	[21]
4	Antigen ELISA Detection Test	Detection of Viral antigen	No case of Cross reactivity with other *alphavirus*	Not available commercially. Performance characteristics not defined	1 day	[7,8,18]
5	Differential Diagnosis	Mainly Based on Antigen Detection	Highly Specific	Costly Not available commercially	-	[2,10, 17,21]
6.	Rapid Detection Kits	Based on Sandwich Immunoassay principle.	Fast Results Cheaper No Specific skill required One step test procedure Bio hazard free, No Instruments required. Excellent Sensitivity & Specificity.	Have limited shelf lives Producing only "yes/no" answers Less sensitive or less accurate Sometimes bit expensive compared to conventional techniques	5-15 minutes depending on the kit	6

Table II

Recently suggested diagnosis technique for CHIKV

Technique	Method	Reference
Western blot detection	Reactivity of recombinant GST-E2 and MBP-E2 antigens against CHIKV patients' sera and normal sera is measured	[9]
Electrochemical CHIKV DNA detection system	Using two-dimensional mos2 Nanosheets based disposable Biosensor	[20]
Immunodiagnostic Assay for CHIKV	Multiple antigenic peptide (MAP) approach using selective epitopes of The E2 protein.	[1]
Antigen Capture ELISA	Monoclonal antibody (Mab) based Antigen capture ELISA to detect Chikungunya virus antigen from the mosquitoes	[5]
Enzyme-linked immunosorbent assay	Using a recombinant envelope protein 2 of CHIKV produced in Escherichia coli system, as a capture antigen.	[11]
Paper based DNA biosensor	The ultra-high charge-transfer efficiency of gold nanoparticles (Au) and biocompatibility associated with Magnetic nanoparticles (Fe3O4) to develop detection platform for Chikungunya virus DNA (CHIKV)	[19]
Rapid Immunochromato graphic Test	Rapid diagnostic test using mouse Mabs that react with CHIKV E1 proteins	[6]
Serum spotted onto filter paper	Analyses were performed from frozen sera and serum spotted onto filter paper provided from 121 Chikungunya suspected cases	[12]
Monoclonal antibodies against chikungunya virus structural proteins	Generation of mouse anti-CHIKV Mabs targeting CHIKV E1 and capsid proteins. These Mabs possessed broad reactivity to all three CHIKV genotypes,	[23]

CONCLUSION

This work discusses and compares the current laboratory diagnosis methods for detection of Chikungunya virus infection. It was observed that although virus isolation, RT-PCR, qPCR, PRNT and serology methods provide accurate results, these methods require mind-numbing steps, expensive requirements, and skilled staff.

ELISA test for antigen and antibody are also discussed which provide results earlier than the conventional techniques. ELISA tests may give false results due to Cross reactivity with other *alphavirus*. Some additional tests after ELISA may confirm the definitive virus.

An antigen detection technique shows excellent sensitivity in detecting CHIKV. More and more antigen based techniques are being presented. Recent researches have shown diagnostic method using recombinant proteins and monoclonal antibodies against Chikungunya virus show promising future in Chikungunya diagnosis.

Rapid detection kits are the need of present. They provide faster results and does not require skilled labor. These approaches have the potential to improve chances of survival, particularly in resource-limited countries. Biosensors have been designed for other viruses .Future diagnostic techniques may include Biosensors for detection of CHIKV.

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