

Original Research Article

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Diagnosis of Chikungunya by a Rapid Immunochromatographic Method in a Tertiary Care Setting

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ABSTRACT

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Vector borne diseases have gained importance in recent decade due to the increasing frequency of sporadic episodes occurring in both endemic and non-endemic areas. The similar clinical presentation and the acute febrile nature results in misdiagnosis often and increases the risk of transmission and morbidity in the vulnerable populations. A study was undertaken to check the prevalence of Chikungunya virus among the febrile patients presenting with body ache or myalgia between June 2014 and July 2015. The serodiagnosis was based on solid immunochromatographic assay to check its feasibility as a reliable and specific test for Chikungunya virus. Out of 92 samples tested, the seropositivity for IgM antibody to Chikungunya was found to be 19.5%. A few clinically suspected cases were not detected by the rapid test. Similarities between the clinical presentation of Dengue fever and Chikungunya were noted. No deaths were reported from the study.

Introduction

Arboviral diseases are increasingly becoming prevalent in areas where the government initiatives have failed to control these vector-borne diseases. Mosquitoes over the decades have become adapted to the various means of eradicating them and pose a great public threat in tropical regions. The temporal changes in the seasons, breaking down of social hygiene, overpopulation, migrating population represented by job seekers, tourist and the medical tourists have made the management of vector-borne diseases beyond an individual government's control.

Aedes mosquito is one of the most adapted of the mosquito vectors (WHO, 2009). Being anthropophilic, its biting and nesting habits

enable it to survive efficiently in the cities and villages. They bite during the day and night (Blacksell *et al.*, 2011).

Two common species of *Aedes*, *A. aegypti* (*Stegomyia aegypti*) and *A. albopictus* (*Stegomyia albopicta*) are responsible for transmitting Chikungunya, Dengue, Sindbis Semliki, forest and eastern equine encephalitis. The mode of transmission is through the bite of infected female *Aedes* mosquito. The seasonality of these diseases and the common vector increases the risk of co-infection of Dengue with Chikungunya in India. In a study, *Aedes albopictus*, was reported as the predominant species for transmitting the infection in the city suburbs,

easily breeding in flower pots and shrubbery while *Aedes aegypti* was more frequently associated in the city. Both *Aedes aegypti* and *Aedes albopictus* are known to be resistant to dichlorodiphenyl-trichloroethane (DDT; 4%) which is commonly used by Indian Municipalities to control the mosquitoes in the cities, but are susceptible to 5% malathion (Dev *et al.*, 2014).

Chikungunya virus is an enveloped, single-stranded RNA virus of the alphavirus genus (family *Togaviridae*). Prior to 2013, the chikungunya viruses were limited to the east Asia, south east Asia, the Indian subcontinent, Africa and few parts of Europe. Since then, the disease has been noted in many countries causing outbreaks (CDC, 2015).

Most patients develop sudden onset of symptoms beginning within 3-7 days of the bite, developing fever and peripheral joint pains. Other symptoms such as headache, body-ache, muscle cramps or rash may be confused with other encephalitic presentations. The disease may sometimes be misdiagnosed as Dengue or Malaria in endemic areas (Murray *et al.*, 2012). The morbidity due to the cramps and joint pains is high which may persist in many patients for few weeks after the treatment is started. The management of the infection is symptomatic including antipyretic drugs, chloroquine and ribavirin. Interferon-alpha, in some studies, have been stated as useful (Briolant *et al.*, 2004).

The seasonality of these diseases and the common vector increases the risk of co-infection of Dengue with Chikungunya in India. The similar clinical presentation and the complications that complicate the management which is usually supportive treatment makes it essential to include the laboratory diagnosis of Chikungunya in all patients who is suspected of pyrexia of unknown origin.

Materials and Methods

The study was conducted in a 100 bedded tertiary hospital between June 2014 and July 2015 in the Mysuru district. Serum samples collected from 92 patients who were suspected of Pyrexia of unknown origin (PUO) were tested for IgM antibodies against Chikungunya. Informed consent was taken from the patients and approval from the Institutional Ethics committee obtained for the study. All patients who presented with acute febrile illness <5 days with joint pains or myalgia or severe body ache were included in the study. Other tests were done as routine requested by clinicians to rule out Dengue, Leptospirosis and Malaria by peripheral smear Weil Felix test and Widal test as part of fever panel.

IgM antibody to Chikungunya virus was detected using SD BIOLINE Chikungunya IgM test. This is rapid qualitative test based on immunochromatographic assay. The card has two pre-coated lines, "T" (Chikungunya recombinant structural protein), and "C" (Goat anti-mouse IgG) on the surface of the device. The monoclonal anti-human IgM is conjugated with colloidal gold. The gold colloid will react specially with IgM antibodies to Chikungunya in human serum, plasma or whole blood. Control line should always appear if the test procedure is performed correctly. Positive result is seen as appearance of "T" line turning purple after 10 mins. A serum sample of 50microliters is added to the card followed by 1 drop of the assay diluent into sample well. The results were correlated with clinical records retrospectively.

A patient with a suspicion of Chikungunya infection was termed as probable case while other patients whose clinical and laboratory criteria satisfied the defined criteria were termed confirmed cases (Directorate of

NVBDCP, 2016). For laboratory confirmation a single test of positive serum sample for Anti-Chikungunya IgM was used to confirm the infection.

Results and Discussion

During the 13 month period, 92 samples were analysed for the presence of the virus specific IgM antibodies. The female to male ratio was noted as 1:1.1 and the age ranged from 19 to 78 years (Table 1)

Of the 92 suspected cases for Chikungunya, 18 samples (19.56%) gave positive results for IgM antibodies to Chikungunya virus, Dengue IgM(15), Rheumatoid arthritis (3) and septic arthritis (4), 3 peripheral smear positive for Plasmodium Vivax, 1 adult Measles, 2 cases UTI, Atypical pneumonia (1) and H1N1(1). 44 samples were negative for tests in the Fever panel including Widal and blood culture (47.8%).

The characteristics of the fever in the 18 confirmed cases was of sudden abrupt onset with temperature ranging from 101-104°C, rarely with chills (2 cases) and intermittent in 4 cases. The rash was of either macular erythema or petechial type described over trunk or abdomen area. Swelling and pain were associated with peripheral joints, commonly bilateral, associated frequently with generalized bodyache or myalgia. Other less frequent clinical findings were headache, nausea, throat pain, muscle cramps, poor appetite, gastritis and dry cough. 8 patients had history of taking NSAIDS before admission.

In all the 92 patients' supportive and symptomatic treatment was administered. Out of 92 patients, 76 of them were administered with either oral or intravenous antibiotics (ceftriaxone, Doxycycline, Augmentin or Levofloxacin), 1 on Tamiflu and 3 on

Antimalarials therapy (inj. Artesunate+ sulfadoxine and pyrimethamine). The fever in the confirmed cases subsided on symptomatic treatment after few days. There were no deaths reported in our study.

Since its first association with an outbreak in Tanganyika in the 1950s, Chikungunya virus had mostly been occurring sporadically in parts of Africa and Asia. The first major outbreak which was documented was in 2004 which spread in the next 4 years from Africa to parts of India and Islands in the Indian Ocean to South East Asia. During that time many countries across Europe and North America reported cases from travelers. (Erin Staples *et al.*, 2009; Taubitz *et al.*, 2007; Panning *et al.*, 2008).

Outbreaks due to Chikungunya virus occur episodically in the Indian subcontinent. Increased contact between the human population and vector due to various factors in both rural and urban places such human displacement to seasonal monsoon failures, deforestation, disorganized urban planning and waste management have all led to increase in vector related arboviral and parasitic infections in the recent years (Mohan, 2006).

Two vectors *Aedes aegypti* and *A.albopictus* share the same epidemiological niches as the endemic zones of Dengue, Japanese encephalitis malaria Chikungunya and in recent years the Zika virus. Co-infections and simultaneous outbreaks of these infections can be explained due to bite of the common vector the female *Aedes* mosquito. (Erin Staples *et al.*, 2009; Ratsitorahina *et al.*, 2008; Moulin *et al.*, 2016).

Dengue fever is characterized by flu-like symptoms accompanied with headache, rash, body-ache and characteristically low platelet count. Chikungunya presents always with

high grade fever, severe bodyache and peripheral arthralgia. Complications of Dengue infections such as encephalitis, hemorrhagic symptoms, severe hematological changes and fatal outcomes are rarely associated with Chikungunya virus. Chikungunya infection however results in prolonged and slow recovery from the polyarthralgias, often persisting for months (Chattopadhyay *et al.*, 2016; Borgherini *et al.*, 2008).

The age group between 40-49 years showed minimally higher preponderance (38.8%) as compared to both 20-29 age group and 50-59(27.7%). In the study, both the sexes were equally affected. This was in agreement with other studies (Chattopadhyay *et al.*, 2016). The study population being small, it is difficult to conclude that age can affect the susceptibility to this infection. In a predominantly agricultural area, vector borne diseases such as Rickettsial infections or Chikungunya can be considered as occupational risks. The panel of tests for the emergency department should contain rapid tests specific for the diseases endemically or sporadically present in that area. The Laboratory should try to get tests repeated after appropriate interval in cases of high suspicion if the tests are negative.

In our study as described in the Table 2, the similarities in the clinical presentation between Chikungunya and Dengue were comparable in most instances (Erin Staples *et al.*, 2009). While the inclusive criteria of the study were the presence of fever and body ache/joint pains, few presentations such as the polyarthralgia, bleeding dyscrasias and hematological changes were the only marked delineating characteristics to clinically differentiate them. In both the infections, patients of various age groups are affected and any underlying cause as Diabetes mellitus, COPD or pregnancy can seriously

affect the outcome for the patient. Many studies have noted the difficulty in clinically diagnosing the vector-borne diseases without the laboratory support.

Laboratory diagnosis of most of the arboviral infections is either ELISA or serological test based on antigen or specific antibody. In acute phase of the febrile illness, most patients may present with similar clinical symptoms and to alleviate, the clinician has to depend on rapid tests. The rapid POC tests are relatively cheaper, widely available, easy to perform, produce faster results but are subjective and can only be used as screening tests due to low specificity (Stacey *et al.*, 2015; Prat *et al.*, 2014). These rapid tests are critical in differentiating Chikungunya from other arboviral infections to institute the correct emergency procedures.

After the onset of symptoms, IgM and IgG antibodies to Chikungunya become detectable after 3rd and 6th day of respectively. IgM antibody levels peak around 3 to 5 weeks and may persist for 3 months (Prat *et al.*, 2014; Labadie *et al.*, 2010). These tests show variable sensitivity and without a more advanced test kit like ELISA being easily available, standardizing the IgM kits in the field becomes difficult. The sensitivity may vary with the progress of the disease. According to one study the sensitivity of the immune-chromatographic tests was found to be 22% in the first week (Rianthavorn *et al.*, 2010). The rapid POC tests are qualitative tests and may not reflect the severity or prognosis of the disease. Therefore the criteria to check for 4 fold rise in titres cannot be fulfilled. A negative result cannot preclude the presence of infection as the antibodies appear after 3 days and are not sensitive to low titres. Other factors such as heterophile antibodies or rheumatoid factors can confuse the result.

Table 1 Age and sex distribution for Chikungunya cases

Range in years	male	female	Positivity rate	Total
<20	1	2	0	3
20-29	13	15	5 (17.8)	28
30-39	8	8	1 (6.25)	16
40-49	10	7	7 (41.1)	17
50-59	10	6	5 (31.2)	16
60-69	4	3	1 (14.2)	7
>70	3	2	0	5
Total	49	43	0	92

Table.2 Comparison of Clinical presentation of Chikungunya IgM positive and other clinical conditions

Clinical and lab parameters n=92	Chikungunya IgM positive -18 (%)	Dengue IgM positive- 15(%)	Other clinical conditions Chik-IgM negative, Dengue IgM negative- 69 (%)
High grade fever	18 (100)	10 (66.6)	32 (46.3)
Polyarthralgia	15 (83.3)	1 (6.6)	4 (5.7)
Rash	8 (44.4)	7(46.6)	6 (8.6)
Muscle cramps	5 (27.7)	2 (13.3)	8 (11.5)
Poor appetite	11 (61.1)	10 (66.6)	54 (78.2)
Bodyache/ myalgia	14 (77.7)	12 (80)	56 (81.1)
Headache with/without nausea	3 (10.6)	12 (80)	27 (39.1)
Leucopenia	10 (55.5)	2 (13.3)	11 (15.9)
Thrombocytopenia < 100 000 cells/ml	3 (10.6)	14 (93.3)	13 (18.8)
Vomiting	1 (5.5)	10 (66.6)	22(31.8)
Hemorrhagic outcomes	-	4 (26.6)	-

A positive result may not distinguish between acute and recent past (<3 months) infection. (Blacksell *et al.*, 2011; Rianthavorn *et al.*, 2010; Burdino *et al.*, 2016).

Cross-reactivity with other arboviral infections as Sindbis and Semliki viruses may produce false positive results (Yiu-Wing Kam *et al.*, 2015). As compared to other tests which require specialized equipment and available space, a POC rapid test can help in

early diagnosis and reduce risk of morbidity in the infected patients. While viral cultures and molecular assays can clinch the diagnosis, in the field or in smaller hospital, a rapid POC kits are more practical and economically feasible.

In conclusion, in high prevalence areas, the rapid IgM based tests may perform poorly as a screening test and need to be supported with immunoassays for confirmation. With the

improvement in medical tourism, expanding and changing epidemiology of the vector-borne diseases, it becomes very essential to differentiate these pathogens using rapid bedside efficient and easy to perform serological tests.

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