Research Article

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COMPARATIVE SENSITIVITY OF RICA AND MAC-ELISA FOR IgM ANTIBODY BASED EARLY DIAGNOSIS OF CHIKUNGUNYA VIRUS INFECTION IN PATIENTS IN KADAPA DISTRICT OF ANDHRA PRADESH, INDIA

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Abstract: Chikungunya (CHIK) is a viral fever that spreads in human population through mosquito bite that is infected with Chikungunya virus (CHIKV). CHIK resembles Dengue fever. Although there is no specific treatment or vaccine available currently, the confirmative rapid diagnosis is based on detection of viral nucleic acid or IgM antibodies in serum, an indication of recent infection, helps in epidemiological monitoring, symptomatic treatment of patients and determining prognosis. In the present study, with the approval of the Kadapa district medical and health officer, 238 blood samples were collected from Chikungunya infection suspected patients, residing in the Kadapa district villages of Andhra Pradesh. Serological detection of anti-CHIKV IgM antibodies was performed using rapid immuno-chromatographic assay (RICA) and IgM-antibody capture enzyme-linked immuno Sorbant assay (MAC-ELISA). Convalescent sera (n = 170) were tested by RICA and 108 of them were found positive for anti-CHIKV IgM antibodies. All 108 anti-CHIKV IgM antibody RICA positive sera were further assayed using MAC-ELISA to compare the sensitivity of both assays for the early diagnosis of disease. Only 45 sera samples gave positive result. The sensitivity of RICA and MAC-ELISA were found to be 100% and 42% respectively.

Keywords: Chikungunya, Rapid Immuno Chromatographic Assay (RICA) and IgM Antibody Capture – ELISA (MAC- ELISA).

INTRODUCTION

Chikungunya virus (CHIKV) is an *Alphavirus* belonging to the family *Togaviridae* (7). 29 *Alphavirus* species are grouped into antigenic complexes on the basis of serological cross-reactivity, CHIKV belonging to the *Semliki Forest Virus* Complex closely related to *O'nyong-nyong virus* (6). CHIKV spread by Culicine mosquitoes (4,1). The main mosquito vectors are *Aedes aegypti* and *Aedes albopictus* although other *Aedes* species have also been reported to transmit the virus (7,15). The common reservoirs for CHIKV are monkeys and other Vertebrates. In the epidemic period, humans also act as reservoir (4,1). The disease symptoms resemble to that of dengue fever. Classical Chikungunya fever in human is characterized by high-grade fever, conjunctivitis, arthritis and skin rash with significant morbidity but rarely life threatening. The infection is severe in infants, elderly and immuno compromised people (12). CHIK is a self-limiting disease and the joint pains resolve in one to three weeks. However in about 12% of the patients, arthritis persisting for upto three years after the onset of illness has been documented (3,10). The deaths have also been reported but are very rare (17).

The entry of CHIKV in India is unknown although Kolkata sea and air roots are believed to be the probable entry points. In India, there is a confirmed history of outbreaks during 1963-64 in Kolkata (18), Pondicherry (1964) (8), Chennai (1965) (19), Andhra Pradesh (1965) (Vishakapatnam, Kakinada and Prakasam districts) (9,16,20), Nagpur (1965) (2). Last epidemic in India was reported from Barsi, Maharashtra in 1973 (14). CHIKV had almost disappeared from India after 1973 and since then, no case was reported till end of 2005 (11,13). In 2005, many suspected fever cases were reported from Coastal parts of Andhra Pradesh and Karnataka (5,12). During 2007-2008 more than 25,000 people affected with CHIK in Kasargod district, Kerala. In 2009 over 140 people affected with Chikungunya in Goa, same year the disease also affected the states of Tamilnadu, Orissa, Andhra Pradesh, Gujarat and Karnataka (Unpublished data). At present Chikungunya became epidemic in the state of Andhra Prasesh. Laboratory diagnosis of the disease can be done by virus isolation, but tedious to perform and requires cell culture facility. Molecular diagnosis is of high cost and facility not available in all laboratories. Antibody based diagnosis found to be cost effective. In the present study we did the early diagnosis of Chikungunya by detecting anti-chik IgM antibodies, indicator of an early infection using rapid immuno chromatographic assay and IgM antibody capture Enzyme linked immuno sorbant assay (MAC-ELISA) to help doctors in determining the prognosis and line of treatment. The two assays were compared for their sensitivity, which would be helpful for the rapid diagnosis of the disease.

MATERIALS AND METHODS

Study population

Health officials from local health centers from Kadapa district (Fig. 1) cooperated in obtaining the list of individuals suffering from disease. About 238 blood samples were collected in between July 2008 and March 2009 (Table 1). Prior to obtaining the samples the patients were asked to complete a questionnaire that included clinical symptoms and demographic information. The most common symptoms of the CHIK were found to be fever for 2-3 days, severe arthralgia, myalgia and swelling of tender joints like wrist, palms, ankles, rash etc., Fig.2. A, B and C shows swelling of joints and rashes observed in our present study.

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Figure 1: Kadapa (Cuddapah) District map marked with regions of sample collection areas

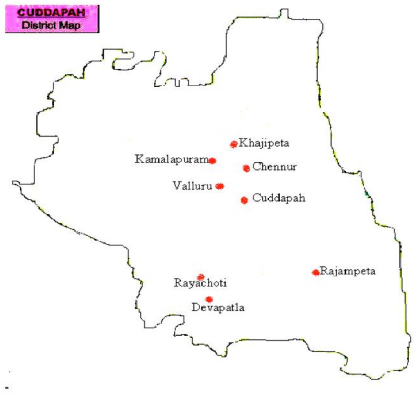

Table 1: Details of Sample collection areas

Sl. No.	Place of survey	Name of PHC in Kadapa district	Date of sample collection	No. of samples collected
1.	Yeguvarajugari palle & Diguvarajugari palle	Devapatla	23.07.2008	33
2.	Rajampeta	Rajampeta	22.09.2008	26
3.	Thudumala dinne village	Khajipeta	14.10.2008	24
4.	Sannapalle	Khajipeta	14.10.2008	11
5.	Thippireddy palle	Mydukur	03.11.2008	6
6.	Mulabatastreet, Mydukur	Mydukur	04.11.2008	7
7.	Dadireddy palle	Pedda cheppali	19.12.2008	15
8.	B.C.Colony, Kamalapuram	Kamalapuram	08.01.2009	30
9.	Kattagutta palli	Devapatla	12.02.2009	24
10.	N.N. Colony, Kadapa	Kadapa (Urban Health Centre)	13.02.2009	49
11.	Pedda Putha Village	Valluru	09.03.2009	13
		Total		**238**

Sample Collection

Approximately 3-5ml-blood sample was collected from each patient in sterile vial and transported under cold conditions to the laboratory. The clotted blood samples were centrifuged to separate serum. Acute phase sera were stored at -20°C whereas the convalescent sera were subjected to serological diagnosis.

Rapid Immuno Chromatographic Assay (RICA)

Serum samples were assayed for the presence of Anti-Chik IgM and Anti-Chik IgG using RICA (SD BIOLINE, South Korea) according to manufacturer's instructions. 25 ul of serum was loaded 1:1 dilution, with diluant provided along with the kit, to the sample well and allowed to pass through the membrane. The result was read within 15 minutes of sample application.

IgM Capture Antibody – Enzyme Linked Immuno Sorbant Assay (MAC-ELISA)

RICA IgM positive sera samples were assayed for the presence of IgM antibodies against CHIK using MAC-ELISA (NIV, Pune) according to manufacturer's instructions.

Ninety - six well microtiter plate pre coated with anti-human IgM antibody was washed, sera for testing were diluted 1:5 in serum dilution buffer and 50ul added to the wells in replicates with two wells serving as positive control and two wells serving as negative control to determine the cutoff value. Following one-hour incubation at 37°C and wash, 50ul CHIK antigen was added to each well. After one-hour incubation at 37°C and wash each well was added with 50ul CHIK B, Chik antigen specific monoclonal antibody. Then after incubation at 37°C/hour and wash each well was added with 50ul Avidin-Horse Radish Peroxidase Enzyme conjugate, incubated for additional half–an hour at 37°C and washed. Anti-human IgM antibody bound Chik IgM antibody- Chik-antigen – Chik- Ag specific antibody sandwich lined with Conjugate was detected by adding 50ul 1:20 diluted TMB substrate. The substrate was allowed to react for 10 minutes in dark at room temperature. Then the reaction was terminated by adding 100ul 1N H_2SO_4. The plate was read at 450nm in ELISA Reader (Model 680 Microplate Reader S/N 19548). The optical density of each serum was determined by subtracting its OD obtained from the negative control OD. The sample was considered positive when its OD is the same or more than the positive control OD obtained and subtracted from negative control OD.

RESULTS AND DISCUSSION

Out of 170 convalescent sera 108 were found positive by the development of a purple color band in the test region along with a band in the control region of RICA strip. The band was absent except in the control region in the negative

RICA strip (Fig. 3). All 108 RICA positive sera were further assayed using MAC-ELISA only forty-five were found positive by the development of color (Fig. 4). Table 2 summarizes the results of analyzed sera using both assays. The sensitivity of RICA was 100% whereas the sensitivity of MAC-ELISA was only 42%.

Figure 2: Clinical symptoms of Chikungunya Virus infected patients on fingers, feet and trunk

A) Swelling of wrist and Small finger joints
B) Swelling of ankle feet
C) Appearance of rash on side posterior area

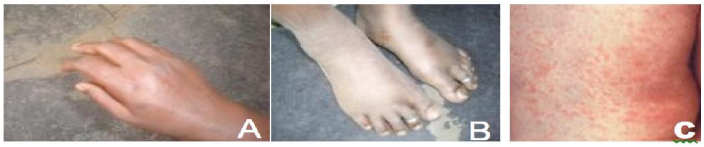

Figure 3: RICA positive and negative test results of convalescent sera

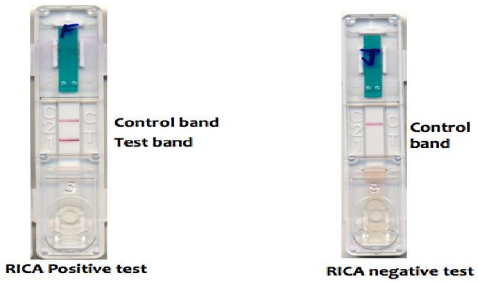

Figure 4: MAC-ELISA positive and negative test result of convalescent sera

Table 2: Anti-chik IgM detection using RICA and MAC-ELISA

Serum number	Vial number	Anti – Chik IgM test result	
		RICA	MAC – ELISA
1.	D1	+Ve	+Ve
2.	D2	+Ve	+Ve
3.	D3	+Ve	+Ve
4.	**D4**	**+Ve**	**-Ve**
5.	D5	+Ve	+Ve
6.	**D6**	**+Ve**	**-Ve**
7.	D7	+Ve	+Ve
8.	D8	+Ve	+Ve
9.	**D9**	**+Ve**	**-Ve**
10.	**D10**	**+Ve**	**-Ve**
11.	D12	+Ve	+Ve
12.	D13	+Ve	+Ve
13.	**D14**	**+Ve**	**-Ve**
14.	**D15**	**+Ve**	**-Ve**
15.	**D16**	**+Ve**	**-Ve**
16.	D17	+Ve	+Ve
17.	D18	+Ve	+Ve
18	**D19**	**+Ve**	**-Ve**
19.	D20	+Ve	+Ve
20.	D21	+Ve	+Ve
21.	**D22**	**+Ve**	**-Ve**
22.	**D23**	**+Ve**	**-Ve**
23.	D24	+Ve	+Ve
24.	D25	+Ve	+Ve
25.	**D26**	**+Ve**	**-Ve**
26.	**D27**	**+Ve**	**-Ve**
27.	**D28**	**+Ve**	**-Ve**
28.	**D29**	**+Ve**	**-Ve**
29	**D30**	**+Ve**	**-Ve**
30.	R2	+Ve	+Ve
31.	R3	+Ve	+Ve
32.	R6	+Ve	+Ve
33.	R8	+Ve	+Ve
34.	R12	+Ve	+Ve
35	**R14**	**+Ve**	**-Ve**
36.	R19	+Ve	+Ve
37.	**R20**	**+Ve**	**-Ve**
38.	**R21**	**+Ve**	**-Ve**
39.	K1	+Ve	+Ve
40.	K3	+Ve	+Ve
41.	**K4**	**+Ve**	**-Ve**
42.	K5	+Ve	+Ve
43.	**K8**	**+Ve**	**-Ve**
44.	K9	+Ve	+Ve
45.	**K10**	**+Ve**	**-Ve**
46.	K11	+Ve	+Ve
47.	**K13**	**+Ve**	**-Ve**
48.	**K14**	**+Ve**	**-Ve**
49	K16	+Ve	+Ve
50.	K18	+Ve	+Ve
51.	K20	+Ve	+Ve
52.	**K21**	**+Ve**	**-Ve**
53.	**K22**	**+Ve**	**-Ve**
54.	K23	+Ve	+Ve
55.	P1	+Ve	+Ve
56.	P2	+Ve	+Ve
57.	**P5**	**+Ve**	**-Ve**
58.	P6	+Ve	+Ve
59.	P7	+Ve	+Ve
60.	**KM1**	**+Ve**	**-Ve**
61.	KM2	+Ve	+Ve
62.	KM3	+Ve	+Ve
63.	**KM4**	**+Ve**	**-Ve**
64.	KM5	+Ve	+Ve
65.	KM6	+Ve	+Ve
66.	KM7	+Ve	+Ve
67.	**KM8**	**+Ve**	**-Ve**
68.	KM9	+Ve	+Ve
69.	KM10	+Ve	+Ve
70.	KM11	+Ve	+Ve
71.	KM12	+Ve	+Ve
72.	**KM13**	**+Ve**	**-Ve**
73.	**KM14**	**+Ve**	**-Ve**
74.	KM15	+Ve	+Ve
75.	**KM16**	**+Ve**	**-Ve**
76.	**KM17**	**+Ve**	**-Ve**
77.	KM18	+Ve	+Ve
78.	KM19	+Ve	+Ve
79.	**KM20**	**+Ve**	**-Ve**
80.	KM21	+Ve	+Ve
81.	**KD2**	**+Ve**	**-Ve**
82.	KD3	+Ve	+Ve
83.	KD5	+Ve	+Ve
84.	KD6	+Ve	+Ve
85.	**KD7**	**+Ve**	**-Ve**
86.	**KD10**	**+Ve**	**-Ve**
87.	**KD11**	**+Ve**	**-Ve**
88.	**KD12**	**+Ve**	**-Ve**
89.	**KA5**	**+Ve**	**-Ve**
90.	**KA8**	**+Ve**	**-Ve**
91.	KA9	+Ve	+Ve
92.	KA10	+Ve	+Ve

93.	**KA14**	**+Ve**	**-Ve**
94.	KA16	+Ve	+Ve
95.	KA17	+Ve	+Ve
96.	KA19	+Ve	+Ve
97.	**KA20**	**+Ve**	**-Ve**
98.	**KA21**	**+Ve**	**-Ve**
99.	KA25	+Ve	+Ve
100.	KA28	+Ve	+Ve
101.	KA31	+Ve	+Ve
102.	**KA34**	**+Ve**	**-Ve**
103.	KA36	+Ve	+Ve
104.	V1	+Ve	+Ve
105.	V3	+Ve	+Ve
106.	V4	+Ve	+Ve
107.	V5	+Ve	+Ve
108.	V8	+Ve	+Ve
Total Positive (+Ve)		**108**	**45**

This article documents the first report on comparative diagnosis of Chikungunya using RICA, easy to perform, cost wise cheap, less time consuming (half an hour) to obtain the result, less laborious and found 100% sensitive for the early diagnosis of anti-Chik IgM antibodies in the sera patients. MAC-ELISA, although previously reported to be effective in diagnosing even the low antibody titer found to be little laborious and takes 5 to 6 hours time to obtain result. Moreover in the present study the sensitivity of MAC-ELISA proved to be only 42%.

CONCLUSION

RICA is easier and rapid to perform than MAC-ELISA and also RICA is more sensitive than MAC-ELISA for the detection of anti-CHIK IgM antibodies in patient serum for the early diagnosis of Chikungunya.

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