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 Barshi, 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in Goa, same year the disease also affected the states of Tamilnadu, Orissa, Andhra Pradesh, Gujarat and Karnataka (Unpublished data).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 Pradesh. 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.
Figure 1: Kadapa (Cuddapah) District map marked with regions of sample collection areas

Table 1: Details of Sample collection areas

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Place of survey</th>
<th>Name of PHC in Kadapa district</th>
<th>Date of sample collection</th>
<th>No. of samples collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Yeguvarajugari palle &amp; Diguvarajugari palle</td>
<td>Devapatla</td>
<td>23.07.2008</td>
<td>33</td>
</tr>
<tr>
<td>2.</td>
<td>Rajampeta</td>
<td>Rajampeta</td>
<td>22.09.2008</td>
<td>26</td>
</tr>
<tr>
<td>3.</td>
<td>Thudumala dinne village</td>
<td>Khajipeta</td>
<td>14.10.2008</td>
<td>24</td>
</tr>
<tr>
<td>5.</td>
<td>Thippireddy palle</td>
<td>Mydukur</td>
<td>03.11.2008</td>
<td>6</td>
</tr>
<tr>
<td>6.</td>
<td>Mubabatstreety Mydrukur</td>
<td>Mydukur</td>
<td>04.11.2008</td>
<td>7</td>
</tr>
<tr>
<td>8.</td>
<td>B.C.Colony, Kamalapuram</td>
<td>Kamalapuram</td>
<td>08.01.2009</td>
<td>30</td>
</tr>
<tr>
<td>10.</td>
<td>N.N. Colony, Kadapa</td>
<td>Kadapa (Urban Health Centre)</td>
<td>15.02.2009</td>
<td>49</td>
</tr>
<tr>
<td>11.</td>
<td>Pedda Putha Village</td>
<td>Valluru</td>
<td>09.03.2009</td>
<td>13</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td>238</td>
</tr>
</tbody>
</table>

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.

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

<table>
<thead>
<tr>
<th>Serum number</th>
<th>Vial number</th>
<th>Anti – Chik IgM test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D1</td>
<td>+Ve</td>
</tr>
<tr>
<td>2</td>
<td>D2</td>
<td>+Ve</td>
</tr>
<tr>
<td>3</td>
<td>D3</td>
<td>+Ve</td>
</tr>
<tr>
<td>4</td>
<td>D4</td>
<td>+Ve +Ve</td>
</tr>
<tr>
<td>5</td>
<td>D5</td>
<td>+Ve</td>
</tr>
<tr>
<td>6</td>
<td>D6</td>
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<tr>
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<tr>
<td>10</td>
<td>D10</td>
<td>+Ve</td>
</tr>
<tr>
<td>11</td>
<td>D12</td>
<td>+Ve</td>
</tr>
</tbody>
</table>

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
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75. KM16 +Ve +Ve
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83. KD5 +Ve +Ve
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87. KD11 +Ve +Ve
88. KD12 +Ve +Ve
89. KA5 +Ve +Ve
90. KA8 +Ve +Ve
91. KA9 +Ve +Ve
92. KA10 +Ve +Ve
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 patient serum and also also 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.

ACKNOWLEDGEMENT

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REFERENCES


