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Research Paper

Detection of Chikungunya Virus IGM Antibodies in Human Serum Samples Using Virus Infected Aedes Aegypti Mosquito Head Squashes

Medical Science

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ABSTRACT

A technique for rapid detection of chikungunya virus IgM antibodies in human serum samples using infected Aedes aegypti mosquito head squashes as immobilized antigen, was developed. Its sensitivity and efficacy was evaluated with two other methods i.e. MAC-ELISA and C6/36 cells. Results showed sensitivity at par with that of C6/36 cells and MAC-ELISA. However, weak positives of MAC-ELISA could not be detected either in the new technique or in C6/36 cells. The technique is simple, rapid and reliable and can be useful in preliminary screening of samples at outbreak areas where ELISA set up is not available.

Chikungunya virus (CHIKV), a member of the genus Alphavirus, family Togaviridae is transmitted by the Aedes mosquitoes and is of considerable public health importance in Africa and South East Asia (Jupp & McIntosh, 1988; Powers & Logue 2007). The viral infection is generally self-limiting and is characterized by sudden onset of fever, headache, nausea, vomiting, myalgia, rash and arthralgia. CHIKV has made an explosive resurgence in 2005-06 in the Indian sub-continent after a gap of 32 years, affecting more than 13 lakh people across the country with a few CHIKV associated deaths (Chandak et al, 2011; Sudeep et al 2011). However, the recent epidemic has been characteristic as it flared up quickly over a large geographical area covering 25 states/union territories (NVBDCP 2014). The sufferings of the patients were enormous as arthralgia persisted for prolonged periods. Many other clinical complications that are not associated with CHIKV infections earlier were also seen during the recent outbreak[3,5] (Chandak et al 2011; Tsetsarkin & Weaver 2012). Virus strain analysis at the molecular level attributed the new strain as East Central South African, reported for the first time in India (Yergolkar et al, 2006; Arankalle et al 2007). In the present communication, we describe a simple and rapid method for detecting CHIKV antibodies using head squashes of CHIKV infected Aedes aegypti mosquitoes as immobilized antigen. The sensitivity and specificity of the test was evaluated with MAC-ELISA and C6/36 cells in detecting the presence of CHIKV antibodies.

Mosquito head squashes: Four to five-day old laboratory-bred male Ae. aegypti mosquitoes were inoculated intrathoracically with approximately 0.2µl of virus suspension (2.1 Log/0.2µl MID_{50}), according to the method described by Rosen and Gubler (1974). Senegal (8914670) strain of CHIKV, received from Yale University, Connecticut, USA, at the 9th mouse brain passage, was used as antigen. Infected mosquitoes were incubated at 28°C with approx 80-90% humidity, harvested on 5th day post infection (PI) and stored at –80°C. Head squashes on clean glass slides were made just before the commencement of the experiment as described by Mourya et al (2001).

C6/36 cells: Virus infected C6/36 cells (48 hr PID) were dispersed with a rubber policeman and seeded in Terasaki plates (150-200 cells/well). Cells were allowed to settle for 2 hr at room temperature, washed once with 1x PBS, fixed in Acetone: Methanol (65:35) mixture for 20 min at –20°C and air dried.

IgM antibody capture (MAC) ELISA: Human sera samples previously tested by MAC-ELISA were used to evaluate the new technique. MAC-ELISA technique was carried out as described by Yergolkar et al (2006). Samples having the optical density (OD) values double or more than that of negative control (averages of OD values obtained from the negative samples in the first test plus standard deviation, positive/negative (P/N) ratio > 2) were considered as positive.

Antibody detection was made using immunofluorescence technique as described by Rosen and Gubler (1974) with certain modifications. Sera samples were diluted (1:100) in 1% solution of skimmed milk in PBS (0.01M) individually and spread on head squashes and C6/36 cells (two wells/sample). After 30 min of incubation at 37°C in a humidity chamber, the slides and Terasaki plates were washed thoroughly with PBS (0.01M). These were probed by affinity purified anti human IgM FITC conjugate (Sigma, USA), and incubated again for 30 minutes at 37°C. After incubation, the head squashes and cells were washed in PBS, counter stained with Evan's Blue and mounted in Glycerol-PBS (1:1) mixture. Head squash slides and the plates were examined under Leica DMIRE2 and Leica DMLA fluorescent microscopes respectively.

Twenty ELISA positive sera samples were tested for CHIKV antibodies simultaneously by mosquito head squash technique (MHQT) and C6/36 cells. All the samples were found positive by both the systems (Table 1). Direct correlation of OD obtained in ELISA test and positivity of samples in MHQT in terms of fluorescence intensity in the infected cells was observed. Both MHQT and C6/36 cell line did not give any false positives. However, two borderline positive samples detected by MAC-ELISA could not be picked up either by MHQT or by C6/36 cells. It was interesting to note that no cross reactivity was observed with other flavivirus positive head squashes. Similarly, the CHIK IgG positive samples also did not show cross reactivity.

The technique described here is simple, rapid and efficient for detection of CHIKV IgM antibodies to determine prevalence of CHIK. It is fast as it needed <2 hr to complete the test and requires only a small quantity of sample (1µl). The virus-infected mosquitoes can be stored at –80°C and squashes can be made as and when required. Mosquitoes stored for >15 days also gave comparable results. However, it is advisable to use head squashes from freshly infected mosquitoes due to better intensity of fluorescence. The possibility of non-specific reactions is ruled out since negative controls were also checked against each sample. The test is completely safe as treatment of head squashes with

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acetone renders the virus inactive. This method may provide quick diagnosis as the serum samples can be tested instantly at the site of the epidemic.

The new technique is found at par in sensitivity to MAC-ELISA and C6/36 cells in the detection of CHIK antibodies. Though MAC-ELISA kits are available commercially, it is expensive and time consuming while MHQT is rapid and can be done at field units where a fluorescent microscope is available. Use of C6/36 cells was found at par with MHQT in the present study; however, the former needs a specialized laboratory facility.

Table: IgM antibodies detection in sera samples using mosquito head squash technique, C6/36 cells and ELISA

	Positive samples (%)*	Undetected samples (%)	Negative samples (%)*
MAC-ELISA	100 (P/N>2)	-	100 (P/N< 2)
MHQT	80	20**	100
C6/36 cell culture system	80	20**	100

*= Sample size (positive-20, Negative - 20); **= Borderline positive samples in MAC ELISA

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