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Original Research Article

The diagnostic utility of commercially available rapid IgM kit tests in comparison with MAC-ELISA IgM in the diagnosis of chikungunya in children

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Abstract

Clinical manifestations of CHIK are non-specific and difficult to differentiate from dengue hemorrhagic fever or other viral exanthema, so it is necessary to confirm by reliable laboratory investigation. The increasing threat of CHIKV emergence in temperate regions and the need to anticipate possible outbreaks of CHIKV infection are presenting a challenge to the current level of diagnostic preparedness. Ethical clearance was obtained from Institutional Review Board. Children who fulfilled the inclusion criteria were enrolled. Informed consent was obtained from all patients or from parents or guardians of all cases. All enrolled children's details of demography, clinical data including symptoms and onset and duration of fever, findings of general as well as systemic examination and laboratory parameters were recorded in a predesigned proforma. The sensitivity of Kit 1 was 0.00% (95% CI 0.0-10.72%) and specificity was 98.53% (95% CIs 92.13-99.74%) with positive predictive value of 0.00% and negative predictive value of 67.68%. The sensitivity of Kit 2 was 3.13% and specificity was 100% with positive predictive value 0f 100% and negative predictive value of 68.69%.

Keywords: Rapid IgM kit tests, MAC- ELISA IgM, chikungunya

Introduction

Chikungunya (CHIKV) is an epidemic viral disease. Asian and African continents bears major brunt of global public health problem of CHIKV.The first reported epidemic occurred in Tanzania in 1952-1953. In Asia, CHIK activity was documented since its isolation in Bangkok, Thailand in 1958.In India it was first detected in 1963 in West Bengal. After quiescence of about three decades, CHIKV re-emerged in India in the states of Andhra Pradesh, Karnataka, Maharashtra, Madhya Pradesh and Tamil Nadu since December, 2005. The important clinical manifestations of CHIKV include high grade fever, severe arthralgia and erythematous maculopapular rash. Rarely, CHIKV infection is associated with neurologic, ophthalmologic, and hemorrhagic disease [1, 2].

Clinical manifestations of CHIK are non-specific and difficult to differentiate from dengue hemorrhagic fever or other viral exanthema, so it is necessary to confirm by reliable laboratory investigation. The increasing threat of CHIKV emergence in temperate regions and the need to anticipate possible outbreaks of CHIKV infection are presenting a challenge to the

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current level of diagnostic preparedness. The routine laboratory diagnosis of CHIKV infection is based on culture and serology followed by identification of viral genome through reverse transcriptase polymerase chain reaction (RT-PCR) Serologically, CHIKV infection can be detected by IgM and IgG capture enzyme linked immunosorbent assay (ELISA). However, confirmation and typing of virus are based on demonstration of 4-fold or greater increase in the virus specific neutralizing antibody titer by plaque reduction neutralization (PRNT) assays [3, 4].

In first 5 days, viremia is present and can be confirmed by viral culture, Polymerase chain reaction (PCR) or antigen detection. CHIKV IgM becomes detectable around 5 days of fever and persists for several months and Immunoglobin G (IgG) is present by 10-14 days.

IgM antibody capture (MAC) ELISA and immunofluorescence are the primary methods and results are available within hours. Point of care ELISA kits are commercially available but accuracy is not well validated.

Methodology

Source of data

All children with suspected chikungunya attending outpatient and inpatient at Dept. of paediatrics.

Type of study: Hospital based cross sectional study.

Inclusion criteria

Children age between 2months to 12yr with acute onset of fever (<7 days duration) and/or severe arthralgia/arthritis with or without skin rash and residing or having left an epidemic area 15 days prior to onset of symptoms.

Methods of collection of data

Ethical clearance was obtained from Institutional Review Board. Children who fulfilled the inclusion criteria were enrolled. Informed consent was obtained from all patients or from parents or guardians of all cases. All enrolled children's details of demography, clinical data including symptoms and onset and duration of fever, findings of general as well as systemic examination and laboratory parameters were recorded in a predesigned proforma. Approximately 3 to 4 ml of blood was collected from each patient enrolled at the time of admission.

Procedure of sample collection for CHIKV testing

Blood collection-Cleaned the area of the skin to be pricked with alcohol, applied iodine and then allow it to dry. Cleaned it again with alcohol. Pricked the skin using 5ml syringe and collected 3-4 ml of blood in a plain fresh pre labeled vaccutainer. Sera were separated from the blood by centrifugation. All serum specimens were screened for CHIKV specific IgM antibodies by ELISA using IgM antibody capture ELISA kit produced by National Institute of Virology (Arbovirus Diagnostic NIV, Pune, India) and 2 commercial rapid kits Kit 1 and Kit 2 according to manufacturer's recommended procedure.

Sample size: Based on previous study sensitivity of Rapid kit test was 83% and proportion of

chikungunya detection in a study was 25.37%.[1] So to estimate true sensitivity of Rapid kit test for chikungunya (in comparision with MAC-ELISA as gold std.) with 15% absolute precision and 95% confidence the required sample size was 96.

Results

Table 1: Outcome of chikungunya by MAC ELISA and Rapid kit tests (n=100)

Kit 1(n=100)	Negative	99	99.0%
	Positive	1	1.0%
Kit 2(n=100)	Negative	99	99.0%
	Positive	1	1.0%
Elisa(n=100)	Negative	68	68.0%
	Positive	32	32.0%

Table 2: Comparision of clinical profile of seropositive and seronegative CHIKV

Parameters	CHIKV Positive (n=32) (%)	CKIKV Negative (n=68) (%)	P-value
Age (mean)	7.6 years	7.4 years	0.697(NS)
M:F Ratio	1.9:1	1.2:1	-
Fever	32(100%)	68(100%)	0.645(NS)
Myalgia	30(93.00)%	58(85.29%)	0.225(NS)
Abdominal Pain	24(75%)	48(70.58%)	0.647(NS)
Arthralgia	20(62.57%)	46(67.64%)	0.612(NS)
Vomiting	23(71.87%)	39(57.35%)	0.163(NS)
Headache	14(43.75%)	43(63.23%)	0.066(NS)
Cough	11(34.37%)	33(48.52%)	0.183(NS)
Restlessness	11(34.37%)	19(27.94%)	0.513(NS)
Rash	6(18.75%)	19(27.94%)	0.322(NS)
Arthritis	7(21.87%)	11(16.17%)	0.489(NS)
Nasal Discharge	3(9.37%)	10(14.70%)	0.460(NS)
Diarrhoea	3(9.37%)	8(11.76%)	0.722(NS)
Photophobia	3(9.37%)	4(5.88%)	0.523(NS)
Others*	8(25%)	18(26.47%)	0.137(NS)
TLC (Mean)	5898	5594	0.727(NS)
Platelet(mean)	136915	118429.00	0.449(NS)
Hess test	9(28.12%)	4(5.88%)	0.009(NS)
Lymphadenopathy	5(15.62%)	13(19.11)	0.451(NS)
Conj. congestion	15(46.87%)	37(54.41)	0.482(NS)

*Epistaxis (4%), Malena (4%)

Clinical features in both seropositive and seronegative group were tabulated in table.11 above, however there is no statistical significance found in both group.

All suspected samples of chikungunya collected were evaluated for their sensitivity and specificity of rapid kit and compared with MAC-ELISA. Among 100 samples 32 were confirmed as chikungunya by MAC-ELISA.

The rapid tests were performed according to the manufacturer's instructions and evaluated. One positive outcome shown by each rapid kit test. Kit 1 showed a false positive outcome.

Table 3: Sensitivity and specificity of Rapid kits

	ELISA				P value
	Positive		Negative		
	Number	%	Number	%	

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Kit 1	Positive	0	0.0%	1	1.5%	0.491
	Negative	32	100.0%	67	98.5%	
Kit 2	Positive	1	3.1%	0	0.0%	0.143
	Negative	31	96.9%	68	100.0%	

Table 4: Validity of kit 1 in comparison to ELISA

Parameter	Estimate	Lower-Upper 95% CIs
Sensitivity	0.0%	0.0, 10.72
Specificity	98.53%	92.13, 99.74
Positive Predictive Value	0.0%	0.0, 79.35
Negative Predictive Value	67.68%	57.95, 76.08
Diagnostic Accuracy	67%	57.31, 75.44

The sensitivity of Kit 1 was 0.00% (95% CI 0.0-10.72%) and specificity was 98.53% (95% CIs 92.13-99.74%) with positive predictive value of 0.00% and negative predictive value of 67.68%.

Table 5: Validity of kit 2 in comparision to ELISA

Parameter	Estimate	Lower-Upper 95% CIs
Sensitivity	3.125%	0.5538, 15.74
Specificity	100%	94.65, 100
Positive Predictive Value	100%	20.65, 100
Negative Predictive Value	68.69%	59, 76.98
Diagnostic Accuracy	69%	59.37, 77.22

The sensitivity of Kit 2 was 3.13% and specificity was 100% with positive predictive value 0f 100% and negative predictive value of 68.69%.
Diagnostic accuracy of Kit 1 was 67% and Kit 2 was 69%.

Discussion

We evaluated two rapid kit tests namely Kit 1 and Kit 2 which were easily available in the local market. The comparision of these two kits were done with Chikungunya IgM antibody capture ELISA kit produced by National Institute of Virology (Arbovirus Diagnostic NIV, Pune, India). The NIV kit was having sensitivity of 95% and specificity of 98% according to manufacturer. However in our study 32% were positive by MAC ELISA. These results are comparable to study done by Johnson *et al*., who used three different MAC ELISA kits and found to have lower specificity and sensitivity(<50%). In, 2009 international evaluation of diagnostic quality of 30 expert laboratories showed that most of the laboratories need more sensitive CHIKV IgM detection assays. The results were correct in only 50.7% cases.

The Kit 1 showed sensitivity of 0.00% and specificity was 98.53% with diagnostic accuracy of 67%. Kit 2 showed sensitivity of 3.13% and 100% specificity with diagnostic accuracy of 69%. But as per manufactures literature sensitivity and specificity of Kit 1 was 97% and 98%. Kit 2 had 97.5% and specificity 99.1% based on test panel of 2000 plasma and serum samples as per manufacturer's literature. Though the reason for this low performance in our study was not clear, however one reason could be due to collection of sample in the early phase of illness.

The results were consistent with studies done by different authors using different rapid kits like Blacksell *et al*. [5] (SD Bioline by SD diagnostics), Rianthavorn *et al*. (SD Bioline) and Arya SC *et al*. (OnSite by CTK Biotech, USA). Study done by Blacksell *et al*. showed sensitivity ranging from 1.9 to 3.9% and specificity 92.5 to 95.0%. Study conducted by

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Rianthavorn *et al*. [6] the sensitivity was 22% and specificity was 88%, however, Arya SC *et al*. observed sensitivity of 71% and specificity 0f 100%. In our study, the commercial Rapid kits that we compared with MAC ELISA, NIV Pune, performed poorly. We evaluated the efficacy of locally available rapid kits with MAC ELISA using small number of sample. However the findings showed poor sensitivity and specificity. This will highlight the importance of evaluating commercial diagnostic kits before we use in our clinical practice.

Conclusion

Two commercial rapid kit tests used in our study showed poor sensitivity with good specificity in comparison to MAC ELISA.

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