EVALUATION OF A RAPID ASSAY FOR DETECTION OF IGM ANTIBODIES TO CHIKUNGUNYA

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Abstract. Chikungunya (CHIK) is a re-emerging disease causing a large negative impact on global health and economics. Clinical manifestations of CHIK are non specific and difficult to differentiate from dengue hemorrhagic fever or other viral exanthema. A rapid, simple and reliable diagnostic assay is necessary for CHIK outbreak control especially in countries with insufficient access to well-equipped laboratories. The aim of the study is to evaluate a commercially rapid, qualitative CHIK diagnostic assay based on specific IgM antibody detection. Performance of the rapid assay was evaluated in comparison with semi-nested RT-PCR and IgM detection by ELISA. The sensitivity of the rapid assay was not constant but positively correlated with duration of symptoms. If the test was conducted within the first week, sensitivity and specificity was 22% and 88%, respectively. If the patients were tested after the first week, sensitivity was increased to 83% while specificity was decreased to 71%. Thus, the rapid assay should not be used as a screening tool during the first week of CHIK due to its low sensitivity.

Key words: sensitivity, specificity, chikungunya, rapid test, ELISA, RT-PCR

INTRODUCTION

Chikungunya (CHIK) did not receive much attention until 2005 when the most recent CHIK outbreak caused a large-scale negative impact on global health and economics as a consequence of prolonged debilitation and absence from work (Simon *et al*, 2008). This outbreak is not yet under control in several countries including Thailand where the outbreak began in the southern part of the country in September, 2008. At present, there have already been more than 10,000 reported cases of CHIK in Thailand.

Important clinical manifestations of CHIK include high grade fever, myalgia, severe arthralgia and erythematous maculopapular rash. It is quite difficult to differentiate CHIK from dengue hemorrhagic fever or other viral exanthema based solely on clinical manifestations (Chhabra *et al*, 2008). A rapid and reliable diagnostic assay is necessary for outbreak control.

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Specific antibody to CHIK and CHIK virus detection are rapid, sensitive and reliable methods but require special equipments and high technician skills, which are unavailable in rural areas where the outbreaks usually occur (Grivard *et al*, 2007). Thus, a rapid diagnostic assay that can be conducted in any laboratory was commercially developed in order to quickly provide a reliable diagnosis.

One Step IgM antibodies to Chikungunya virus Test (SD BIOLINE, Kyonggi-do, Korea) is a solid phase immunochromatographic assay for rapid, qualitative detection of IgM antibodies to CHIK in human serum or plasma. The assay can be performed without any requirement for special equipments or technical skills. The result is readily available within 10 minutes and can be easily interpreted. The analytical sensitivity and specificity of the rapid assay according to the manufacturer's data is 97.1% and 98.9%, respectively, when using a commercial CHIK IgM capture ELISA as a gold standard. However, the validity of the rapid assay has never been studied in an actual clinical situation. Thus, this study was aimed at evaluating the performance of this rapid assay in diagnosing CHIK during an outbreak.

MATERIALS AND METHODS

From the 2008 CHIK outbreak in South Thailand, 527 sera were obtained from patients who presented at Narathiwatratchanakharin Provincial Hospital with symptoms compatible with CHIK. The symptoms included fever, arthralgia or arthritis, skin rash and flu-like symptoms. Duration of illness reported by each patient was recorded at the time of serum collection. Chikungunya infection was investigated at the Center of Excellence in Clinical Virology, Chulalongkorn University.

Semi-nested RT-PCR was performed to detect CHIKV RNA as follows. CHIKV RNA was extracted from 50 μl of serum by the guanidium-isothiocyanate method as described elsewhere (Cha *et al*, 1991), and subsequently reverse transcribed into cDNA using random hexamer primers. For the first PCR, DVRChkF 5' ACCGGCGTC TACCCATTCATGT 3'(nt 10237-10258) and CU3-CHIKR 5'TCGCTRCAGCACACRG CACC 3' (nt 10741-10760) were used as forward primer and reverse primer, respectively. PCR was performed in a thermocycler (Perkin Elmer Cetus Model 9600, Norwalk, CT). Cycling conditions included initial denaturation at 95ºC for 3 minutes, followed by 40 amplification cycles of 1 minute at 95ºC, 1 minute at 55ºC and 1 minute at 72ºC, followed by 10 minutes at 72ºC for final extension. Then, semi-nested PCR was performed using CU1CHIKF 5' GCATCAG CTAAGCTCCGCGTC 3' (nt 10378-10398) as an inner forward primer. After electrophoresis in 2% agarose gel and staining with ethidium bromide, the expected 532-bp band was visualized on a UV trans-illuminator.

Each serum was then tested using One Step IgM antibodies to Chikungunya virus Test (SD BIOLINE, Kyonggi-do, Korea). In brief, 50 μl aliquot of serum was added into the sample well of the test device. Then, 1 drop of provided assay diluent was added. The result was interpreted after 10 minutes. The presence of only a "C" line within the result window indicated a negative result. The presence of both "C" and "T" lines within the result window indicated a positive result. If a "C" line was absent or only a "T" line was present, then the result was considered invalid and the specimen was retested. Serum samples were also tested using a commercial ELISA a kit (SD BIOLINE, Kyonggi-do, Korea). CHIK

Table 1
Number of patients diagnosed with and without CHIK in relation to duration of symptoms. The results from the rapid assay were compared with the results from viral detection by semi-nested RT-PCR and specific IgM antibody detection by ELISA.

Duration of symptoms (days)	Patients with CHIK						Patients without CHIK			
	RT-PCR +	IgM +	RT-PCR + AND IgM +	Rapid test +	Rapid test -	Total	Rapid test +	Rapid test -	Total	Total patients
1	10	1	0	1	10	11	0	5	5	16
2	47	3	2	5	43	48	0	29	29	77
3	46	7	2	8	43	51	5	37	42	93
4	31	7	2	7	29	36	5	39	44	80
5	17	4	1	3	17	20	3	23	26	46
6	8	4	2	4	6	10	3	18	21	31
7	7	4	1	5	5	10	1	13	14	24
8	4	5	2	6	1	7	7	15	22	29
9	6	8	1	8	5	13	5	2	7	20
10	7	10	3	11	3	14	4	9	13	27
11	0	9	0	9	0	9	0	4	4	13
12	3	11	1	11	2	13	2	6	8	21
13	2	6	1	6	1	7	2	3	5	12
14 and more	4	17	3	16	2	18	3	17	20	38
Total patients	192	96	21	100	167	267	40	220	260	527

antibodies detection by both ELISA and rapid test was performed at 25ºC.

RESULTS

From a total of 527 patients, 267 were diagnosed with CHIK. CHIKV RNA was detected in 192 specimens by semi-nested RT-PCR. IgM antibodies for CHIK were detected in 96 specimens by ELISA and 21 patients were positive for both CHIKV RNA and IgM antibodies. Based on the rapid assay, there were 140 positive and 387 negative specimens. Comparison between the results of the rapid test (positive or negative) and the status of CHIK infection (disease or without disease) in relation to the duration of symptoms is shown in Table 1. Sensitivity and specificity of the rapid test was 37% and 85%, respectively. Positive and negative predictive value was 71% and 57%, respectively.

Sensitivity of the rapid assay was positively correlated with the duration of symptoms (Fig 1). The sensitivity was markedly increased after having symptoms of more than 1 week. Hence, the patients were divided into 2 groups based on duration of illness (symptoms within 1 week and symptoms more than 1 week) in order to study the sensitivity and specificity of the rapid test in relation to duration of fever. For sera obtained when symptoms were within 1 week, sensitivity and specificity of the rapid test was 22%

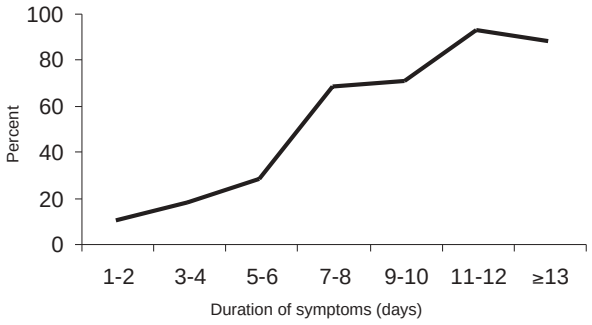

Fig 1–Sensitivity of CHIK rapid assay in relation to duration of symptoms.

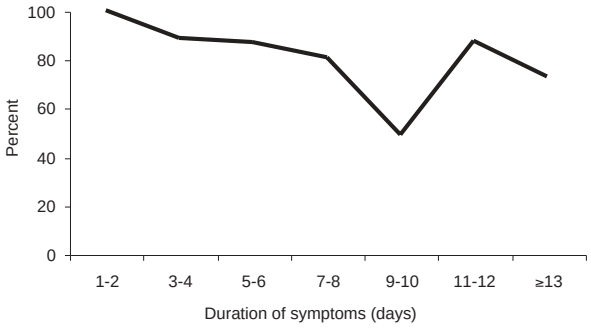

Fig 2–Specificity of CHIK rapid assay in relation to duration of symptoms.

and 88%, respectively. For sera obtained when the duration of symptoms were more than 1 week, sensitivity was increased to 83% whereas the specificity was slightly decreased to 71%. The specificity of the test in relation to the duration of symptoms is shown in Fig 2.

DISCUSSION

The clinical sensitivity of the rapid test determined during a CHIK outbreak was significantly lower than that reported by the manufacturer and dependent on the duration of illness (Fig 1). The discrepancy could be attributable to different gold standards and the normally delayed increase of IgM antibodies to a detectable level. Taubitz *et al* (2007) reported negative IgM antibody by immunofluorescence technique in 60% of patients with CHIK if the test was done during the first week of illness and specific IgM antibodies were never detected within the first 3 days. They concluded that at an early stage of infection, viral detection appears to be a more sensitive method for diagnosis (Taubitz *et al*, 2007).

In this study, the results of the rapid assay were compared with CHIK status diagnosed by either RNA or IgM antibody detection. During the first week of fever, more patients were diagnosed by RNA detection than by IgM antibody detection. When using the rapid test, whose principle is to detect IgM antibodies sensitivity of the test was inevitably low during the first week of illness when the antibodies are not yet developed. IgM antibodies to CHIK started to rise after the first week of illness, as previously reported (Taubitz *et al*, 2007). Thus, patients were diagnosed by IgM antibody detection more often than RNA detection. When using the rapid test after the first week of illness, the sensitivity of the test was unsurprisingly improved.

The limitation of this study was that the duration of symptoms was presumptively used to define the time between the onset of the disease and the time of specimen collection for diagnostic purposes. However, symptoms may not accurately represent the onset of virus inoculation which is difficult to determine in clinical settings. Moreover; some patients may have developed asymptomatic infection and thus were not enrolled in this study. Nevertheless, the rapid test was sensitive for diagnosis of CHIK when used on a patient with more than 1-week history of illness. However, the rapid test should not

be used as a screening tool especially during the first few days of illness.

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