An Early Evaluation on the Usefulness of NS1 Antigen-Capture ELISA versus IGM ELISA Tests for the Diagnosis of Acute Dengue Infection

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ABSTRACT: Accurate and early diagnosis of dengue is vital for patient's management and control of the disease in dengue endemic region with limited resources. In this study, we evaluated the performance of non-structural 1 (NS1) antigen-capture enzyme-linked immunosorbent assay (ELISA) test against IgM ELISA test for confirmation of dengue virus infection in 311 single acute serum samples. Dengue NS1 antigen and dengue IgM antibodies were detected by using Panbio Dengue Early ELISA and Panbio Dengue IgM Capture ELISA kit, respectively. The tests were performed and interpreted according to manufacturer's instruction. NS1 antigen and the IgM test had 41.15 % and 37.62% positive detection rates, respectively. However, overall detection rates increased to 52.73% when both tests were combined. Notably, in samples with negative IgM results, 47 were positive by NS1 antigen-capture ELISA test (24.22%). In this study it was found that the combined use of NS1 and IgM ELISA would provide better and early confirmation of acute dengue infection.

Keywords: dengue NS1, dengue IgM, dengue serodiagnosis, ELISA

Introduction

Dengue, a mosquito-borne viral infection, continues to be a major public health problem worldwide, notably in tropical and subtropical areas (Idrees and Ashfaq, 2012). Globally, dengue virus ((DENV) imperils 2.5 billion people, nearly 900 million people who inhabit urban tropical and sub-tropical countries of south and south-east Asia, the Caribbean, Central and South America, and more recently in Africa (Schwartz *et al*., 2000; Guzman *et al*., 2010). Fifty to 100 million dengue cases appear every year and approximately 500,000 people develop dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS), with a case-fatality rate of 2.5% (Castro-Jorge *et al*., 2010). Over the last two decades, the incidence, distribution, and clinical severity of DF/DHF have increased dramatically, but unfortunately no licensed vaccine or specific therapies are available to combat the disease (McBride, 2009; de la Cruz Hernández *et al*., 2012). Therefore, reliable and quick diagnostic methods are necessary for surveillance and proper treatment of dengue (Pok *et al*., 2010).

Dengue infection is diagnosed by one or a combination of four basic diagnostic tests: detection of either virus via culture, viral nucleic acid by reverse transcriptase polymerase chain reaction (RT-PCR), dengue NS-1 protein or specific anti-dengue IgM/IgG antibodies. Dengue IgM test is the most widely used tests in hospitals due to its cheap cost and ease of handling. However, the test is more suitable for diagnosis of dengue after five days of infection (late infection). For early infection, isolation of the virus or detection of viral genomic material or NS-1 antigen is the most sensitive assay. However, the first two tests are time consuming, expensive and require well trained personnel (Sadon *et al*., 2008; Castro-Jorge *et al*., 2010; Watthanaworawit *et al*., 2011). On the other hand, the NS1 ELISA test is inexpensive and easy to handle. Combining NS1 and IgM tests would be ideal to confirm dengue during both early and late infection. However, it is quite difficult to afford both tests at the same time in hospitals with a constrained budget. Numerous retrospective studies have been conducted to evaluate the use of NS1 antigen test versus IgM ELISA using mostly well characterized stored serum samples (Bhattacharya *et al*., 2013). However, in this study, we tested real uncharacterized serum samples sent for serodiagnosis of dengue. The aim of this study was to evaluate the use of NS1 test against IgM assay as the most suitable test for laboratory confirmation of dengue at primary health care settings where prompt diagnosis is required.

Material and Methods

Serum sample collection

In the current study, 311 samples from dengue suspected patients presenting to the outpatient clinic in Hospital Universiti Sains Malaysia and sent to Serology Laboratory, Department Medical Microbiology and Parasitology, Universiti Sains Malaysia (USM) were collected during April 2013 to August 2013. The patients presented with fever ≥38◦C and may be accompanied with clinical symptoms like abnormal bleeding, eye redness, headache, myalgia, swollen glands or rash. The samples were collected randomly with unknown fever duration. After collection, samples were allowed to clot at room temperature and then were separated and stored in a -70^0C refrigerator. This study has received approval from the USM Human Ethics Committee (USM/JPeM/270.4. (1.3).

Dengue NS1 antigen detection

Detection of NS1 antigen from serum sample was performed by Panbio Dengue Early (Panbio Diagnostics, Brisbane, Australia). Enzyme-linked immunosorbent assay (ELISA) detection of dengue NS1 antigen was carried out according to the manufacturer's instructions. The principle of the test was based on a one-step sandwich format microplate enzyme immunoassay for the detection of dengue virus NS1 antigen in human serum.

Anti-dengue IgM antibody detection

Serological assay for the detection of anti-dengue IgM present in acute serum samples were carried out using a commercial IgM-capture ELISA kit (Panbio Diagnostics, Brisbane, Australia). Test procedures and interpretation of results were carried out in accordance with the manufacturer's instructions.

Statistical analysis

The study analysed only the sensitivity of both assays, in terms of either a single or a combined usage of the tests since previously published studies have demonstrated a

consistently high specificity for both assays (Lima *et al.*, 2010; Hunsperger *et al*., 2009). Statistical analysis was calculated using SPSS statistical package (SPSS, Chicago, IL), (McNemar test). Equivocal or indeterminate results were regarded as negative for sensitivity analysis.

Results

Of the 311 dengue suspected serum samples tested with dengue IgM capture ELISA, 117 samples were positive for dengue with a detection rate of 37.62%. However, when the same samples were tested with NS1 antigen-capture ELISA test, the detection rate increased to 41.2% **(Table 1)**. Interestingly, detection rate improved significantly to 52.73 % (McNemar, $p< 0.05$) when both tests were applied simultaneously to the dengue suspected serum samples and the effect of combined test were compared with each individual test **(Table 2 A** and **2B).**

It is interesting to note that from 117 serum samples that were tested positive by dengue IgM ELISA, 81 (69.32%) samples were also positive for NS1 antigen ELISA. Of the 128 samples tested positive by dengue NS1 antigen-capture ELISA, 81 (63.28%) samples were also positive by IgM ELISA. The detection rate of dengue infection by NS1 antigen-capture ELISA in the presence of IgM ELISA was 69.32%, while in the absence of IgM ELISA was 24.22%.

Table 1: Comparison of results obtained for 311 sera tested using the Panbio early dengue ELISA and Panbio IgM capture ELISA

			Panbio Early Dengue ELISA		**Total**
			Negative	**Positive**	
Panbio IGM capture ELISA	**Negative**	Count	183	11	194
		% of Total	58.8%	3.5%	62.4%
	Positive	Count	0	117	117
		% of Total	0.0%	37.6%	37.6%
Total		Count	183	128	311
		% of Total	58.8%	41.2%	100.0%

Table 2: Comparison of detection rates of A) Panbio IgM capture ELISA versus combined tests and B) Panbio early dengue ELISA versus combined tests

A)

		Combined test		**Total**
		Negative	**Positive**	
Panbio IgM capture ELISA	**Negative**	147	47	194
	Positive	0	117	117
Total		147	164	311

B)

		Combined test		**Total**
		Negative	**Positive**	
Panbio Early Dengue ELISA	**Negative**	147	36	183
	Positive	0	128	128
Total		147	164	311

Discussion

With the increasing number of dengue cases in Malaysia in the past few years, the provision of timely information is indeed contributing significantly to the good management of patients with acute dengue infection as well as early public health control of dengue outbreak in the country. Currently, the two tests, i.e. dengue NS1 antigen-capture ELISA and IgM ELISA are available for dengue serodiagnosis. The latter test is the most widely used assays in many hospitals because of the low cost and ease of handling. It is common for some hospitals to only offer one test rather than both tests due to budget constraint. However, dengue IgM ELISA has a limitation since antibody usually detectable 3-5 days after onset of symptoms and this could lead to false negative results to patients during early dengue infection. Thus, the patient would need to give a second serum sample after a few days for confirmation of diagnosis. This hinders and delay effective patient management and public health control. Incorporating dengue NS1 antigen assay would provide early dengue detection since NS1 antigen present in the blood up to the ninth day of fever (McBride, 2009) but at the expense of doubling the cost of dengue serodiagnosis per patient. Therefore, in this study, we investigated the impact of combined tests rather than solely depending on either dengue NS1 antigen or IgM tests for diagnosis of dengue.

Most of previous studies studying the usefulness of dengue NS1 antigen versus IgM test used well characterised stored serum samples (Osorio *et al*., 2010; Blacksell *et al*., 2012). Unlike those studies, we were interested to investigate the utility of both tests in real serum samples sent to the lab for dengue diagnosis to obtain the best view on real world practice. In this study, a total of 164 (52.73%) out of 311 dengue suspected patients were laboratory confirmed when tested with dengue NS1 antigen ELISA and/or IgM ELISA. However, the rate of dengue suspected cases confirmed by NS1 antigen ELISA was comparatively higher (41.15 %) than the detection rate of IgM test (37.62%), suggesting that NS1 antigen test was a slightly more sensitive assay, a similar finding to that observed by Huang and co-workers (2013), who also used real serum samples. Of 164 laboratory confirmed patients, 128 (78.04%) were detected by NS1 antigen-capture ELISA, while 117 (71.34%) samples by IgM ELISA. On the other hand, in the presence of dengue IgM antibody, the detection ratio of NS1 test was 69.23%, a similar finding to Lima *et al.*, 2010, while in the absence of dengue IgM antibody, it was 24.22%, suggesting that NS1 detection is perhaps a more sensitive and

suitable test for the diagnosis of dengue in outpatient clinics. More importantly, in the current study, it was found that the combined use of NS1 antigen /IgM antibody detection test yielded a significantly higher detection rate of dengue ($p<0.05$) than individual test, which is in agreement with other previous studies (Dussart *et al.*, 2008; Tricou *et al.*, 2010b). Huang and co-workers (2013) reported that the combined NS1 antigen and IgM tests had increased the laboratory confirmed dengue cases from between 68-40% to 82% in their study population suggesting that relying on a single test may be inappropriate.

There are several reasons for incorporating the NS1 antigen test for an early diagnosis of dengue infection in primary health care settings. Although reverse transcriptase polymerase chain reaction (RT-PCR) is a more sensitive and direct detection method for acute dengue virus infection but conventional PCR is less sensitive than real time PCR (rt-PCR). However, rt-PCR is quite expensive diagnosis method and is not affordable in limited resource countries. On the other hand, the NS1 antigen detection test is less expensive than RT-PCR and user friendly. In addition, NS1 antigen is detectable in blood from the first day and up to day nine after the onset of fever. Furthermore, the NS1 antigen could be detected even when viral RNA is not detected in RT-PCR and in the presence of IgM antibodies (McBride, 2009). Additionally, it also eliminates the usage of paired samples. Another advantage of using a dengue NS1 test is that the NS1 protein is highly conserved among all four serotypes of dengue virus; therefore, unlike dengue IgM antibody, it is a more specific detection method with a low false-positive rate reported (Castro-Jorge *et al.*, 2010; Huang *et al*, 2013).

There are several limitations in this study. First, the duration of fever for all the samples was not known, limiting the analysis of sensitivity of NS1 antigen versus IgM antibody test. Additionally, the status of infection, that is, whether patients were having a primary or secondary infection was also not known as that it has been shown previously that status of dengue infection could affect the sensitivity of the NS1 assay (Polk *et al*., 2010). Although including NS1 ELISA test with IgM ELISA would double the operation cost, a detection rate of 24.22% of acute dengue infection by NS1 ELISA in the absence of IgM antibodies suggests that this particular test is very useful in providing early laboratory diagnosis of dengue. This is especially pertinent during a dengue outbreak as experienced currently in Malaysia where there is a need of a prompt diagnosis of dengue so that timely patient care

and vector control could be initiated. In addition, further study should carry out to evaluate the efficacy of both tests with larger sample size.

In conclusion, our findings suggest that the NS1 antigen-capture ELISA is very useful and specific tool for the diagnosis of acute dengue infection. However, the sensitivity of the NS1 assay is dependent on the level of viremia and host humoral immune response. Therefore, a combined use of NS1 antigen with dengue IgM test could significantly improve diagnostic sensitivity of dengue infection.

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