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ORIGINAL ARTICLE

Evaluation Of Dengue IgA Antibody and NS1 Antigen Rapid Tests As Early Diagnostic Tests For Dengue Virus Infection*

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The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, have met the requirements for authorship.

ABSTRACT

Background: Dengue is a major health problem. The lack of data on the usefulness of rapid diagnostic tests for early detection of dengue has generated interest in determining their validity.

Objectives: This research aimed to determine the validity of dengue IgA antibody versus NS1 antigen test as rapid diagnostic tests for early detection of dengue using Hemagglutination Inhibition test (HI) as standard reference.

Methodology: This study included 51 pediatric patients being evaluated for dengue in a private hospital from March 01, 2012 to October 30, 2012. Paired serum samples from patients suspected of dengue and had fever of not more than seven days were examined. Initial blood samples were collected on the first day of consult and tested for dengue IgA antibody, dengue NS1antigen, and dengue HI tests. Second blood samples for HI were collected seven days after the initial extraction.

Results: The 51 serum samples used in this study came from 29 males and 22 females. From these samples, sensitivity of dengue IgA antibody was 80% with 95% CI (70-90) while specificity was at 50% with 95% CI (36-64) while dengue NS1 antigen which showed sensitivity of 27% with 95% CI (15-39) and specificity of 67% with 95% CI (54-86). IgA rapid test demonstrated 71% positivity in detecting acute primary dengue infection and 82% for acute secondary infection. NS1 detected 43% of primary infection and 24% of secondary infection.

Conclusion: Dengue IgA antibody rapid test was more sensitive than NS1 antigen test for early diagnosis of dengue and had better performance in detecting primary and secondary dengue.

KEYWORDS:
dengue IgA rapid test, dengue NS1antigen rapid test, dengue rapid diagnostic test

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INTRODUCTION

Dengue has become a major international public health problem due to high morbidity and mortality. In January 2012, World Health Organization (WHO) reported that over 2.5 billion people or 40% of the world's population are at high risk of having dengue.[1] Further, dengue may affect 50–100 million people each year with 500,00 people with severe dengue infection requiring hospitalization. A large proportion of these occur in children, 2.5% of whom die from the disease.[1]

In the Philippines, the latest data from the Regional Epidemiology and Disease Surveillance Unit (RESU) of the Department of Health (DOH) reported 32,193 cases of dengue from January to June 2, 2012.[2] Almost 39,000 were confirmed dengue cases as of July 23, 2012, which was 3.89% higher than last year's 30,889 cases within the same period.[2]

To date, many of Filipino doctors rely mainly on dengue NS1 antigen rapid test in the early detection of dengue infection particularly during the first four days of fever. In many dengue endemic settings, including the Philippines, simple rapid diagnostic tests (RDTs) are much needed to provide accurate diagnosis of acute dengue infection so that appropriate treatment and patient management may be administered.

Due to lack of data on the usefulness of RDTs in the early detection of dengue infection in the Philippines, this study seeks to validate which among the two tests, NS1 antigen or IgA antibody, can be utilized as an optimal diagnostic tool for early diagnosis of dengue with HI assay as standard reference.

METHODOLOGY

Research design

This is a prospective and cross-sectional study that was carried out over a period of eight months, from March 01, 2012 to October 30, 2012.

Subjects

Included in the study were pediatric patients who were 18 years old and below and had fever of not more than seven days from at the out-patient department or admitted in the Pediatric ward of a private tertiary hospital in Metro Manila.

Excluded were those who had fever exceeding seven days, were immunocompromised and were receiving chemotherapy or radiotherapy secondary to malignancy, and were diagnosed with chronic conditions like nephrosis, liver cirrhosis, connective tissue diseases, and congestive heart failure.

Sample size calculation

The *Stat Calc method* was used for sample size calculation with sensitivity and specificity of the measurement at 95% level of confidence and a relative error of 5%. Using a 95% confidence level and 80% power of the study, with an estimated 20% difference between dengue IgA antibody and NS1 antigen tests at least 30 subjects were needed.

Description of Procedure for Data Collection

Two blood samples were extracted from patients who fulfilled the inclusion criteria. The first sample (S1) was taken on the day of consult and tested for dengue NS1 antigen, IgA antibody and HI assay. The second sample (S2) was taken seven days after the first blood extraction and tested for HI. After collection of the 51 paired sera, specimens were sent and analyzed for HI at RITM (Research Institute of Tropical Medicine).

Data Analysis

Diagnostic assays were evaluated in terms of sensitivity and specificity that are calculated using a 2 × 2 cross-tabulation Count Matrix where a reference standard result was compared with the rapid test to determine diagnostic accuracy.

RESULTS

From a total of 51 patients, 29 were males and 22 were females. The most commonly affected group came from the 15-to-<19 year olds, with male preponderance.

Among those tested HI, 45 (88%) patients were confirmed to have dengue infection while six (12%) patients did not have the infection. (Table 1)

Table 1. Confirmed cases of Dengue Infection with HI as Standard Reference

Result of HI	Value	Percent
Positive dengue infection	45	88% (45/51)
Negative dengue infection	6	12% (6/51)

Only 36 patients had positive IgA from those tested for the IgA antibody. Sensitivity of IgA was noted at 80% with 95% CI (70-90); while positive predictive value pegged at 92%, 95% CI (90-94). The specificity was 50% and 95% CI (36-64); and a negative predictive value at 25%, 95% CI(13-37). (Table 2, 4)

Table 2. Analysis of sensitivity and specificity of dengue IgA

	HI positive	HI negative	Total
IgA positive	36	3	39
IgA negative	9	3	12
Total	45	6	51

Among the 45 confirmed cases of dengue, there were only 12 patients who had positive NS1 antigen with a sensitivity of 27%, 95% CI(15-39); and a positive predictive value of 86%, 95% CI (76-96). Among the six confirmed cases with no dengue infection by HI, only four patients tested negative for NS1 with a specificity of 67%, 95% CI (54-80); and a negative predictive value of 11%, 95% CI (11-19). (Table 3,4)

Table 3. Analysis of sensitivity and specificity of dengue NS1.

	HI positive	HI negative	Total
NS1 Positive	12	2	14
NS1 Negative	33	4	37
Total	45	6	51

Table 4. Comparative performance of dengue IgA against dengue NS1.

	Sensitivity(%)	Specificity (%)	PPV	NPV
IgA	80	50	92	25
IgA 95%CI	70-90	36-64	90-94	13-37
NS1	27	67	86	11
NS1 95%CI	15-39	54-80	76-96	11-19

From those subjects tested for HI, there were six patients who were confirmed for negative dengue infection. Out of the 45 HI confirmed dengue cases, there were seven patients interpreted to have acute primary dengue infection at 43% on day 1-2; 29% on day 3-4; and 29% on day 5-7 as compared to those with positive IgA at 67%, 100%, and 50% on days 1-2, 3-4 and 5-7, respectively. On the other hand, positivity of NS1 by day of illness showed 33% on day 1-2; 100% on day 3-4; but failed to detect dengue infection on day 5 onwards.

With regard to secondary infection, IgA detected true infection at 40% on day 1-2; 93% on day 3-4; and 84% on day 5-7. Meanwhile, NS1 had lower yield of positivity at 20% on day 1-2; 21% on day 3-4; and 26% on day 5-7. (Table V, VI)

Table 5. Comparative performance of dengue IgA versus dengue NS1 by day of illness and HI results.

HI Interpretations	Day of illness	No. of samples with (+)HI	No. of samples with positive RDTs	
			IgA	NS1
Primary dengue infection	1-2	3	67% (2/3)	33% (1/3)
	3-4	2	100% (2/2)	100% (2/2)
	5-7	2	50% (1/2)	0
Secondary dengue infection	2	5	40% (2/5)	20% (1/5)
	3-4	14	93% (13/14)	21% (3/14)
	5-7	19	84% (16/19)	26% (5/19)

Table 6. Comparative performance of dengue IgA versus dengue NS1 by HI results.

HI result	Ig A		NS1	
	Positive	Negative	Positive	Negative
Acute secondary dengue infection (n=38)	82% (31/38)	18% (7/38)	24% (9/38)	76% (29/38)
Acute primary dengue infection (n=7)	71% (5/7)	29% (2/7)	43% (3/7)	57% (4/7)
No evidence of dengue infection(n=6)	50% (3/6)	50% (3/6)	33% (2/6)	67% (4/6)

DISCUSSION

The Dengue virus (DENV), from the genus flavivirus, is mostly found in tropics and subtropics and is transmitted by Aedes aegypti mosquito bite. Patients with primary DENV infections typically present with sudden onset of fever lasting for three-to-seven days, and may have accompanying nonspecific signs and symptoms which include severe headache with retro-orbital pain, body aches, joint pain and rash. Secondary DENV infection is caused by a second exposure to a different DENV serotype and may lead to dengue hemorrhagic fever or dengue shock syndrome.

For many years, the HI was the standard method used in dengue virus diagnosis due to its high degree of sensitivity and being of great help to differentiate primary from secondary infections. The high levels of antibodies remain constant for two to three months. The main disadvantages of the HI test is its lack of specificity, the need for paired samples, and the inability to identify the infecting virus serotype.9

The development of Non-Structural Protein 1 (NS1) antigen detection in the Enzyme-linked immunosorbent assay (ELISA) and rapid lateral flow platform has proved to be a very promising tool for early diagnosis during the febrile phase of the disease. However, its performance in detecting secondary cases of dengue was reported to be not as good as in primary infection.3 Contrary to this, a study done in Bangkok and Oxford by Stuart D Blacksell in 2012 has shown that IgA response is 3.5 times greater than that of NS1 in secondary infection.4

The use of dengue IgA as an RDT has been explored. Study done by Talarmin A and team in 1998 showed that anti-dengue IgA typically appears a day after IgM and disappears within 45 days following detection.5 Dengue IgA rapid test is an immunochromatographic test device intended for the detection of IgA antibodies to dengue virus in human blood, plasma, and serum, within 20 minutes.6 The advantages of IgA rapid test compared with other diagnostic tests are the following: it is able to detect dengue infection on the first day of illness; it is highly sensitive in secondary infection; it has higher response in detecting the presence of dengue; it only needs 25 microliter sample of serum, plasma and whole blood; it is easier to interpret using intensity scale; and the results are available within 15-20 minutes.7

The overall sensitivity and specificity of dengue IgA RT were 86.7% (n=233) and 86.05% (n=681), respectively which detected 77.42% primary and 92.86% secondary cases. NS1 antigen test has 89.25% sensitivity and 20% specificity. Using 125 paired samples, dengue IgA RT showed a sensitivity of 84.08% in acute phase and 99.2% in the convalescent phase with a specificity of 92% in both phases.6

On the other hand, NS1 test is helpful for diagnosis especially in first four days of illness whereas IgM antibody is recommended to be used from the fifth day of fever onwards. A study by Shera et al. in 2011 showed that NS1 had significantly higher sensitivity

for primary infections at 94.7% than for secondary infection at 67.7%.8

In this study, we compared the performance of dengue IgA and NS1 rapid tests for early diagnosis of dengue using HI as standard reference. In previous comparative studies done by Chuansumrit.et al in India in 2008, the sensitivity of dengue IgA (91.7%) was consistently higher than dengue NS1 (16.7%). [7] Similar trend was seen in this study with recorded sensitivity at 80% and specificity of 50% for dengue IgA while dengue NS1 at 27% and 67%, respectively.

Our results showed that dengue IgA and NS1 can detect the presence of dengue virus infection during the first seven days of illness. Both RDTs showed 100% sensitivity in detecting dengue infection between the third and fourth days of illness. However, detection rate of IgA decreased from days five-to-seven while NSI could no longer detect infection within the same period. These results were congruent to the study done in 2011 at Central Tropical Medicine in Oxford and Thailand by Blacksell which showed that dengue IgA could detect presence of dengue infection in the blood from the first day up to tenth day of illness more than dengue NS1 with sensitivity limited only from days 1 to 4.4

In this study, IgA rapid test demonstrated 71% positivity in detecting acute primary dengue infection and 82% for acute secondary infection. These results were also demonstrated in a study done by Ahmed in Bangladesh detecting 100% primary and 92.9% secondary infections using Dengue IgA rapid test.9 On the contrary, NS1 was able to detect only 43% of primary dengue infection and 24% of secondary dengue infection (Table 6). Comparable to our results, Shera and colleagues in 2011 showed NSI to have higher positivity for primary infection at 94.7% and secondary infection at 67.7%.8 In 2007, another study by Kumarsamy et al showed NSI performance in detecting dengue secondary cases to be not as good in detecting primary infection.3

The dynamics of dengue virus infection have potentially large influence on the interpretation of RDT (Figures 2 and 3). Virus remains detectable in the blood for up to 2 to 12 days after the onset of symptoms.10,11 During the viraemic phase, NS1 antigen is produced concomitantly during the virus replication and hence is an excellent tool for acute dengue diagnosis.12,13 Difference in the persistence of soluble NS1 antigen in serum between primary (6-12 days) and secondary dengue infections (5-6 days post-onset of illness) has been noted and soluble NS1 antigen peaks at second day of illness and declines earlier to almost undetectable levels by days 5-6 of illness.14 The presence of anti-NS1 antibodies in dengue secondary infections, results to formation of antigen-antibody complexes, that impede the ability to detect free NS1 antigen.15,16 This is the reason that the turnout of NS1 positivity in dengue secondary infections is much lower than the primary infections. NS1 peaks at days 2-3 of illness and starts to decline by days 4, and may still persist but almost undetectable from days 8-9, which holds true with our results of negative NSI from days 5-7 in acute primary infection.

Dengue IgM antibodies are a reliable marker of recent infection but not necessarily acute infection. In primary dengue infections, IgM antibodies develop following the decline of viraemia between days 3-to-5 after the onset of infection17,18 and reach peak levels approximately 2 weeks later.19 Persistence of IgM antibodies following primary infection has been estimated at 179 days (95%CI, 155 to 215 days).20 In secondary infections, IgM antibodies may be detectable as soon as after 2-3 days of infection21-23 and peak IgM antibody levels are usually lower than in primary infections.10,24 Persistence of IgM antibodies following secondary infection is shorter than that of primary infections at 139 days (95%CI, 119 to 167 days),20 and estimates of IgM antibody persistence range from 2 months to 6 months.10,25 The IgG antibody response develops a few days after the onset of the IgM antibody response and may persist for many years. In secondary infections, IgG antibodies are detectable at days 4-5 of illness18 which is much sooner than primary infection. Dengue IgA antibodies have been reported between days 8 and 11 after onset of fever.19 However, in primary dengue infection, the onset of detectable levels of IgA antibodies has been reported on average at 5.5 days after onset of fever and in secondary infection, IgA antibodies increased slowly during the first days of the study.24 The rates of positivity for IgA antibodies in

in serum were reportedly significantly higher in secondary infections than in primary infections (100% versus 84.6%).26

There is a clear need in dengue infections to have prognostic details of disease severity. Prognostic indicators of clinical severity would provide direction for patient management. Patients with secondary or later dengue infections are considered to have an increased risk of the more severe forms of the disease, and therefore the accurate detection of primary and secondary at presentation to a clinical facility may become a promising patient management tool. In this regard, our results showed that IgA had better performance in the detection of acute primary dengue (50% IgA versus O% NS1) and acute secondary infections (84% IgA versus 26% NS1) during the 5-7 days of illness. Overall performance of IgA showed superiority over NSI at any time during the first seven days of illness in both acute primary and secondary infections. Therefore, results in this study infer that IgA is a better tool than NS1 for detection of dengue infection regardless if acute primary or secondary infections; and for prognostication of disease severity as well.

The specificity of each test was comparable. In any case, a negative result for IgA or NS1 in a single sample does not confirm dengue, therefore, the impact of false negative results in the routine clinical setting should be assessed.

Predictive values depend on the prevalence of the disease but their trend here showed that all tests were comparable. In a scenario where a clinician's interest is to confirm dengue diagnosis, any of the tests is likely to be useful but he should be aware that a negative test does not rule out the disease.

The limitation of the present study was the small number of patients with negative results for dengue (6/51), hence specificity was not statistically significant (IgA 50% and NS1 67%).

CONCLUSION AND RECOMMENDATION

Dengue IgA antibody was a more sensitive RDT than NS1 antigen in the early diagnosis of dengue infection from days 1 to 7 of illness. NS1 diagnostics were only sensitive in the early phase of dengue infection (days 1-4), and therefore, not a suitable and reliable test where late manifestations of dengue may occur. IgA test had better performance than NS1 in detecting both primary and secondary dengue infection that may help the clinicians in the prognostic details of disease severity.

In addition, clinicians must also be aware that a negative result does not rule out dengue. To further assess the usefulness of these tests in a clinical setting, a bigger sample size with varied days of illness be subjected to both IgA and NS1 with viral isolation as the gold standard.

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