ORIGINAL ARTICLE

A STUDY OF RELIABILITY OF RAPID TEST IN ACUTE DENGUE FEVER AND ITS IMPLICATION

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HOW TO CITE THIS ARTICLE:
Vijay Kumar B. A, Yogesh Kamshetty, Ashish Kumar Sharma, Laxman S. J, Deepa V. A, Vijay Kumar S. "A Study of Reliability of Rapid Test in Acute Dengue Fever and its Implication". Journal of Evidence Based Medicine and Healthcare; Volume 1, Issue 8, October 15, 2014; Page: 1115-1119.

ABSTRACT: OBJECTIVES: Rapid diagnosis of dengue infection is essential to patient management and disease control. In a rural tertiary health setting and diagnostic laboratories, dengue suspect cases were assessed with a rapid (15 minutes) immunochromatographic tet and compared to an IgM capture enzyme-linked immunosorbent assay (ELISA) (2-3 days) and the reliability of the rapid test was tested. **METHODOLOGY:** The objectives were to evaluate dengue rapid test against dengue IgM capture ELISA and to assess the scopes of such rapid tests in peripheral setting. A rapid immunochromatographic card test was compared with an IgM capture ELISA (National Institute of Virology, Pune India) as the reference gold standard. The among 158 dengue suspects. **RESULTS:** The rapid test showed good sensitivity in the diagnosis of both primary and secondary dengue infection. The rapid test as confirmed by IgM capture ELISA was found to have specificity of 98.4% and sensitivity of 96.4%. The positive predictive value was 93.1% and negative predictive value of 99.2%. The positive likelihood ratio worked out to be 62.6, with negative likelihood ratio was 0.036, signifying large impact. **CONCLUSION:** The rapid tests may be useful aid in screening in case of clinical diagnosis of dengue infection, particularly valuable in peripheral health setting, where it can hasten the initiation of first line of management; while the ELISA has a place in central testing laboratories, aiding in resource optimization.

KEYWORDS: Immunochromatographic Rapid test, dengue virus, enzyme-linked immunosorbent assay (ELISA) screening test.

INTRODUCTION: Dengue is an endemic viral disease affecting tropical and subtropical regions around the world. Dengue fever (DF) and its more serious forms, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), are becoming important public health problems and were formally included within the disease portfolio of the United Nations development Programme/World Bank/World Health Organization Special Program/ for Research and Training in Tropical Disease by the Joint Coordination Board in June 1999.[1] Dengue virus is a mosquito-borne flavivirus and the most prevalent arbovirus in tropical and subtropical regions of the world.[2] There are four distinct serotypes of the dengue virus, serotypes 1 to 4. Infection induces a life-long protective immunity to the homologous serotype but confers only partial and transient protection against subsequent infections by the other three serotypes.[3-5]

At present, the three basic methods used by most laboratories for the diagnosis of dengue virus infection are viral isolation and characterization, detection of the genomic sequence by nucleic acid amplification technology assay, and detection of dengue virus-specific antibodies.

After the onset of illness, the virus is found in serum or plasma, circulating blood cells, and selected tissues especially those of the immune system, for approximately 2 to 7 days, roughly corresponding to the period of fever.[6] Serological diagnosis remains the main stay of diagnosis during the epidemic since viral isolation is laborious, expensive and is only available in reference laboratories.

Two patterns of serological response can be observed in patients with dengue virus infection: Primary and secondary antibody responses, depending on the immunological status of the infected individuals. A primary antibody response is seen in individuals. A primary antibody response is seen in individuals who are not immune to flaviviruses. A secondary antibody response is seen in individuals who have had a previous flavivirus infection. For acute- and convalescent-phase sera, serological detection of antibodies based on capture immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA) has become the new standard for the detection dengue virus infections.[6-8]

This is important, since a sensitive and reliable assay for the detection and differentiation of primary versus secondary or multiple dengue virus infection is critical for the analysis of data for epidemiological, pathological, clinical, and immunological studies. But in rural peripheral health setting with dengue outbreak, rapid assessment is of great importance with a balanced reliability. In this context, this study was carried out to evaluate the performance of a rapid immunochromatographic test (ICT) device for the detection of IgM and IgM antibodies to dengue virus against the IgM and IgG antibodies to reference standard at a rural tertiary care hospital in India.

MATERIAL AND METHODS: The study was carried out among 100 dengue suspect cases presenting to a rural tertiary care hospital, over a period of 3 years. Dengue suspects were defined as patients presenting with acute febrile illness, rashes, bleeding tendencies, leucopenia and or thrombocytopenia were evaluated according to WHO criteria[5] for probable dengue infection. Acute phase sera were collected after 5 days of the onset were initially tested with immunochromatography card test, a rapid test device, taking about 15 minutes. It simultaneously detects IgM and IgG antibodies to dengue virus in human serum.

Specific human IgM and the nitrocellulose membrane as two individual test bands (IgM and IgM) in the test window. As the test sample flows through the membrane assemble within the test device, the coloured- dengue specific recombinant antigen – colloidal gold conjugate complexes with specific antibodies (IgM and IgG) to dengue virus, if present in the sample, forming coloured band confirming positive results. Appearance of coloured bands in the test window is interpreted as a negative test result. Bands appearing in both IgG and IgM regions in the test wind along with control band are indicative of acute secondary dengue infection. If in addition to the control band, coloured band appears only at the IgM test region, it is taken as reactive for acute primary infection.

Appearance of no bands is interpreted as invalid test. Consequently the samples were checked with IgM capture ELISA (MACELISA) by national institute of Virology, Pune, as the reference standard. The MACELISA) is a 2-day test that requires about 4 hours of a technician's time. This test is designed to detect IgM antibodies to any one or three of the flaviviruses

prevalent in India (DEN-2, JE and West Nile). The procedure starting with the coating of anti-IgM was performed according to protocol provided. The intensity of colour/optical density (OD) is monitored at 450nm. The OD values are directly proportional to the amount of virus specific IgM antibodies present in the sample. Samples are declared reactive based on the ratio of absorbance of sample to negative control, with OD value of sample tested exceeding the OD of negatives control by factor 2.1. Samples are considered negatives when this rations greater than 1.9 but less than 2.1 were considered indeterminate.

OBESERAVTION:

Year	No. of suspect samples	No. of samples positive for dengue	
		IgM ELISA	Rapid immunochromatography
2011	47	14	14
2012	35	2	1
2013	18	3	3
Total	100	19 (18.3%)	18 (17.7%)

Table 1: O DF Cases Diagnosed at the tertiary care center, 2011 to 2013

Test	Specificity	Sensitivity	Positive predictive value (PPV)	Negative predictive value (NPV)	Positive likelihood ratio (+LR)	Negative likelihood ratio (-LR)	Diagnostic accuracy (%)
Rapid ICT	98.4%	96.4%	93.1%	99.2%	62.6	0.036	99.3%

Accuracy = TP+TN+/TP+TN+FP+FN (TP- true positive, TN-true negative, FP-false positive FN-false negative

Table 2: Accuracy indices of the Rapid immunochromatographic test

A total of 158 dengue suspect cases formed the study subject, with 28 samples testing reactive for either primary or secondary dengue infection, and 130 being non-reactive, which were initially tested with rapid ICT devices were used for serological diagnosis of dengue virus infections. Results obtained with the rapid device were verified for accuracy with IgM capture ELISA as the reference standard. Validation results of the rapid test are depicted in Tables 1 and 2 depicting the sample obtained over the study period respectively. Higher false negative results as compared to ELISA were obtained with both the kits. The rapid test revealed a prevalence rate of dengue viral infection to be 17.7% compared to 1803% with ELISA indicating a slightly lower accuracy for rapid test results, which was not found to be statistically significant.

DISCUSSION: Different studies[9.10] have compared different immunoassay methods to detect IgM dengue antibodies including ELISA, dot ELISA, dipstick assay, dot blot assay and ICT devices. Some studies have tested the diagnostic accuracy of various commercially available rapid test devices. The results of the present study revealed specificity compared to the sensitivity and higher negative predictive value then the positive predictive value. The present study returned

high likelihood ratio signifying a good impact. The observation of Blacksell et al[10] who conducted an elaborate prospective study where they compared 8 commercially available immunochromatographic rapid device test using a panel of references samples, the results revealed vary low sensitivity where 6/8 rapid device test had sensitivity of less than 50% (6-50%). However specificity in this study was higher. The authors advocate that currently available the diagnosis of acute dengue infection is unlikely to be useful for patient management.

The same group carried out meta-analyses of published peer reviewed studies which racy and evaluated eleven studies.[10] They reported highly heterogenous results with sensitivity ranging between 45-100% and specificity range of 57-100% similar to our findings the study by Vaughn et al[11] report diagnostic test device (Pan-Bio, Brisbane, Australia) for diagnosis of DF when they compared the results of a rapid diagnostic test deice in 98 cases of primary and secondary DF cases with haemagglutination assay and enzyme immunoassay. Sang et al[12] compared the results revealed 98% sensitivity for the immunochromatographic device suggesting it as a useful diagnostic tool. Using same reference standard of haemagglutination assay Kittigul and Suankeow[13] reported sensitivity of 79% for the immunochromatographic rapid test device but a higher specificity of 95%.

As revealed in this study, with a high negative predictive value, the rapid tests may prove to be useful aids in screening in the clinical diagnosis of dengue infection, more so in the resource poor peripheral health setting. I can prove to be a useful tool to hasten the imitation of first line of management and thereby can be of great help to the healthcare providers in the rural areas, ELISA (IgM) with its higher precision accuracy and elaborate processing has a place in the central testing laboratories aiding in resource optimization and acting as a fall back for the rapidly conducted tests for dengue diagnosis.

REFERENCES:

1. World Health Organization – Strengthening implementation of the Global Strategy for Dengue Fever and Dengue Haemorrhigic Fever, Prevention and control; report of the informal consultation, October 18-20th 1999 Geneva: WHO 1999.
2. Guzman MG, Kouri G, Bravo J, Soler M, Vazquez S, Morier l – Dengue hemorrhagic fever in cuba, 1981: a retrospective seroepidemiologic study. Am J Trop Med Hyg 1990; 42: 179-84.
3. Halstead SB, Shotwell H, Casals J – Studies on the pathogenesis of dengue infection in monkeys. Ii: clinical laboratory responses to heterologus infection. J infect Dis 1973; 128: 15-22.
4. Halstead SB- Pathogenesis of dengue: challenge to molecular biology. Science 1988; 239: 476-81.
5. World Health Organisation – Dengue Haemorrhagic Fever Diagnosis, Treatment, Prevention and Control. 2nd ed Geneva; WHO, 1997.
6. Gubler DJ – Serologic diagnosis of dengue/ dengue haemorrhagic fever. Dengue Bull 1996; 20: 20-03.
7. Innis BL, Nisalak A, Nimmannitya S, Kusalerdchariya S, Chongswasdi V, Suntayakorn S, et al – An enzyme-linked Immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. Am J Trop Med Hyg 1989; 40: 418-27.

8. Branch SL, Levett PN – Evaluation of four methods for detection of IgM antibodies to dengue virus. Clin Diagn Lab Immuno 1999; 6: 555-7.
9. Groen J, Koraka P, Veizing J, Copra C, Osterhous, AD Evaluation of six immunoassays for detection of dengue virus specific Igm and IgG antibodies. Clin Diagn Lab immuno 2000; 7: 867-71.
10. Blacksell SD, Neuton PN, Bell, D Kelly J, Mammen MP Jr, Aughn DW, et al – The comparative accuracy of 8 commerical rapid immunochromatographic assays for the diagnosis of acute dengue virus infection. Clin Infect Dis 2006; 42: 1127-34.
11. Vaugn DW, Nisalak A, Kalayanarooj S, Solomon T, Dung NM, Cuzzobo A, et al – Evaluation of a rapid immunochromatographic test for diagnosis of dengue virus infection. J Clin Microbiol 1998; 36: 234-8.
12. Sang TC, Hoon LS, Cuzzubbo A, Devine P=-Clinical evaluation of a rapid immunochromatographic test for the diagnosis of dengue virus infection. Clin Diagn Lab Immuno 1998; 5: 407-9.
13. Kittigul L, Suankeow K – Use of a rapid immunochromatographic test for early diagnosis of dengue infection. Eur J Clin Microbiol infect Dis 2002; 21: 224-6.

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Date of Submission: 22/09/2014.
Date of Peer Review: 23/09/2014.
Date of Acceptance: 13/10/2014.
Date of Publishing: 15/10/2014.