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Dengue Duo Rapid Test: A Valuable Screening Test for Dengue Outbreak

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Dengue is a major health problem in many parts of the tropical world presenting as both epidemic & endemic forms. It is a mosquito borne illness caused by one of the serotypes of dengue viruses. Mortality rate of dengue in untreated patients is 10%-12%. The present study aims to determine whether the rapid test SD dengue duo (IgG, IgM, and NS-1 Ag detection) can be used as screening test in dengue outbreak when compared to ELISA. 226 serum samples were tested in patients clinically suspected Dengue. All the 226 samples were subjected to IgG, IgM Microlisa test. The same were put on rapid SD bioline Dengue duo rapid test and was compared with ELISA. 150 samples were tested positive with ELISA (either positive for IgG, IgM). When compared with ELISA, Rapid test showed sensitivity of 80.6% specificity and positive predictive value of 100% & zero false positive rates. Efficiency of the test was 87.16% SD Dengue duo rapid test should be a valuable screening test for dengue fever outbreak which can be interpreted easily. Results were comparable to ELISA. It provides additional diagnostic investigation of primary and secondary dengue that compliments NS-1 antigen detection.

Key words: Dengue, ELISA, rapid test, NS-1 antigen.

Dengue is a major health problem in many parts of tropical world. Dengue is caused by infection with one of the four serotypes of dengue virus (DEN 1- 4) which are arboviruses belonging to the Flaviviridae family and are transmitted by mosquito principally Aedes aegypti. In terms of morbidity, mortality and economic costs, dengue virus infection is the most important mosquito borne virus disease in the world with an estimated 100 million cases per year [1,2].

There are several reasons why early and accurate diagnosis of dengue outbreak is important. First, an early and accurate diagnosis can assist in patient management by directing clinical attention to the appearance of major warning signs of severe or even life threatening complications, e.g. rapidly rising hematocrit, poor peripheral perfusion. Second, dengue diagnosis prevents unnecessary and possibly expensive antibiotic usage. Third,

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prompt diagnosis of index cases can facilitate vector control activities in the community so as to mitigate further transmission. Lastly, the expanded use of accurate dengue diagnostics provides important data on the epidemiology and health burden of dengue and in doing so can inform and guide public health policy, particularly as dengue vaccines and anti- virals make their way through development pipelines [3-5].

The major diagnostic methods currently available are viral culture, viral RNA detection by reverse transcriptase PCR (RT-PCR) & serological tests such as IgM MAC-ELISA and IgG MAC-ELISA. However early dengue diagnosis still remains a major problem as all these assays have their own pitfalls. The first two assays have restricted scope as a routine diagnostic procedure. The MAC- ELISA which is a commonly used assay has low sensitivity in first few days of illness [6,7].

Now- a- days detection of NS-1 Ag on rapid tests offer an even faster route to a presumptive dengue diagnosis. NS-1 (Non structural protein) is a highly conserved glycoprotein that is essential for the viability of Dengue virus & is produced both in membrane associated & secretory forms by the virus. The detection of secretory NS-1 protein represents a new approach to the diagnosis of dengue infection [7,8,10].

The present study aims to determine whether the rapid test SD dengue duo (IgG, IgM, and NS-1 Ag detection) can be used as screening test in dengue outbreak when compared to ELISA.

MATERIAL AND METHODS

Case Definition

- In children experiencing febrile illness consistent with dengue fever and clinically suspected cases dengue fever (according to World Health Organization criteria).

Serological Definition

Primary dengue virus and Secondary dengue virus infections were defined as those serum samples positive to IgM antibodies and IgG antibodies respectively.

Patients and study design

226 blood samples were screened from clinically suspecting cases of dengue from July 2010 to August 2010 from the Pediatric outpatient department, SS Institute of Medical Sciences and Research Centre.

Two serum samples were collected from each patient, one at the day of enrollment and second 7-14 days after the fever onset.

The samples were subjected to SD Bioline Dengue Duo rapid test and Dengue IgG and IgM Microlisa.

SD Bioline dengue Duo rapid test is an invitro immunochromatographic, one step assay designed to detect IgG and IgM antibodies to dengue virus in human serum and NS-1 antigen. The test was read after 20 minutes.

Interpretation of the SD Bioline Dengue duo rapid test :

The presence of each one color line (control) within the result window indicates a negative result.

The control line (C) and IgM line (M) are visible on the test device. This is positive for IgM antibodies to Dengue virus and indicates primary dengue infection.

The control line and IgG line (G) are visible on the test device. This is positive for IgG antibodies and indicates of secondary or past dengue infection.

The control line, IgM line (M) and IgG line (G) are visible on the test device. This is positive for both IgM and IgG antibodies and indicates late primary or early secondary dengue infection.

The control line, NS-1 Ag line is visible on the test device. This is positive for NS-1 antigen and indicates of early acute dengue infection.

The same samples were again tested for IgG and IgM dengue antibodies by IgG and IgM capture Microlisa. The ELISA was performed as per the Manufacturer's instructions.

RESULTS

A total of 226 samples were studied from July 2010 to August 2010 from the Pediatric Out patient department, SS Institute of Medical Sciences and Research Centre, Davangere.

All the 226 samples were subjected to IgG, IgM Microlisa test. In this 124 samples were positive to IgM (Primary Dengue infection) and 26 samples were positive to IgG (Secondary Dengue infection). No negative results were reported in

Table 1. Comparison of RAPID test with ELISA test ELISA (IgG & IgM)

Rapid test (IgG, IgM & NS-1)	Positives	Negatives	Total
Positives	121(true positives)	0(false positives)	121
Negatives	29(false negatives)	76(true negatives)	105
Total	150	76 22	

Rapid test
Sensitivity = 80.66%
Specificity = 100%
Positive Predictive Value = 100%
Negative Predictive Value = 72.4%
False Positive Rate = 0.
Efficiency of the test = 87.16%

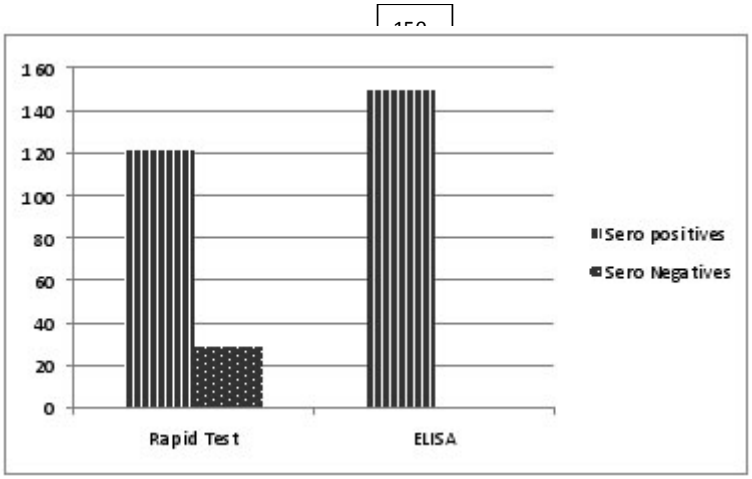

Fig. 1. Seropositivity in ELISA and Rapid test

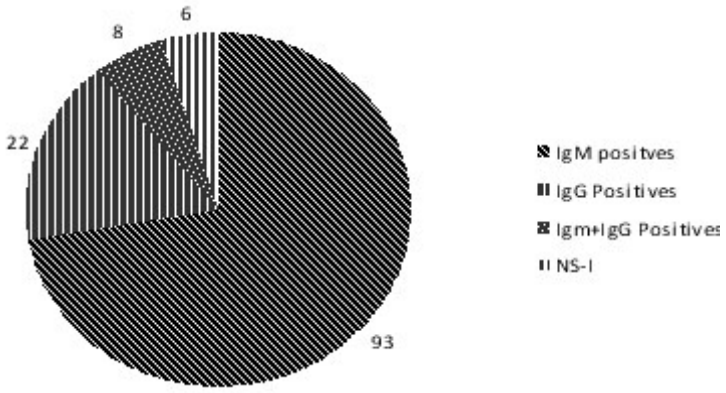

Fig. 2. Pie diagram showing Seropositivity in rapid test (SD Bioline Dengue Duo test)

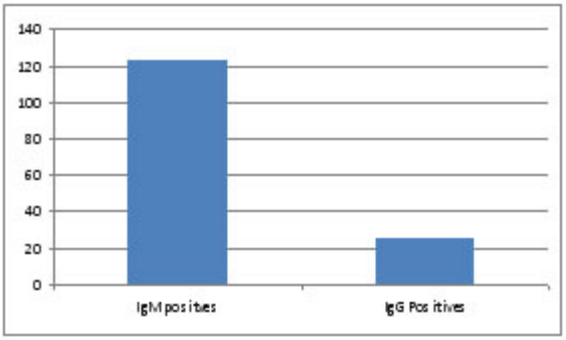

Fig. 3. Seropositivity Detected in ELISA

ELISA test. Total of 150 seropositive cases were detected. (124 IgM + 26 IgG) (Fig. 3).

Out of 150 seropositive cases from ELISA, the rapid Bioline Dengue duo test showed 93 IgM positives and 22 IgG positives. 8 samples were positive to both IgG and IgM. 6 samples were positive to NS-1 antigen. 29 samples were negative to all IgM, IgG and NS-1 antigen (Fig. 1,2).

When rapid test results were compared with ELISA test, out of 226 samples 121 were true positives, 76 were true negatives whereas there were 29 false negatives but no false positives in rapid test (Table 2).

Elisa
IgM
Positives 124
IgG
Positives 26

DISCUSSION

All ELISA positives (IgG and IgM positives) were analyzed and evaluated with the rapid SD bioline Dengue duo rapid test which is an in vitro immuno chromatography test. Sensitivity for primary dengue infection was 75% & for secondary dengue infection 85% which were similar to the results obtained by different authors [6,9].

We made a comparative evaluation of both ELISA and Rapid test. Rapid test had a sensitivity of 80% and specificity 100% when compare to ELISA. The variations in sensitivity and sensitivity are comparable with previously published data and this might be caused by different principles of the assays, different antigens, conjugates. Lam S et al reported sensitivity and specificities of the rapid tests varied between 80 – 98% [12-15].

A rapid and accurate method for the diagnosis of the dengue fever is important for the both clinician and patient. The commercially available dengue rapid test is suitable for the detection of anti dengue IgM and IgG antibodies and NS-1 antigen with results available in just 20 mins with a positive predictive value and negative predictive value of 100%, 72.4% respectively[4]. The combined use of IgM and IgG has been shown to increase the sensitivity in detection of dengue virus infection [5].

Caution should be applied in interpreting tests that are positive to dengue virus IgM or IgG only in areas where dengue virus co circulates with other flavi viruses [8]. This might be the probable reason for the false negative rate (19.3%) of rapid test & negative predictive value (72.4%) in our study. As the patients are referred to our hospital (tertiary care hospital) from the peripheries the chances of detecting NS-1 antigen was low. The mean duration of illness of patients in our study was between 5-14 days after fever onset.

In this regard NS-1 antigen detection by ELISA method may be useful. NS-1 is postulated to contribute to the pathogenesis of dengue. First, in children elevated NS-1 plasma concentrations early in illness are associated with more severe disease, possibly reflecting higher viral burdens in these patients. The potential for early NS-1 concentrations to predict clinical outcome has been postulated but not assessed[4,5]. It has been suggested that high NS-1 levels may activate complement by directly binding endothelial cells, and may establish foci for immune complex formation leading to complement activation, endothelial damage and capillary leakage [11].

Efficiency of the rapid test in our study is 87.16%. The use of IgM & IgG test parameters with NS-1 antigen detection is rational as it will likely provide improved presumptive diagnostic coverage towards the end of acute illness when NS-1 levels are declining but dengue virus specific IgM & IgG titres are climbing[7]. Studies claim that in addition to early diagnosis, NS-1 antigen may be indicator of severity of the disease [8].

CONCLUSION

Prompt diagnosis of index cases can facilitate vector control activities in the community so as to mitigate further transmission. The commercially available SD dengue duo rapid test described in the study should be a valuable screening test for dengue fever. It is rapid and can easily be performed and interpreted early and has an extended shelf life. This test can also be used in field settings or in laboratories without adequate equipment or electricity. The strength of the SD dengue duo rapid test is that dengue IgG and IgM test windows provides additional diagnostic investigation that compliments NS-1 antigen detection.

We conclude that rapid test is an effective tool, if when used in combination of NS-1 MAC ELISA has the ability to improve the diagnostic algorithm contributing significantly to clinical treatment and control dengue viral infections.

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