DENGUE: SEROPREVALENCE, COMPARISON OF RAPID TEST WITH ELISA

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ABSTRACT

Introduction: - Dengue is a major health problem in many parts of the tropical world. It is a mosquito borne illness caused by one of the serotypes of dengue viruses.

Aims and objectives: - The present study was done to know the common clinical features and Seroprevalence of dengue in our region. An attempt was made to compare rapid test SD dengue duo (IgM, IgG & NS-1 Ag detection) with capture ELISA (IgM, IgG Microlisa Dengue).

Materials & Methods: - 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.

Results:- 226 serum samples were tested in patients clinically suspected Dengue before noting common clinical signs and symptoms. 150 samples were tested positive with ELISA (either positive for IgG, IgM or both). Seroprevalence of 66% were reported. 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%

Conclusion:- High prevalence rate in our region particularly in premonsoon & monsoon season gives an alarm to the doctors regarding early and accurate diagnosis of dengue virus infection. SD Dengue duo rapid test should be a valuable screening test for dengue fever which can be interpreted easily. Results were comparable to ELISA. It provides additional diagnostic investigation that compliments NS-1 antigen detection.

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

INTRODUCTION

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 [1,2] and are transmitted by mosquito principally Aedes aegypti. Infection with dengue virus may be clinically apparent or may be present as a nonspecific febrile illness, Classic dengue fever or Dengue Hemorrhagic Fever (DHF) [3].

The major diagnostic methods currently available are viral culture, viral RNA detection by reverse transcriptase PCR (RT-PCR) & serological tests such as IgM Capture & IgG Capture 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[4]. Viral isolation by Immunoflourescence though a gold standard cannot be used as a routine diagnostic procedure due to its low sensitivity, laborious procedure & time consumption. The MAC- ELISA which is a commonly used assay has low sensitivity in first few days of illness.[5-7]

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 & secretary forms by the virus [8]. The detection of secretary NS-1 protein represents a new approach to the diagnosis of dengue infection.

The present study aims to determine the common clinical features and Seroprevalence of Dengue virus infection in Davangere & an attempt was made to compare the rapid test SD dengue duo (IgG, IgM, and NS-1 Ag detection) with Capture ELISA (IgG, IgM) (Microlisa Dengue)

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MATERIAL AND METHODS

Case Definition:- In children experiencing febrile illness consistent with dengue fever and clinically suspected cases of 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:- Blood samples from 226 clinically suspecting cases of dengue were screened from June 2009 to May 2010 in Paediatric OPD SSIMS & RC Davangere. 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 Dengue IgM and IgG Microlisa and SD Bioline Dengue Duo rapid test.

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

SD Bioline dengue Duo rapid test is an invitro immunochromatographic, one step assay designed to detect IgM and IgG antibodies to dengue virus in human serum & 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.

RESULTS

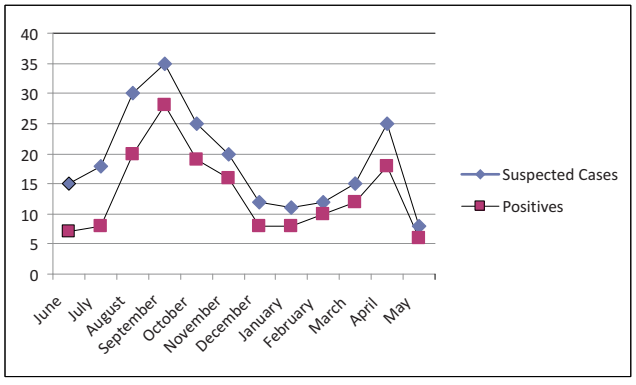
Total of 226 samples from June 2009 to May 2010 were studied. We observed maximum number of clinically suspected cases of dengue in August, September, October months (Graph-1). There was male patient predominance over female with maximum cases between the ages 6 – 10 yrs.

We tried to note the symptamatology of dengue cases in our region. The most common symptoms apart from fever were vomiting, Abdomen pain, Rashes, Malaena and Coldness of feet & common signs were Hepatomegaly, Splenomegaly, and Jaundice. (Graph -2)

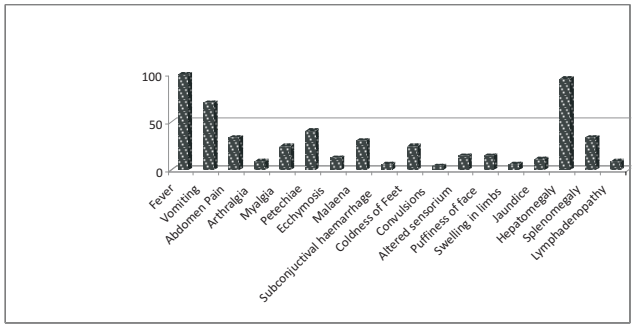
All the 226 samples were subjected to IgM, IgG Microlisa test. In this 124 samples were positive to IgM (Primary Dengue infection) and 26 samples were positive to IgG (Secondary Dengue infection). Total of 150 seropositive cases were detected. (124 IgM + 26 IgG).

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.(Graph-3&4)

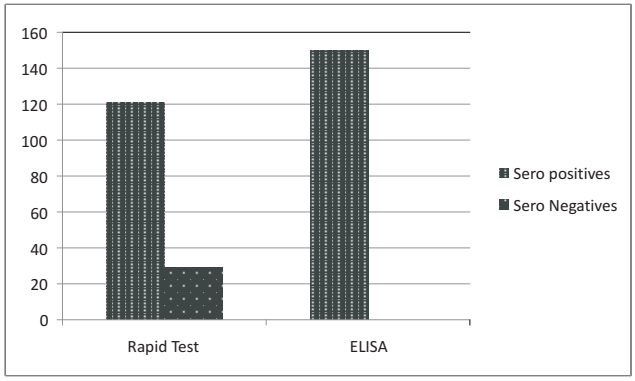
When rapid test results were compared with ELISA test, out of 226 samples 121 were true positives, 76 were true negatives whereas there were 29 false negatives but no false positives in rapid test. (Table-1)



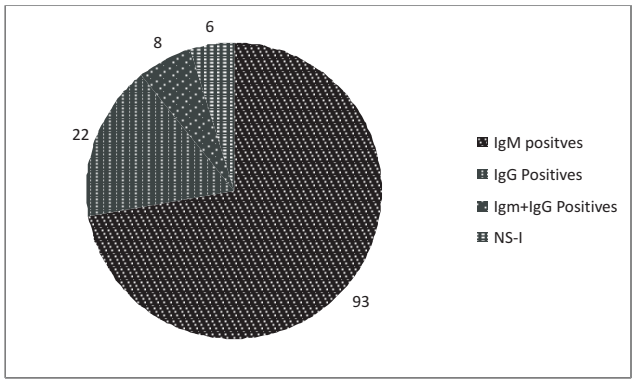
Graph – 1 : Seasonal variation of dengue



Graph – 2 : Clinical Features in dengue suspected cases



Graph – 3 : Seropositivity in ELISA and Rapid test



Graph – 4 : Pie diagram showing
Seropositivity in rapid test (SD Bioline Dengue Duo test)

Rapid test (IgG, IgM & NS-1)	Positives	Negatives	Total Cases
Positives	121 (TRUE POSITIVES)	0 (FALSE POSITIVES)	121
Negative	29 (FALSE NEGATIVES)	76 (TRUE NEGATIVES)	105
Total	**150**	**76**	**226**

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

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%

DISCUSSION

In the present study, maximum number of cases were between 6- 10 yrs. Gomber S et al in a similar study had similar findings and this could be attributed to the health care seeking behavior of the patients, endemic nature of the infection [10].

12-18 % of the positive cases occurred during the months of July, August, September. This is in comparison with similar pattern of month-wise case distribution seen with authors Rasul CH et al in 2002, Narayan et al in 2002, Gomber et al in 2001 [11-14]. The reasons may be due to the geographical region with prime occupation of the people being agriculture. July and August months are paddy sowing months which needs large stores of water. Also the breeding of Aedes aegypti is highest during pre and post monsoon period. But sporadic cases extend up to December which indicates endemicity of the infection up to December.

ELISA test showed 82.6% of cases as primary dengue and 17.3% as secondary dengue. High dengue primary infection is due to the virulence of the infecting serotype of the virus.

We made a comparative evaluation of both ELISA and Rapid test. Rapid test had a sensitivity of 80% and specificity 100% when compared to ELISA. The variations in sensitivity and specificity are comparable with previously published data[14] & this might be caused by different principles of the assays, different antigens, conjugates.

A rapid and accurate method for the diagnosis of the dengue fever is important for both the clinician and the 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]. 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 [15]. This might be the probable reason for the false negative rate (19.3%) & negative predictive value (72.4%) of rapid test in our study.

The role of NS1 Ag for early detection of Dengue virus infection is currently being evaluated by many investigators. As there was time lag in the patients who were referred to our hospital which is a tertiary care hospital from the peripheries, the chance of detecting NS-1 antigen was low. In this regard NS-1 antigen detection by ELISA method may be useful[5]. The mean duration of illness of patients in our study was between 5-14 days after fever onset.

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 would 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 [5].

CONCLUSION

High prevalence rate in our region particularly in pre monsoon and monsoon season gives an alarm to the doctors regarding early and accurate diagnosis of dengue virus infection and its complications. 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, easily be performed, interpreted early and has a extended shelf life. The strength of the SD dengue duo rapid test is that dengue IgM and IgG 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 with NS-1 MAC ELISA in single sample of suspected cases, has the ability to improve the diagnostic algorithm contributing significantly to clinical treatment and to control dengue viral infections.

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