Comparative evaluation of a rapid test with ELISA for the detection of Dengue Infection

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Abstract
Background: Dengue is a global health issue. The clinical illness ranges from an asymptomatic febrile illness to dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS).
Objectives: The present study was undertaken to evaluate sensitivity and specificity of SD (BIOLINE) Dengue Duo (NS1 Ag & Ab combo) rapid test in comparison to capture ELISA for early diagnosis of dengue infection.
Material and Methods: It was a tertiary hospital based retrospective cross sectional study. The data was collected by reviewing the records of 250 patients who attended the OPD or were admitted to the hospital with the suspicion of dengue infection, from May 2015 to March 2016. The blood samples were routinely processed in central clinical laboratory and were tested by both, SD (Bioline) Dengue Duo rapid test and ELISA.
Results: Out of 250 samples 69 were found to be reactive for Dengue infection by ELISA. Out of these 69 seropositive samples rapid test was reactive in 55 samples. There were 18 samples in which only NS1 Ag was positive by both rapid test and ELISA. Overall sensitivity of rapid test in comparison with ELISA was found to be 79.71% and specificity was 100%.
Conclusion: Early diagnosis and treatment of the patient suffering from the infection is important. SD (Bioline) Dengue Duo rapid test can be a useful tool in initial diagnosis of the infection as it detects NS1 Ag in addition to the antibodies. Its results are comparable to ELISA.

Keywords: Dengue, NS1 Ag, Rapid test, ELISA

Introduction
Dengue is an arthropod borne viral illness caused by one of the 4 serotypes of the Dengue virus (DEN1-4). It is worldwide in distribution especially in tropical and sub-tropical regions and is transmitted by mosquito Aedes aegypti (mainly) and Aedes albopiticus.[1] The clinical manifestations ranges from an asymptomatic febrile illness to more severe forms of infection i.e. dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS).[2]

In India first outbreak was reported in Kolkata in 1963-64 and gradually it spread to other parts of the country.[3] NVBDCP website data shows that dengue is endemic in 16 states of India and Punjab is one of them.[4] Over past two decades there has been increase in the number of Dengue cases and also a change in the trends of epidemiology is seen.

Current diagnostics modalities available are detection of RNA of the virus with reverse transcriptase PCR (RT-PCR) in the serum of the patient which is a Gold standard test; viral isolation and serological tests like IgM capture ELISA (MAC ELISA).[5] However viral isolation and RT-PCR both are time consuming, labour intensive and expensive therefore cannot be used as routine diagnostic procedures. MAC ELISA is easy to perform so it is the most widely used serological test for the diagnosis of Dengue infection. But this test also has low sensitivity in the first week of the infection as it take 4-8 days for IgM levels to be detectable by MAC ELISA.[6] Nowadays a variety of rapid diagnostic tests (RDTs) and ELISA targeting NS1 antigen (Ag) are available commercially.[7-9] NS1 is a highly conserved Dengue virus specific non- structural protein and is detectable early during the acute phase (Day 0 to 9 of fever) of both primary and secondary Dengue virus infection.[10]

The present study was undertaken to evaluate the sensitivity and specificity of rapid SD (bioline) Dengue Duo (NS1& Ab combo) test in comparison to ELISA (NS1, IgG, and IgM).

Material and Methods
Study design & Data collection: It was a retrospective cross sectional study carried out in a tertiary care hospital attached to S.G.R.D Institute of Medical Science and Research, Amritsar, North India. The data was collected by reviewing the records of 250 patients who attended the OPD or were admitted to the hospital with the suspicion of dengue infection, from May 2015 to March 2016.

The blood samples of these patients were routinely processed for dengue infection in central clinical laboratory of the hospital. Serum was separated and each sample was tested with both SD Bioline Dengue Duo rapid test as well as ELISA. SD Bioline Dengue Duo test is an in vitro immuno-chromatographic rapid which provides results within 20 minutes. It is a two test device in which one side is for qualitative

determination of NS1 antigen and other side is designed for simultaneous differential detection both of IgG and IgM antibodies to dengue virus. For detection of NS1 antigen the test device contain a pre-coated membrane strip, with anti-dengue NS1 Ag capture antibody on region showing test band. Its result window has two lines, 'T' line is NS1 test line and 'C' control line for procedural control. Dengue test device for IgG/IgM has 3 pre coated lines, 'G' (Dengue IgG test line), 'M' (Dengue IgM test line), and 'C' control line in result window. ELISA was used as a reference standard and all the samples were also tested for detection of NS1 by ELISA and for IgG and IgM antibodies with capture micro ELISA using manufacturer's instructions.

Ethical approval: The study was conducted after getting ethical approval from the ethical committee of the institution.

Statistics analysis: The data obtained was statistically analysed by using Chi square test with SPSS version 17.0 software to know the association between the variables.

Results

A total of 250 samples of the patients suspected of Dengue infection were tested from June 2013 to October 2014 by SD Bioline Dengue Duo test and ELISA. Male to female ratio was found to be 2:1 and the age of seropositive patients ranged between 11- 60 years but commonly affected age group was between 19-42 years.

Out of 250 samples 69 were found to be reactive for Dengue infection by ELISA. Out of these 69 seropositive samples rapid test was reactive in 55 samples. There were 18 samples in which only NS1 Ag was positive by both rapid test and ELISA and in rest seropositive samples either IgM or IgG was positive. Rapid test results were compared with ELISA test, out of 250 samples 55 were true positives, 181 were true negative. Sensitivity, specificity, and agreement were also calculated (Table 1 & Fig. 1).

Table 1: Results of rapid test in comparison to ELISA

True positive	False positive	True negative	False negative	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
55	0	181	14	79.71	100	100	92.82

N= 250

Accuracy of the test = 94.40%

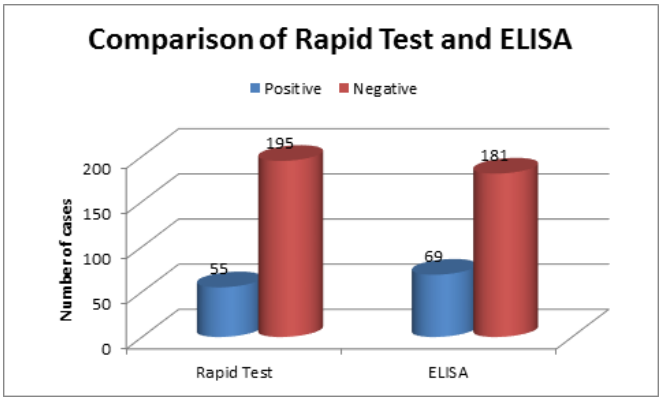

Fig. 1: Graph showing comparison of Rapid test and ELISA results

Discussion

Dengue infection usually presents like any other viral illness but its clinical spectrum ranges from asymptomatic febrile illness to DHF or Dengue shock syndrome (DSS) which has high mortality rate. This is why early diagnosis and treatment of the infection becomes important. RDTs like SD Bioline Dengue Duo test are simple assays which can provide results within 20 minutes and does not require sophisticated laboratory equipment's or trained staff. Therefore these tests can be used as screening tests for initial diagnosis of the infection.

In our study out of 250 serum samples of the patients suspected of Dengue fever, 69 were seropositive by ELISA and 55 were positive by the rapid test. Mean duration of illness was 3-10 days. There were 18 samples which were positive for NS1Ag only, by both rapid test and ELISA. We could have easily missed these cases if we had performed only MAC ELISA for IgM which is a popular test for dengue. Overall sensitivity of rapid test in comparison to ELISA was 79.71% and specificity was 100% which is comparable to other studies.[8,11-14] Positive predictive value came out to be 100% and negative predictive value was 92.82%. Since the PPV in our study was high so there was no need for confirmatory testing. Studies have shown high PPV of these rapid tests in area with high endemicity ranging from 86%-100%.[8] But the

sensitivity of rapid test was less than that of ELISA since there were 14 cases which were missed by rapid test. So those cases which have high clinical suspicion but are negative with rapid test should always be retested with ELISA or RT-PCR. Also in those areas where infections with other Flavi viruses are also common, the results should be interpreted with caution. There can be variation in positive and negative predictive values.

The main limitation of our study was that we could not take RT-PCR which is more sensitive than ELISA, as reference method.

Conclusion

Since dengue cases are on rise globally so early diagnosis and treatment of the patients suffering from infection is important for both patient and the community. Diagnosis of the index cases can prevent an outbreak and help boost up affective vector control measures. Advantage of SD Dengue duo rapid test is that it detects NS-1 antigen in addition to the antibodies and its results are also comparable to ELISA. So it can be a useful screening test during early dengue infection.

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