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Assessment of a rapid test with ELISA done for the detection of dengue infection: A comparative study

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Abstract
Background: There had been a dramatic rise in the global incidence of dengue in the past few decades. Timely clinical intervention, etiological investigation, and disease control of the dengue infection is caused by the early diagnostic confirmation of dengue infection. Nowadays, market is flooded with a variety of rapid diagnostic tests (RDTs) and NSI antigen (Ag) targeting enzyme-linked immunosorbent assay [ELISA]. Hence; we undertook the present study to comparatively evaluate the efficacy of rapid SD (bioline) Dengue Duo test and ELISA for the detection of dengue infection.
Materials & Methods: The present study included assessment of records 160 patients who were suspected for dengue infection. In the central pathology laboratory of the intuition, blood samples of these were collected and were routinely processed for the identification of dengue infection. Testing with both SD Bioline Dengue Duo rapid test and ELISA was done after separating the serum from all the samples. All the results were recorded separately and analyzed and compared.
Results: Mean age of the patients of the present study was 28.5 years. Out of 160 patients, 110 were males while 50 were females. Out of 160 samples tested by rapid card test, 82 samples were found to be positive while 78 samples were found to be negative. Out of 82 samples tested by ELISA, positive results were obtained in 46 samples while negative results were obtained in 36 samples.
Conclusion: In addition to the identification of antibodies, Dengue rapid test also provides additional advantages of detecting NS1 antigens.

Keywords: Dengue, ELISA, Rapid

1. Introduction
In the recent few decades, there had been a dramatic rise in the global incidence of dengue. In more than one hundred countries of Africa, America and South East Africa, the disease is now considered to be endemic. [1, 2] As per the stats of the World Health Organization (WHO), more than 2.5 billion people are subjected to the risk of development of dengue infection with 50–100 million cases occurring annually. There are four dengue serotypes (DENV-1, DENV-2, DENV-3, DENV-4), which can cause illnesses in humans ranging from the self-limiting to the life-threatening dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS). [3] Generally considered as self-limiting, Classical dengue fever (DF) is characterized by fever and a variety of non-specific signs and symptoms such as headache, malaise, weakness, rash and body aches. The DHF is distinguished from DF by the onset of plasma leakage, marked thrombocytopenia, and a bleeding diathesis. [4]
Timely clinical intervention, etiological investigation, and disease control of the dengue infection is caused by the early diagnostic confirmation of dengue infection. [5] hence; priority should be given to the diagnosis of dengue disease during the acute phase. Several approaches have been applied for laboratory diagnosis of dengue virus infection. These methods include detection of the virus, detection of virus antigen. However, for a confirmed dengue diagnosis, DENV should be identified by isolation or nucleic acid detection or there should be a 4-fold rise in antibody titer with paired sera in patients presenting with signs and symptoms that are consistent with dengue virus infection. Recently, commercially available kits have been developed for the rapid detection of dengue infections. [6, 7]
Nowadays, market is flooded with a variety of rapid diagnostic tests (RDTs) and NSI antigen (Ag) targeting enzyme-linked immunosorbent assay [ELISA]. Being detected in the acute phase of the infection, NS1 is a rapid diagnostic tests (RDTs) of the dengue infection. [8] Hence; we undertook the present study to comparatively evaluate the efficacy of rapid SD (bioline) Dengue Duo test and ELISA for the detection of dengue infection.

2. Materials & Methods
The present study was conducted in the tertiary heath care centre and included assessment of records 160 patients who attended the OPD or were admitted to the hospital because of suspected dengue infection. Ethical approval was taken from the institutional ethical committee in written after explaining in detail the entire research protocol. By reviewing the records of 160 patients, data was collected. In the central pathology laboratory of the intuition, blood samples of these were collected and were routinely processed for the identification of dengue infection. Testing with both SD Bioline Dengue Duo rapid test and ELISA was done after separating the serum from all the samples.

2.1 SD Bioline Dengue Duo rapid test
It is an in-vitro immuno-chromatographic rapid which shows results in twenty minutes time. It is a two test device which performs following two functions:
- Qualitative determination of NS 1 antigen

- Differential detection of both Ig G and Ig M antibodies against dengue virus.

It contains a pre-coated membrane strop for the detection of NS 1 antigen. Two lines are shown in its result window, NS1 test line shown by the 'T' line and for carrying out procedural control, the 'C' control line. For the detection of IgG/IgM in the dengue test service, three coated lines are present in the result window:

- 'G' line: Dengue IgG test line
- 'M' line: Dengue IgM test line
- 'C' line: Control line

For IgG and IgM antibodies detection, ELISA was performed following manufacturer's instructions. All the results were recorded separately. Analysis of all the recorded data was done by SPSS software. Chi-square test and student t test were used for the assessment of level of significance.

3. Results

Mean age of the patients of the present study was 28.5 years. Out of 160 patients, 110 were males while 50 were females (Fig 1). Out of 160 samples tested by rapid card test, 82 samples were found to be positive while 78 samples were found to be negative (Table 1). Out of 82 samples tested by ELISA, positive results were obtained in 46 samples while negative results were obtained in 36 samples (Table 1, Fig 2). Table 2 shows the results of rapid test in comparison to ELISA. The specificity among 160 patients in the present study was found to be 92% whereas the senstivity was found to b e 94%.

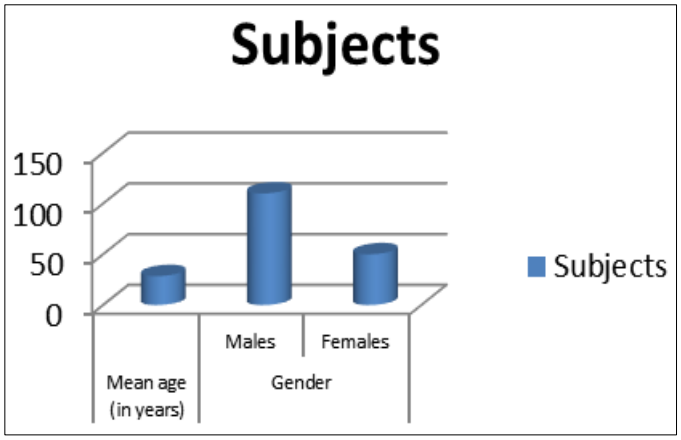

Fig 1: Age and gender distribution of patients

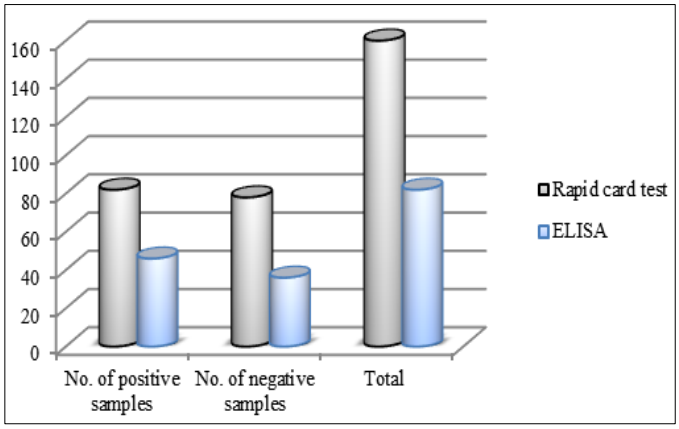

Fig 2: Showing results of Rapid card test and ELISA test

Table 1: Results of Rapid card test

Test	No. of positive samples	No. of negative samples	Total
Rapid card test	82	78	160
ELISA	46	36	82

Table 2: Results of rapid test in comparison to ELISA

Parameter	Value
No. of patients	160
Specificity (%)	92
Sensitivity (%)	94

4. Discussion

Diagnosis of dengue infection can be done by rapid dipstick test, the Dengue NS1 Ag Strip which allows reaching the results within 15 minutes. [9] NS1 is a highly conserved non-structural glycoprotein secreted by virus-infected cells during the acute phase of dengue, and it is essential for virus viability. [10-12] Hence; we undertook the present study to comparatively evaluate the efficacy of rapid SD (bioline) Dengue Duo test and ELISA for the detection of dengue infection.

In the present study, we observed that 82 patients out of 160 were positive by rapid card test while negative results were exhibited in 78 patients (Table 1). Out of 82 patients tested by ELISA, positive results were obtained in 46 patients while 36 patients exhibited negative results (Table 1). Gill *et al.* evaluated sensitivity and specificity of SD Dengue Duo rapid test in comparison to capture ELISA for early diagnosis of dengue infection. They collected data by reviewing the records of 250 patients who attended the OPD or were admitted to the hospital with the suspicion of dengue infection. The blood samples were routinely processed in central clinical laboratory and were tested by both, SD (Bioline) Dengue Duo rapid test and ELISA. 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%. 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 [5].

Vajpayee *et al.* evaluated three commercial assays for detection of antibodies to dengue virus, to assess their performance in a diagnostic laboratory. Sera from 58 patients collected during a febrile outbreak in New Delhi in 1997 were studied. The methods evaluated were MRL Diagnostic Dengue Fever Virus IgM Capture ELISA, Pan Bio Dengue Duo IgM and IgG Capture ELISA and Pan Bio Rapid Immunochromatographic test. The MRL ELISA correctly identified 97.8% (43 of 44) of samples as dengue positive while the Pan Bio Duo ELISA and Pan Bio RIT identified 95.45% (42 of 44). The sensitivities of both Pan Bio Duo ELISA and Pan Bio RIT for primary dengue and secondary dengue were 100% and 93.54% respectively. The specificity of three assays were MRL IgM ELISA 100%, Pan Bio Duo ELISA 92.8% and Pan Bio RIT 85.7%. [13] Pal *et al.* evaluated these tests using a well-characterized panel of clinical samples to determine their effectiveness for early diagnosis. Overall,

the sensitivity of the rapid diagnostic tests (RDTs) ranged from 71.9%–79.1% while the sensitivity of the ELISAs varied between 85.6–95.9%, using virus isolation as the reference method. Most tests had lower sensitivity for dengue virus (DENV)- 4 relative to the other three serotypes, were less sensitive in detecting secondary infections, and appeared to be most sensitive on Day 3–4 post symptom onset. The specificity of all evaluated tests ranged from 95%–100%. ELISAs had greater overall sensitivity than RDTs. In conjunction with other parameters, the performance data can help determine which dengue diagnostics should be used during the first few days of illness, when the patients are most likely to present to a clinic seeking care. [14] Moorthy *et al.* evaluated the performance of a rapid immunochromatographic test (ICT) device in detecting antibodies to Dengue virus (DENV) in a tertiary hospital in South India. Sera from hospital attendees, with requests for DENV antibody testing, were tested with the Panbio Dengue Duo Cassette and a reference antibody capture assay for the detection of IgM and IgG antibodies. The ICT results were compared with results of antibody capture tests for the detection of the IgM and IgG antibodies, respectively. Accuracy indices for IgM and IgG detection, respectively were -- sensitivity 81.8% and 87.5%, specificity 75.0%, and 66.6%, positive predictive value (PPV) 61.0% and 72.9% and negative predictive value (NPV) 89.6% and 83.9%. The device performs poorly in detection of IgM and IgG antibodies to DENVs and is not recommended for use as a stand-alone diagnostic test. [15] Parida *et al.* comparativly evaluated ELISA with a commercial Rapid PanBio Immunochromatographic test (IC test) for detection of DENV-specific IgM and IgG antibodies in patient sera. Among crude and purified viral antigens prepared from mouse brains or cell cultures, a DENV type 2 antigen purified from cell cultures by sucrose density gradient centrifugation was found superior in terms of the signal/ noise (S/N) ratio in the assay system. The sensitivity of detection of the virus by specific IgM antibody was improved by removal of IgG from patient sera prior to testing. The evaluation of the Dipstick ELISA by use of 156 serum samples revealed an overall accordance of 96% and 93% with the IC test in detection of IgM antibodies to DENV (IgM antibodies) and IgG antibodies to DENV (IgG antibodies), respectively. The sensitivity of the Dipstick ELISA and the IC test with reference to the mu-capture ELISA was 83% and 87%, respectively, with a specificity of 98% in both cases. The sensitivity of the Dipstick ELISA with reference to the IC test in detecting IgM and IgG antibodies was 84% and 94%, respectively, and the specificity of the Dipstick ELISA was 98% and 92%, respectively [16].

5. Conclusion

From the above results, the authors concluded that in addition to the identification of antibodies, Dengue rapid test also provides additional advantages of detecting NS1 antigens.

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