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RESEARCH ARTICLE

LABORATORY EVALUATION OF THE SD BIOLINE- DENGUE DUO RAPID IMMUNODIAGNOSTIC TEST KIT FOR DETECTION OF NS-1 ANTIGEN AND IGM ANTIBODY DURING DENGUE OUTBREAK IN DELHI

***[1]Kumar S Abhishek and [2]Anita Chakravarti**

[1]Ex- Post Graduate Student, Department of Microbiology, Maulana Azad Medical College, New Delhi
[2]Ex- Director Professor, Department of Microbiology, Maulana Azad Medical College, New Delhi

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**Corresponding author*

ABSTRACT

Background: Dengue a mosquito borne viral fever is caused by Dengue virus of family Flaviviridae. It has a wide spectrum of sign and symptoms ranging from acute self-limiting infection to fatal complications in few cases like Dengue Hemorrhagic Fever (DHF), Dengue Shock Syndrome (DSS). Several Rapid immuno-chromatographic and ELISA based diagnostic kits are available for laboratory diagnosis in clinically suspected cases. Due to threat of developing fatal complications, rapid diagnosis becomes important in order to prevent such complications.
Objective: Evaluation of Rapid immuno-chromatographic test kits taking ELISA (NS-1 Ag and IgM Antibody) as reference.
Materials and Method: Cross-sectional study. ELISA and SD BIOLINE- Dengue Duo Rapid immunodiagnostic test kit as per manufacturer's instruction.
Result: Sensitivity and Specificity for NS1 antigen 89.6% and 98% and for IgM antibodies 94.7% and 100% respectively were observed using SD BIOLINE- Dengue Duo Rapid immunodiagnostic test kits taking NS-1 antigen and IgM anti-dengue antibody ELISA as reference.
Conclusion: During an outbreak where rapid diagnosis is needed, rapid diagnostic kits are good alternative to ELISA based tests.

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INTRODUCTION

Dengue is an acute viral infection with fatal complications occurring in few cases. Dengue virus belongs to positive strand enveloped RNA virus of family *Flaviviridae* and there are four serotypes of the virus referred as DENV-1, DENV-2, DENV-3 and DENV-4, recently a possible fifth serotype (DENV-5) was reported (Normile, 2013). Dengue virus is transmitted by the bite of an infected female mosquito. The primary vectors are *Aedes aegypti* and *Aedes albopictus,* other species though less common *Aedes polynesiensiscan* can also transmit the infection (Lam, 1995). The RNA genome is composed of three structural protein genes (Core (C) protein, a membrane associated (M) protein, an enveloped (E) glycoprotein) and seven non-structural protein genes (NS-1, NS-2a, NS-2b, NS-3, NS-4a, NS-4b and NS-5). Dengue fever is a major public health challenge worldwide; with around 2.5 billion people at risk and more than 100 million cases with 25,000 deaths annually (Mustafa, 2011). In north India, epidemiology of dengue is changing rapidly and most of the cities have become hyper-endemic. Delhi is one of the dengue endemic states in India (Gupta, 2012).

It has witnessed several outbreaks during past 100 years, *viz.* 1920, 1982, 1988, 1996, 2003, 2006, 2010 and 2013 (Kumar, 2015). In 2015 with highest number of dengue cases in last five years (Bagcchi, 2015). There is always a risk of incidence with fatal dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) cases, which are medical emergencies (Dash, 2006). It has been observed that with the severity there is increased level of circulating inflammatory and anti-inflammatory Interleukins like IL-10, IL-2 and others which could be the markers for early detection of cases that might develop in to fatal and complicated dengue (Abhishek, 2017). Dengue usually follow an incubation period of 2-7 days and may include a wide spectrum of signs and symptoms (Kalayanarooj, 2011). There is no specific treatment for dengue but early diagnosis and supportive management can decrease the mortality or severe dengue diseases (WHO, 2009). The laboratory diagnosis of dengue includes serological, viral isolation and molecular techniques. Viral isolation generally takes long time while molecular methods are expensive. Considering this, serological methods prove to be the most promising for early diagnosis. Enzyme linked

immunosorbent assay (ELISA) is most often used in the diagnostic method in India. This test aims to detect specific dengue antibodies such as Immunoglobulin (Ig) M, IgG, IgA or dengue antigen particularly NS-1 glycoprotein (Wang, 2011). NS-1 glycoprotein secreted by dengue virus infected mammalian cells (Gunzman, 2010). Being soluble, NS-1 can be detected in the bloodstream. Several rapid immunochromatographic tests (ICTs) have become available. Our study aimed to determine the performance of rapid dengue ICT kit as its performance has been noted to vary with different countries. Once its performance is verified, it can aid to the rapid bedside diagnosis of dengue infection, even in the regions where there is shortage of trained personnel.

MATERIALS AND METHODS

The study was conducted at the Virology laboratory in the Department of Microbiology of Maulana Azad Medical College (MAMC), New Delhi during the year 2015. 5ml of Venous blood were collected in the plain vial from the dengue suspected patients coming with complain of fever and other possible signs and symptoms of dengue to the Emergency Medicine OPD of Lok Nayak Hospital, New Delhi. The same samples were subjected to the Dengue NS-1 antigen ELISA and Dengue IgM antibody capture ELISA after performing rapid test using SD-BioLINE Dengue Duo (Standard Diagnostics, INC. Korea) kit. Dengue NS-1 antigen ELISA (J. Mitra & Co. Pvt. Ltd. DENGUE NS1 Ag MICROLISA) and Dengue IgM antibody capture ELISA (NIV DEN IgM Capture ELISA) were taken as the reference methods. All reference testing procedures were performed and interpreted as per the manufacturer's instructions and with trained personnel.

The SD-BioLINE Dengue Duo (Standard Diagnostics, INC. Korea) kit contains:

- Dengue NS-1 antigen and Dengue IgG/IgM combo device.
- Assay diluent for Dengue IgG/IgM antibody test.
- 10µl capillary pipette for Dengue IgG/IgM antibody test.
- Disposable dropper for Dengue NS-1 antigen test.
- Test kit is made up of two cartridge containing immunoreactive test strips.

Dengue NS-1 antigen cartridge test strip included; Gold conjugates (as main component) : Mouse monoclonal anti-dengue NS-1 – gold colloid (0.27±0.05µg), Test Line (as main component) : Mouse monoclonal anti-dengue NS-1 (0.72±0.14µg) , Control Line (as main component) : Goat anti-mouse IgG (0.72±0.14µg).

Dengue IgG/IgM antibody cartridge test strip included; Gold conjugates (as main component): Recombinant Dengue virus envelope protein – gold colloid (1±0.2µg), Test Line "G" (as main component): Mouse monoclonal anti-human IgG (5±1µg), Test Line "M" (as main component): Mouse monoclonal anti-human IgM (5±1µg), Control Line (as main component): Rabbit anti-Dengue IgG (2.5±0.5µg). The SD-Dengue Duo test kit is capable of detecting Dengue NS-1 antigen/ Dengue IgG/IgM antibody from human serum, plasma and whole blood, but we preferred using serum. Total of 230 serum samples (serum were separated by centrifuging the blood sample at 1500 rpm for 5 minutes) were tested using SD-BioLINE Dengue Duo following manufacturer's instructions. Out of 230 serum samples tested, 1 test failed to give valid control line and an additional 2 tests did not absorb the serum sample on test strip of Dengue NS-1 antigen component and were excluded from the study. So, we were left with 227 specimens that were included in the final analysis.

RESULTS

The Dengue NS-1 component of SD-BioLINE Dengue Duo test gave 69 true-positive results and 3 false-positive results, 8 false-negative and 147 true-negative results (Table-01). 8 false-negative and 3 false-positive results were found to be positive and negative respectively by reference test (Dengue NS-1 antigen ELISA).

Table 1. Laboratory performance for detection of Dengue NS-1 antigen by using SD-Bio Line Dengue Duo rapid test

Dengue NS-1 Antigen ELISA

SD-Bioline dengue duo rapid test.	Positive	Negative	Total
positive	69	03	72
negative	08	147	155
total	77	150	227*

*For 230 specimens tested, three gave an invalid test and therefore were not included in the analysis.
Sensitivity :(69/69+8) x 100 = 89.6%
Specificity : (147/147+3) x 100 = 98%
PPV : (69/69+3) x 100 = 95.8%
NPV : (147/147+8) x 100 = 96.7%

Table 2. Laboratory performance for detection of Dengue IgM Antibody by using SD-Bio Line Dengue Duo rapid test.

Dengue NS-1 Antigen ELISA

SD-BioLine Dengue Duo rapid test.	Positive	Negative	Total
Positive	18	00	18
Negative	01	209	209
Total	19	208	227*

*For 230 specimens tested, three gave an invalid test and therefore were not included in the analysis.
Sensitivity : (18/18+1) x 100 = 94.7%
Specificity : (208/208+0) x 100 = 100%
PPV : (18/18+0) x 100 = 100%
NPV : (208/208+1) x 100 = 99.5%

The sensitivity estimate of the Dengue NS-1 antigen component was 89.6% and specificity estimate was 98%. The Positive predictive value (PPV) and Negative predictive value (NPV) estimates were 95.8% and 96.7% respectively. For the Dengue IgG/IgM antibody component, the test produced 18 true-positive results and 0 false-positive result, 1 false-negative result and 208 true-negative results (Table-02). 1 false-negative result was found to be positive by reference test (Dengue IgM antibody capture Elisa). The sensitivity and specificity estimated were 94.7% and 100% respectively. The PPV and NPV estimates were 100% and 99.5% respectively. Out of 227 specimens, 11 specimens were positive for both Dengue NS-1 antigen and Dengue IgM antibody by SD-BioLINE Dengue Duo. All these 11 samples result were included in the Dengue NS-1 antigen component of rapid test and none of them proved to be false-positive by reference test.

DISCUSSION

Dengue is a serious threat for the population especially in the endemic areas like Delhi. Diagnosis of dengue is largely dependent on dengue virus antigen and anti dengue IgM

antibody detection. Gold standard test for the antibody and antibody detection is ELISA. But performing the ELISA is time consuming, cumbersome and require technical expertise which is a major issue in the diagnosis of dengue especially in remote areas. Rapid immunochromatographic tests offer a very good solution to this problem as they are cost effective, easy to perform, do not require technical expertise and detects both antigen and antibody in a single setting within 10 to 15 minutes. But the major drawback of the rapid immunochromatographic tests is its unreliable sensitivity and specificity. Low sensitivity of a rapid diagnostic kit may give false negative results whereas the kits with low specificity may give many false positive results. To curb this problem all such rapid diagnostic kits should be introduced only after strict verification and approval of the national and international testing authorities. This study was subjected to some limitations, due to study sample size. In order to get the first-hand insight of the performance of dengue rapid diagnostic kit we compared the results of rapid kits with ELISA. Sensitivity and specificity for NS1 antigen detection by rapid immunochromatographic tests were 89.6% and 98% and for IgM antibodies were 94.7 % and 100% respectively. The results are comparable to the study done by Vickers et al. Who found the sensitivity for NS1 antigen to be 90 %. But for IgM antibody detection, sensitivity of the kits were much higher than the (Vickers et al, 2015) (49.3%). Considering the dynamics of the IgM antibody response we performed IgM testing only after five days of the onset of the fever, this may explain the higher sensitivity of IgM rapid testing in our study.

Conclusion

Conclusion from this study was that, in case of an outbreak of Dengue, where early diagnosis is the need of time to minimize hospitalization and to prevent fatal complications arising out of undiagnosed Dengue infection, and also where there is lack of trained personnel, Rapid immunodiagnostic test kits are of great value considering its comparison with Enzyme Linked immunosorbent Assay.

List of Abbreviations

DENV: Dengue virus
DHF: Dengue hemorrhagic fever.
DSS: Dengue shock syndrome.
ELISA: Enzyme linked Immunosorbent Assay.
IL: Interleukin
NPV: Negative Predictive Value
PPV: Positive Predictive Value

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