ISSN 2515-8260 Volume 09, Issue 06, 2022

Title - Elisa vs Rapid card : Comparitive evaluation for dengue diagnosis

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ABSTRACT

Background - Dengue diagnosis is routinely carried out by detection of dengue virus (DENV) antigen NS1 or anti-DENV IgM antibodies using enzyme-linked immunosorbent assays and rapid diagnostic tests. This study was aimed at evaluation of quality of diagnostic assays currently in use in India for the identification of DENV infection.

Methodology

Study design - Retrospective Study.

Study Centre **-** Microbiology LaboratoryBundelkhand Medical College, Sagar, Madhya Pradesh

Study duration **-** June to November 2021

Methods and material- Serum samples from patients (n=1003) seeking dengue diagnosis were tested using J Mitra dengue NS1 antigen Microlisa kit andMedSourcedengue NS1rapid antigen diagnostic kits. The presence of NS1 antigen was taken as evidence for dengue-positive diagnosis.

Result – In this record review J Mitra ELISA identified 91% patients as dengue NS1 positive. J Mitra ELISA taken as reference, the Medsourcedengue NS1 test kit was found to be less sensitive (82.4%) and less specific (87.8%).

Interpretation &conclusions - These results confirmed superiority of ELISAs forNS1 antigen dengue diagnosis, and emphasized on improvement in sensitivity of RDTs.

Keywords - Dengue virus, enzyme-linked immunosorbent assay, NS1, rapid diagnostic test.

INTRODUCTION

Dengue virus (DENV) is the most common arbovirus worldwide. Dengue is a vector-borne disease, found in tropics and subtropics areas. Dengue is endemic in more than 100 countries in Africa, Eastern Mediterranean, South America, Western Pacific and Southeast Asia. Almost 2.5 billion people are at risk of infection with dengue virus[1]. In 2013 according to the Global Burden of Disease dengue incidence are doubled every decade from 1990 to 2013[2]. In India many epidemics has been reported; 1963- Calcutta, 1964- Visakhapatnam, 1968- Vellore 1969- Ajmer , 1969- Kanpur, 1985- Rajasthan, 2002- Chandigarh, 2004- Mumbai, 2007- Ludhiana, 2010- Delhi[3-11].According to WHO revised 2009 case definition, dengue is classified into dengue and Sever dengue. Dengue is defined as fever with two or more of the following: nausea/vomiting, rash, aches and pain, positive tourniquet test, leukopenia or any warning sign and criteria for SD

ISSN 2515-8260 Volume 09, Issue 06, 2022

are evidence of severe plasma leakage, bleeding or organ involvement [12]. Dengue can be diagnosed clinically and confirmed by a variety of methods, the methods used by laboratories for the dengue virus infection diagnosis like detection of viral genomic sequence by nucleic acid amplification technology assay (RT-PCR), viral isolation, Antigen detection, particularly NS1 and the detection of specific IgM antibodies by the enzyme linked immunosorbent assay (ELISA) or the rapid immunochromatographic test (ICT). ELISA and rapid diagnostic tests (RDTs) are available commercially. Early diagnosis is important for appropriate treatment and surveillance of disease for proper interventions, disease control and clinical care, particularly for complicated cases and reducing the expenses of investigations and treatments. Different diagnostic methods have different sensitivity and specificity. There is diagnostic uncertainty in between and within countries depending on there protocol for different tests. This study aimed for Comparative evaluation of enzyme-linked immunosorbent assay & rapid diagnostic tests used for dengue diagnosis.

MATERIALS AND METHODS

This is a record based study conducted in a tertiary care hospital (Bundelkhand Medical College and Hospital) for a period between june – November 2021. . In this study evaluation of rapid immune chromatographic test with ELISA for detection of NS1 antigen was done .All age group patients who had clinical signs and symptoms of acute dengue like illness were included in the study. Serum samples from patients (n=1003) seeking dengue diagnosis were tested using J Mitra dengue NS1 antigen Microlisa kit and MedSource dengue NS1 rapid antigen diagnostic kits. The presence of NS1 antigen was taken as evidence for dengue-positive diagnosis. A survey was done before conducting the study, about the use of commercially available rapid diagnostic kits by various laboratories which do not have ELISA facilities for diagnosis of dengue fever, and based on survey results, following are used :- 1. Dengucheck Combo (Tulip Diagnostics, India). 2. Dengue day 1 test (J Mitra and Co, India). 3. Medsource dengue NS1 test kit. In which Medsource dengue NS1 test kits are selected. Dengue NS1 antigen test uses the human serum or plasma by immunchromatography for qualitative detection NS1 antigen of dengue virus. The membrane strip of the device is pre-coated with anti-dengue NS1 monoclonal antibody on test region (T) and goat anti- mouse IgG is pre-coated on control region (C). During testing, if the sample have dengue NS1 Ag, complex of the antibody-dengue NS1 Ag-gold conjugate moves laterally on the membrane by capillary action. The pink-purple line will appear on the membrane in test line (T). To serve as a control, an additional line of Goat anti-mouse IgG has been immobilized on the card. If the test is performed correctly, there will be formation of pink purple line upon contact with the conjugate as a control line. J mitra dengue NS1 ELISA kit uses one enzymatically amplified, two-step sandwich-type immunoassay to detect levels of NS1 in serum. In this Dengue NS1 ELISA kit, controls and unknown serum samples are diluted in sample dilution buffer containing secondary antibody and incubated in micro titration wells. These wells on the Dengue NS1 ELISA kit have been coated with a NS1 antibody. NS1 antigens present in the samples on the ELISA kit are then sandwiched between the capture and secondary antibodies. The presence of NS1 antigen on this Dengue NS1 ELISA kit is confirmed by the

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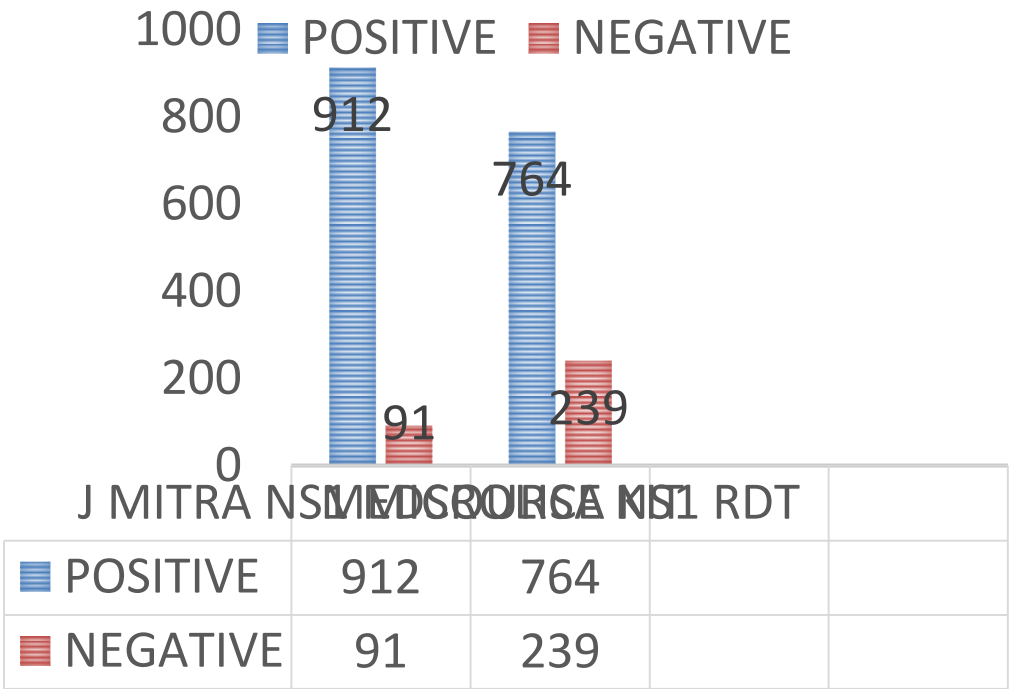
colorimetric response obtained using an enzyme-conjugate-HRP and liquid TMB substrate. The patients tested positive for NS1 Ag by ELISA were taken as confirmed cases. The tests were strictly performed according to the kit manufactures instructions Management and analysis of data were done by using Microsoft excel.

RESULTS

In this present study, during study period total 1003 dengue suspected sample were tested for the presence of NS1 antigen. J Mitra ELISA identified 91% patients as dengue NS1 positive. J Mitra ELISA taken as reference, When the rapid ICT test for NS1 Ag was compared with the NS1 Antigen capture ELISA, it showed a sensitivity of 82.4% and specificity of 87.8%, the Medsource dengue NS1 test kit was found to be less sensitive and less specific.

Table 1. - Comparison of result by different diagnostic assays

		J MITRA NS1 MICROLISA KIT		
		POSITIVE	NEGATIVE	TOTAL
MedSource dengue NS1 rapid antigen kits	POSITIVE	752	12	764
	NEGATIVE	160	79	239
	TOTAL	912	91	1003



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DISCUSSION AND CONCLUSION

There is no preventive vaccine for dengue, Early diagnosis and treatment is the key for preventing complications and for disease control in the endemic areas. Along with difficulties in prevention, proper diagnosis of of dengue infection is also difficult because of its symptoms are not specific, especially in the early stage. The diagnosis of dengue infection can be done through viral isolation, viral RNA detection by RT-PCR, but this methods is time consuming and not available in most of the tertiary care hospitals, so diagnosis is based on the detection of dengue specific NS1 antigen via rapid kits or ELISA. The sensitivity of the rapid ICT tests for NS1 Ag in our study was more than 80%, and specificity more than 85% when compared to ELISA. Hence, the probability of patient suffering from acute dengue infection if the tests are positive is almost same as ELISA based tests. Findings of this study are similar with other studies, which have shown the results of rapid ICTs (Pal et al.,2014[13]; Groen et al., 2000[14]; Shih et.al.,2016[15]). Few Indian and foreign studies with similar background are tabulated in Table 2.The rapid ICTs have a advantage that they are easy to perform, need less expertise and are completed within minutes (Chaterji et al., 2011[16]) For making ELISA cost effective, large number of samples need to be processed at same time. For ELISA test, lab needs to be equipped with instruments like ELISA washer and reader. Comparison to ELISA, rapid ICT need very less technical expertise to perform and the time for the results is within minutes. The main advantage of the rapid ICT is that a single sample can be run without waiting for the samples. The sensitivity and specificity of different kits available in the market in a developing country like india vary widely and this should be kept in mind while performing the dengue diagnostic tests. Countries lacking infrastructure for the diagnostic labs in the rural areas, the rapid dengue ICT tests can be used in early diagnosis and management of acute dengue infection. At the end , serology is the method of choice for diagnosis. The main motive must be disease prevention . Effective disease prevention and control programs depends on vector control methods and sustained community involvement.

Table 2. - Comparative table of sensitivity and specificity of various rapid diagnostic test kits in different studies

Kit used	Serological target	Sensitivity	Specificity	Place of study	Reference number
Bioline Dengue Duo	NS1	70	73	Bangkok	17
	NS1	58	-	Mexico	18
	NS1	76	98	Malaysia	19
	NS1	81	98	Singapore	20
Dengue Day 1 test	NS1 only evaluated	99	96	India	30

Source of funding – None.

Conflict of interest – The author declare that there is no conflict of interest.

Acknowledgment – Heartfelt thank to our technical staff.

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