Original Research Article

Comparison of Techniques for Dengue Detection: A research study in a medical College of Rajasthan

Abhineet Mehrotra[1*], Vipul Mathur[2]

[1]Professor, Department of Microbiology, Government Medical College, Dungarpur, Rajasthan, India
[2]Senior Demonstrator, Department of Microbiology, Government Medical College, Dungarpur, India

Received: 15-10-2021 / Revised: 28-12-2021 / Accepted: 14-01-2022

Abstract
Early diagnosis of dengue can benefit timely clinical intervention by correct followups, etiological investigations and timely making aware the community at large. Detection of viral nonstructrural glycoprotein(NS1), Immunoglobulins IgM by rapid card test and or ELISA test has been found to be useful biomarker for this. A number of rapid diagnostic tests (RDTs) and enzyme-linked immune sorbent assays (ELISAs) targeting these NS1 antigen (Ag) and IgM are now commercially available. Here we evaluated these tests using a well-characterized panel of clinical samples to determine their effectiveness for early diagnosis by finding the sensitivity and specificity of RDT and ELISA and hence also comparing the two techniques for the most accurate results by these parameters. A total of 300 samples of patients who were clinically found to be having symptoms similar of dengue were taken and run on Rapid NS1 & IgM along with ELISA plate reader in a medical college hospital of Rajasthan state which has this virus in all rainy season.

Keywords: dengue, virus antibody.

Introduction

When dengue virus exposure occurs for the first time in a person, the patient gives response of primary antibody with characteristically a slow and specific antibody response. The NS1 is a highly conserved glycoprotein that is present at high concentrations in sera of dengue-infected patients during the early clinical phase of disease, and is found from Day 1 and up to Day 9 after onset of fever in sample of primary or secondary dengue-infected patients[1,2]. IgM appears first in day 3-5 of the disease in 50% of the cases with 80% in days more and even from 5th day and finally upto 99% of cases by day 10 or after. IgM levels peak about two weeks after the onset of symptoms and then decline generally to undetectable levels over 2–3 months. Anti-dengue serum IgG is generally detectable at low titres at the end of the first week of illness, increasing slowly thereafter, with serum IgG still detectable after several months, and probably even for life[3].

IgG appear by the fourteenth day and persist for life. Secondary infection shows that IgG rises within 1 to 2 days after onset of symptoms, simultaneously with IgM antibodies. Therefore, patients with secondary infections will have a positive IgG result, usually, but not always with a positive IgM result[4-5]. The chief immunoglobulin IgG which shows high level of detection not only in acute phase but may lasts for a period from 10 months to life long

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). Classical dengue fever (DF) is generally self-limited and 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[6].

*Correspondence
Dr. Abhineet Mehrotra
Professor, Department of Microbiology Government Medical College, Dungarpur, Rajasthan, India.
E-mail: abhineetmehrotra@gmail.com

With increasing incidence od dengue cases, early diagnostic confirmation of the cases holds the key for clinical intervention, etiology based investigation and disease control.

These methods include detection of the virus (by cell culture, immunofluorescence), detection of virus antigen (by enzyme-linked immunosorbent assay [ELISA]), detection of anti-dengue virus antibody (by hemagglutination inhibition [HI], complement fixation test [CF], neutralization tests, ELISA), and detection of virus nucleic acid (by real-time reverse transcription-polymerase chain reaction [RT-PCR])[6].

But for the routine investigation of the dengue in a resource crunch lab dengue rapid card test and semi automatic ELISA plate reader is enough to detect accurately and notify the disease to the concerned authorities.

Overall, the sensitivity of the 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 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%[7].

Material and Method

Rapid Card Test of Jay Mitra and ELISA plate reader and washer of Transasia's ERBA LifeScan M is used .All the samples are collected at room temperature and then centrifuged to isolate serum from the samples and then transferred in small alicots. These are then stored in refrigerator till all 300 samples which were covered in duration of over a period of 3 months are collected and are brought to room temperature before processing the samples in RDT and for ELISA. RDT require one drop of serum in each of NS1 slot and IgM slot which are on the card .Further one drop of buffer solution is added in IgM slot after a drop of serum and whole test is read after 20 minutes if there is T line along with C then the result is positive otherwise only C is negative with neither T without C or no line is invalid.

In ELISA a series of buffer and HRP conjugate along with serum and TMB A and TMB B are used as indicated In the kit insert of NS1 and also same in IgM with incubation of almost more than 2 hours during

the procedure to complete the procedure with washing in between the procedure and lastly after the whole procedure to read on plate reader. All 300 samples were split into three parts. First part tested for IgM& NS1 by standardised rapid card test and then in next step same were tested by ELISA plate reader machine of the medical college lab after fulfilling all necessary standard protocals. Third part of the samples were sent to another medical college lab for ELISA fully automatic machine testing as a reference testing to access the quality of the results given by the ELISA plate reader machine of our medical college.

Result

The results which came were unprecendeted as the result demonstrated that the ELISA plate reader of our medical college and of another medical college were exactly the same, that is 180 samples were positive out of 300 samples in both ELISA machines of our as well as in another medical college for IgM and 120 positives for NS1 but rapid card tests were found to be only 72 IgM positive and 60 NS1 positive .This is only 40% and 50% of the total positives of IgM and NS1 given by ELISA machine.

Table 1: Total Specimen

	Total specimen =300	
	RDT	**ELISA**
IgM	72	180
NS1	60	120

Conclusion

In research on comparison of the two techniques of RDT and ELISA done elsewhere it was found that the sensitivity of the RDTs ranged from 71.9%–79.1% while the sensitivity of the ELISAs varied between 85.6–95.9%, .The specificity of all evaluated tests ranged from 95%–100%[7].

But in our case we found that both IgM & NS1 have an unprecendented much lower senstivity of 40% and 45% of IGM and NS1 respectively. Since Dengue in India is a notifiable disease and in endemic areas even where there resource crunch labs it is advised to test minimum by ELISA plate reader before notifying the results to the government health authorities.

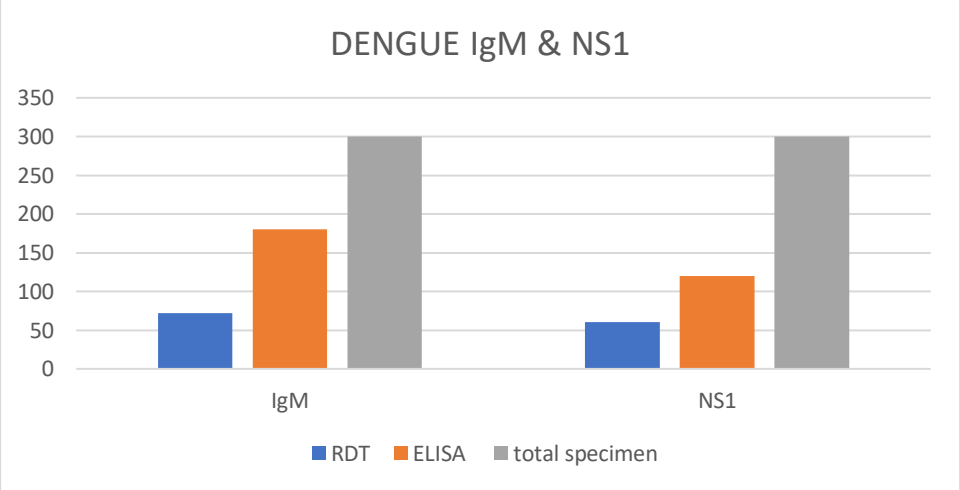

Fig. 1: Dengue IgM & NS1

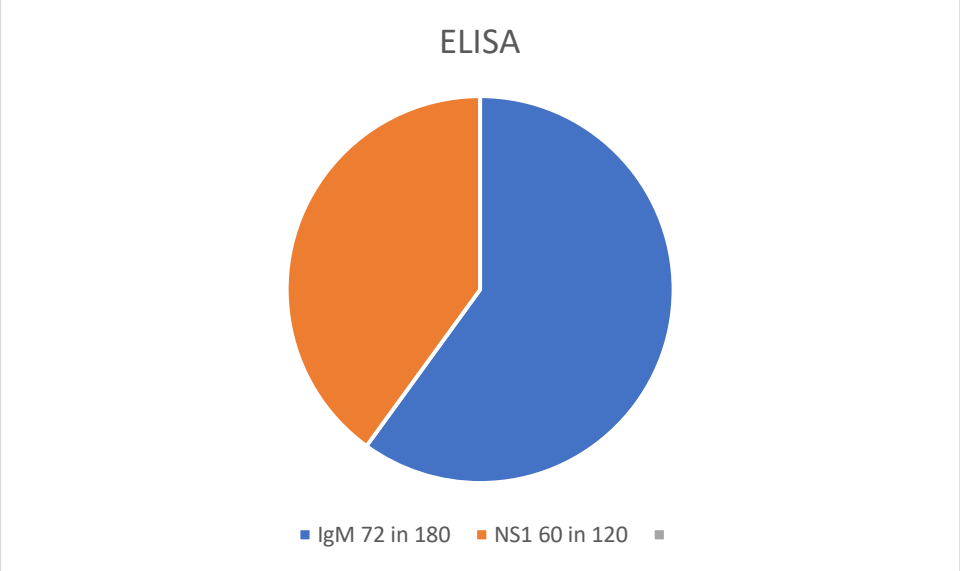

Fig. 2: ELISA

References

1. Young PR, Hilditch PA, Bletchly C. An antigen capture enzyme-linked immunosorbent assay reveals high levels of the dengue virus protein NS1 in the sera of infected patients. J Clin Microbiol. 2000;38:1053–1057.
2. Alcon S, Talarmin A, Debruyne M, Falconar A, Deubel V, Flammand M. Enzyme-linked immunosorbent assay specific to dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in blood during the acute phase of disease in patient experiencing primary or secondary infection. J Clin Microbiol. 2002;40:376–381.
3. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control: New Edition. Geneva: World Health Organization; 2009. 4, LABORATORY DIAGNOSIS AND DIAGNOSTIC TESTS
4. Shu P, Huang J. Current advances in dengue diagnosis. Clin Diagn Lab Immunol. 2004;11:642–650.
5. Gubler DJ. Serological diagnosis of dengue haemorrhagic fever. Dengue Bull. 1996;20:20–23.
6. Wang SM, Sekaran SD. Early diagnosis of Dengue infection using a commercial Dengue Duo rapid test kit for the detection of NS1, IGM, and IGG. Am J Trop Med Hyg. 2010;83(3):690-695.
7. Subhamoy Pal, Allison L. Dauner, Indrani Mitra, Brett M. Forshey, Paquita Garcia, Amy C. Morrison, Eric S. Halsey, Tadeusz J. Kochel, Shuenn-Jue L. Evaluation of Dengue NS1 Antigen Rapid Tests and ELISA Kits Using Clinical SamplesWuPLoS One. 2014; 9(11): e113411.

Conflict of Interest: Nil Source of support: Nil