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EVALUATION OF NS1, IGG AND IGM RAPID DENGUE TEST VERSES IGG AND IGM ELISA IN ACUTE DENGUE FEVER INFECTION.

Clinical Microbiology

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ABSTRACT

Introduction: Early diagnosis of dengue virus infection improves clinical outcomes by helping to initiate appropriate supportive therapies and raising awareness to the potential of haemorrhage or shock. Non-structural glycoprotein-1 has proven to be a useful biomarker for early diagnosis of dengue. Dengue IgM MAC ELISA is for the qualitative detection of anti-dengue IgM antibody with high degree of sensitivity and specificity. A number of rapid diagnostic kit tests and enzyme-linked immunosorbent assay targeting NS1 antigen, IgM antibody and IgG are commercially available. Objectives: Comparing the results of Dengue Rapid kit tests with the ELISA results of NS1, Ig M and Ig G. **Material and Methods**: A total of 86 serum samples received at department of microbiology, BMCRI hospitals, positive for IgM Dengue ELISA were selected, in the time period of September 2017 and October 2017. The NS1 and IgG ELISA for the same samples were done as well. IgM ELISA was performed by MAC-ELISA kits provided by NIV, Pune while IgG and NS1 ELISA were performed by PanBio kits. The RDTs were performed by kits from Alere. **Results:** Out of 86 IgM ELISA positive samples only 11(13%) were Ig M positive in RDTs. Of the 65 IgG positive in ELISA; 51(78%) were positive in RDTs and among 47 NS1 positive in ELISA; 20 (43%) were positive in RDTs. **Summary:** RDTs that can be performed near the patient's bedside are being adopted worldwide for their utility in initial diagnosis as well as they are easy to perform and give rapid results on spot. But the initial results of RDT's should not be depended upon if negative and an ELISA confirmation should be desired therafter.

KEYWORDS

Dengue, NS1 Ag, IgM, IgG, ELISA

INTRODUCTION:

Dengue virus is an important vector borne disease and is found largely in areas of tropics and subtropics. The disease has been now endemic in >100 countries in Africa, South America, Eastern Mediterranean, Southeast Asia, and the Western Pacific.

Billions of people stand at risk of dengue with 50 to 100 million as infected[1] every year with no treatment or definitive vaccines available. Early diagnosis of dengue virus (DENV) infection is very important. It helps to bring down the mortality and morbidity of 20-30% in acute dengue fever infection to less than 1% with just supportive care and treatment. Supportive therapies are the main stay of treatment for Dengue infections[2].

Symptoms of this mosquito borne disease vary from mild asymptomatic illness to dengue shock syndrome and at times are too vague to reach at any differential diagnosis[5].

The dengue virus has four distinct but antigenically related serotypes and infection produces a spectrum of clinical illness, ranging from an asymptomatic or mild febrile illness to classic dengue fever to the most severe form of illness, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Frequently seen symptoms are fever, headache, myalgia, joint pains, which encompass a panel of viral infections.

Accurate and affordable tests are thus very much required to combat the mortality of dengue worldwide. Early diagnosis can alarm the physicians to monitor the platelet counts and helps to predict further complicating symptoms of haemorrhage and dengue shock syndrome[2].

Current diagnostics modalities are detection of RNA of the virus with reverse transcriptase PCR in the serum of the patient which is a Gold standard test; viral isolation and serological tests like IgM capture ELISA (MAC ELISA)[3]. However, viral isolation and PCR studies are time consuming, expensive and need expertise and are mostly done for research and epidemiological purposes.

IgM MAC-ELISA is easy to perform and is comparatively cheaper but rate of IgM detection is low during first 4-8 days of illness. NS1 is a highly conserved Dengue virus specific non- structural protein and is detectable during the acute phase of illness. Figure 1 shows gene structure of Dengue virus.

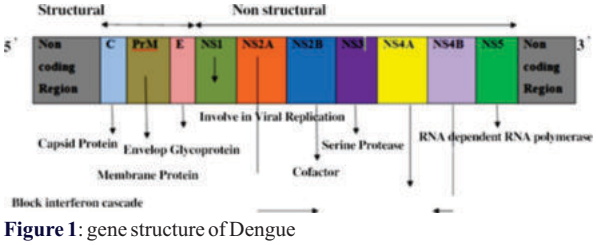


Figure 1: gene structure of Dengue

OBJECTIVE:

The present study was done to evaluate the sensitivity and specificity of rapid Dengue Duo (NS1& Antibody combo) test kit(RDT) in comparison to ELISA (NS1, IgG, and IgM).

MATERIALS AND METHODS:

A total of 283 blood samples received at Department of microbiology, BMC &RI hospitals were selected, in the time period of September and October 2017. The blood sample was centrifuged at 3000 rpm for 10 minutes and serum was separated. The serum was tested for rapid kit tests and results were noted at the end of 20 minutes. The RDTs were performed by kits from Alere Pvt Ltd(figure 2). The RDTs were performed by kits from Alere Pvt Ltd. Each kit has a result window with T- line and C- line. C-line is seen to be positive in all kits as per standards and T- line shows the test results.

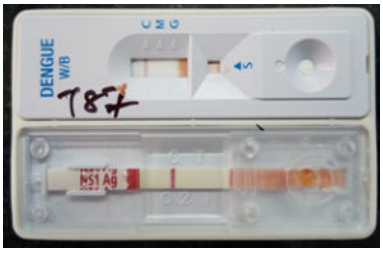


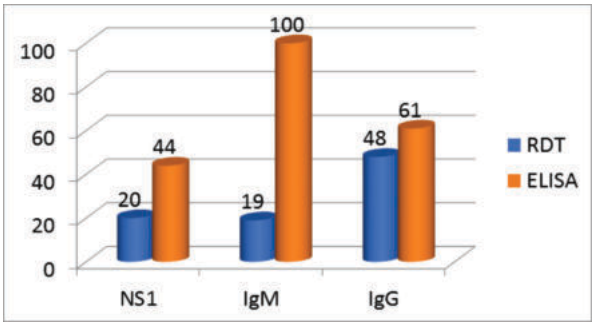
Figure 2 : Rapid Dengue Kit

The serum was then subjected to ELISA testing. IgM MAC-ELISA was performed with MAC-ELISA kits provided by NIV, Pune while IgG and NS1 ELISA were performed by kits from PanBio Pvt Ltd. ELISA reading was done in 450 nm wave length in ELISA Reader. The tests were carried out according to manufacturer's instructions.

Tabulation, management, and analysis of raw data were carried out using Microsoft Excel. A 2 × 2 table was made in which the predetermined results of test serum was tabulated with the tested RDT and ELISA to define true-positive, false-positive, false-negative, and true-negative values.

RESULTS:

A total of 87 tests were positive on IgM ELISA. 51 tests were positive in rapid tests. On comparison of Elisa test results with RDTs, for NS1 RDTs showed 80% sensitivity and 98% specificity with positive predictive value (PPV) of 73% and Negative predictive value (NPV) of 98%. Sensitivity of IgM rapid test was 23%, while specificity was 100%. The PPV was 100% and (NPV) was 37%. For IgG ELISA, the rapid tests showed sensitivity of 86.4% and specificity of 97% while PPV was 86% and NPV 96%. The serotyping for these 87 samples positive for MAC-ELISA (IgM) studied showed 30 positive for virus .



DISCUSSION:

Dengue virus is single-stranded RNA virus that belongs to the family Flaviviridae. The Flaviviridae family also includes other important vector-borne viruses such as West Nile virus, Yellow fever virus, Japanese Encephalitis virus, and St. Louis encephalitis virus. Dengue viruses are arboviruses transmitted primarily to humans through the bite of an infected female Aedes mosquito (tiger mosquito). Dengue infection has a wide spectrum of symptoms and has a high mortality and morbidity rate, which makes it important to find an important and correct method for an early diagnosis and treatment of the disease. RDTs that are rapid and they need least expertise, give bedside results are helpful in the initial diagnosis. They usually give results in 15-30 minutes, and so are considered useful in resource limited settings. In a study in Raipur, 33% serologically positive dengue patients showed 30% of both NS1+IgM postive and only 15% NS1 positive while 54% were IgM positive, so they suggest that combined results of NS1 and IgM could prove to be useful in detecting cases of dengue[6]. In our study NS1+IgM combined by rapid gives 57% positive, while NS1+IgG combined 59% positive detection rate. Pal S, et al. quotes that sensitivity of RDTs vary with serotype of dengue and overall sensitivity being higher for DENV-1 (>90%)[2] , also sensitivity of kits vary with different kits (2%-26%) while ELISA assays are more reliable to detect antigen as well as antibody.

CONCLUSION:

Positive Predictive value for RDTs are more than 80%, which makes it a good screening test but sensitivity for RDTs is low (NS1-80%, IgM 30% and IgG-84%) which tells us to reconfirm cases with ELISA if settings is available. ELISA can be made cost effective if many samples are processed at a time.

Conflicts Of Interest: None.

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