Retrospective study and evaluation of rapid and ELISA tests for diagnosis of dengue in a tertiary care hospital

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Abstract:

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In recent years, Dengue has been emerging as a global health problem with approximately 2.5 billion people being affected by it. For a diagnosis of dengue to be made, a fourfold rise in acute and convalescent sera is required. There are several methods in use for diagnosis of dengue. These include detection of virus by cell culture or immunofluorescence, detection of virus antigen by ELISA or rapid kits, detection of Dengue virus antibody by haemagglutination inhibition, complement fixation, neutralization tests or ELISA and detection of virus nucleic acid by reverse transcriptase (RT-PCR) or real time PCR. However, most of these tests are expensive and time consuming and require expertise to carry out. This retrospective study was carried out in MYH, department of microbiology, M.G.M. medical college and M.Y. hospital, Indore, from June 2014 to May 2015 in 329 patients to evaluate performance of rapid and ELISA kits for diagnosis of Dengue infection. .Out of total 329 samples tested by rapid method, 66 samples were positive only for NS1 antigen, 263 samples were positive for either IgM alone or with IgG antibodies. Out of 66 samples positive for NS1 antigen, only 22 samples were positive by capture ELISA for IgM. Out of 263 samples which showed positive antibodies either IgM or IgG by rapid test, 203 were positive by capture ELISA for IgM, 60 samples showed negative results. For early detection of disease, antigen detection by rapid method is a good choice. But the sensitivity of ELISA in detecting antibodies is good. Thus rapid diagnostic kits used for detection of NS_1 antigen can be helpful in acute stage of Dengue infection and for detection of antibodies ELISA is the method of choice.

Keywords: Dengue, ELISA, NS1 antigen, IgG, IgM

Introduction:

In recent years, Dengue has been emerging as a global health problem with approximately 2.5 billion people being affected by it. People living in urban areas of tropical and subtropical regions are at increased risk of contracting dengue infection (1). Increased urbanization and migration of people from rural to urban areas with lack of proper sanitation has led to an upsurgence of dengue infection in India (2). There is no specific treatment modality or vaccine for dengue so prevention and control of the disease is the mainstay of healthcare in India.

Dengue is a mosquito borne flavivirus belonging to the family flaviviridae. It has four distinct serotypes DENV -1, DENV-2, DENV-3 & DENV-4, which are distinguished from each other by serological and molecular assays (3). The NS_1 protein is a 50 KDa glycoprotein which is rich in amino acids and has a nucleotide homology among flavivirus(4).

NS_1 does not form part of the virion but is released from the virus infected cells, studies reveal that NS_1 antigen may be involved in viral RNA replication and has been found in acute phase sera of patients of dengue so it can be used as a suitable marker for early dengue infection (5).

Early diagnosis of dengue virus infection is important for early initiation of therapy and to avoid complications like Dengue hemorrhagic fever (DHF)and Dengue shock syndrome(DSS).Dengue virus specific IgM antibodies appear as early as three days of dengue viral fever and can persist for 30-60 days, whereas IgG antibodies appear around the 7th day of fever with a peak at 2-3 weeks and persist for life (6) NS1 antigen can be detected from as early as first day of fever and onset of symptoms and is positive upto 18 days (7).Dengue IgM antibody is a marker of recent infection but it has a croosreactivity between other circulating Flaviviruses.

For a diagnosis of dengue to be made, a fourfold rise in acute and convalescent sera is required. There are several methods in use for diagnosis of dengue. These include detection of virus by cell culture or immunofluorescence, detection of virus antigen by ELISA or rapid kits, detection of Dengue virus antibody by haemagglutination inhibition, complement fixation, neutralization tests or ELISA and detection of virus nucleic acid by reverse transcriptase (RT-PCR)or real time PCR. However, most of these tests are expensive and time consuming and require expertise to carry out.

In the present study, we evaluated a one year data of dengue patients tested by both rapid(SD BIOLINE) and ELISA(NIV DEN MAC ELISA) kits and compared the performance of both the kits in a tertiary care hospital.

Materials and methods:

This was a retrospective study in which sera of total of 329 patients were used for evaluating the performance of rapid and ELISA kits for detecting dengue infection. These patients were enrolled between June 2014 to May 2015 (Figure 1).

The patients were both males and females with age range from less than 1 year to more than 40 years. The criteria for enrollment in the study was history of fever $\geq$ 38 degree Celsius, headache, joint pain that was suggestive of dengue infection, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Serum samples were tested for dengue IgM. IgG and NS_1 antigen by rapid (SD BIOLINE) method and simultaneously for IgG and IgM antibodies by ELISA method (NIV DEN MAC ELISA KIT).WHO criteria was used for including a case of dengue infection in our study (8).

Results:

Out of 329 samples analyzed from June 2014 to May 2015, > 80 % of the samples were positive in the month of October and November. Generally dengue infection is post monsoon infection but as monsoon started late, so most of the patients were seen in winter season. Rarely any patient was positive from January 2015 to May 2015. 185 were male patients and 144 were female patients. Most of the patients were between 10 – 30 years of age. But we found 1 patient of less than a month and two patients 7 month of age. In this study, first line of testing in out lab is rapid method, which tested NS1 antigen, IgM and IgG antibody. Samples showing positivity for NS1 alone or combined with IgG, IgM were subjected to ELISA testing for IgM antibody thrice a week

provided by NIV, Pune. Out of total 329 samples tested by rapid method 66 samples were positive only for NS1 antigen, 263 samples were positive for either IgM alone or with IgG antibodies. Out of 66 samples positive for NS1 antigen, only 22 samples were positive by capture ELISA for IgM. Out of 263 samples which showed positive antibodies either IgM or IgG by rapid test, 203 were positive by capture ELISA for IgM, 60 samples showed negative results. IgG + IgM positive 50 % patients were of secondary dengue infection. In 66 NS1 positive cases by rapid test, ELISA method detected IgM antibody in 22 patients, which was negative by rapid test. Out of 245 patients tested by rapid method which showed positive results either IgM alone or IgG + IgM only 185 were positive by ELISA.

The combined use of IgM and IgG has been shown to increase sensitivity in the detection of dengue virus infection. For early detection of disease, antigen detection by rapid method is a good choice. But the sensitivity of ELISA in detecting antibodies is good 30 % of patients who were not detected by rapid method, showed positive result with ELISA method.

Discussion:

India has seen periodic upsurge of dengue infection in the past decade (9). This may be partly attributed to rapid unplanned urbanization, construction and poor sanitation facilities which are a good breeding ground for Aedes mosquitoes to breed and multiply. However increased awareness among health care workers and general public about the disease has lead to increased serological testing and timely diagnosis. This has increased the detection rate of dengue considerably in the last few years (10).

Diagnosis of dengue fever based on symptoms alone is not reliable and requires confirmatory laboratory tests. In the past decade, there has been a rise in Dengue fever cases in various parts of India with DHF and DSS developing in patients who were not diagnosed in the early stage of the disease.

Dengue virus is an enveloped positive sense virus. There are four serotypes of Dengue (DENV I to DENV 4) that are antigenically related. Recovery from infection by one serotype can confer lifelong protection against that serotype only. Partial transient immunity is provided against other three serotypes. Progression from DF to DHF to DSS usually occurs after a second infection with a different serotype which is due to immune mediated enhancement of infection known as antibody mediated enhancement (11).

A seasonal variation of data in our study revealed maximum cases in October (106 / 329) and November (133 / 329), followed by December (41 / 329). A gradual increase was observed from August with a peak in October / November and tapering off after December. This is because this season is favorable for dengue vector (Aedes Aegypti) to breed (12). Dengue infection was highest in the active age group i.e. 21 – 30 years (106 / 329) followed by 11 – 20 years (95 / 329).

The results obtained from our study are consistent with the studies conducted by many others who have shown that detection of NS1 antigen is more useful in the acute phase of the dengue infection and that its sensitivity drops as the concentration of antibody rises(13-16).

Conclusion:

Early laboratory diagnosis of acute dengue viral infection is important so that timely treatment can be started and complications such as dengue hemorrhagic fever and Dengue shock syndrome can be avoided. At present, the most common techniques for dengue diagnosis are virus isolation, detection of dengue specific IgM and IgG antibodies and NS_1 antigen. Although, viral isolation is a gold standard method, it is expensive and time consuming. Similarly ELISA methods also take relatively more time than rapid methods.

NS_1 antigen is detected from day 1 up to 9th day of onset of fever whereas IgM antibodies in primary infection are detectable from day 3 to day 5 and in secondary infection from day 1 to day 2 after onset of fever(17). In our study, the combined use of IgM and IgG has been shown to increase sensitivity in the detection of dengue virus infection.

For early detection of disease, antigen detection by rapid method is a good choice. But the sensitivity of ELISA in detecting antibodies is good.30 % of patients who were not detected by rapid method, showed positive result with ELISA method. Thus rapid diagnostic kits used for detection of NS_1 antigen can be helpful in timely detection of dengue infection and for detection of antibodies ELISA is method of choice.
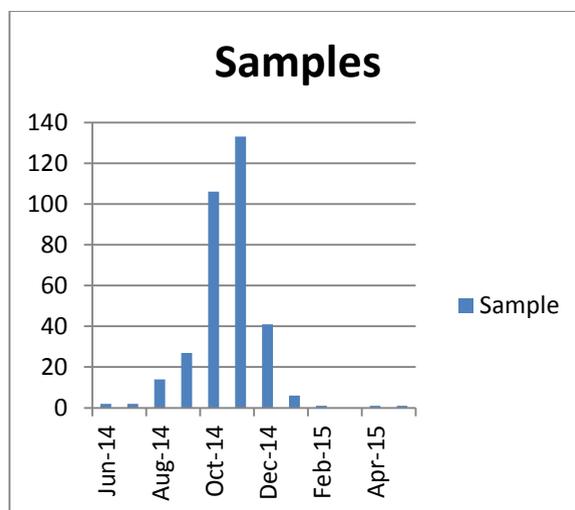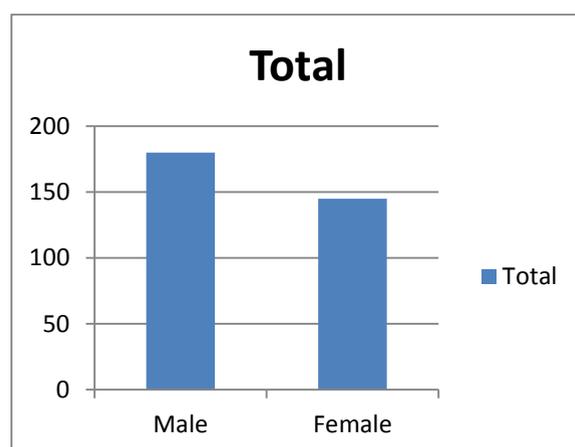

Figure 1: Total samples monthwise

Figure 2: Patient demographics

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