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Research Article

Study of Rapid Serological Tests for Diagnosis of Dengue

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Abstract: Dengue is one of the rapidly emerging global threats. Many outbreaks are being noticed nowadays all around the world. In situations of epidemic, early diagnosis is the key to successful management of dengue cases. Many diagnostic kits are available commercially for the same purpose. But their validity is unknown. The gold standard in such situations is IgM capture ELISA, though it is more time consuming. In present study, the test results of one of the commercially available rapid immunochromatographic card test are compared with IgM capture ELISA as gold standard. Probable dengue cases were diagnosed as per the WHO criteria and rapid immunochromatographic card test and IgM capture ELISA were conducted on the same serum sample. Results are analyzed. The study was conducted for a period of one year. A total of 66 probable dengue cases were selected. 16 cases were found to be positive for dengue rapid immunochromatographic test, whereas 14 cases were found to be dengue positive by IgM capture ELISA. The sensitivity & specificity of rapid test along with positive predictive value and negative predictive value were deducted and compared with other studies. The study shows that the sensitivity of rapid card test is less but has a good specificity. In situations of epidemic, the card test can be used for screening but with the support of IgM capture ELISA. Highly suspicious cases should be subjected to investigations with higher sensitivity & specificity, though the results take little more time.
Keywords: Dengue, Immunochromatography, Antibody, NS1 protein, dengue virus.

INTRODUCTION

Dengue is the most rapidly spreading mosquito-borne viral disease in the world [1,2]. It is becoming a global public health emergency. Though disease is usually seen in endemic areas, many epidemics involving continents or even globe have been witnessed by the world. One more such epidemic was being experienced in India while this study was being performed.

Dengue manifests in three forms, Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS), the latter being the most serious form of illness. Early diagnosis is the key to successful management of all these forms of dengue fever. For confirmed diagnosis, there are following methods which can be used – (1) Serology, (2) Viral isolation, & (3) RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction). After the onset of illness, the dengue virus is found in serum or plasma & circulating blood cells for approximately 2 to 7 days, roughly corresponding to the period of the fever[3,4]. This can be considered an ideal time for viral isolation and RT-PCR method to detect the viral infection. But serological diagnosis remains the mainstay of diagnosis during the epidemic since viral isolation is laborious, expensive and is only available in reference laboratories. Serological detection of antibodies based on capture IgM (immunoglobulin M) ELISA (Enzyme Linked Immuno-Sorbent Assay) has become the new gold standard for the detection of dengue virus infections[5,6].

In peripheral health settings, like the one where present study was being conducted, with dengue outbreak, rapid assessment is of great importance with a balanced reliability. In this context, this study was carried out to evaluate the performance of a rapid immunochromatographic test device for the detection of IgM, IgG and NS1 response to dengue infection against the serological detection of antibodies based on capture IgM ELISA taken as the reference standard, at HSK hospital, Bagalkot.

MATERIALS AND METHODS

Dengue cases

This one year study was conducted to assess the validity of commercially available dengue rapid test kit. For that purpose, suspected dengue cases, which are defined as probable dengue cases by WHO [1] (World Health Organization), were selected. A proforma was prepared and details of patients were entered.

Dengue rapid immunochromatographic card test

The commercially available kit "SD Bioline Dengue Duo, Dengue NS1 + Ab combo" (manufactured by Standard Diagnostics, Inc. Korea) was used to detect

the primary result of suspected dengue cases. It is a rapid (15 minutes) test (in vitro immunochromatographic, one step assay) to detect both, NS1 (Non-Structural) antigen and dengue IgM, IgG antibodies. Positive results were recorded irrespective of the component detected (IgM/IgG/NS1).

Dengue IgM capture ELISA

For performing this test, blood is collected from the probable dengue cases on day 5 or more of the illness (IgM titres rise after the 5th day of illness)[1] in a plain bulb and serum is separated by centrifusion and stored for less than 14 days (as directed in the technical literature), so that maximum number of tests should be performed at a time.

The test kit used for this purpose was "EUROIMMUN", produced by a Germany based company[7].. According to the literature provided with the test kit, the kit has 98% specificity and 100% sensitivity. All necessary precautions were taken while performing tests, like avoiding contamination of sample, storage at specified temperature, preparation of controls etc.

Statistical analysis

Statistical analysis was done using chi-square method and other relevant equations using SPSS software.

For selection of cases, the classification suggested by WHO was used and "probable" dengue cases were included in the study. Cases with confirmed diagnosis of other febrile illnesses like malaria, enteric fever, urinary tract infection etc, were excluded from the study. Study was designed for a period of one year and all the suspected cases were subjected to both tests. Care was taken to take the samples on 6th day of illness as IgM antibodies appear on the 5th day of illness. NS1 antibodies can be found from 2nd to 9th day of illness. The same serum samples were subjected for both tests.

RESULTS

A total of 66 serum samples were tested for the presence of either NS1 antigen or IgM/IgG antibodies with the rapid immunochromatographic test. The same serum samples when used for IgM antibody detection, obtained results are depicted in table 1. Out of 66, 18 samples were positive for dengue by rapid test and 16 were positive by IgM capture ELISA. The sensitivity & specificity of the rapid test along with other values for validation were calculated and are depicted in table 2.

Table 1: Dengue positive cases by different methods

Total no of samples	Dengue positive by rapid test	Dengue positive by MAC-ELISA
66	18	16

Table 2: Parameters of interest for dengue rapid card test

Sensitivity	Specificity	Positive predictive value	Negative predictive value
68.75%	86%	61.11%	89.58%

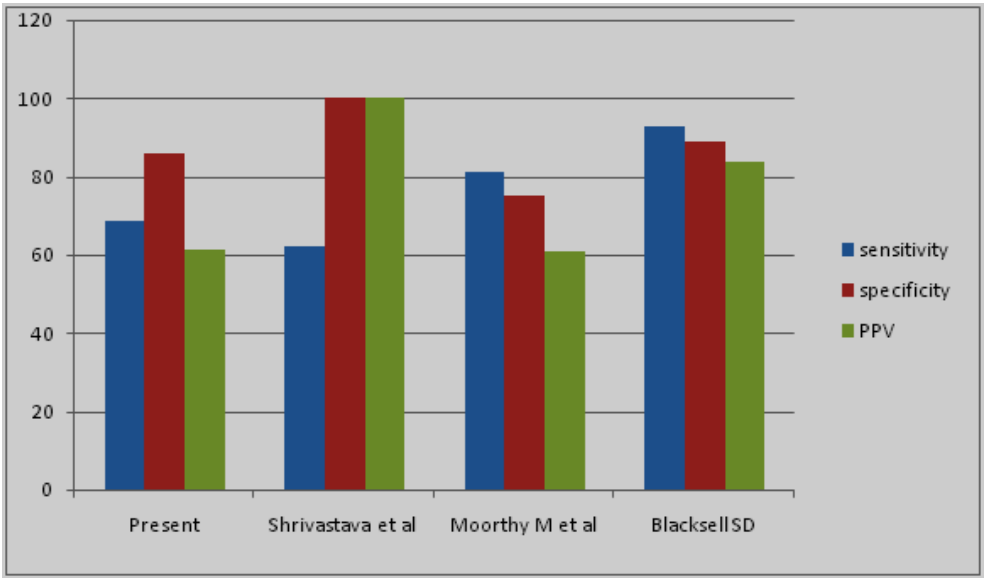

Fig. 1: Comparison of studies

DISCUSSION

Diagnosis of dengue virus infection based on clinical syndromes is not reliable and should be confirmed by laboratory studies. For a diagnosis of confirmed dengue, dengue virus should be identified by isolation or there should be a 4-fold rise in antibody titre. Isolation of viruses can take 7 to 10 days, and serological tests depend on the demonstration of the presence of IgM antibody or a rise in IgG antibody titre in paired acute- and convalescent-phase sera. Serological tests are generally the tests of choice to diagnose acute flavivirus infections, with most utilizing IgM capture ELISA formats. Commercial kits for the measurement of antibodies include the ELISA kits, a dipstick, and a rapid dot blot assay. These kits do not require specialized training but their sensitivity and specificity is very variable. The choice of a test, therefore, depends on the availability of facilities and human resources and, also, the time of sampling.

With the escalating incidence of dengue infections and the absence of vaccines for the prevention of this disease, early diagnostic confirmation of dengue virus infections in patients is needed, as it allows for timely clinical intervention, etiologic investigations, and disease control. Hence, diagnosis of dengue disease during the acute phase should be a priority for patients and for public health reasons. In-house IgM capture ELISAs have been the mainstay of dengue diagnosis in many laboratories throughout the world.

Though, the IgM capture ELISA is used as the standard test of reference in this study, higher rates of sensitivity and specificity are evident with tests like RT-PCR and viral isolation. But in epidemics, their usefulness and cost effectiveness is questionable.

Many studies have been performed to evaluate the rapid test with different reference standards. Few Indian and foreign studies with similar background and results are discussed here. The study characteristics are mentioned in table3.

The sensitivity calculated in present study correlates well with the sensitivity of dengue rapid test found in a study conducted by Shrivastava *et al.* [8], but the specificity and positive predictive value are 100%. This may be due to different kits used to perform the tests. They have also used RT-PCR, a better reference standard for evaluation.

The positive predictive value in present study is comparable to that observed in a study by Moorthy M *et al.* [9]. but the sensitivity is higher and specificity way lower than what we observed in this study. Again the different test devices can be a factor, but also timing of the test should also be considered. Usually IgM antibodies start to appear in serum after the 5^{th} day of illness. Earlier serum samples may give false negative results, thereby decreasing the specificity of the test. The specificity we observed in our study matches closely to a standard study by Blacksell SD *et al.* [10]. In that study, various tests available (six) commercially were evaluated against the standard reference of maximum sensitivity and specificity like RT-PCR. The rapid test kit used in our study was also a component of the study performed in UK. The main difference lies in the sensitivity value, which is less in our study (fig. 1). The reason may be evaluation against IgM capture ELISA which has a lesser sensitivity than RT-PCR.

One more thing to be noted here was that; the rapid test used for this study was a combination of both, antigen (NS1) and antibodies (IgM/IgG). The positive result was considered irrespective of antigen or antibody. The combination is expected to perform better than isolated detection of antigen or antibody. More studies are required to assess the performances of combo test devices and that too, on a bigger sample size.

Nowadays, ratio of IgM/IgG is used to differentiate between primary and secondary dengue infection. Though the rapid test gives a clue about primary or secondary infection, quantitative information is lacking. Hence IgG capture ELISA is also advocated in highly suspicious secondary dengue infections.

Recommendation

The dengue immunochromatographic rapid card test has a good specificity but very less sensitivity. The test should not be used as a standalone device for diagnosing dengue cases. Test results need confirmation with tests of higher sensitivity and specificity values.

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