USE OF A RAPID TEST KIT AND ELISA FOR DETECTION OF NS1 ANTIGEN AND IgM ANTIBODY IN SUSPECTED DENGUE FEVER

Susanta Kumar Sahu¹, Sudhir Kumar Ghosh¹, Sulin Kumar Behera¹, Dibya Prasana Mohanty¹, Satyabrata Thakur¹

¹Department of Microbiology V S S Medical College, Burla, Odisha, PIN- 768017

Correspondence should be addressed to Susanta Kumar Sahu

Received January 09, 2015; Accepted January 27, 2015; Published February 16, 2015;

Copyright: © 2015 Susanta Kumar Sahu et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.


ABSTRACT

Incidence of dengue infection has been increasing since last few years. Diagnosis of dengue mainly depends upon detection of NS1 antigen, IgM antibodies or a rising titre of IgG antibodies in patients' blood by ELISA. This study was undertaken to evaluate a rapid test kit for detection of NS1 antigen and anti-dengue IgM antibodies in suspected dengue fever taking NS1 ELISA and MAC ELISA as reference standard. A total number of 1102 serum samples collected from patients having fever for 5 days or less were tested with NS1 ELISA and 1548 serum samples from patients with fever for more than 5 days were subjected for MAC-ELISA. All samples were tested by a rapid diagnostic test (RDT) kit that detects NS1 antigen, IgM and IgG antibodies. The RDT kit showed a sensitivity and specificity of 95.97% and 99.43% respectively for NS1 antigen detection and 93.90 and 99.53% for IgM.

KEY WORDS: Dengue, Immunochromatographic Test (ICT), MAC-ELISA, NS1 Antigen

INTRODUCTION

Dengue is the most rapidly spreading vector-borne viral disease in India. In eastern part of the country the disease is sporadic till 2009. Since 2010 it has spread in outbreak proportions affecting more than 17457 reported cases and claiming 59 deaths in an eastern state.[1] early diagnosis and management is the key to reduce the fatal outcome of dengue fever and dengue haemorrhagic fever. The WHO guidelines for diagnosis of dengue relies on virus culture, viral RNA detection, viral antigen detection in tissues by immune-chemistry in patients or rising titres of IgG antibody in convalescent patients. Detection of IgM antibody by ELISA in acute cases also gives a probable diagnosis of dengue.[2] But, in resource constrained peripheral health care facilities these tests are remotely available and may compromise with the management of the patients. The rapid test kits available are based on detecting the NS1 antigen, IgM & IgG antibodies; but do not fulfi the WHO guideline.[2][4] Keeping these in view an attempt has been made to compare the rapid test results with ELISA test results in suspected Dengue cases.

MATERIALS & METHODS

A retrospective study, comprising 2650 consecutive serum samples tested between August 2011 to December 2013 in a tertiary care teaching hospital were analysed. Out of these 1102 cases have history of fever within 5 days (group A) and the rest 1548 have fever history of more than 5 days (Group B). All the samples in group A are subjected to NS1 ELISA (Panbio Dengue Early ELISA manufactured by Standard Diagnostic Inc., Republic of Korea) and those in group B are subjected to MAC ELISA (manufactured by National Institute of Virology, Pune)
All 2650 samples were tested with a commercial rapid immune-chromatographic test (ICT) combo test kit that detects NS1 antigen, IgM and IgG antibodies simultaneously in a single device (Dengue Day 1 test manufactured by J mitra & co. Pvt Ltd., New-Delhi). The results were tabulated and statistically analysed.

RESULTS

In group A out of the total 1102 sera tested 397 showed positive result by NS1 ELISA and 385 are NS1 positive by ICT. Out of the ICT positive cases 381 are concordant with the ELISA result and 16 cases were discordant, but showed positive IgM response in ICT. Moreover 4 cases which were negative for NS1 in ELISA showed positivity by ICT. (Table 1)

The statistical values obtained were as follows: sensitivity 95.97%, specificity 99.43%, PPV 98.96%, NPV 98.31% and efficiency of the test 98.18%.

Out of the 1548 early convalescent sera (group B) tested for IgM, 279 were positive by MAC-ELISA and 268 were positive for IgM in ICT. 262 of the ICT positive cases were concordant with MAC-ELISA. The rest 17 cases are discordant. Six ICT positive cases which showed negative result in MAC-ELISA. The test showed sensitivity 93.90%, specificity 99.53%, PPV 97.76%, NPV 98.67% and efficiency 98.51%. (Table 2)

While correlating the different parameters in the ICT combo test kit it was seen that of 1102 acute phase sera 19 showed both NS1 and IgM positivity and 4 showed only IgM positivity. These 4 cases were positive by NS1 ELISA, but negative by ICT. On the other hand in the convalescent group 24 cases showed positivity both in NS1 and IgM and 6 cases gave negative result for IgM, but positive result. (Table 3)

DISCUSSION

Rapid ICT kits for the detection of dengue virus non-structural protein 1 (NS1) antigen, IgM, IgG, and IgA antibodies have been developed by a number of commercial manufacturers and have found wide application because of their ease of use and rapidity of results. These require no specialized equipment or training, and the results are available within 10 to 30 minutes, making them ideal for low-technology environments; however, these have limitations like subjective variation in reading and substandard performance for the diagnosis of acute cases. This demands large scale evaluation of these kits by independent agencies.[4][5]

The viremic phase lasts for 2 to 21 days after dengue infection. During this period soluble NS1 circulates in the serum of patients and hence is an excellent diagnostic target for acute dengue diagnosis.[2][6][7] Dengue IgM antibodies appear in the blood of patients 2 to 5 days post infection. It is hypothesised that the presence of anti-NS1 antibodies complexes with the soluble NS1 antigen which impedes the ability of the test to detect free NS1 antigen.57 Kaylan D, et.al have recorded a higher positivity with ICT than ELISA while testing for IgM antibodies in acute dengue cases. However most other studies including the present, have recorded a higher detection rate with ELISA. By combining the results of NS1 and IgM assay the diagnostic sensitivity and efficacy can be increased, particularly during the acute phase of the disease. This has been shown in our study and similar observations have been made by different researchers.[7][8]

Though many studies have shown variable sensitivity (48.5 to 98.7%) and specificity (71.42 to 100%) of ICT based RDTs compared with ELISA, [9][13] in the present study

Table 1: Group A: Comparison of NS1 ELISA and ICT for NS1 antigen

<table>
<thead>
<tr>
<th>NS1 ICT +ve</th>
<th>NS1 ICT -ve</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS1 ELISA +ve</td>
<td>381</td>
<td>4</td>
</tr>
<tr>
<td>NS1 ICT -ve</td>
<td>16</td>
<td>701</td>
</tr>
<tr>
<td>397</td>
<td>705</td>
<td>1102</td>
</tr>
</tbody>
</table>

Table 2: Group B: Comparison of MAC ELISA and ICT for IgM

<table>
<thead>
<tr>
<th>IgMICT +ve</th>
<th>IgM ICT -ve</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAC-ELISA +ve</td>
<td>262</td>
<td>6</td>
</tr>
<tr>
<td>IgM ICT -ve</td>
<td>17</td>
<td>1263</td>
</tr>
<tr>
<td>279</td>
<td>1269</td>
<td>1548</td>
</tr>
</tbody>
</table>

Table 3: Comparison of IgM and NS1 antigen assay results in ICT test

<table>
<thead>
<tr>
<th>Type of sera</th>
<th>NS1 Positive</th>
<th>NS1 negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM+ve</td>
<td>IgM -ve</td>
<td>IgM +ve</td>
</tr>
<tr>
<td>Acute phase</td>
<td>19</td>
<td>362</td>
<td>4</td>
</tr>
<tr>
<td>Convalescent</td>
<td>24</td>
<td>6</td>
<td>238</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>368</td>
<td>242</td>
</tr>
</tbody>
</table>
we have got a sensitivity and specificity of 95.97%, and 99.43% respectively in detecting NS1 antigen in acute phase serum and 93.90% and 99.53% in detecting IgM in early convalescent sera. The sensitivity can further be increased if acute phase sera are also observed for presence of IgM antibodies and convalescent sera are observed for NS1 antigen. The RDT we have used is a combo test kit allowing simultaneous testing for NS1 antigen, IgM antibody and IgG antibody. We suggest the use of this kit in lieu of ELISA, particularly in small laboratories where facility for the latter is wanting.

REFERENCES


[12] Sharmin R, Tabassum S, Jahan M, Nessar A, Mamun KZ. Evaluation of an immunochromatographic test for early and rapid detection of dengue virus infection in the context of Bangladesh World Health Organization, Regional Office for South-East Asia; World Health Organization, Regional Office for the Western Pacific 2011