Evaluation of an immunochromatographic test for early and rapid detection of dengue virus infection in the context of Bangladesh

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Abstract

Early, accurate and rapid diagnosis of dengue virus infection is important for early case management and for reducing its associated complications, DHF/DSS. In this study, an early and rapid diagnosis of dengue virus infection was performed from single serum samples by two serological methods. Blood samples collected from a total of 201 clinically-suspected dengue fever patients were tested for IgM and IgG antibodies by a rapid immunochromatographic test (ICT), and also by IgM and IgG antibody Capture ELISA. Of these, 126 (62.7%) patients tested positive for dengue antibodies by ICT, of which 70 (55.6%) were primary and 56 (44.4%) were secondary cases. By ELISA, 137 (68.2%) tested positive for dengue antibodies, of which 80 (58.4%) were primary and 57 (41.6%) were secondary cases. Before 5 days of fever, 20.2% primary and 10.1% secondary dengue infections were detected by ICT, while 30.3% primary and 12.6% secondary dengue infections were detected by ELISA. At day 5 of fever, ICT detected 42.8% cases as primary and 34.7% as secondary dengue infections, but ELISA detected 51.0% primary and 32.6% secondary infections. After 5 days of fever, ICT detected primary dengue infection in 45.2% cases and secondary infection in 42.5% cases, while ELISA detected 42.5% primary dengue infection and 42.5% secondary infection. When compared with ELISA, ICT showed 86.7% sensitivity and 96.5% specificity for IgM detection, whereas for IgG it was 94.7% and 98.6% respectively.

Keywords: Dengue fever; Dengue haemorrhagic fever; ICT; ELISA; Bangladesh.

Introduction

Dengue fever/dengue haemorrhagic fever (DF/DHF) continues to be the most important arboviral disease of mankind. Compared with nine reporting countries in the 1950s, today the geographical distribution of dengue has spread to more than 100 countries worldwide,
with South-East Asia and the Western Pacific regions being the most seriously affected areas.\cite{3,4} Two-fifths of the world’s population is now at the risk of dengue, with approximately 50 million new cases occurring annually.\cite{5}

The first reported outbreak of dengue in Bangladesh was called the “Dacca fever” recorded in 1964.\cite{6,7} Subsequent reports suggested that DF and DHF may have been occurring sporadically in Bangladesh.\cite{8-11} Dengue virus infections may be asymptomatic or may cause undifferentiated febrile illness (viral syndrome), dengue fever (DF), or dengue haemorrhagic fever (DHF) including dengue shock syndrome (DSS).\cite{2,3}

Primary infection with one of the four serotypes confers life-long immunity to that serotype. Secondary infection with a different serotype is associated with an increased risk of DHF. Primary dengue virus infection is characterized by elevation in specific immunoglobulin M (IgM) levels 3-5 days after the onset of symptoms and subsequent rise for the next 1-3 weeks. This particular IgM can persist in blood for more than 2-3 months.\cite{12,13} Immunoglobulin G (IgG) is detectable at low titres at the end of the first week of illness, increasing slowly and may persist for life in low titre. In secondary infection, approximately 5% of patients do not produce detectable levels of specific IgM, and the IgM titre rises slowly. However, in secondary infection, IgG appears approximately two days after the symptoms appear and is detectable at significantly higher titres which may persist for 10 months to the rest of life.\cite{13,14,15}

Since the prevalence of dengue has increased dramatically in recent decades, its early and rapid diagnosis will obviously lead to a better management of affected patients. The laboratory diagnosis of dengue infection is based on three approaches, namely, virus isolation, serology and molecular techniques, e.g. the polymerase chain reaction (PCR).\cite{12,15,16} Serology is the mainstay for the diagnosis of dengue infection in most routine laboratories in developing countries as it is rapid, easier to perform and is less costly.\cite{12,17} ELISA has been successfully applied for years to detect and distinguish IgG and IgM antibodies to dengue and other flaviviruses\cite{14,16} and is the most effective diagnostic method in large outbreaks.\cite{14,18} Recently, IgM and IgG Capture ELISA have been modified into immunochromatographic formats in which the results of the assay are detected by a colour change visible to the naked eye. Rapid immunochromatographic test (ICT) relies on both immunoglobulin M (IgM) and immunoglobulin G (IgG) detection to diagnose active dengue virus infection\cite{12,19} and has the potential for use at the point of care or in laboratories where the volume of testing is less or sporadic and where appropriate equipments such as, ELISA, PCR, cell culture, etc., are not available.\cite{20}

**Materials and methods**

This study, conducted in 2008, covered 201 clinically-suspected dengue fever patients selected from different hospitals of Dhaka city, Bangladesh, and from patients visiting the Department of Virology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka,
for dengue antibody testing. Dengue fever cases were selected on the basis of “WHO criteria for case definition of dengue fever, dengue haemorrhagic fever, dengue shock syndrome” (National Guidelines for Clinical Management of Dengue Syndrome, Bangladesh, 2000). Blood samples were collected from July to October. All serum samples were tested to detect dengue virus-specific IgM and IgG antibodies by ICT and antibody Capture ELISA.

Capture ELISA

For dengue IgM and IgG Capture ELISA, Dengue IgM and IgG Capture ELISA kits (Panbio Diagnostics, Australia, Catalog No. E-DEN01M and E-DEN02G) were used according to the manufacturer’s instructions.

Immunochromatographic test

Panbio Dengue Duo Cassette (Panbio Diagnostics, Australia, Catalog No. R-DEN03D) was used according to the manufacturer’s instructions, and both IgM and IgG antibodies were determined using a capture assay format.

Data analysis

Data obtained from the study were analysed and the significance of difference was estimated by using the computer-aided statistical package (SPSS) version 15. Comparison between groups was done by chi-square test and correlation coefficient test as applicable. Probability less than 0.05 was considered as significant.

Results

The serological diagnosis among the 201 clinically-suspected dengue fever patients by ICT detected 126 (62.7%) and the ELISA test detected 137 (68.2%) dengue antibody-positive cases. Of the 126 positive cases detected by ICT, 70 (55.6%) were positive for only IgM antibody, 5 (3.9%) were positive for only IgG antibody, and 51 (40.5%) were positive for both IgM and IgG antibodies. Among the 137 ELISA-positive cases, IgM was detected in 80 (58.4%) patients, IgG in 2 (1.5%) patients, and both IgM and IgG was detected in 55 (40.1%) patients (Table 1).

Of the 79 patients tested before 5 days of fever, primary dengue infection was detected in 16 (20.2%) and secondary dengue infection in 8 (10.1%) cases by ICT. However, by ELISA, 24 (30.3%) cases were detected as primary and 10 (12.6%) cases as secondary dengue infections. Out of the 49 patients who were tested at day 5 of fever, 21 (42.8%) were detected as primary and 17 (34.7%) as secondary dengue infection by ICT, whereas by
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**Table 1:** IgM and IgG antibodies determined by ELISA and ICT in dengue fever patients in Dhaka, Bangladesh, 2008

<table>
<thead>
<tr>
<th>Type of antibody</th>
<th>ELISA</th>
<th>ICT</th>
</tr>
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<tbody>
<tr>
<td>Only IgM</td>
<td>80 (58.4%)</td>
<td>70 (55.6%)</td>
</tr>
<tr>
<td>Only IgG</td>
<td>2 (1.5%)</td>
<td>5 (3.9%)</td>
</tr>
<tr>
<td>Both IgM &amp; IgG</td>
<td>55 (40.1%)</td>
<td>51 (40.5%)</td>
</tr>
<tr>
<td>Total positive</td>
<td>137 (68.2%)</td>
<td>126 (62.7%)</td>
</tr>
<tr>
<td>Negative</td>
<td>64 (31.8%)</td>
<td>75 (37.3%)</td>
</tr>
</tbody>
</table>

ELISA, 25 (51.0%) were detected as primary and 16 (32.6%) as secondary dengue infections. Among the 73 patients tested after 5 days of fever, ICT detected primary dengue infection in 33 (45.2%) cases and secondary dengue infection in 31 (42.5%) cases, while ELISA detected 31 (42.5%) primary dengue infection and 31 (42.5%) secondary dengue infection (Table 2). No significant difference was observed between primary and secondary dengue cases with regard to the duration of fever by ICT and ELISA (*p* = 0.136 for ICT; *p* = 0.446 for ELISA).

With the rapid ICT, 119 (59.3%) samples tested positive for dengue IgM antibody and 56 (27.9%) samples tested positive for dengue IgG antibody. Using the focus ELISA as the gold standard, the sensitivity, specificity and positive predictive values and the negative predictive value determined for IgM were 86.7%, 96.5%, 98.3% and 75.3% respectively, while for IgG, these were 94.7%, 98.6%, 96.4% and 97.9% respectively (Table 3). A positive correlation was observed between ELISA and ICT for IgM (r=0.768) and IgG (r=0.753) respectively (Figure).

**Table 2:** Relation of duration of fever with antibody detection among primary and secondary dengue cases, Dhaka, Bangladesh, 2008

<table>
<thead>
<tr>
<th>Duration of fever</th>
<th>Dengue ICT</th>
<th>Dengue ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary</td>
<td>Secondary</td>
</tr>
<tr>
<td>Total (n=201)</td>
<td>70 (34.8%)</td>
<td>56 (27.9%)</td>
</tr>
<tr>
<td>&lt; 5 days (n=79)</td>
<td>16 (20.2%)</td>
<td>8 (10.1%)</td>
</tr>
<tr>
<td>5 days (n=49)</td>
<td>21 (42.8%)</td>
<td>17 (34.7%)</td>
</tr>
<tr>
<td>&gt; 5 days (n=73)</td>
<td>33 (45.2%)</td>
<td>31 (42.5%)</td>
</tr>
</tbody>
</table>

*p* value = 0.446 for ICT and *p* value = 0.136 for ELISA.

*Chi-square test was done to measure the level of significance.*
**Table 3**: Results of ICT compared to ELISA for detection of IgM and IgG antibodies, Dhaka, Bangladesh, 2008

<table>
<thead>
<tr>
<th>ICT</th>
<th>ELISA (IgM)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>117</td>
<td>2</td>
<td>86.7%</td>
<td>96.5%</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>18</td>
<td>55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ICT</th>
<th>ELISA (IgG)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>54</td>
<td>2</td>
<td>94.7%</td>
<td>98.6%</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>3</td>
<td>142</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PPV - Positive predictive value.
NPV - Negative predictive value.

**Figure**: Correlation between the results of IgM and IgG by ICT and ELISA. Here, result for ELISA was quantitative and for ICT categorical variable (0 = negative and 1 = positive). Positive r value indicates positive correlation

**Discussion**

Dengue fever is a major public health problem throughout the world. The severe form of the disease is a leading cause of hospitalization and death among children and adults in many south-east Asian countries including Bangladesh. Therefore, there is an urgent need
for a rapid, reliable and early diagnostic test for dengue surveillance, especially in countries where dengue is endemic. Due to higher mortality associated with secondary dengue cases, it is important to use diagnostic assays that are able to differentiate between primary and secondary dengue infections. As primary and secondary dengue infections show markedly different immunological responses, detection of antibodies is a valuable procedure to diagnose and differentiate between dengue infections.\cite{2,13,14}

In this study, Panbio ELISA IgG/IgM and Panbio ICT were used to evaluate early and rapid diagnosis of dengue virus infection. A comparison was also done between rapid ICT and IgM and IgG antibody Capture ELISA for dengue virus-specific IgM and IgG antibodies from serum samples. The two commercial tests used in this study are both suitable for the detection of anti-dengue IgM and IgG antibodies. ELISA is more appropriate for routine diagnostic laboratories where large numbers of samples are tested, while the rapid test may have greater utility in peripheral health settings where relatively fewer specimens are processed. Both tests use the combined determination of IgM and IgG antibodies in dengue diagnosis according to the manufacturer’s instructions, and interpretations are made as primary, secondary or ‘no dengue’ infection. The values of these methods have been reported previously.\cite{21} Total assay time for the rapid test is 15 min, while the ELISA takes just over 3 hours to complete. The IgM and IgG antibody Capture ELISA is very quick compared to other dengue ELISAs reported previously.\cite{14,22,23} In Capture ELISA, the incubation of serum in the anti-human antibody plate is done simultaneously when peroxidase conjugated monoclonal antibody with antigen is left at room temperature. This decreases the number of assay steps and speeds up the diagnosis.\cite{21} Furthermore, both the rapid ICT and ELISA are convenient to use as antigen is provided in a stable dry form and all reagents are provided in the ready-to-use form.

In the combined use of IgM and IgG Capture ELISA, the cut-off value of the IgG ELISA is generally set to differentiate between the high levels of IgG characteristic of secondary infections and the lower IgG levels characteristic of primary or past dengue infections. With this combination, the majority of secondary dengue virus infections are detected on the basis of IgG, and most of them also show an elevation of IgM. In contrast, the majority of primary dengue virus infections show an elevation of IgM but not of IgG.\cite{18,22,24} In rapid ICT, the IgG test line is set to detect high levels of IgG characteristic of secondary virus infection (HI≥1:2,560) and hence is able to distinguish between secondary and primary and past dengue infections. The IgM test line is set to detect IgM levels characteristically present in primary dengue virus infections and in the majority of secondary dengue virus infections.\cite{21,22}

In our study, 62.7% of patients were positive for dengue antibodies by ICT and 68.2% were positive by ELISA. A total of 55.6% primary and 44.4% secondary dengue cases were detected by ICT, whereas 58.4% primary and 41.6% secondary dengue cases were detected by ELISA. Previous studies from Bangladesh have detected 71%, 65% and 78% secondary dengue...
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Infections and remaining 29%, 35% and 22% primary infections respectively by ELISA.[8,11]. Identification of secondary infection early during an acute phase of illness is valuable for the clinician as proper management can be started early, thereby decreasing the risk of progression to life-threatening DHF and DSS and hence reducing the case-fatality rate.

The detection rate of dengue in this study was relatively less before five days of fever by both ICT and ELISA, but ELISA detected more primary cases (30.3%) than ICT (20.2%). However, the detection rate of secondary dengue infection was almost the same by the two methods. Failure to identify dengue-specific IgM or IgG antibodies during the first 5 to 7 days of illness does not eliminate dengue virus as the etiology of the illness, and, as such, follow-up testing is important.[25] Although the majority of patients in our study developed dengue-specific antibodies from day 5 and onwards of illness,[22] A study from Thailand observed that nearly 80% of patients with dengue virus infection were detected four days after the onset of symptoms, and this rose to over 90% by day 5.[26] Similarly, other studies have also detected that most dengue patients produced dengue-specific antibodies by day 5 of illness.[12,22,27] Therefore, these cases would have been interpreted as negative if they were not re-tested after five days. Thus, for the detection of dengue-specific antibodies, patients should be tested from day 5 of fever and onwards.[28]

The rapid ICT showed good sensitivity and specificity in our study which is comparable to IgM and IgG Capture ELISA. Moreover, a positive correlation was observed between ELISA and ICT. While some studies have reported very high (99%–100%) sensitivity and (88%–96%) specificity of rapid test (ICT) for dengue diagnosis,[22,29] other studies have reported 45.8%–67% sensitivity and 33.3%–53.8% specificity.[28,30] In another study using Panbio ICT, IgM showed 67.3% sensitivity, 91.7% specificity, 89.7% positive predictive value and 72.1% negative predictive value, while IgG showed 66.4% sensitivity, 94.4% specificity, 97% positive predictive value and 51.0% negative predictive value.[19] Other studies also offer a conclusion in favour of rapid ICT.[29,31] Thus, dengue rapid ICT may be a useful tool in the diagnosis of dengue fever as it is rapid, easy to perform and can be used in settings where laboratory equipments such as ELISA, PCR or cell culture are not available.

Our study showed that most patients were not very keen to visit the hospital again to be re-tested as they recover within seven days of their first test. Therefore, early and rapid diagnosis of dengue virus infection from a single serum sample is extremely important. Single serum samples are convenient for identifying most of the dengue cases by both ELISA and ICT methods. However, for early diagnosis, and where laboratory equipments are available, ELISA is more suitable than ICT.
Acknowledgments

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References


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