Original Research Article

Evaluation of NS1 Antigen Detection as Point of Care Test against Other Dengue Markers

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A B S T R A C T

Early and accurate diagnosis is need for hour in cases of dengue virus infection. The present study was conducted to evaluate the usefulness of the dengue NS1 antigen with other dengue biomarkers. A total of 306 samples collected from the fever of unknown origin (FUO) during the period July 2014 to March 2015 were tested for dengue fever parameters such as dengue NS1 antigen, dengue antibody tests (IgM, IgG) by rapid immunochromatography, RT-PCR for the presence of dengue virus RNA and CBC. Out of 306, 47(15%) were positive for dengue virus infection by RT PCR, among them 89.4% were infected with single DENV serotype and 10.6% had concurrent infection. 45 samples were positive for NS1 antigen test, of which 43 were positive in samples which were positive for RT PCR and 2 samples were positive in RT PCR negative samples. The sensitivity and specificity of NS1 antigen detection was 91.5% and 99.2% in the early diagnosis of dengue fever. It doesn’t require any special instruments and it is easy to perform and to interpret. It can be used easily in the peripheral laboratories for the early diagnosis of dengue fever where the facilities are minimal.

Keywords
Dengue fever, NS1 antigen, RT-PCR, Dengue antibodies

Introduction
Dengue has become a major cause of human morbidity and mortality in International Public Health. Globally, more than 2.5 billion people live in areas where there are risks of exposure to dengue virus infection and worldwide WHO estimates about 50 million
people are infected with dengue virus every year (WHO, 2000). Controlling and the rapid diagnosis of dengue infections is the major challenge faced by the stakeholders. Dengue virus infection with any one of four serotypes produces a spectrum of clinical illness ranging from asymptomatic to its most severe form like dengue hemorrhagic fever and dengue shock syndrome.

In view of high mortality and morbidity rates, it is imperative to have rapid and sensitive laboratory assay for early detection of the disease. For a diagnosis of “confirmed dengue,” dengue virus should be identified by isolation or there should be a 4-fold rise in paired serum antibody titer. Several methods have been applied for laboratory diagnosis of dengue virus infections. These approaches include detection of the virus, detection of virus antigen, and detection of anti-dengue virus antibody by hemagglutination inhibition, complement fixation test, neutralization test, or ELISA, and detection of virus nucleic acids by reverse transcription PCR or real-time RT-PCR (Huhtamo, 2010). Recently, commercially available kits for the detection of dengue virus NS1 antigen have been developed, and studies have shown that dengue virus NS1 antigen could be useful for the detection of early stages of dengue virus infections (WHO, 2000; Chippaux, 1991; Guzman, 2004; Alcon, 2002; Blackshell, 2008).

In this study, we describe the evaluation of Immunochromatographic technique that is designed to detect the presence of dengue virus NS1 antigen and dengue virus antibodies in samples from patients with dengue virus infection

**Materials and Methods**

A total of 306 sera were collected from patients with suspected dengue infection during the period of July 2014 to March 2015. These patients were selected based on a clinical diagnosis of dengue infection and fulfilled the WHO case definition for dengue fever. Dengue NS1, IgM and IgG was performed by using Immunochromatographic kit (J Mitra diagnostics). The manufacturer’s instructions were followed in the procedure and results were interpreted as reactive and nonreactive.

Viral RNA was extracted from serum samples using the QIAamp Viral RNA mini kit (Qiagen, Germany) according to the manufacturers’ instructions. Extracted RNA was stored at −70°C or immediately used for RT-PCR. RT and PCR were performed in one tube using a universal primer and a one-step RT kit (QIAGEN, GmbH, Hilden, Germany); the reaction was then placed in a thermal cycler (Eppendorf). The preliminary product was further used for nested PCR in another reaction tube (Yamada, 1999). Nested PCR was performed with a thermal cycler. The secondary PCR product was subjected to agarose gel electrophoresis using a 2% agarose gel (Bangalore Gene) in Tris—borate buffer, followed by staining with ethidium bromide and visualization on a UV transilluminator at 302 nm (Eles 2005).

**Statistical analysis**

Assessment of efficiency, sensitivity, specificity, positive and negative predictive values of NS1 antigen was calculated using SPSS software version 16.

**Results and Discussion**

Median age of the study population was 10 years and the sex ratio (male/female) was 1.4.

Out of 306 samples subjected to RT-PCR, 47 tested positive for dengue virus, among
them 89.4% were infected with single DENV serotype and 10.6% had concurrent infection. DENV 2 dominated the outbreak among the single DENV serotype and among the concurrent infection DENV 2 and DENV 3 combination was more prevalent.

45 samples were positive for NS1 antigen test, of which 43 were positive in samples which were positive for RT PCR and 2 samples were positive in RT PCR negative samples (Table 1).

The results of dengue NS1 antigen detection were also compared to the results of dengue antibody tests (IgM and /or IgG). A total of 45 samples positive for NS1 antigen, 30 cases were positive along with dengue antibody tests (Table 2).

Sensitivity of NS1 antigen in detecting dengue fever was 91.5%, specificity 99.2%, positive predictive value is 95.6%, and negative predictive value is 98.5%.

Platelet count less than 40,000 was observed in 29 (61.7%) patient and a platelet count between 40,000 to 80,000 was observed in 13 (27.7%) and a platelet count of >100,000/mm$^3$ was observed in 5 (10.6%) (Table 3). Hematocrit value greater than 20 was observed in 20 RT-PCR positive cases. Liver function tests were derailed in 51.1% and renal function test showed higher value in 31.9% of dengue cases.

The sensitivity and specificity of NS1 antigen detection was 91.5% and 99.2% and positive predictive value was 95.6% and negative predictive value was 98.5%.

Efficient and accurate diagnosis of dengue is of primary importance for clinical care i.e. early detection of severe case, case confirmation and differential diagnosis with other infectious diseases. As the initial symptoms of dengue mimic those of malaria, typhoid and leptospirosis which are endemic in the country, availability of a rapid and differential diagnosis at an early stage of infection is utmost importance for better patient management (Wiwanitikit, 2010). For long time detection of dengue specific antibody has been main stay of diagnosis of dengue infection (Hu, 2011; Guzman, 2010).

**Table 1** Correlation of Dengue NS1 antigen test compared to RT-PCR

<table>
<thead>
<tr>
<th>RT-PCR</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>43</td>
<td>02</td>
<td>45</td>
</tr>
<tr>
<td>Dengue NS1</td>
<td>04</td>
<td>257</td>
<td>261</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>259</td>
<td>306</td>
</tr>
</tbody>
</table>

* Sensitivity: 91.5%
* Specificity: 99.2%
* Positive Predictive value: 95.6%
* Negative Predictive value: 98.5%
Table 2: Correlation of Dengue NS1 antigen test compared to dengue antibody tests

<table>
<thead>
<tr>
<th>Antibody test (IgM and /or IgG)</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dengue NS1 Positive</td>
<td>30</td>
<td>15</td>
<td>45</td>
</tr>
<tr>
<td>Dengue NS1 Negative</td>
<td>04</td>
<td>257</td>
<td>261</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>272</td>
<td>306</td>
</tr>
</tbody>
</table>

Table 3: Laboratory Investigations in patient with dengue fever

<table>
<thead>
<tr>
<th>Investigations</th>
<th>Numbers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit ≥ 20%</td>
<td>22 (46.8)</td>
</tr>
<tr>
<td>Platelet Count ≤40,000/mm³</td>
<td>29 (61.7)</td>
</tr>
<tr>
<td>Platelet Count 40,000 – 80,000/mm³</td>
<td>13 (27.7)</td>
</tr>
<tr>
<td>Platelet Count &gt;100,000/mm³</td>
<td>5 (10.6)</td>
</tr>
<tr>
<td>Aspartate Aminotransferase &gt;40 U/L</td>
<td>24 (51.1)</td>
</tr>
<tr>
<td>Alanine Aminotransferase &gt;40 U/L</td>
<td>24 (51.1)</td>
</tr>
<tr>
<td>Creatinine Kinase &gt;175 U/L</td>
<td>15 (31.9)</td>
</tr>
<tr>
<td>Partial thromboplastin time &gt;2 fold</td>
<td>16 (34.0)</td>
</tr>
</tbody>
</table>

Serological IgM-ELISA and IgG-ELISA are currently widely used for dengue fever diagnosis in routine laboratories (Young, 2002; Dussart, 2006). The tests are user friendly and robust; however, the sensitivity of these tests does not become acceptable until five days after the onset of fever. Accuracy of these tests is dependent on the timing of their sample collection. Moreover, a single serological detection of IgM is merely indicative of recent dengue virus infection and a paired second serum sample is required for confirmation (Kumarswamy, 2007). Additionally, IgM detection is not always conclusive because of cross reactivity with other flaviviruses. Therefore, early laboratory diagnosis (within 1–2 days of illness) will be more important. Virus isolation is carried out only by reference laboratories and is a time consuming and expensive technique. The use of dengue RT-PCR in most laboratories is currently difficult due to the cumbersome procedure, difficult interpretation and the time taken (Conceicao, 2010).

Recently, an up-to-date test for early diagnosis of dengue infection is dengue NS1 antigen detection. This is possible due to the presence of antigens in the early phase of infection. Viremia in DENV infection generally lasts from 4 to 5 days and DENV antigens remain detected in patients with both primary and secondary dengue infection up to 9 days. There are both ELISA and immunochromatographic test
(rapid test) formats available in the market for the detection of NS1 antigens (Ramirez, 2009; Zainah, 2009; Datta, 2010). These diagnostic test kits, especially the immunochromatographic strip kits, can help early diagnosis and shorten the turnaround time (Watthanaworawit, 2011). NS1 protein is highly conserved for all dengue serotypes, circulating in high levels during the first few days of illness. There is no cross reaction of dengue NS1 protein with those of other related flaviviruses (Guzman, 2010). Plasma viremia levels would associate with the detection of plasma NS1; since NS1 like virions is product of infected cells. Srivastava et al. (2011) have reported that viremia levels significantly higher in patients who were NS1 positive versus those who were NS1 negative.

In the present study evaluation of the NS1 assay indicated moderately high sensitivity and very high specificity to dengue infection. Out of 45 NS1 positive cases, 15 cases were detected on the day 2 of the dengue fever and all these 15 cases were negative for dengue antibodies. While correlating various dengue specific parameters with thrombocytopenia, we found that out of 47 RT-PCR positive cases, 42 cases had the platelet count less than 100,000/ml. Out of 45 exclusive NS1 positive cases, thrombocytopenia was evident in 39 cases.

The results obtained from this study allow the laboratory to consider a combination of tests that are suitable to increase the sensitivity. Since it is not possible to set up a PCR laboratory in the rural settings, it is advisable to use the NS1 antigen detection as an alternative method for the speedy diagnosis of dengue fever. This study doesn’t advocate replacing the current antibody test used in the laboratory, but NS1 could be used as complement. The combination of tests definitely increases the sensitivity and the specificity for the early diagnosis of DENV infection.

Acknowledgement

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References


