NS1 Antigen and Immunoglobulin M detection in the Acute and Early Convalescent Stages of Dengue Fever

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Abstract

Background: To evaluate the efficacy of NS1 Antigen assay and Ig M in the diagnosis of dengue virus infection in its acute and early convalescent stages

Methods: This prospective study was conducted in the Department of Medicine, DHQ Teaching Hospital Rawalpindi from 1st October 2010 to 10th November 2010. It included all 105 patients who presented with dengue fever during this period. Serum samples of all patients were sent for detection of NS1 Ag, Ig M and Ig G besides other routine investigations. The first four days of clinical illness were taken as the acute stage of the disease while the period from day 5 to day 10 was designated as the early convalescent stage. The efficacy of NS1 Ag and Ig M antibody in both stages was evaluated.

Results: The 105 patients comprised of 74 males and 31 females (male to female ratio 2.4:1). Age of patients ranged from 14 to 80 years. Mean age was 33.34 years. Thirty one patients presented in the acute stage of illness while 72 were seen in the early convalescent stage. NS1 antigen was detected in 29 out of 31 patients presenting in the acute stage of dengue infection (93.55%) while Ig M was positive in 7 patients (22.58%). In the early convalescent stage, NS1 antigen was detected in 12 of the 72 patients presenting in this period (16.6%). Ig M was positive in 64 out of 72 patients (88.88%) in this stage.

Conclusion: NS1 antigen assay is a very reliable test to diagnose dengue fever in the acute stage of illness. Detection of immunoglobulin M is a dependable test for diagnosis in the early convalescent period of the disease.

Key Words: Dengue Fever, NS1 Antigen, Immunoglobulin M, Acute Stage, Early Convalescent Stage.

Introduction

Dengue fever (DF) is the most important arthropod-borne viral disease in terms of human morbidity and mortality. Each year over 100 million dengue infections occur leading to 500,000 hospitalizations and an estimated 50,000 deaths. According to World Health Organization (WHO) estimates, its incidence has increased by a factor of 30 over the last 50 years. It is now endemic in more than 100 countries and poses a threat to more than 2.5 billion people. No effective therapy exists.

Dengue virus (DV) is transmitted to humans by *Aedes aegypti* (a.k.a. *Stegomyia aegypti*) mosquitoes. The clinical spectrum ranges from a self-limiting infection to more severe forms like “Dengue Haemorrhagic Fever” (DHF) or “Dengue Shock Syndrome” (DSS) where a mortality rate of up to 40% may be seen. The viral genome is approximately 11 kilobases long and contains a single open reading frame encoding a polyprotein precursor of about 3400 amino acids. Co- and post translational processing of the polyprotein by cellular and viral proteases gives rise to three structural proteins, designated C (core) protein, M (membrane) protein and E (envelope) protein as well as seven non-structural proteins, NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5.
Detection of secretory NS1 protein is a recent development and a new diagnostic tool for the diagnosis of acute DV infection. The purpose of this study was to evaluate the efficacy of NS1 Ag assay and Ig M in the diagnosis of dengue virus infection in its acute and early convalescent stages.

Patients and Methods

This prospective study was conducted in the Department of Medicine, DHQ Teaching Hospital Rawalpindi, a tertiary care referral centre of Rawalpindi Medical College. It included all 105 patients presenting with dengue fever from 1st October 2010 to 10th November 2010. All were aged over 14 years and presented with an acute febrile illness. Dengue fever was suspected if two or more of the following features, in addition to fever were present: headache, retro-orbital pain, myalgias, arthralgias, scarlet/macular rash, vomiting/abdominal pain and haemorrhagic manifestations. Laboratory investigation criteria were also laid down. Patients with acute febrile illness having leucopenia (TLC < 4000/ cmm) and/or thrombocytopenia (platelets < 150000/ cmm) and deranged transaminases were also worked up for dengue fever. Blood samples for dengue virus Ig M were sent to National Institute of Health, Islamabad and our own hospital laboratory where the tests were performed by standardized ELISA method.

Samples for detection of NS1 Ag in sera were sent to a reliable laboratory offering this test. Immunochromatography method (ICT/Tan Bio Company) was employed. In this immunological assay, NS1 antigen present in the patient’s sample binds with colloidal gold complexes containing anti-NS1 antibody. A procedural control is included to indicate that the assay has been performed correctly. Sensitivity and specificity of the assay is 80% and 95% respectively. A blood complete picture, liver function tests, serum urea and creatinine, prothrombin time and urine R/E were also obtained. Considering the overlapping signs and symptoms and endemic nature of typhoid and malaria, all patients also underwent appropriate smear or serological tests for these diseases (Typhidot and malarial parasite smears).

Once a definite diagnosis of dengue fever was established, only symptomatic treatment was advised and the patient monitored for DHF and DSS using current guidelines of WHO and Pan American Health Organisation16.

Dengue haemorrhagic fever was diagnosed when the patient fulfilled the following criteria: fever, any haemorrhagic manifestations and thrombocytopenia (platelet count 100,000/cmm or less). Objective evidence of increased capillary permeability, incipient and frank circulatory failure was taken to represent Dengue shock syndrome.

The “Acute Stage” of dengue viral infection was defined as the period from day 1 to day 4 of the clinical illness while the period from day 5 to day 10 was designated as the “Early Convalescent Stage”. The efficacy of NS1 Ag and Ig M antibodies in both the stages of dengue infection was evaluated.

The clinical presentations and laboratory findings were recorded on a proforma especially designed for the purpose. Descriptive analysis was performed using SPSS v 10.0 programmer.

Results

The 105 patients studied comprised of 74 males and 31 females (male to female ratio 2.4:1). Age of patients ranged from 14 to 80 years. Mean age was 33.34 years. The highest incidence occurred in those aged between 16 to 41 years, with 71 patients (68%) figuring in this age range.

Thirty one patients presented in the acute stage of the illness while 72 were seen in the early convalescent stage. Two patients came on the 14th and 15th day of the clinical illness. Both were Ig M positive and NS1 Ag negative for dengue fever.

<p>| Table 1: NS1 Ag and Ig M antibody detection in the “Acute Stage” (n=31) |
|-----------------|------------------|------------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Day</th>
<th>Patients</th>
<th>NS1 Ag Positive</th>
<th>Ig M Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>2</td>
<td>2 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Two</td>
<td>5</td>
<td>4 (80%)</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>Three</td>
<td>11</td>
<td>11 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Four</td>
<td>13</td>
<td>12 (92.3%)</td>
<td>6 (46.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>29</td>
<td>7</td>
</tr>
<tr>
<td>Positivity</td>
<td>93.55%</td>
<td>22.58%</td>
<td></td>
</tr>
</tbody>
</table>

NS1 antigen was detected in 29 out of 31 (93.55%) patients presenting in the acute stage of dengue infection while Ig M was positive in 7 (22.58%) patients in this stage (Table 1). One patient who was Ig M positive on day 2 was also Ig G positive for dengue.

In the early convalescent stage NS1 antigen was detected in 12 of the 72 (16.6%) patients presenting in
this period. Ig M was positive in 64 out of 72 (88.88%) patients in this stage (Table 2).

Table 2: NS1 antigen and Ig M antibody detection in the "Early Convalescent Stage" (n=72)

<table>
<thead>
<tr>
<th>Day</th>
<th>Patients</th>
<th>NS1 Ag Positive</th>
<th>Ig M Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Five</td>
<td>21</td>
<td>10 (47.6%)</td>
<td>15 (71.4%)</td>
</tr>
<tr>
<td>Six</td>
<td>14</td>
<td>0</td>
<td>14 (100%)</td>
</tr>
<tr>
<td>Seven</td>
<td>24</td>
<td>2 (8.3%)</td>
<td>23 (95.8%)</td>
</tr>
<tr>
<td>Eight</td>
<td>7</td>
<td>0</td>
<td>7 (100%)</td>
</tr>
<tr>
<td>Nine</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ten</td>
<td>6</td>
<td>0</td>
<td>5 (83.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>12</td>
<td>64</td>
</tr>
<tr>
<td>Positivity</td>
<td></td>
<td>16.6%</td>
<td>88.88%</td>
</tr>
</tbody>
</table>

NS1 antigen detection registered a sharp fall on day 5 of the clinical illness when it was positive in 10 out of 21 patients (46.7%). However Ig M positive patients quickly rose to 71.4% the same day reaching 100% on day 6, being positive in all 14 patients tested on that day of illness (Table 2).

Thirty five of our 105 patients developed clinical features of DHF. They were managed with platelet concentrates and blood transfusions. None developed signs of DSS. There was no mortality in this time period with all 105 patients recovering including those with DHF.

Discussion

This is not the first time that dengue fever has assumed epidemic proportions in Pakistan. The previous outbreak on a country-wide scale occurred in the last three months of 2006. During that period 220 patients were managed by the present authors in Rawalpindi General Hospital (now Benazir Bhutto Hospital) Rawalpindi. These included 22 patients with DHF. All patients subsequently recovered.17

To limit disease expansion and bring down the significant mortality rate associated with DF, it is essential to have a rapid and sensitive laboratory test for early detection of the disease. NS1 Ag circulates uniformly in all serotypes of dengue virus and it circulates at high level during the first few days of illness.18 Antigen levels as high as 2 to 10 mcg/ml may be found in acute stage sera4 while in the convalescent stage these may be 0.4 mcg/ml or less12. This is the likely explanation for the much higher detection rate of NS1 Ag in acute phase serum samples. It is possible that with production of specific immunoglobulins in the early convalescent stage, immune complexes are formed, resulting in low NS1 Ag positivity.

Our study demonstrated a high detection rate of NS1 Ag (93.55%) in the acute stage of illness. Rates above 90% have also been confirmed by other researchers10, 19. Datta et al11 noted a rapid decline in NS1 Ag positivity in the early convalescent stage with only 28.57% of their patients testing positive. This was even lower (16.6%) in our study. NS1 Ag may also be an indicator of disease severity18. In one study, very high levels, observed within 72 hours of illness, identified patients at risk of developing DHF.14 A quantitative estimation of NS1 Ag was not carried out in this study.

Ig M positivity in excess of 90% in the early convalescent stage has been demonstrated by various authors10, 11, which is in conformity with our findings (88.9%). Ig M positivity in the acute phase of DF has been found to be as low as 6.38%11, whereas it was 22.58% in our study. However, most of our Ig M positivity in the acute stage became evident on day 4 of DF and most of these patients also tested positive for dengue Ig G. We infer that Ig M rises earlier in Ig G positive patients.

Day 5 of clinical disease seems to be important from a diagnostic viewpoint since NS1 Ag serum levels start to fall whereas Ig M levels register a rise. Thus a negative NS1 Ag test at this juncture, coupled with a negative Ig M does not necessarily rule out DF. In such patients, we recommend that Ig M be tested again after 48 to 72 hours since antibody levels become detectable in most cases around this period.

The short time frame of our study impeded us from assembling any control group of consequence. However, seven patients with typhoid seen during this period did undergo dengue serological tests. Both Ig M and NS1 Ag were negative in all cases. Studies done on large control groups have established 100% specificity for both NS1 Ag and Ig M detection serological tests.10, 11. Dengue serology is also negative in yellow fever, a disease to which it bears many a similarity10.

In conclusion, NS1 Ag assay is a very reliable test to diagnose DF in the acute stage of illness. Detection of immunoglobulin M is a dependable test in the early convalescent period. A diagnostic strategy combining NS1 Ag testing of serum samples collected within 5 days of the onset of fever and Ig M testing of serum samples obtained in the early convalescent stage seems to be the best policy to diagnose dengue fever in the vast majority of patients.

References