PRESENCE OF BOTH DENGUE SPECIFIC NS1 ANTIGEN AND IMMUNOGLOBULIN M ANTIBODY IN CONFIRMED DENGUE PATIENTS IN KOLKATA

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Abstract
Purpose: The object of this study was to identify patients with diagnosed dengue infection, who were positive for both dengue-specific NS1 antigen and IgM antibody.

Method: From January 2013 to December 2016, in Central Kolkata in West Bengal in India, patients with symptoms of dengue infection, were sent to the laboratory by the physicians for confirmatory diagnosis of dengue infection. A total of 4762 patients were seen, and serum samples tested and distributed into seven panels, according to the investigations requested. 1436 patients were tested positive.

Results: 1053 cases were tested for both NS1 and IgM antibody, 835 for dengue-specific NS1 antigen, IgM and IgG antibodies and 218 for NS1 dengue-specific antigen and IgM antibody. Of these, dengue was confirmed in 34.3%, with 16.6% positive for both NS1 antigen and IgM antibody. Eleven were diagnosed, with late dengue infection, thirty-nine with late primary infections and ten with late secondary dengue infection.

Conclusions: Many of the patients were reactive for both NS1 antigen and IgM antibody, and they required proper attention and strict vigilance with effective monitoring and treatment, not of early dengue infection, but of late dengue infection. Unless the serological tests for IgM and IgG antibodies, and the dengue specific viral antigen NS1 are performed simultaneously, these types of cases would not all be detected.

Keywords: Dengue, NS1 antigen, IgM and IgG antibodies, Primary and Secondary Dengue, Early Dengue, Late Primary, Late Secondary and Late Dengue

Introduction
Dengue is endemic in Kolkata. Dengue haemorrhagic fever first occurred in India in the city of Kolkata from 1962 to 1965, when about 100,000 persons were hospitalised, out of whom 200 succumbed (1,2). In the new millennium, in 2005, a dengue outbreak occurred in an epidemic form involving all the districts of West Bengal. Altogether 6293 persons were serologically diagnosed to be suffering from dengue through detection of IgM antibodies, with 27 (0.42%) deaths. In Kolkata alone, 3967 persons were infected, with 14 deaths (0.35%) (3).

In a monitoring centre in central Kolkata, in the years 2005 to 2011, the percentage of serologically confirmed dengue cases was reported to be between 9.2%, to 24.9% (4). In 2012, the incidence had increased to 38.3%, with 725 found positive out of 1892 suspected cases (5). Outbreaks of dengue were occurring in Kolkata often and with the worrying peak of 2012. During the transmission season, from July to November patients suspected with dengue were referred to the clinical laboratories to confirm the diagnosis (6).
The different methods of diagnosis of dengue as stated by WHO were the direct method of virus isolation, genome detection and antigen detection with the nonstructural protein 1 (NS1); and the indirect serology method with IgM and IgG detection. When diagnostic tests were compared according to confidence in the test and its accessibility, the confidence level gradually decreased from virus isolation, genome detection and NS1 antigen to IgM and IgG antibody with the least confidence in IgG; while accessibility gradually increased from virus isolation to the serological estimation of IgG (7). For virus isolation and genome detection, highly specialised laboratories were required, and the methods were time-consuming. On the other hand, the rapid antigen test for dengue-specific NS1 and the serological tests for IgM and IgG antibodies became extremely popular dengue diagnostic tests in laboratory practice in India.

The dengue-specific NS1 antigen could be detected in the serum from the onset of illness (8-11). For this reason, this test was most often used to detect dengue at an early stage, facilitating better monitoring and treatment. However, according to WHO (12), NS1 antigen could be detected up to nine days after the onset of the disease (8). Dengue-specific IgM antibody usually appeared five days after the onset of illness in 80% of the cases (12, 13) and its presence in the blood indicated that the patient was in a late stage of infection.

A recent trend in clinical practice was to examine for only NS1 antigen in the suspected early cases of dengue, and in the suspected late cases, examination of IgM and IgG antibodies or NS1 antigen, and IgM antibody was advocated. The patients in Kolkata had been advised for either all three tests for NS1 antigen, IgM and IgG antibodies together or only NS1 antigen and IgM antibody tests, as confirmed dengue cases from these two groups could be both NS1 and IgM reactive.

The study aimed to determine the proportion of confirmed dengue cases in which both dengue specific NS1 antigen and IgM antibody were present.

**Methods**

The study was conducted in the Gautam Laboratories in Central Kolkata. The study period was from January 2013 to December 2016. Patients with suspected dengue infection were sent to the laboratory by the physicians, for a specific diagnosis of dengue. For detection of dengue-specific NS1 antigen and IgM and IgG antibodies, the ELISA method was employed (5), and the procedure of Bhattacharyya et al. (5) was followed. BIO-RAD kits were used for this purpose (14). Vircell kits were used to perform MAC ELISA (15) to demonstrate IgM antibodies. For the detection of IgG antibodies, BIO-RAD kits were utilised (14). Instructions of the manufacturers were meticulously followed while performing the tests.

According to the different serological tests, either in combination or alone, the samples were placed in different panels, as suggested by Bhattacharyya et al. (5). The panels were classed as Panel I for NS1 antigen, Panel II for IgM antibody, Panel III for NS1 antigen, IgM & IgG antibodies, Panel IV for only IgM and IgG antibodies, Panel V for NS1 antigen and IgM antibody, Panel VI for NS1 antigen and IgG antibody, and Panel VII for IgG antibody. With these panels of I, II, III, IV, V, VI and VII, there were 3044, 174, 835, 485, 218, 4 and two patients respectively for a total of 4762 samples.

The ethics approval was duly obtained from the ethical committee of the organisation to conduct this study.

**Results**

During the four year study period from 2013 to 2016, serum samples from 4762 patients with a provisional diagnosis of dengue, was distributed into the seven panels, according to the different serological tests results. Dengue was diagnosed in 30.2% of the suspected cases. The rate of infection varied from 18.3% in 2013 to 36.8% in 2015 (Table 1).

In the study, the physicians advised 64.0% of the patients (3044) to test only for NS1, 17.5% (835) to test for NS1, IgM and IgG, 10.2% (485) to test for IgM and IgG, 4.6% (218) to test for NS1 and IgM and 3.7% (174) to test for IgM. Of these, 28.7% (872/3044) in Panel I, 34.5% (60/174) in Panel II, 34.7% (290/835) in Panel III, 29.5% (143/485) in Panel IV, and 32.6% (71/218) in Panel V were positive for dengue.

To determine the incidence of both dengue-specific NS1 antigen and IgM antibody in the serum samples, the panels III and V were analysed. There were 361 confirmed dengue cases altogether, and both NS1 antigen and IgM antibody were found to be present in 16.6% or 60 of the patients. In the years 2013, 2014, 2015 and 2016, the numbers of such cases were nine, six, twenty-eight, and seventeen respectively. Of these sixty cases, forty-nine and eleven were detected in the panel III and panel V respectively; thirty-nine, ten, and eleven were classed as late primary, late secondary and late dengue cases respectively (Table 2).

**Discussion**

Out of a total of 4762 suspected cases of dengue infection, in the four year study period, dengue infection was confirmed in 1436 patients, with an incidence of 30.2%, indicating a high activity of the virus in the area. 18.3% (78 out of 426) in 2013, 29.2% (179 out of 611) in 2014, 36.8% (606 out of 1648) in 2015 and 27.6% (573 out of 2077) in 2016 were found to be infected with dengue. Compared with the first two years of the study period, major outbreaks of dengue occurred in Kolkata in the last two consecutive years of 2015 and 2016, after 2012.

In the present series, of the 1436 dengue-infected patients, 25.1%, in panels III and V, tested positive for both NS1 antigen and IgM antibody, 49 patients in Panel III and 11 patients in Panel V were positive for both. 39 (79.6%) and 10 (20.4%) of these patients were suffering from primary and secondary dengue respectively.
Table 1: Dengue diagnostic panel (serological) in suspected cases in Kolkata (2013-2016)

<table>
<thead>
<tr>
<th>Year</th>
<th>Panel:</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>Total</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NS1</td>
<td>IgM</td>
<td>NS1+IgM+IgG</td>
<td>IgM+IgG</td>
<td>NS1+IgM</td>
<td>NS1+IgG</td>
<td>IgG</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td>426</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>611</td>
</tr>
<tr>
<td>2015</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1648</td>
</tr>
<tr>
<td>2016</td>
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<td></td>
<td></td>
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<td></td>
<td>4762</td>
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</tbody>
</table>

Legend: E = Examined, R = Reactive, ND = Not a case of dengue, LD = A late case of dengue, O = Old case of dengue, EP = Early primary case, ES = Early secondary case, LP = Late primary case, LS = Late secondary case, ED = A dengue case, detected in early stage, ESD = An early secondary dengue case, NOD = Not an old dengue case
The present study identified 16.6% (60/361) dengue patients with both dengue-specific NS1 antigen and IgM antibody, indicating a late stage of dengue infection, of a least a duration of five days where complications might develop. Developing complications would also be more frequent in secondary cases than in primary cases. Warning signs of complications could range from abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleeding, to liver enlargement, with a laboratory increase in packed red cell volume (PCV), concurrent with a rapid decrease in platelet count (7). Patients with warning signs would require strict observation and medical intervention (7).

The criteria for severe dengue would be severe plasma leakage leading to shock with the dengue shock syndrome (DSS). There could be fluid accumulation with respiratory distress and severe bleeding with severe organ involvement, a rise in liver enzymes (AST or ALT > 1000), impairment of the central nervous system (CNS), and heart and other organs involvement (7).

Unless all three available tests for the dengue-specific NS1 antigen, the IgM and the IgG antibodies were done simultaneously, the stage of dengue infection, whether in early stage or in late stage, would not be properly ascertained. Furthermore, it would also not be possible to judge whether the patient was suffering from a primary or a secondary dengue infection. In the study, only 17.5% of 4762 suspected dengue cases were advised by their physicians to have all three serum tests performed. Such knowledge will be invaluable in treating the patient.

Table 2: Distribution of both NS1 antigen and IgM antibody reactive dengue patients (2013-2016)

<table>
<thead>
<tr>
<th>Year</th>
<th>Panel III</th>
<th>Panel V</th>
<th>Panel III</th>
<th>Panel V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Late primary</td>
<td>Late secondary</td>
<td>Late dengue</td>
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<tr>
<td>2013</td>
<td>8</td>
<td>1</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
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<td>6</td>
<td>0</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>2015</td>
<td>24</td>
<td>4</td>
<td>28</td>
<td>18</td>
</tr>
<tr>
<td>2016</td>
<td>11</td>
<td>6</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>11</td>
<td>60</td>
<td>39</td>
</tr>
</tbody>
</table>

Diagnosis of dengue infection in early or late stage and information about primary or secondary infection are two important aims of laboratory investigation of dengue suspected cases. The medical history of the patients related to the initiation of fever in days might not always be reliable. Under these circumstances, the standard protocol should be to perform all the three laboratory tests simultaneously in suspected dengue patients, to provide all the required information to meaningfully monitor and treat the patients.

**Conclusion**

Diagnosis of dengue infection in early or late stage and information about primary or secondary infection are two important aims of laboratory investigation of dengue suspected cases. The medical history of the patients related to the initiation of fever in days might not always be reliable. Under these circumstances, the standard protocol should be to perform all the three laboratory tests simultaneously in suspected dengue patients, to provide all the required information to meaningfully monitor and treat the patients.

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**Competing interests**

The authors declare that they have no competing interests.

**References**