Role of NS1 Antigen in the Diagnosis of Dengue Viral Infection

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Abstract

Background: Dengue is widely distributed in subtropical and tropical areas of the world; it affects all age-groups and is classified as a major global health threat by the World Health Organization. Detection of dengue virus-specific antibodies is commonly used for routine diagnosis.

Methodology: 81 consecutive serum samples were tested for dengue NS1 antigen in addition to dengue IgM and IgG antibodies and the results were analyzed with other parameters.

Results: Out of 81 samples tested for both antigen and antibody, 53 were positive for dengue infection. NS1 antigen was detected in 12 patients. In five of these cases, neither IgM nor IgG was detected.

Discussion: Viral isolation and RT-PCR are time consuming and expensive. Therefore, their use in routine labs is not feasible. In our study, there were more number of secondary dengue infections than primary dengue cases. Among the 13 cases of primary dengue infection, NS1 antigen was the only marker detected in five cases.

Conclusion: Dengue NS1 Ag detection test is highly appropriate for field-testing conditions, particularly for early acute-phase samples, as its sensitivity and specificity far exceeds those of the MAC-ELISA for such samples

Keywords: Dengue infection, NS1 antigen, Dengue IgM and IgG

Background

Dengue is a mosquito-borne viral disease in humans. It is an increasing public health problem in both rural and urban areas, causing lot of mortality and morbidity.

It occurs in tropical areas and affects nearly 100 million people each year. Initial infection with one of the four serotypes of dengue virus may lead to a self-limiting, febrile illness: dengue fever. In some cases, the disease may be associated with more severe manifestations, such as dengue hemorrhagic fever (DHF) and/or dengue shock syndrome.¹ The pathogenesis of DHF is unclear, but it is thought that secondary infection with a different serotype in patients with heterologous dengue antibodies increases the risk of DHF. There has been an increase in dengue infection over the years.²,³ Detection of dengue virus-specific antibodies is commonly used for routine diagnosis. Dengue viremia occurs prior to onset of fever and symptoms and peaks in 2–3 days after onset of symptoms, which is 2–3 days before defervescence. Immune

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responses include production of IgM antibodies produced by the 5th day of symptoms and persist for 30–60 days. IgG antibodies appear by the 14th day and persist for life. Secondary infections often result in high fever and, in many cases, with hemorrhagic events and circulatory failure. Secondary infections show that IgG antibodies rise within 1–2 days after the onset of symptoms, simultaneously with IgM antibodies. Therefore, patients with secondary infections will have a positive IgG result usually, but not always with a positive IgM result.6 Recently, detection of circulating dengue virus non-structural protein NS1 has been described as an alternative method for early diagnosis.7

The objective of our study was to evaluate the role of NS1 antigen in the diagnosis of dengue virus infection.

Table 1. Serological Profile of Dengue Patients

|                | Ag  | IgG | IgG+IgM | Ag+IgG+IgM | Ag+IgG | Ag+IgM | IgM |
|----------------|-----|-----|---------|------------|--------|--------|-----|
| Primary No.:   | 13  | 5   |         |            |        |        |     |
| Sec No.:       | 40  | 8   | 28      | 2          | 3      | 5      |     |

Among the 81 cases tested for NS1 antigen and dengue antibodies, only 53 were positive for dengue fever.

Methodological Protocol of Dengue Patients

Methodology

Institutional ethical clearance was obtained for the study. Of the serum samples that were sent for diagnosis of dengue infection for IgM and IgG ELISA, 81 consecutive samples were tested for dengue NS1 antigen by dengue early ELISA (Panbio) and were compared with IgM and IgG antibodies. Correlation was done with other parameters like platelet count, liver enzymes and clinical features. The procedures for detection of dengue antigen and dengue antibodies were done as per manufacturer’s guidelines.

Results

Out of 81 samples tested, 53 were positive for dengue infections. NS1 antigen was detected in 12 patients. In five of these, neither IgM nor IgG was detected. Of the 53 positive cases, majority were in the 11–20 age group (Fig. 1). Out of the 53 positive cases, 40 (75%) were identified as secondary dengue based on presence of either only IgG or both IgG and IgM antibodies. In these, 4 (10%) were positive for NS1 antigen also. Among the secondary dengue cases, 20 (50%) were of less than 13 years of age. Primary dengue infections were identified in 13 cases based on the presence of IgM antibodies alone or along with antigen also. Among these 13 cases, in 5 (38%) the NS1 antigen was the only parameter detected and 4 (31%) were of <12 years of age. Platelet counts were <1 lakh in 6 (46%) among the primary dengue cases, and in 31 (78%) among the secondary dengue cases. In the 5 cases in which NS1 antigen was detected alone, platelet counts were <1 lakh in all cases. Among the 28 negative cases, four had a low platelet count of <1 lakh. In these patients, two had proven malaria, one had drug-induced aplastic anemia, and one was proven enteric fever.

![Distribution of positive cases among different age groups](image_url)

Figure 1. Distribution of positive cases among different age groups
Discussion

Currently, serological MAC-ELISA for dengue antibodies is routinely used for dengue fever diagnosis; the sensitivity of this test does not become acceptable until 5 days after the onset of fever. Viral isolation is carried out only in reference laboratories and is a time-consuming and expensive technique. The use of dengue RT-PCR is now well documented. However, its use in most laboratories is currently difficult, largely due to the stringent requirements concerning storage temperature, transportation, time between collection and extraction, and laboratory workflow. These two techniques are therefore largely restricted to surveillance systems and research. In our study, dengue cases were prevalent in all age groups, more common in less than 30 year-old patients. Platelet count correlated well with both secondary and primary dengue infections and is similar to that of other studies in India and abroad. In the four cases with low platelet count, but which were negative for dengue parameters, demonstrated other reasons such as malaria and aplastic anemia.

In 5/13 cases of primary dengue, antigen was not detected probably because the blood was collected after the first week. Antigen was detected in low titers in four of the secondary dengue cases. Alcon et al. reported that the NS1 antigen was found circulating from the first day after the onset of fever up to day 9: NS1 levels ranged from 0.04 to 2 μg/mL in acute-phase serum samples (from day 0 to 7), and the level for a convalescent-phase serum (day 8 and later) was 0.04 μg/mL. In secondary infection, NS1 levels ranged from 0.01 to 2 μg/mL and were not detectable for convalescent-phase sera. Another study showed that the NS1 antigen, limited to dengue virus serotype 1, could be detected until day 18 after the onset of symptoms. NS1 antigen and IgM antibodies were detected concomitantly during the acute phase, but from day 1 to day 3, NS1 antigen showed a more sensitive detection. There is an overall increase in secondary dengue infections as compared to primary dengue cases and this indicates the circulation of multiple serotypes, similar to other studies. This leads to more serious DHF/DSS similar to other studies in India. Most of our patients who were diagnosed as cases of dengue presented with fever, body ache and a few with rashes and hepatomegaly. They also had a low platelet count and raised liver enzymes on liver function test. Among the 53 positive cases, 40 were secondary dengue cases. Among the 13 cases of primary dengue infection, NS1 antigen was the only marker detected in five cases. The dengue antigen was detected best when the sample was collected within the first 3 days of symptoms. This study confirms that the detection of dengue virus NS1 antigen using dengue NS1 Ag detection kits is useful for the rapid, early biological diagnosis of dengue disease. Earlier reports observed similar fall in sensitivity of NS1 antigen as days progressed. Had we not used antigen detection, we would have failed to diagnose five cases in the laboratory.

Dengue is one of the major public health problems which can be detected early in the course of the infection and can be controlled with active participation of the community.

Conclusion

This evaluation of the dengue diagnostic tool dengue NS1 Ag test indicates that this test is highly appropriate to field testing conditions, particularly for early acute-phase samples, as its sensitivity and specificity far exceeds those of the MAC-ELISA for such samples. NS1 antigen detection has great potential value for use in epidemic situations, as it could facilitate the early screening of patients and limit disease spread.

Conflict of Interest: None

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