Seroprevalence of Dengue Viral Infection in Patients Attending to a Tertiary Care Hospital in Western Maharashtra and Usefulness of NS1antigen Detection in Diagnosis

Jayawant A.C[a]., Chate S.S. [a]
[a]Department of Microbiology, MIMER Medical College Talegaon Pune, Maharashtra, India

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Abstract

Dengue virus infection has emerged as a public health problem in the last few years in terms of mortality and morbidity associated with it. In the present study, an attempt has been made to know the seropositivity of dengue infection in patients attending BSRTH hospital, Talegaon, Pune. A total number of 282 patients suspected to be suffering from dengue were included in the study. Serum samples were tested for the presence of Dengue IgM and IgG antibodies and Dengue Non structural protein1 (NS1) antigen by using commercial rapid visual Dengue Day 1 test. Of the 282 samples tested, a total of 53 (18.79%) samples tested positive for either one or more of the three markers (NS1,IgM and IgG). Among the sero positives, 20(37.73%) patients were positive for NS1 only,11(20.75%) positive for IgM only; while 13(24.52%) patients had only IgG. More than one marker was detected in the remaining 9 samples. Inclusion of NS1in the diagnosis of Dengue increased the detection rate significantly. Additional 20 cases were detected by NS1antigen test. Therefore, the dengueNS1 antigen test can be used in combination with antibody tests to increase the diagnostic efficiency.

KEYWORDS: NS1antigen, dengue virus, IgM antibody, IgG antibody

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Introduction:

Dengue virus infection is one of the most important human arboviral infections in India. It is spread through the bite of infected Aedes aegypti mosquito. The spectrum of disease ranges from self limited Dengue fever to more severe forms in recent years such as Dengue Haemorrhagic fever/ Dengue Shock Syndrome (DHF/DSS)[1]. Prompt, early diagnosis and treatment are essential to prevent the complications and minimize the mortality rate. Dengue viraemia is of short duration and during this period, the virus, its nucleic acid and circulating NS1 viral antigen can be detected.[2] IgM antibodies can be detected by 3-5 days after the onset of illness. IgG antibodies can be detected in low levels by the end of first week which increase steadily and remain for many years. Several laboratory methods such as virus isolation, genomic RNA, antigen and antibody detection methods are available to diagnose dengue infection. However, methods such as virus isolation and genomic RNA detection (PCR) need a specialized laboratory, well trained laboratory personnel, which are not widely available in hospital settings. Specific antibody detection has been the mainstay of diagnosis which is prone for both false positive and false negative results[3]. The newer parameter NS1 antigen appears to be highly specific and reliable for diagnosis of dengue infection from the first day of fever[4]. It has gained a lot of interest as a new biomarker for early diagnosis. Recently many commercial rapid serological test –kits for detection of NS1 antigen and anti –dengue IgM and IgG antibodies are available.

They are rapid, easy to perform, require less time (result within 10 minutes), does not require skilled personnel and can be used in peripheral setups .

This study was undertaken to know the seropositivity of dengue infection among clinically symptomatic patients attending BSTRH Talegaon, Pune by using Rapid diagnostic test and to see whether the diagnostic efficiency increases by combination of NS1 antigen and antibody tests.

Material and Methods:

The study was conducted at a tertiary care teaching hospital from November 2012 to November 2014. Permission from the institutional ethical committee was taken. A total number of 282 patients suspected to be suffering from Dengue infection were included based on clinical symptoms. The study population comprised individuals of all age groups. Blood samples were collected and the sera were separated for testing Dengue IgM and IgG antibodies and for Dengue Non-structural protein-1 antigen(Dengue Day 1 Test kit J Mitra and Co. Pvt Ltd, New Delhi, India). The tests were performed strictly as per the manufacturer's instructions on all 282 samples. Dengue Day1 test is a rapid solid phase immune-chromatographic test for the qualitative detection of Dengue NS1 antigen and differential detection of IgM and IgG antibodies to dengue virus in human serum/plasma. This is an in vitro test used for the early diagnosis of dengue infection and presumptive diagnosis between primary and secondary dengue infection.

Results

In this study, all serum samples were tested for the presence of dengue NS1 antigen, IgM antibodies and Ig G antibodies. From the 282 samples tested, NS1alone or in combination with either IgM or IgG was positive in 53 (18.79%) cases. Among the sero positives, 24.52% (13/53) were IgG positive; 20.75% (11/53) were IgM positive and 37.73% (20/53) were NS1positive. 9.43% (5/53) were positive for both IgM and IgG. In 5.66% (3/53) cases there was simultaneous detection of NS1antigen, IgM and IgG antibodies. One sample (1/53; 1.88%) was positive for both, NS1 and IgM. Out of 53seropositive samples, 36 (67.92%) samples were of male patients and 17 (32.07%) samples were of female patients. Compared to females, higher seropositivity was found in males. Out of 53 seropositive samples, 3 (5.66%) samples belonged to age group 0-15; 33 (62.26%) samples belonged to patients in the age group 16-40 and 17 (32.07%) samples were of patients above 40 years. Higher seropositivity was seen in the age group 16-40 years.

Discussion

Dengue is one of the rapidly emerging global threats caused by four serotypes of dengue virus, namely DEN-1, DEN-2, DEN-3 and DEN-4 belonging to family Flaviviridae. In India, Dengue is widespread and endemic in most major cities. In this study, 18.79% patients were serologically positive for dengue infection. Study conducted by Garg et.al. has also shown similar type of prevalence (19.7%) [5]. When we compared the prevalence of Dengue infection in our two year study, there was an increase in the number of cases in 2014 as compared to 2013. There was an increase in the number of cases in the months of August, September, October and November i.e. soon after monsoon. Thus the correlation between occurrence of dengue and monsoon season is clearly evident in the study. Increase in prevalence is due to the

breeding of vector mosquito in stagnant water after rainfall. Similar findings have been noticed by Bhat et.al.[6] and studies from Delhi and Chandigarh.[7,8] There was a male preponderance in the seropositives in our study; which correlates with many other studies.[9, 1, 5] The most affected age group was 16-40 years of age. Other Indian studies have also reported 15-45 years as the most affected age group.[10, 11] Among the 20 NS1 positive patients, none was positive for IgM and those would have been missed otherwise had we not included NS1in the test panel. These patients were suffering from a Primary infection in the early phase of illness and were viremic, capable of transmitting the virus if bitten by a mosquito. The NS1 antigen in dengue day-1 test helped in diagnosing additional 33 (20NS1and13 IgG positives) otherwise IgM negative cases. These cases would have been labelled negative if dengue IgM alone had been used. 3 cases that were positive for NS1, IgM and IgG (triple positives) were in the late stage of either a primary or a secondary infection and could be infectious for mosquitoes. The NS1 negatives included 11 IgM positives that had a primary infection presenting a later phase of illness. Among 258 cases that were NS1 negative, there were 229 triple negative cases. It would not be possible to rule out dengue infection in the negative cases without specific tests such as viral replication in cell culture / molecular methods etc for which the facilities are not available in our hospital. In endemic areas, if antibody detection is used for the diagnosis of infection, then one has to rely on rising titres. However, sending repeat samples for demonstrating rise in titres is hardly followed in clinical practice. There is no need of repeat testing if NS1is positive since it is highly specific marker of Dengue infection[12]. Ours is a tertiary care teaching hospital. Being resource poor, we do not have viral culture set up and other facilities. In such circumstances, the laboratory must function with minimum available resources and still provide optimum results to the clinicians for management of dengue patients.

Conclusion

Accurate diagnosis of dengue is very important for clinical care, early detection of severs cases, case confirmation and differential diagnosis. As specific treatment for dengue is not available, rapid immune chromatographic test for detection of NS1antigen and IgM antibodies is very useful for early diagnosis. We found Dengue Day 1 Test kit to be efficient for capturing NS1antigen from infected patients. We could detect and confirm additional 20 cases by using NS1antigen kit which would have been missed otherwise if we had relied on antibody detection methods alone. Moreover, the test was easy to perform, results could be obtained in 10 minutes and did not require any special equipment. Inclusion of NS1in the diagnosis of dengue increased the detection rate significantly.

We therefore feel that the commercial dengue NS1antigen test kits may be used in laboratories with limited resources, lacking viral culture/RT-PCR facilities for the early diagnosis of dengue infection. The combination of the Non- structural protein1 antigen, IgM and IgG antibody test in a duo kit increases the diagnostic efficiency for the early treatment.

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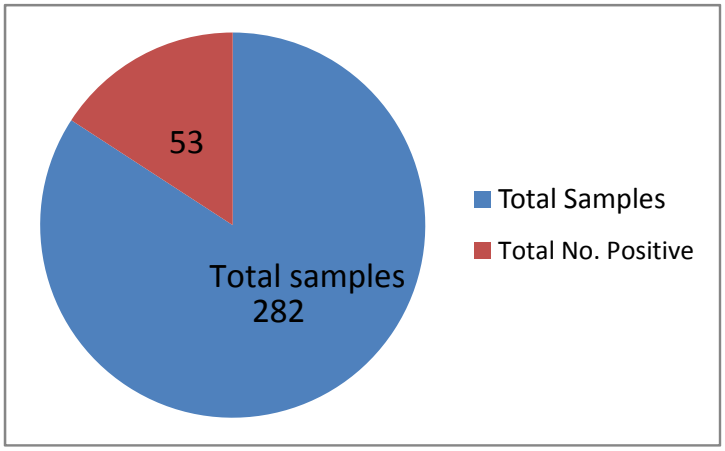
SEROPREVALENCE (Fig 1)



Seropositivity by NS1, IgM and IgG tests (Fig2)

(Total no. of seropositive samples - 53)