Early Diagnosis of Dengue Infection Using Immuno-Chromatographic Technique

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ABSTRACT

Background: Dengue fever is an important mosquito-borne viral disease of humans. Nowadays detection of IgM and IgG antibodies along with NS-1 antigen by immunochromatographic method offers rapid option for presumptive diagnosis of Dengue. Therefore, the present study was conducted to detect the rate of dengue virus infection in clinically suspected cases as well as to know the age, sex and season related seropositivity of Dengue infection.

Material and methods: The study was carried out from January to December 2014 at S.R.T.R. Govt. Medical College, Ambajogai. The clinically suspected cases for dengue were included in this study. Serum samples from such patients were collected and were subjected for detection of NS1 antigen, IgM and IgG antibodies by in vitro solid phase immunochromatographic technique. The test was read after 20 minutes. Details of the patient like age and sex were recorded.

Results: Out of 403 serum samples received during period of one year, 169 (41.94%) were positive. Maximum number of seropositive cases (109) was in pediatric age group. The rate of infection was more common in males. Highest number of positive cases was recorded in September to November.

Conclusion: Immunochromatographic method detecting NS-1, IgM and IgG can give result in only 20 minutes. This helps in early diagnosis and timely intervention that may reduces risk of complications such as DHF and DSS, especially in areas where dengue is endemic.

Key Words: Dengue, NS-1, IgM, IgG, Immunochromatography.
Dengue virus infection is endemic in many parts of the India, and epidemic outbreaks have been frequently reported from Rajasthan, Tamil Nadu, West Bengal, Maharashtra, Punjab, Madhya Pradesh and Delhi. [3] The Nonstructural-1 (NS1) is a highly conserved glycoprotein that is present at high concentrations in sera of dengue-infected patients during the acute phase of the disease. It is found from Day 1 to Day 9 after onset of fever in serum of primary or secondary dengue-infected patients. The IgM antibody can be detected on Day 3 to 5 of illness in case of primary dengue infection and persist for 2 to 3 months, whereas IgG antibody appear by the fourteenth day and persist for life. IgG antibody increases in secondary infection within 1-2 days after onset of symptoms. [4,5]

With increasing incidence of primary and secondary dengue infection, the early laboratory diagnosis may lead to timely clinical intervention and disease control. Nowadays detection of IgM and IgG antibodies along with NS1 antigen by immunochromatographic method offers rapid option for presumptive diagnosis of Dengue compared to other laborious, expensive and time consuming serological and molecular techniques. Therefore, the present study was conducted to detect the rate of dengue virus infection in clinically suspected cases as well as to know the age, sex and season related seropositivity of Dengue infection.

MATERIALS AND METHODS

Type of study: Prospective study

This study was carried out from January to December 2014 at S.R.T.R. Govt. Medical College and Hospital, Ambajogai. The clinically suspected cases for dengue (according to World Health organization criteria) were included in this study. [6]

Serum samples from such patients were collected and were subjected for detection of NS1 antigen, IgM and IgG antibodies by in vitro solid phase immunochromatographic test (J. Mitra & Co. Pvt Ltd). This is a one step assay for qualitative detection of Dengue NS-1 antigen and differential detection of IgM and IgG antibodies to dengue virus in serum. The test was read after 20 minutes. The procedure and interpretation of the test was carried out according to the manufacturer’s instructions.

Details of the patient like age and sex were recorded. Appropriate statistics were applied in the results obtained.

RESULTS

A total of 403 serum samples were received during period of one year out of which 169 were positive by immunochromatographic test for NS-1 antigen, IgM and IgG antibodies singly or in combination (Graph-1, Table no 1).

Table-I: distribution of cases positive by Immunochromatographic test

<table>
<thead>
<tr>
<th>Type of Antigen/Antibody</th>
<th>No. of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS-1 only</td>
<td>103</td>
</tr>
<tr>
<td>NS-1+IgM</td>
<td>11</td>
</tr>
<tr>
<td>NS-1+IgG</td>
<td>9</td>
</tr>
<tr>
<td>NS-1+IgM+IgG</td>
<td>5</td>
</tr>
<tr>
<td>IgM only</td>
<td>11</td>
</tr>
<tr>
<td>IgG only</td>
<td>27</td>
</tr>
<tr>
<td>IgM+IgG</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>169</td>
</tr>
</tbody>
</table>

Graph-I

Distribution of Positive cases
Maximum number of seropositive cases (109) was noted below age of 12 years, i.e. in pediatric age group. (Graph-3)

The rate of infection was more common in males 96(57%) than in females 73(43%). (Graph-4)

Highest number of positive cases were recorded in September (29), October (24) and in the month of November(29). (Graph-5)

**DISCUSSION**

Dengue fever and its severe forms like DHF and DSS have become an important public health problem in many parts of India. Laboratory diagnosis of Dengue infection is very important as clinical presentations not always help in making of accurate diagnosis.

In this study, overall rate of infection was 41.94% by using solid phase immunochromatographic test comprising the detection of NS-1, IgM and IgG antibodies. Jaysimha V.L. et al (2010) have reported rate of seropositivity as 53.54%. [1] Seroprevalence of 52.2% was reported by Quader Ahmed Jailily et al (2013) by using IgM and IgG only in their methodology. [7]

In our study we have observed NS-1 antigen only reactive in 25.56%. Lata patel et al (2013) and Vickers et al (2015) have reported NS-1 antigen positive rate as 16.3% and 32.79% respectively. [8,9] The NS1 is a highly conserved glycoprotein present at high concentrations in sera of dengue-infected patients during the early clinical phase of disease, and is found from Day 1 to Day 9 after onset of fever in serum of
primary or secondary dengue-infected patients.\[^5,10\]

We have also found IgM and IgG antibody positive individually in 11 (2.73%) and 27 (6.70%) serum samples respectively. Dengue virus specific IgM antibodies tend to appear in 3 days after infection and remains in circulation for 1-2 months. IgG antibodies arise at about 7 days, reaches a peak at 2-3 weeks and persist for life.\[^11\] To distinguish primary and secondary dengue infections, IgM/IgG antibodies ratio is nowadays used commonly.\[^12\]

Our study has also reported 11(2.73%) cases positive for NS-1+ IgM suggesting acute or primary dengue infection. Also there were 9 (2.23%), 5 (1.24%) and 3 (0.74%) serum samples positive for NS-1+IgG, NS1+IgM+IgG and IgM+IgG respectively indicating late primary or secondary dengue infection. Similar findings also noted by Arya SC et al (2011) and Stephan et al (2015).\[^13,14\]

Dengue is serologically diagnosed by IgM antibody in acute primary infection and IgG antibody in late primary and secondary infections. Also IgM and IgG shows cross reactivity with other Flavivirus group viruses.

Among the serological markers, NS1 antigen appears to be a better and earlier marker appearing between 1 and 9 days. The NS1 antigen is also highly sensitive and specific which does not cross react with Japanese B encephalitis and yellow fever. Therefore, NS-1 antigen in combination with IgM and IgG antibody enhances detection rate of Dengue virus infection with better sensitivity and specificity.\[^15\]

We observed maximum number (82) of positive cases in September, October and November. Cecilia et al (2012) have reported maximum number of cases in October in their 6 year study in Pune, while Stephen et al (2015) found number of cases peaked in July to October, only few in March.\[^14,16\]

Seasonality of Dengue infection is more during cooler months, with increase in prevalence during post monsoon season. Presence of stagnant water after rainfall increases chances of breeding of mosquito, resulting peak in dengue cases in this season. Therefore vector control measure should be implemented during this duration.

Our study also reported maximum number of cases in age group 0 to 12 years. Our finding matches with studies done at other places.\[^17,18\] Though Dengue infection has been seen as a pediatric health problem it can affect humans of all age group. Studies describing maximum infection in other age group have been also reported.\[^3\]

In this study a higher seropositivity was found in males compared to females. This preponderance might be due to greater outdoor activity of males compared to females. This finding also has been mirrored in studies done by Ukey PM et al (2010) and Quader Ahmed Jalily et al (2013).\[^7,19\]

**CONCLUSION**

Dengue viral infection has been gained a lot of notoriety in these days because of havoc it has caused in last few years. Though highly sensitive and specific methods like Molecular techniques, Viral isolation, Immunofluorescence technique, ELISA etc are available for diagnosis, they are expensive, time consuming and unavailable at peripheral set up. Immunochromatographic method detecting NS-1, IgM and IgG can give result in only 20 minutes. This helps in early diagnosis and timely intervention that may reduces risk of complications such as DHF and DSS, especially in areas where dengue is endemic.

Also combining NS-1 antigen along with detection of IgM and IgG antibodies in rapid test has increased the sensitivity of detecting early/acute infection. These tests can be used successfully for presumptive diagnosis of Dengue infection.
Increase in seropositivity in post monsoon season may give idea to public health personals for prevention and control management of vectors transmitting Dengue viral infection.

REFERENCES


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