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Corelation of immunological markers, platelet count and significance of NS1 antigen in dengue fever

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Abstract
Background: Dengue fever is one of the most important arboviral diseases in man with outbreaks in South East Asia including India. Detection of NS1 antigen, IgM and IgG specific antibodies have been the most important method of diagnosing dengue fever. Dengue viral infection leads to reduction in platelet count. Here we have evaluated the serological markers like NS1 antigen, IgM and IgG antibodies in relation to platelet counts in dengue infection.
Method: Serum samples from clinically suspected dengue fever cases were tested for Dengue NS1 antigen, IgM & IgG antibodies by immuno chromatography based test. Platelet counts were done for the dengue suspected cases and the results were correlated.
Results: Of the 1320 samples tested, 554 were found to be positive for one or more of the immunological markers. Of the 554, 227 were positive for NS1, 12 for IgM and 77 for IgG only; 69 were positive for NS1and IgM, 47 for IgM and IgG, 43 for NS1, IgM and IgG, 10 for NS1 and IgG. Thrombocytopenia was mostly associated with NS1 antigen positive cases i.e., in 63.53%.
Conclusion: NS1 is one of the most important parameters in diagnosis of dengue infection. Thrombocytopenia correlates well with NS1 positive and NS1+IgM positive cases.

Keywords: dengue fever, NS1 antigen, viral fever

Introduction

Dengue is one of the most important arboviral diseases in man with outbreaks in South East Asia including India. It is caused by four serotypes of Dengue virus, namely DEN-1, DEN-2, DEN-3 and DEN-4 belonging to genus Flavivirus and family Flaviviridae1. It is spread through the bite of infected *Aedes aegypti* mosquito. Detection of NS1 antigen, IgM and IgG antibodies have been the most important method of diagnosing Dengue fever. Dengue viral infection leads to reduction in platelet count2.

Dengue is almost endemic throughout India. It is known that early and specific diagnosis of Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS) followed by supportive therapy reduces mortality and morbidity1. Detection of dengue specific antibodies IgM/IgG has been the mainstay of diagnosis of Dengue. Of late, non structural protein 1 (NS1) is available for diagnosis of Dengue fever. NS1 detection is reported to both sensitive and highly specific as well. Apart from dengue specific parameters, platelet count is the only accessory laboratory test available in peripheral areas that can support the diagnosis of DHF or DSS1. Here we have evaluated the serological markers like NS1 antigen, IgM and IgG antibodies in relation to platelet counts in dengue viral infection.

Material and Method

The study was conducted at dept Medicine, Dr RML Institute of Medical Sciences, Lucknow from Apr17 to Dec 201. Serum samples from 1320 clinically suspected Dengue fever cases were tested for Dengue NS1 Ag, IgM & IgG antibodies by immuno chromatography (ICT)

Platelet counts of all the positive cases were recorded. The Dengue specific parameters were correlated with thrombocytopenia**.**

Results

Of the 1320 samples tested, 554 were found to be positive for one or more of the immunological markers. Of the 550, 277 (50%) were positive for NS1 only, 12
(2.16%) for IgM only and 77 (13.8%) for IgG only. 69 (12.4%) were positive for NS1+IgM, 47 (8.4%) for IgM+ IgG and 43 (7.7%) for NS1+IgG. Thrombocytopenia was found in 535 (96.5%) of the seropositive cases. Of the 535, 184 were found to be < 5o,ooo/ ml, 159 were 50,000 – 1lakh/ml and 192 were 1lakh to 1.5 lakh/ml (Table 1). Of the 535 seropositive cases, 357(65%) were showing the onset of thrombocytopenia by 5th or 6th day of onset of fever. Of the 535 seropositive cases, 294(55%) showed normal platelet counts after 5 days of platelet transfusion.

Table 1: Comparison of immunological parameters of Dengue and platelet counts

Parameter	Total	%	<50,000/ml (%)	50,000-1 lakh/ ml (%)	1lakh-1.5lakh/ ml (%)
NS1(p<0.001)	277	50	97(35.01)	79(28.5)	101(36.4)
IgM	12	2.16	6(50.0)	5(41.6)	1(8.3)
IgG	77	13.8	37(48.05)	15(19.48)	25(32.46)

NS1+IgM(p<0.001)	69	12.4	13(18.84)	30(43.47)	26(33.76)
IgM+IgG	47	8.4	19(40.4)	13(27.6)	15(31.9)
NS1+ IgM+IgG	43	7.7	9(20.9)	13(30.2)	21(48.8)
NS1+ IgG	10	1.8	3(30)	4(40)	3(30)
Total	554	100	184	159	192

The chi-square test showed significant correlation (p<0.001) between NS1 and NS1+IgM positive cases and thrombocytopenia.

Discussion

In our study we found that NS1 is one of the most important parameters in diagnosis of Dengue infection. It is followed by IgG, NS1+IgM, NS1+IgM+IgG, IgM and NS1+IgG. The Chi square test showed a very high significant association between the NS1 and NS1+IgM with thrombocytopenia. The role of antibody in the pathogenesis of dengue is well known. Therefore, better association of platelet count with detection of antibody is consistent. NS1 antigen detection method has been proved to be a rapid, easily applicable method.

For a long time detection of dengue specific IgM/ IgG has been the main stay of diagnosis of dengue infection. The dengue specific antibodies begin to appear only around 5th day of fever in primary infection3. Even in secondary infections, both the IgM and IgG antibodies cannot be recorded before 3rd day4. Therefore, there is always a window period, both in the primary and secondary cases when only antibodies are tested. The new parameter, now available for diagnosis of dengue infection, the NS1 antigen, is detectable from day 1 of fever in both primary and secondary infections.

This study has been carried out at a tertiary care teaching hospital. It is worth mentioning here that most tertiary care hospitals lack in viral culture set up. Dengue is in infection that is present in urban, semi- urban and rural areas. Viral cultures and other investigations are available in research and high end laboratories. It is important to conduct studies at peripheral centres, where the laboratories have to function without great technological backup and still provide reasonable opinion to clinicians in the management of infections like dengue. It is in this context that simple and rapid ICT tests for NS1, IgM and IgG are valuable in the diagnosis of dengue.

The limitation of the present study was ELISA and PCR could not be used. ELISA has higher sensitivity than ICT-based tests5. Inclusion of ELISA in this study could have given us an opportunity to comment on the efficacy of ICT to identify dengue infection. In our study precise day of fever at the time of conducting the tests was not identified as majority of cases were referred to us from various places. In spite of this, NS1 was positive in 50% of cases. Given an opportunity to test every case of fever on day 1, more number of cases could have been picked up by NS1. It is shown that titers of NS1 is directly proportional to viral load and viral load is related to complications6. It can be logically inferred that in complication prone cases with high viral load, detection of NS1 will be easier because of higher NS1 levels. We therefore infer that inclusion of NS1 in test panel along with other parameters for evaluation of cases of fever in both endemic and non- endemic areas.

Conclusion

In our study we found that NS1 is one of the most important parameters in diagnosis of dengue infection. Thrombocytopenia is found to correlate well with NS1 and NS1+IgM positive cases.

Conflict of Interest: None

Source of Funding: None

Ethical Clearance: Ethical clearance was taken by Ethical Committee.

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