

Utility of NS1 antigen as an early serological marker in the diagnosis of dengue

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Abstract

Introduction: Dengue infection is one of the most common arboviral infection in India transmitted by the aedes mosquitoes. Early diagnosis of the infection is often missed if only the antibodies are tested. In this scenario detection of the NS1 antigen has gained importance which has very high specificity and can be detected from the first day of fever. Rapid immunochromatographic tests for the detection of both NS1 antigen as well as the IgM and IgG antibodies have been extensively used in the diagnosis of dengue infection because of the ease of performing when compared to ELISA testing. The present study aims in identifying various dengue markers in the early diagnosis of dengue fever.

Materials and Methods: All the suspected dengue samples obtained in the Department of Microbiology were tested by rapid immunochromatography test for the detection of NS1 and IgM/IgG antibodies. Platelet counts were detected by automated analyzer and history and duration of fever has been taken.

Results: A total of 1021 samples were tested for dengue infection out of which 158(15.4%) were positive for one or more of the dengue serology markers. Out of the 158, 85(53.79%) were positive only for NS1 antigen indicating the infection in the early stage, 60(37.97%) samples showed only IgM dengue antibodies and 6(3.79%) samples showed only IgG antibodies. Two serological markers are seen in 7 samples, out of which 4(2.53%) samples were positive for NS1+ IgM antibodies and the remaining 3(1.89%) samples for IgM+IgG antibodies. Majority of the cases were in the age group of 16-30 years and males were predominantly affected when compared to females. Decreased platelet count was observed in 62 out of 158 cases (39.2%). Maximum NS1 positivity was observed from 2nd to 7th day of fever where as IgM positivity was maximum between 7th to 9th day.

Conclusion: Early diagnosis of dengue infection is warranted by the detection of NS1 antigen. Detection of NS1 antigen along with the IgM/IgG will identify the maximum number of dengue cases. Rapid immunochromatography tests have been extremely useful in the diagnosis of these dengue markers.

Keywords: NS1 antigen, Dengue, Serological markers.

Introduction

Dengue is one of the most important arboviral infections in India. It belongs to the flaviviridae and has four serotypes. Dengue hemorrhagic fever and dengue shock syndrome are the two most important fatal complications of dengue. Frequent epidemics are very common in India especially during rainy season because of the socioeconomical factors and ineffective vector control measures.^{1,2} This disease has no vaccine as of now and also no antiviral drugs are available for treatment. Early diagnosis and appropriate symptomatic management of the infection is the mainstay of controlling the epidemics and associated mortality with it. Though reverse transcriptase PCR or isolation of virus are considered the gold standard methods for the diagnosis of dengue infection, none of them can be routinely practiced in the laboratories because of the complexity and practical difficulties associated with them. In this scenario antibody detection tests based on ELISA and rapid diagnostic immunochromatographic method have been widely available for the serological diagnosis of dengue infection.^{3,4} These serological tests detect either IgM and/or IgG antibodies or both. Antibody detection tests have low sensitivity for the early diagnosis because even the IgM antibodies start appearing after the 5th day of fever which delays the diagnosis and increases the mortality. Diagnosis of infection during the first week of fever is extremely important in the clinical management of the patient and to

prevent potential outbreaks. In order to do so, antigen detection tests came to existence which detect NS1 antigen. This antigen is a non structural protein which is a highly conserved protein which can be detectable during the very early phase of the disease. The levels of NS1 antigen in the circulation is very high in the acute phase of the disease and can be detected as early as day 1 of the fever. NS1 antigen is also highly specific in the diagnosis of dengue infection.⁵⁻⁸ With this background, the present study highlights the importance of NS1 antigen in the early diagnosis of dengue fever.

Material and Methods

This prospective study was done in SVS medical college and hospital for a period of 6 months from JUL-2017 to DEC 2017. A total of 1021 samples from clinically suspected patients were screened for dengue serological markers during the study period. All the serum samples were screened for NS1 antigen and IgG and IgM antibodies by using lateral flow immunochromatographic rapid test. (J MITRA & CO). Platelet counts were detected by automated analyzer and duration of fever has been noted down.

Results

Of the 1021 samples tested, 158(15.4%) were positive for one or more of the dengue serology markers. Out of the 158, 85(53.79%) were positive only for NS1 antigen indicating the infection in the early stage, 60(37.97%) samples showed

only IgM dengue antibodies and 6 (3.79%) samples showed only IgG antibodies.

antibodies and the remaining 3(1.89%) samples for IgM+IgG antibodies.

Two serological markers are seen in 7 samples, out of which 4 (2.53%) samples were positive for NS1+ IgM

Table 1: Prevalence of dengue markers in the positive cases

Dengue marker	Number	Percentage (%)
NS1	85	53.79%
IgM	60	37.97%
IgG	06	3.79%
NS1+IgM	04	2.53%
IgM+IgG	03	1.89%
Total	158	100%

Out of 158 positive samples obtained males were predominant accounting for 69% followed by females 31% and the male to female ratio was 2.2:1

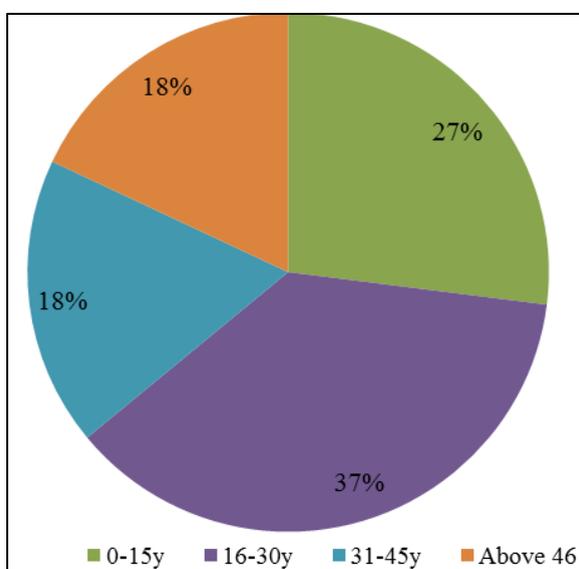


Fig. 1: Age wise distribution of dengue positive cases:

Majority of the dengue positive cases were observed among the 31-45 years age group (37%) followed by below 15yrs age group (27%)

Table 2: Comparison of various dengue specific parameters in the diagnosis of dengue infection (age wise)

Age	NS1 only	IgM only	IgG only	NS1+IgM	IgM+IgG
0-15y	41%	8%	17%	25%	nil
16-30y	45%	30%	33%	25%	67%
31-45y	9%	28%	33%	50%	33%
Above 46	5%	34%	17%	nil	nil

Table 3: Comparison of platelet counts with dengue parameters

Parameter	Platelet Count	Percentage
	<100000/ML	%
NSI [n=85]	30	48.38%
IgM [n=60]	24	38.70%
IgG [n=06]	4	6.45%
NSI + IgM [n=4]	2	3.22%
IgM + IgG [n=3]	2	3.22%
Total 158	62	100%

Table 4: Distribution of dengue positive cases according to the number of days of fever

Day of fever Dengue Markers (no of cases)	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	9 th	10 th	11 th	12 th	13 th	14 th	Total Number of patients (n=158)
NS1	6	10	14	14	12	4	12	5	4					4	85
IgM			6	4	4		18	10	6	8				4	60
IgG					2	4									6
NSI+IgM		1	2		1										4
IgM+IgG						1	1	1							3

Discussion

Dengue infection has long been diagnosed by serological markers which include detection of IgG or IgM antibodies by either ELISA or rapid immunochromatographic tests. Majority of the complications of the dengue infection occurs during the first week of fever and to prevent the epidemics, early diagnosis of infection in the first week of fever is essential. Antibody detection by conventional methods delays the diagnosis as they will be detectable only after 5-7 days of fever. With the introduction of NS1 antigen detection methods recently, it has become much easier to diagnose the cases in the early acute phase (after day 1 of fever). Detection of both NS1 antigen and IgM/IgG antibodies is essential in distinguishing primary dengue from secondary. Though NVBDCP recommends ELISA as the gold standard for the diagnosis of dengue infection, it is not widely practiced as it is a time consuming method and needs expertise and because of cost constraints single samples cannot be tested. Further many studies have evaluated the performance of rapid diagnostic kits of dengue with respect to the ELISA tests and found satisfactory results with good sensitivity and specificity.^{9,10} ELISA is also not feasible in primary healthcare settings because of which alternatives like rapid immunochromatographic tests have gained importance in the diagnosis of these markers.

In the present study out of 158 positive cases, NS1 alone or in combination with either IgM or IgG was positive in 89 cases in our study which is similar to kulkarni et al¹¹ study where 40.6% cases were positive for NS1 alone or in combination with either IgM or IgG.

Only NS1 antigen was positive in 85(53.8%) of the cases. Considering very high specificity of NS1 we might have missed 30% cases if we have not included NS1 in the test procedure in studies by. Datta and Shrivastava NS1 was positive in 140 out of 600(23.3%) and 15 out of 91(16%) cases respectively.^{5,6}

In the present study IgG alone was positive in 6 cases (3.79%). And IgM only was positive in 37.97%. These findings are similar to kulkarni et al¹¹ where IgG alone was positive in 3% And IgM only was positive in 50% of cases. Where as in Laxmi et al¹² study IgM alone was seen only in 5% of cases and IgG in 24%.

Majority of the dengue infections can be subclinical but will raise the IgG antibodies titres which persist for years and hinder the diagnosis of infection. Because of which IgG detection alone is a less reliable marker in the diagnosis. In

dengue endemic countries like India IgG levels could be very higher because of frequent bites from mosquitoes.

On the other hand detection of dengue-specific IgM is a good indicator for the detection of recent infection. It is detectable in both primary and secondary dengue. But frequent cross reactions occur in unrelated infections and false positive results can be encountered during the detection of both IgG and IgM antibodies and weak bands in the rapid test kits are not uncommon which needs repeat testing. In contrast when NS1 is positive, there is no need of repeat testing as it is a highly specific marker of dengue infection unlike the antibody detection.

In the present study thrombocytopenia was observed in 62(39.2%) of the total 158 positive cases. This is comparable to the findings of Kulkarni et al and Laxmi et al^{11,12} study where the thrombocytopenia was observed in 69% and 78% respectively.

Together NS1 along with IgM and IgG detection will detect the maximum number of dengue cases with good sensitivity and specificity which helps in the better management of patients and in undertaking appropriate prevention measures of the spread of dengue infections.

Conclusion

In the present study NS1 antigen is the most commonly detected serological markers in dengue suspected cases. If only antibodies are screened which is commonly done in majority of the health care setups in India, many of the dengue cases will be underdiagnosed (or) misdiagnosed and will be treated inappropriately resulting in increased mortality and morbidity.

Conflict of Interest: None.

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