Original research article

STUDY OF NS1 ANTIGEN DETECTION AND ASSOCIATION OF PLATELET COUNT WITH VARIOUS DENGUE SEROLOGICAL MARKERS IN DENGUE INFECTION: AN INSTITUTIONAL EXPERIENCE

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Abstract

Introduction: Dengue is a mosquito-borne disease caused by dengue virus. Annually, it affects up millions of people worldwide. An early and accurate diagnosis of dengue in the acute phase of illness is important for identifying an epidemic and for initiation of therapy. Detection of the secreted NS1 protein is a new approach that aid in the early diagnosis. Platelet count is the only non-dengue parameter that can support the diagnosis of the dengue shock syndrome and dengue. This study was done to detect dengue parameters and correlate them with the platelet count.

Material and Methods: This study was conducted from September 2016 to November 2016. Total 205 blood samples were collected from clinically suspected dengue cases. Serum was separated and tested for NS1 antigen, IgM and IgG using the immunochromatography kit. The platelet count was also recorded in all samples.

Results: Out of 205 samples tested total 92(44.9%) specimens were positive for one or more dengue parameters. Among 92 specimens 45(49%) were positive for only NS1 antigen and 20 were positive for IgM only. Thrombocytopenia was recorded in 73(79%)out of 92 dengue positive cases.

Conclusion: Inclusion of NS1 in the diagnosis of dengue increases the detection rate significantly in early phase of the infection.

Keywords: NS1 antigen, platelet count, dengue serological marker

INTRODUCTION

Dengue is an acute febrile illness, endemic to the Indian subcontinent. It is caused by the Dengue virus, and is one of the most significant mosquito borne viral disease.[1,2] The Dengue virus (DENV) belongs to the family Flaviviridae, and it is transmitted to humans by the bite of Aedes aegypti mosquitoes. Dengue virus is a RNA virus, consisting of four serotypes (1, 2, 3 and 4) all of which cause infection. Infection with one serotype does not confer cross-protection against the other serotypes, instead can cause a severe form of infection.[3] Globally, WHO has estimated that around 3 billion people reside in areas where there are risks of exposure to dengue virus and nearly 50 million people are infected with dengue virus every year.[4,5]

In most of the cases, serologic tests are used to detect IgM and IgG antibodies by ELISA. During the acute phase, the presence of IgM antibodies indicates primary infection and it appears after viremia ends or after fever subsides. However, in secondary infections, IgG antibodies rise to high levels within the first week of infection and reduce over 3 to 6 months.[6] Recently, detection of nonstructural protein 1 (NS1) antigen during the acute phase of disease in patients having primary and secondary infections has been studied in various laboratories across the world.[7,8] NS1 is a highly conserved glycoprotein for all the serotypes and produced in both cell membrane-associated secreted forms.[2,5,8] It is an early indicator for virus viability or replication. The non dengue parameter thrombocytopenia (platelet count<100000/ml) serves as a predictive marker to promote the early diagnosis of dengue infection. Platelet count is the accessory laboratory test available in the peripheral areas that can support the diagnosis of Dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS). We detected NS1 antigen along with IgM and IgG antibodies in the suspected dengue patients & also tried to correlate the NS1 positive samples and platelet count.

MATERIAL AND METHODS

This retrospective study was carried out in the Department

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of Microbiology, Shri Ram Murti Smarak Institute of Medical Sciences (SRMSIMS), Bareilly from September 2016 to November 2016 after receiving permission from the institutional ethical committee. A total of 205 serum samples were collected from the suspected dengue patients. Study was done using dengue day1test for detection of NS1 antigen and IgG/ M antibodies manufactured by J. Mitra and company pvt. Ltd. New Delhi, India. The samples were tested for NS1 antigen, IgM, and IgG antibodies using the immunochromatography test kit according to manufacturer' s instructions. The platelet count was recorded in all dengue positive and negative cases. Statistical analysis was done by using Chi-square test.

RESULTS

Out of total 205 samples tested, total 92(44.9%) specimens were positive for either one or more dengue markers (NS1, IgM, IgG). Among 92 positive samples total 72(78.26%) were NS1 antigen positive either alone or in combination with other parameters and rest 20(21.73%) samples were positive for IgM only. Out of 72 NS1 Antigen positive cases 45 (49%) showed only NS1 antigen and 27 specimens showed more than one dengue markers. (Table-1)

Table-1: Distribution of various serological parameters in dengue infection

Pt. Count	Dengue parameters positive	Dengue parameters negative
<1.0 lakh/ml	73	28
>1.0 lakh/ml	19	85
Total	92	113

Platelet count of all samples were recorded. Among 92 positive dengue cases 73(79.3%) patients showed thrombocytopenia (platelet count <100000/ml) and of the 113 negative samples for dengue 28 (24.77%) showed thrombocytopenia. (Table-2 & 3).

Table-2: Distribution of platelet count (<100000) among dengue positive parameters

Parameter	Number	Pt. Count (%)
NS1 only	45	35(77.7)
IgM only	20	16(80)
NS1 and IgM	8	8(100)
NS1 and IgG	2	2(100)
NS1, IgM and IgG	17	12(70.6)
Total	**92**	73(79.3)

Table-3: Correlation of platelet count with dengue parameters

Parameter	Number	Percentage%
NS1 only	45	49
IgM only	20	21.7
IgG only	0	0
NS1 and IgM	8	8.7
NS1 and IgG	2	2.2
NS1, IgM and IgG	17	18.5
Total	**92**	

DISCUSSION

Dengue infection has symptoms similar to other viral infection, therefore in order to provide timely information for the management of patients and early public health control of dengue outbreaks, it is important to establish the diagnosis of acute dengue virus infection during the first few days of manifestation of the clinical symptoms.[8]

There are various methods available for diagnosis of dengue virus infection like isolation of virus genome sequence by nucleic acid amplification, detection of antibodies and rapid dengue ICT for NSI antigen. Although virus isolation and characterization are considered as the gold standard for laboratory diagnosis of acute denguevirus infection, it is expensive and takes at least six to ten days for the virus to replicate in a cell culture or laboratory mosquitoes. Detection of the viral genomic sequence by Real Time Polymerase Chain Reaction is also an expensive method and is not available in most hospital diagnostic laboratories.[7] Detection of NS1 has been a promising test to diagnose dengue in its early febrile stage, due to its long half-lifein blood.[7,9]

In the present study there were 92 seropositive cases (44.8%) out of 205, other studies have shown seropositivity of 15.2% by Kulkarni RD et al[10]and 38.5% by Badave GK et al.[11] In our study out of 92 cases NSI only antigen, in which no antibodies were detected, were positive in 45(49%) patients. These 45 cases positive for the specific marker NS1 Ag had been missed if NS1 was not recorded in early days of the infection. This was in concordance with study done by Badave GK et al[11] (42.9%) and Jyothi P et al (56%).[12] Higher values of 60% and lower values of 23% have also have been recorded by Santosh Tathe et al[13] and Shrivastava A et al[7] respectively.

Among 92 dengue positive cases, 20 (21.7%) were positive for IgM only. Such patients presented after taking primary treatment in some health care center during early infection.

Our study found 27 cases which were positive for NS1 antigen along with antibodies either IgM / IgG or both. A recent meta-analysis for NS1-based test as a diagnostic utility for dengue infection supported the use of single NS1-based test with improved sensitivity of detection when combined with an IgM test.[14]

Platelet counts are decreased in several other conditions like some viral infections other than dengue, drug induced thrombocytopenia, collagen vascular diseases, idiopathic thrombocytopenia etc.[15] In the present study thrombocytopenia (platelet count <100000/ml) was

recorded in 73 (79.3%) out of 92 seropositive patients. This was supported by other studies done by Santosh Tatheet al[13] and Kulkarni RD et al[10].

We recorded Platelet count of <100000/ml in 35(77.8%) out of 45 NS1 only positive samples. Similar values were shown in studies by Kulkarni RD et al[10] and Badave GK et al[11]. Higher number of such cases had been seen in study by Santosh Tathe et al.[13]

In correlating the platelet count with dengue parameters we found that thrombocytopenia (pt. count<100000/ml) was present in 79.3% of the seropositive cases. Applying chi-square test the association between platelet count and dengue parameters was statistically significant.

CONCLUSION

The remarkable increase in the worldwide burden of dengue has gained increased awareness in developing improved diagnostics for dengue infections.

As the NS1 antigen is detectable in blood from day one after onset of fever, its assay is an effective tool for early diagnosis so as to avoid complications of dengue infection. The ease, speed and dependability of ICT tests make them an effective technique in addressing this potentially fatal, epidemic prone infection.

Apart from these dengue specific parameters, platelet count is the other accessory laboratory test available that support the diagnosis of dengue infection. Compared with conventional ELISA, rapid immunochromatography test results are available within 20 min. This will be very helpful in initiating instant treatment and minimizing the serious complications and mortality of dengue infection.

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