

Correlation of thrombocytopenia and serological markers in early diagnosis of dengue infection with special reference to NS1 antigen

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Abstract

Background: Dengue is an acute viral infection with potential fatal complication. In view of the high mortality rate and to reduce the disease burden, early and specific diagnosis of Dengue Hemorrhagic Fever or Dengue Shock Syndrome followed by supportive therapy reduces morbidity and mortality. This study was undertaken to evaluate the efficacy of NS1 antigen its comparison with platelet count.

Method: A total of 358 serum samples were collected from clinically suspected cases of dengue fever, serum samples were tested immediately for NS1, IgM and IgG by immunochromatography based test and correlated the results.

Results: Of the 358 serum samples tested, 135(37.70%) were positive for either one or more of the dengue parameters. Among 135 positive cases, 45 (33.33%) showed positivity for NS1 antigen. Out of remaining 90 cases, predominantly 57(63.33%) cases showed positivity for IgM antibody followed by 7 (5.1%) cases showed positivity for IgG. The result of dengue specific parameters were compared against platelet count. Statistical analysis were done by using chi-square test and Z-test.

Conclusion: Rapid immunochromatography test is an excellent tool in addressing this potentially fatal epidemic prone infection which is an important public health problem of our country.

Keywords: Dengue, NS1 antigen, IgM antibody, IgG antibody, Immunochromatography, Thrombocytopenia, Dengue hemorrhagic fever, Dengue shock Syndrome.

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Introduction

Dengue an emerging arboviral and arthropod-borne disease caused by dengue virus belonging to the family flaviviridae and genus flavivirus.¹ It is one of the most serious mosquito-borne viral disease in humans affecting tropical and subtropical regions of the world.² Depending on characteristic antigenicity feature virus has five serotypes, namely DENV-1, DENV-2, DENV-3 and DENV-4.³ While the fifth type was announced in 2013.⁴ It affects 100 million people annually with 5,00,000 cases of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) and around 30,000 deaths mostly among children.⁵

Clinical manifestation of dengue range from asymptomatic or mild febrile illness to classic dengue fever (DF) to most severe form of illness like DHF associated with plasma leakage and a hemorrhagic diathesis, typically after 5 days of fever.⁶ In severe DHF, morbidity and mortality are the results of hypotension and shock at times accompanied by severe coagulation abnormalities and bleeding.

As the disease is associated with high mortality and morbidity, a rapid and accurate diagnosis is essential for early appropriate management and for prevention of complications.

The 'gold standard' tests for the identification of Dengue infection (DI) are not within the reach of peripheral and even most tertiary care laboratories. Currently we have viral culture, viral RNA detection by reverse transcriptase Polymerase Chain Reaction (RT-PCR) and serological tests such as an immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (MAC-ELISA) for diagnosis and confirmation of dengue viral infection. However, early dengue diagnosis still remains a problem as all these assay have their own pitfalls. Detection of dengue specific IgM/IgG antibody supports in the diagnosis of DI.⁷ NS1 (Non-structural protein1) is a highly conserved glycoprotein, its detection is sensitive as well as specific because it is essential for viability of dengue virus, produced both in membrane-associated and secretory forms by the virus. Hence detection of secretory NS1 protein represents a new approach to the diagnosis of acute dengue virus infection.^{8,9}

In peripheral areas at tertiary center, these newer diagnostic method are not available or feasible, only tool in their hand is measuring platelet count, and it is the only accessory laboratory test that can support the diagnosis of DHF or DSS. Keeping in mind the logistic constraints of healthcare system in the peripheral areas, aims of the study was to correlate the platelet count

with various dengue specific serological markers by immunochromatography based dengue serologic test.

Materials and Method

This study was conducted at a tertiary care hospital and medical college from June 2014 to December 2015. A total of 358 serum samples were collected from suspected cases of dengue fever. The samples were

Results

Among the suspected 358 serum samples tested at central clinical laboratory, 135 cases were showed positivity for one or more markers like NS1, IgM or IgG. In 135 cases, majority of 57 (63.33%) cases were positive for IgM, followed by NS1 and IgG with 45 (33.33%) and 7 (5.1%) cases respectively. [Table 1] All the suspected cases were screened for platelet count. Among them platelet count less than 1,00,000/cmm were noticed in 114(84.44%), [Table 2] and compared them with positive dengue cases as shown in Table 3, showing comparison of dengue cases with platelet counts.

tested immediately for NS1, IgM an IgG by immunochromatography test (ICT) based tests supplied by J Mitra & co. Pvt. Ltd, 'DENGUE DAY1 TEST'. The test were performed strictly as per directives given in the literature provided by the manufacturer. Platelet counts of all these cases were noted. Statistical analysis was done by using Chi-square and Z-test.

Table 1: Comparison of efficacy of various Dengue Specific Parameters (n= 135)

Dengue specific parameters	Total positive serum sample	Percentage (%)
NS1 only	45	33.33
IgM only	57	63.33
IgG only	7	5.1
NS1 + IgM only	19	14.07
NS1 + IgG only	17	12.59
NS1 + IgM + IgG	14	10.37
IgM + IgG	21	15.55

Table 2: Comparison of Platelet count with various Dengue Specific Parameters

Dengue specific parameters	Total positive serum samples	Platelet counts (<1,00,000/cmm)	Percentage (%)
NS1 only	45 (33.33%)	30	66.66
IgM only	57 (63.33%)	41	71.92
IgG only	7 (5.1%)	4	57.14
NS1 + IgM	19 (14.07%)	15	78.94
NS1 + IgG	17 (12.59%)	2	11.76
NS1 + IgM + IgM	14 (10.37%)	7	50
IgM + IgG	21 (15.55%)	15	71.42
Total	135	114	84.44

Table 3: Study of Dengue cases with platelet counts (n=358)

Platelet range	Dengue positive cases	Dengue negative cases
Platelet count less than 1,00,000/cmm	114	7
Platelet count more than 1,00,000/cmm	21	216
Total	135	223

Discussion

Efficient, rapid and accurate diagnosis of Dengue is crucial for preventing complications of DHS and DSS, it also helps not only in confirmation of diagnosis but also to rule out differential diagnosis other infectious disease which mimics like dengue fever. This also helps healthcare researcher to implement control measures to prevent the epidemics of dengue fever in community. As the initial symptoms of dengue mimic those of malaria, typhoid and leptospirosis which are

endemic in the country. Recently it has observed that numbers of cases of dengue are increasing in our country, but fact is that we are lacking most of diagnostic testing facility at primary level. This can be eliminated by implementing newer rapid diagnostic test for clinically suspected cases dengue fever and preventing further complication.

For a long time detection of dengue-specific IgG/IgM has been the mainstay of diagnosis of dengue infection. In a patients of primary infection the dengue

specific antibodies begins to appear in their blood around fifth day of fever.⁷ The new parameter, NS1 antigen is detectable from day 1 of fever both in primary and secondary infection due its specificity and characteristic absent cross-reactivity of NS1 protein with other groups of flavivirus.

The DENV IgM as well as IgG antibodies show some cross-reactivity with other members of the flaviviridae family.¹⁰ In our study out of 135 cases, 45(33.33%) cases showed positivity for NS1 antigen. Only RD Kulkarni et al have been shown NS1 was positive in 95(30%) cases out of 320 samples.¹¹ Datta (2010) and Shrivastava (2011) have shown that NS1 was positive in 140 (23.3%) out of 600 cases and 15 (16%) out of 91 cases respectively.^{12,13}

From 135 seropositive cases, 57(63.33%) were positive for IgM only this finding were in concordance with previous published studies of RD Kulkarni et al, 2011 and Santosh Tathe et al, 2013.^{11,14}

In the present study, a significant correlation were noted between NS1 antigen and thrombocytopenia(Badave GK et al 2015).¹⁵ Decreased platelet count can be seen in condition which can mimics dengue infection like malaria, viral infections, drugs causing bone marrow suppression, collagen vascular diseases, idiopathic thrombocytopenia etc.¹⁶

In our country, currently available 'gold standard' tests for diagnosis of DI are not feasible for peripheral and even most of the tertiary care laboratories. These centers has to function without great technological backup. In such remote areas, correlation of platelet counts and ICT-based serological tests for NS1 antigen and antibody detection helps in early detection and management of this vulnerable group.

Conclusion

Dengue is endemic to Indian subcontinent. Currently no specific antiviral therapy is available, whatever newer diagnostic modalities available, not feasible or out of reach to the peripheral and tertiary care center. Only in hand tool is measuring platelet count and use the ICT-based test to detect the NS1 antigen, which plays vital role in early diagnosis, management and implementing the control measures in community to avoid spread of disease.

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