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ORIGINAL ARTICLE

IMPORTANCE OF CONCURRENT TESTING OF DENGUE SPECIFIC SEROLOGICAL MARKERS AND PLATELET ENUMERATION

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ABSTRACT: INTRODUCTION: Dengue infection can result in dengue shock syndrome which is associated with mortality. So it is imperative to have rapid diagnostic test for its diagnosis. Recently NS1 determination from serum samples has emerged as a diagnostic tool for early diagnosis. Other serological markers are antibodies like IgG & IgM**. MATERIAL AND METHODS**: Serum samples were tested for Dengue NS1, IgM and IgG using the single-step immunochromatograhic assay. Platelet counts of all seropositive cases and 100 seronegative cases were recorded. Results of dengue serological markers were compared against platelet counts. Statistical analysis was done using chi square test and by determining p value**. RESULTS:** Of the 233 patients screened with the SD Dengue Duo, 78 cases were seropositive for one or more markers. Of 78 samples ,NS1 antigen was detected alone in 26 cases, IgG+IgM in 7cases, NS1 +IgG in 7 cases, NS1+IgG+IgM in 27 cases. Thrombo-cytopenia was more significantly associated in NS1+IgM+IgG positive cases (p value<0.05). **CONCLUSION**: So inclusion of NS1 in test panel increased the detection of additional seropositive cases. Thrombocytopenia was significantly associated with dengue positive than dengue negative cases. It was significantly associated when NSI was detected along with IgM and IgG.
KEYWORDS: Dengue virus, IgM, IgG, Viral nonstructural protein NS1, Platelet count.

INTRODUCTION: Dengue is an acute febrile arboviral disease found largely in the tropics and subtropics. There are four distinct but antigenically related serotypes of dengue virus and its transmission is by mosquito principally Aedes aegypti.[1] Infection with one serotype induces lifelong immunity against reinfection by the same serotype but only partial protection against other serotypes.[2,3] Dengue infections vary in severity ranging from asymptomatic infection to dengue fever and the severe disease such as dengue hemorrhagic fever/dengue shock syndrome.[4] It carries a significant mortality if the diagnosis and treatment are delayed.[5] So it is imperative to have a rapid, sensitive and easy to use diagnostic assay for early detection of the disease.[6]

Laboratory confirmation relies on isolation of virus in cell culture, identification of viral RNA but these methods have restricted scope as routine procedure. Serological tests based on determination of dengue specific IgG/IgM antibodies have been the mainstay of diagnosis because of their ease of use. But the sensitivity and specificity of these assays is strongly influenced by the quality of antigen used.[7] Also these assays have poor sensitivity and specificity in first four days of illness.[8] Recently detection of secreted NSI antigen in the bloodstream has emerged as sensitive and specific diagnostic tool during acute phase of illness.[9]

In peripheral health clinics of resource poor countries, platelet count is also considered to be the reliable parameter to support the diagnosis of dengue infection as this can be determined by microscopy.[10] Considering this fact the present study was conducted to determine the association between dengue specific serological markers and platelet counts.

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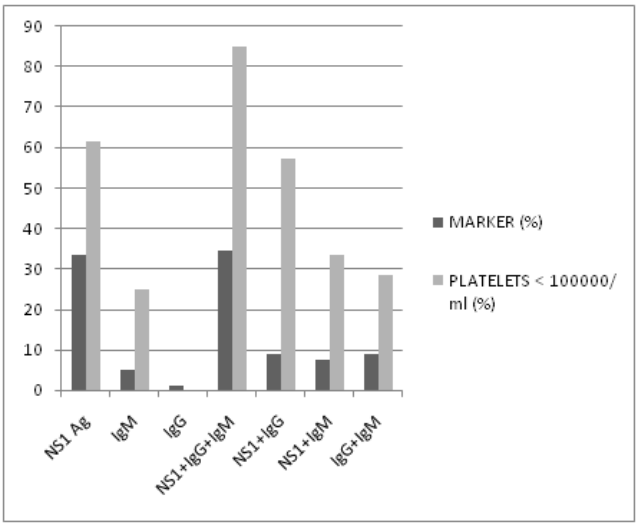
MATERIAL AND METHODS: The present study was conducted at a tertiary teaching care center from September 2011 to December 2011 after receiving approval from institutional ethical committee. A total of 233 serum samples were screened from clinically suspected cases of dengue fever. The dengue serology was performed according to manufacturer instructions using SD Dengue Duo by standard diagnostics (Korea). This is an invitro immunochromatographic one step assay to detect NSI antigen and differential IgG/ IgM antibodies to dengue virus. The individual reports were available in 1-2 hours. Platelet counts of all positive cases were recorded. Platelet counts of all 100 dengue negative cases were recorded as well. Apart from inbuilt controls in the test; no independent controls were used for IgG/ IgM and NSI antigen.

RESULTS: Of the total 233 serum samples tested, 78 samples turned positive for one or more of three serological markers. Of 78 samples NS1 antigen was detected alone in 26 cases (33.33%), IgG+IgM was detected in 7 cases (8.97%), NS1+IgG in 7 cases (8.97%), NS1+IgG+IgM in 27cases (34.6%). Platelet count less than 100000/ul was detected in 48 cases (61.53%).Out of the 100 cases that were negative for dengue infection thrombocytopenia was detected in 28 % cases.

Dengue Specific Serological Marker	Total Cases (%)	Platelet Count Less Than 100000/ul (%)
NS1 antigen	26(33.33%)	16(61.5%)
IgM	4(5.12%)	1(25%)
IgG	1(1.28%)	0
NS1+IgG+IgM	27(34.6%)	23(85.1%)
NS1+IgG	7(8.9%)	4(57.14%)
NS1+IgM	6(7.69%)	2(33.3%)
IgG+IgM	7(8.9%)	2(28.5%)

Table 1: Association of dengue specific serological marker with platelet count



Graph showing the percentage distribution of serological markers and platelet count

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DISCUSSION: In this study two parameters were studied. First the distribution of dengue specific serological markers was analyzed. In this study, out of total 78 positive cases, 26 cases were exclusively positive for NSI antigen. These were the cases with primary infection and could transmit the infection if bitten by mosquito. Srivastava et al also reported the NSI antigen positivity to be around 23.3 %.[11] Similarly there were 7 cases that were positive for NS1+IgG. Without NSI screening these would have been overlooked and labeled as negative. This is because IgG alone is considered to be less reliable marker for dengue infection and its positivity is due to subclinical infection and its levels are higher in endemic areas [12]. So inclusion of NS1 antigen testing in test panel helped in detecting these 33 cases (26 NSI antigens, 7 IgG+NSI antigen) which would have been missed without NSI antigen screening. NSI testing does not require repeat testing due to its high specificity and can reliably diagnose the disease from day 1 of illness.

There were 4 cases who were exclusively IgM positive, these patients had a primary infection presenting during later phase of illness. Similarly there was 1 case that was positive for IgG only, probably the patient presented during secondary infection. 7 Cases were positive for IgG+IgM; these were cases with primary and secondary infection who presented in later stage of illness. Furthermore there were 155 cases that were negative for serological markers. In such patients dengue infection should be ruled out by testing for viral replication by cell culture or RNA detection methods, immunofluorescence, immunohistochemistry.[13]

Secondly in this study correlation between platelet count and dengue serological marker was studied. Thrombocytopenia occurred in 61.5% cases with acute dengue infection. Platelet counts were low in NS1 positive cases. The counts were lowest in patients with NSI+IgM+IgG. Statistical analysis of NSI positive cases only (33.33%) with NSI+IgG+IgM positivity (34.6%) showed that thrombocytopenia was significantly associated with NSI+IgG+IgM positive cases than with NS1 only ($\chi 2$ =3.84, p value=0.049).The role of antibodies is well defined in causing disease pathogenicity and thrombocytopenia.

The platelet counts were low in 28% with negative triple serological marker. But association of thrombocytopenia with dengue positivity was more significant than in dengue negative cases ($\chi 2$=20.15, p value=0.0001). Thrombocytopenia is observable in other viral diseases, collagen vascular disorders, drug induced thrombocytopenia.[14]

The various limitations in the study were that no gold standard test was used for authentication as facilities for these methods were not available in our study. Also precise details about the duration of fever were not available which would have helped us to pick additional NSI positive cases.

So we conclude that concurrent detection of NSI antigen with IgG/IgM antibodies will help in detection of additional dengue positive cases which escape detection and its testing must be included in test panel. The use of one step ICT based test would enable rapid and easy detection of dengue infection in resource poor settings. Also simultaneous estimation of thrombocytopenia will help clinician to offer appropriate therapy.

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