

IMPORTANCE OF PLATELET COUNT AND SEROLOGICAL MARKERS IN DIAGNOSING DENGUE INFECTION WITH SPECIAL REFERENCE TO NS1 ANTIGENSaurabh G. Agarwal¹, Atul R. Rukadikar², Avinash Laghawe³, Apoorva Tripathi⁴**HOW TO CITE THIS ARTICLE:**

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ABSTRACT: INTRODUCTION: Dengue is an acute viral infection with potential fatal complications. Specific antibody detection has been the mainstay of diagnosis which is prone for both false positive and false negative reactions. The newer parameter NS1 appears to be highly specific and reliable for diagnosis of dengue infection from the first day of fever. Platelet count is the only accessory test for diagnosis of dengue infection in the peripheral laboratories. Therefore, study was done to evaluate the importance and association of platelet counts against NS1 and IgM/IgG in dengue infections. **MATERIALS AND METHODS:** Serum samples from clinically suspected dengue cases were tested for NS1, IgM and IgG by immunochromatography-based test. Platelet counts were obtained for all positive cases and dengue seronegative cases of fever served as controls. Test results of dengue-specific parameters were compared against platelet counts. The results were subjected to statistical analysis. **RESULTS:** Of 159 samples tested, 17 were positive for one or more dengue parameters. Of the 17, 05 were positive for NS1 only, 06 showed IgM only while 01 showed IgG only. More than one marker was detected in the remaining 05 samples. Thrombocytopenia was more consistently associated whenever NS1 was detected compared to antibody detection (P value <0.001). **CONCLUSIONS:** Inclusion of NS1 in the diagnosis of dengue increases the detection rate significantly. In cases of fever, thrombocytopenia is more consistently found in dengue positive rather than dengue negative subjects. It correlates well when NS1 and IgM are detected simultaneously. **KEYWORDS:** Dengue, IgM, IgG, NS1, thrombocytopenia.

INTRODUCTION: Dengue is an acute, potentially fatal viral infection that can culminate into dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). It is caused by four serotypes of dengue virus (DV), namely DEN-1, DEN-2, DEN-3 and DEN-4 belonging to genus Flavivirus and family Flaviviridae. It is spread through the bite of infected *Aedes aegypti* mosquito.¹ Most primary infections are uneventful. Infection with one serotype confers an individual life-long immunity to that serotype and cross-reactivity to the other serotypes. The complications like DHF and DSS are usually attributed to this cross-reactivity.^{1,2} Dengue is almost endemic throughout India. The resource poor health care system has to depend upon simple to perform and easy to interpret laboratory tests for diagnosis. It is known that early and specific diagnosis of DHF or DSS followed by supportive therapy reduces morbidity and mortality.³ The variability in the clinical illness associated with dengue infection (DI) cannot be accommodated in a single clinical definition. However, confirmation of DI is the most essential pre-requisite in the management of complications.⁴ The 'gold standard' tests for identification of DI are not within the reach of peripheral and even most tertiary care laboratories. Detection of dengue specific IgM/IgG has been the mainstay of diagnosis of DI. Antibody detection is

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an indirect method of diagnosis and, therefore, is prone to false positive as well as false negative results.⁵ Of late; non-structural protein 1 (NS1) detection is available for diagnosis of DI.⁶ NS1 detection is reported to be sensitive as well as highly specific.⁷ Apart from the dengue specific parameters, platelet count is the only accessory laboratory test available in the peripheral areas that can support the diagnosis of DHF or DSS.⁸ Keeping in mind the logistic constraints of healthcare system in the peripheral areas, we tried to correlate the platelet counts and immunochromatography (ICT)-based dengue serology tests.

MATERIAL AND METHODS: The study was conducted at Chirayu Medical College and Hospital, Bhopal from January 2013 to December 2013 after receiving permission from the institutional ethical committee. A total of 159 serum samples from clinically suspected dengue fever irrespective of age and sex were collected. Since our laboratory works round the clock, the samples were tested immediately for NS1, IgM and IgG by Immunochromatographic test (ICT). The tests were performed strictly as per the manufacturer's instructions. Platelet counts of all the cases positive for any of the dengue parameter were recorded. Platelet counts were also recorded in cases of fever that were negative for any of the dengue parameter. No healthy controls were included in the study.

RESULTS: Of the 159 serum samples tested, a total of 17 specimens were tested positive for either one or more of the three markers (NS1, IgM and IgG) tested. Of the 17 serum samples, 05 (29.41%) patients were positive for NS1 only, 06 (35.29%) positive for IgM only, while 01(05.88%) patients had only IgG. More than one marker was detected in the remaining 05 (29.41%) samples (Table 1).

Platelet count less than 1, 00, 000/ml was noticed in 12 cases (70.58%). Of the 142 cases presenting with fever that were negative for any of the dengue-specific parameters by ICT, none had showed thrombocytopenia. When the results were subjected to statistical treatment, this difference was found to be statistically significant, P value being less than 0.001. (Table 2) (Table 3).

DISCUSSION: For a long time detection of dengue-specific IgG/IgM has been the mainstay of diagnosis of DI. The dengue-specific antibodies begin to appear only around fifth day of fever in primary infection.⁵ Even in most secondary infections, both the IgM and IgG type antibodies cannot be recorded before third day.⁹ Therefore, there is always a window period, both in primary and secondary DI when only antibodies are tested. The new parameter, NS1 antigen, is detectable from day 1 of fever both in primary and secondary infections. It is important to note that NS1 is shown to be highly specific viral marker making it extremely reliable parameter for the diagnosis of DI from day 1 of the fever.¹⁰

The distribution of various dengue specific parameters is shown in (Table 1). Of the 159 cases, 05 (29.41%) were positive for only NS1 antigen. This finding is consistent with the study done by Kulkarni RD et al¹¹ and Datta S et al¹⁰ in which 30% and 23.3% cases were positive for only NS1 antigen respectively. Only IgM was positive in 06 (35.29%) cases. This is in consistent with the study conducted by Ukey PM et al¹² in which 31.3% patients were serologically positive for dengue infection. This indicates that, dengue-specific IgM is a very good indicator of recent infection. IgG was positive in only 01(05.88%) case which showed past infection with dengue. NS1 in combination with IgM were positive in 01 (05.88%). No sample was found to be positive for both NS1 and IgG. Both IgM and IgG were found to be positive for 04 (23.52%) cases indicating acute on chronic infection.

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We tried to find the association of dengue parameter positivity with thrombocytopenia. The comparison of platelet counts with different dengue specific parameters is shown in (Table 2). Of the 17 cases, 12(70.58%) showed thrombocytopenia. In 05 cases that were positive for NS1, thrombocytopenia was evident in 04 (80.00%) cases. In contrast, when IgM and IgG antibodies were considered for the diagnosis of DI, thrombocytopenia was noted only in IgM antibody (83.33%). Association of thrombocytopenia with NS1 and IgM was found to be 100% while association of thrombocytopenia with IgM and IgG was evident in 50% cases.

Platelet counts are decreased in several other conditions like some viral infections other than dengue, drug induced thrombocytopenia, collagen vascular diseases, idiopathic thrombocytopenia etc.³ We therefore, tried to correlate platelet counts in cases of fever that tested negative for any of the dengue parameters (Table 3). In 142 cases of fever, in which none of the dengue parameters was positive, thrombocytopenia was not noted. Applying Statistical analysis, it was found that association of thrombocytopenia and dengue parameter was significantly higher with p value less than 0.001.

This study has been carried out at a tertiary care teaching hospital. It is worth mentioning here that most tertiary care teaching hospitals lack in viral culture setup. Therefore, applying gold standard tests in studies related to viral infections is out of reach of these centers. Dengue is an infection that is present in urban, semi-urban and rural areas. Our healthcare system is extremely resource poor. Top class technological backup is available only at very few elite laboratories situated in big cities. It is important to conduct studies in the peripheral centers where the laboratory has to function without great technological backup and still is expected to provide reasonable opinion to the clinician in the management of infections like dengue.¹¹

The limitation of the present study was that enzyme linked immunosorbent assay (ELISA) for qualitative or quantitative detection or Polymerase Chain Reaction (PCR) could not be used. ELISA has higher sensitivity than ICT-based tests.¹³ Inclusion of ELISA in this study could have given us an opportunity to comment on the efficacy of ICT to identify DI. Most of the cases coming to our hospital were referred from various places and had received a few days treatment before reaching this hospital. The precise day of fever at the time of conducting the test could not be obtained in a large number of cases. In spite of this, NS1 only was positive in 30% 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 the titres of NS1 represent the viral load and the viral load is directly proportional to complications.⁷ It can be logically inferred that in complication prone cases, i.e. having higher viral load, detection of NS1 will be easier because of higher NS1 levels. This would reduce the chances of false negativity by a less sensitive test like ICT.¹¹

CONCLUSION: Inclusion of NS1 must be included for evaluation of all cases of fever, either in endemic or non-endemic areas. The ease, speed and dependability of ICT make it an excellent tool in addressing this potentially fatal, epidemic prone infection that has become an important public health problem in our country. One can never forget the fact that dengue often breaks out in resource poor peripheral areas where ICT-based tests could be the only support available.

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PARAMETER	TOTAL	PERCENTAGE
NS1 only	05	29.41%
IgM only	06	35.29%
IgG only	01	05.88%
NS1 & IgM	01	05.88%
NS1 & IgG	00	00.00%
IgM & IgG	04	23.52%
Total	17	100.00%

TABLE 1: COMPARISION OF VARIOUS DENGUE SPECIFIC PARAMETERS IN DIAGNOSING DENGUE INFECTION

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PARAMETER	TOTAL	PLATELET COUNT LESS THAN 1, 00, 000/ML	PERCENTAGE
NS1 only	05	04	80.00%
IgM only	06	05	83.33%
IgG only	01	00	00.00%
NS1 & IgM	01	01	100.00%
NS1 & IgG	00	00	00.00%
IgM & IgG	04	02	50.00%
Total	17	12	70.58%

TABLE 2: COMPARISON OF PLATELET COUNT WITH VARIOUS DENGUE PARAMETERS

	DENGUE POSITIVE CASES	DENGUE NEGATIVE CASES	TOTAL
PLATELET COUNT LESS THAN 1, 00, 000/ML	12 (100%)	00 (00.00%)	12 (07.54%)
PLATELET COUNT MORE THAN 1, 00, 000/ML	05 (03.40%)	142 (96.59%)	147 (92.45)
TOTAL	17 (10.69%)	142 (89.30%)	159 (100%)

p value <0.001 (Highly Significant), Chi Square (χ^2) = 108.4

TABLE 3: COMPARATIVE EVALUATION OF DENGUE CASES AND ASSOCIATED PLATELET COUNTS

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