Original Research Article

Use of Immunochromatographic Test in Association with Thrombocytopenia for Early Diagnosis of Dengue Infection

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A B S T R A C T

Dengue is an acute viral infection with fatal complications and noted as endemic in India. Since the clinical presentation of dengue is nonspecific, dengue diagnosis would benefit from a sensitive rapid diagnostic test (RDT) to reduce the disease burden and with supportive therapy reduces morbidity and mortality. Platelet count is the only accessory test for diagnosis of dengue infection in the peripheral laboratories. Therefore, in this study association of platelet counts and Ns1 antigen, (IgM, IgG) antibody is evaluated in dengue infections. This study is to correlate the platelet count and (IgM/IgG) antibody, NS1 antigen in the acute stage of dengue infection. A total of 775 serum samples were collected from clinically suspected dengue cases and were included in the study from Jan 2018 to Dec 2018 at tertiary care hospital. Serum samples were tested for NS1, IgM and IgG by Immunochromatographic-based test (ICT kit). The platelet count was recorded in dengue parameter-positive and -negative cases. Out of 775 samples screened in our study, 208 were positive for dengue parameters, 567 were negative. Out of 208 positive cases, 151 (72.5%) cases were positive for NS1antigen either alone or in combination with antibodies. 128 (61.53%) cases were exclusively positive for NS1 antigen. Primary infection (positive for NS1 Ag, IgM, NS1 + IgM) was seen in 202 (97.1%) cases and secondary infection (positive for IgG, NS1 + IgG, IgM + IgG, NS1+ IgM+ IgG) was seen in 6 cases (2.9%). Out of 208 positive cases, thrombocytopenia was observed in 108 (51.9%) of patients while 100 (48.07%) had platelet count within normal range.

Introduction

Dengue is an acute febrile illness caused by the Dengue virus (flavivirus), and is one of the mosquito-borne (*Aedes aegypti* mosquitoes) viral diseases.[1,2,3] Four serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) have been found on the basis of the neutralization assay data.[4] Primary infections are mostly uneventful. There is presence of lifelong immunity for the serotype which causes infection to the patient and cross-reactivity to the other serotypes will occur in primary dengue infection. Complications like dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are usually due to

cross-reactivity.[5,6,7] Thrombocytopenia is more prominent in DHF than in dengue fever.[8,9] Today there is no effective antiviral treatment or a licensed vaccine to prevent dengue infection. Judicious fluid management and continuous monitoring for signs of complications is currently the only option available. Earlier case fatalities due to dengue infection have been reported to be around 2.5% to 5.4%.[10,11,12] Organ impairment and shock are most important factors that lead to mortality in dengue infection.[10,13,14] Better fluid management, greater awareness for severe dengue, fatality rates have dropped drastically in many dengue endemic countries.[15] However, for early detection of those who can develop severe dengue, the clinical and laboratory parameters are measured frequently in all patients admitted to the hospital with dengue infection. Therefore, such intense monitoring has caused a great strain to resource poor health care facilities of endemic developing countries. Since India is noted as endemic area and due to resource poor health care system we have to depend upon simple to perform and easy to interpret laboratory tests for diagnosis. Keeping in mind the logistic constraints of healthcare system in the peripheral areas, we tried to correlate the platelet counts and immunochromatographic (ICT)-based dengue serology tests.

The main objectives of this work to study the number of NS1 Antigen and IgM/IgG Antibody positivity in suspected dengue infection and Evaluate the association of low platelet counts against NS1 Antigen and IgM/IgG Antibody in suspected dengue infection.

Materials and Methods

This retrospective study was carried out in the Department of Microbiology in a Tertiary Care Hospital, Pune, Maharashtra, for a period of one year, from January 2018 to December 2018. During the study period serum samples were collected from patients presenting with dengue-like illness and tested for dengue specific parameters. A total number of 775 serum samples were collected from the suspected dengue fever patients. The samples were tested for NS1 antigen, IgM, and IgG antibodies using the ICT (Immunochromatographic test) kit. The platelet count was recorded in dengue parameter-positive and negative cases.

Results and Discussion

A total of 775 serum samples were collected from suspected Dengue fever patients. 208 samples tested positive for one or more Dengue-specific parameters (Table 1 and Figure 1). In the study, Analysis of thrombocytopenia in dengue parameter positive cases (Table 2 and Figure 2). Out of 208 cases, thrombocytopenia was seen in 108 (51.9%). Out of 128 cases that were positive for NS1, thrombocytopenia was observed in 88 cases (68.75%), whereas, when the antibodies alone were considered, thrombocytopenia was observed in 10 out of 57 cases (17.54%). When NS1 plus IgM antibodies were considered, thrombocytopenia was observed in 9 out of 20 cases (45%).

When the platelet count was completed in 100 dengue parameter-negative fever patients (controls), thrombocytopenia was observed in 25% of the patients. The association of thrombocytopenia in dengue parameter-positive cases was well co-related, as compared to thrombocytopenia in dengue parameter-negative patients.

In order to control outbreaks of dengue infection and provide timely intervention for management of patients to prevent conversion into severe forms of dengue, it is important to establish the diagnosis of acute dengue virus infection during the first few days of illness.[16]

Virus isolation is considered as the gold standard for laboratory diagnosis of acute dengue virus infection, it is not only expensive but takes at least 6 to 10 days for the virus to replicate in a cell culture or laboratory mosquitoes. Detection of the viral genomic sequence by RT-PCR is not available in most hospital diagnostic laboratories.[17] The NS1 antigen is considered a specific marker of dengue infection, because there is no cross-reaction of the dengue NS1(Non-structural protein antigen), with other infections. Detection of NS1 antigen has been important component of test to diagnose dengue infection in early febrile stage, as it has long half-life in blood.[1] The IgM as well as IgG antibodies for DENV show some cross-reactivity with other *Flaviviridae* infections. Thus overestimation of the infection rates, more commonly during secondary dengue infection.[2]

In a study conducted by Kulkarni *et al.,*[18] the NS1 alone and with IgM correlated well with thrombocytopenia with 29.68% patients were positive for NS1, 50.3% positive for IgM, while 3% out of 320 positive patients had only IgG. Low platelet count for positive patients was 68.75%. In similar study by Agarwal SG *et al.,*[5] 29.41% patients were positive for NS1, 35.29% positive for IgM, while 05.88% patients had IgG with low platelet count in positive patients was 70.58%.

Similar findings were observed in a study by Datta S *et al.,*[20] In this study, 61.53% patients were positive for NS1 only, 25.96% positive for IgM only, while 1.44% patients had IgG. Thrombocytopenia in positive patients was observed in 51.9% and there is correlation between Dengue seromarkers and thrombocytopenia in Dengue parameter-positive cases, as it is in the above studies.

The probable reason for high positivity rate for NS1 Ag in this study is because ours is a tertiary care center located in dengue endemic suburban area having patients who directly come from periphery as well as patients who are being referred from other centers.

Parameswarappa *et al.,*[1] 62.9% were positive for the NS1 antigen, 11.3% were positive for IgM, and 4.9% were positive for IgG with low platelet count was observed in 51.6%. in their study there is no correlation between Dengue seromarkers and thrombocytopenia in Dengue parameter-positive cases. This may be because; the level of NS1 depends on the viral load. When antibodies start to appear, the NS1 antigen is concealed into immune complexes.[19] The other reasons for non-correlation are: in a tertiary care center, patients are sent after few days of treatment from primary and secondary health care centers. More NS1-positive cases would have been detected if the test was done in the first three to four days of fever. Moreover, the sample size in their study is comparatively less.

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 done. ELISA has higher sensitivity than ICT-based tests. 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 74% cases. Titers of NS1 represent the viral load which is directly proportional to complications.[21] It can be 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.

Table.1 Various dengue specific parameters in suspected cases

POSITIVE TEST RESULT	NUMBER	PERCENTAGE
NS1	128	61.53%
IgM	54	25.96%
IgG	3	1.44%
NS1 & IgM	20	9.62%
NS1 & IgG	0	0%
IgG & IgM	2	0.96%
NS1 & IgM & IgG	1	0.48%
TOTAL	208	100%

Table.2 Comparison of platelet counts with parameters of ICT

POSITIVE TEST RESULT	NUMBER OF SEROPOSITIVITY	PLATELET COUNT (<1,50,000/ml)	PERCENTAGE
NS1	128	88	68.75%
IgM	54	7	12.96%
IgG	3	3	100%
NS1 & IgM	20	9	45%
NS1 & IgG	0	0	0%
IgG & IgM	2	1	50%
NS1 & IgM & IgG	1	0	0%
TOTAL	208	108	51.92%

Fig.1 Showing various parameters of ICT in dengue positive cases
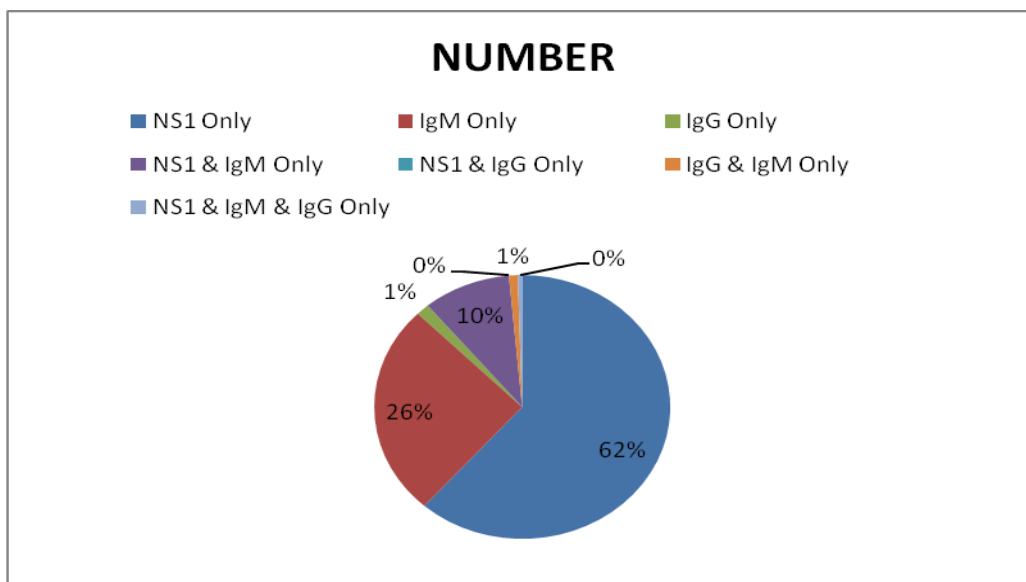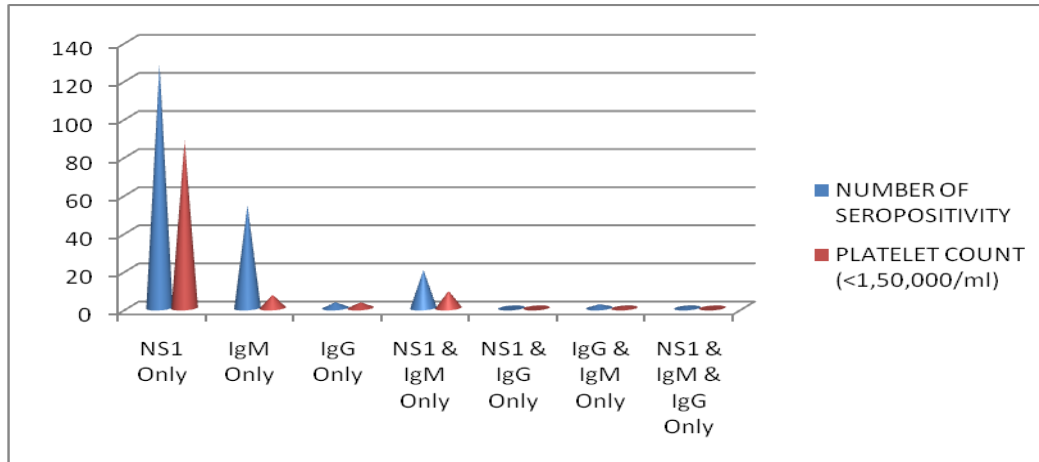
Fig.2 Percentage of thrombocytopenia associated with parameters of ICT

In conclusion, immunochromatographic test is a point of care test and can be done at remote areas with less sample load and not much of expertise is needed. Association of thrombocytopenia in dengue parameter-positive cases was highly significant with comparison to thrombocytopenia in dengue parameter-negative cases. In a country like India hospitals have constraint of resources, ELISA, viral culture, and PCR less likely be done for the diagnosis of dengue infections, though the sensitivity of these tests is more than immuno-chromatographic test. 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 should not forget the fact that dengue often breaks out in resource poor peripheral areas where ICT-based tests could be the only support available. The antibodies take nearly one week to appear in the blood; therefore, antigen detection by the immunochromatographic test is a useful means of diagnosis of dengue infections in the first few days of fever, which helps in management of complications like DHF and DSS.

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