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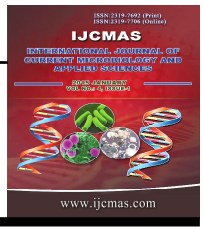

Original Research Article

Importance of NS1 antigen detection and its association with platelet count for early diagnosis of dengue virus infection

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Dengue, NS1 antigen, Ig G antibody, Ig M antibody, Thrombocytopenia, Dengue hemorrhagic fever, Dengue shock syndrome, Immuno-chromato graphy

A B S T R A C T

Dengue fever is an arboviral disease caused by dengue virus belonging to the family *Flaviviridae* and the genus *flavivirus*. Clinical manifestations of dengue range from undifferentiated viral fever to fatal forms like dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). In many dengue endemic settings, laboratory diagnostic resources are limited and simple rapid diagnostic tests (RDTs) provide the opportunities for point-of-care diagnosis. In the present study, we detected NS1 antigen by rapid immunochromatography test and correlated the results with platelet counts. A total of 327 serum samples were collected from clinically suspected cases of dengue fever. The samples were tested immediately for NS1 antigen, IgM and IgG antibodies by using the immunochromatography test kit - Dengue NS1 Ag and Ab Combi Card supplied by J Mitra and Co. Pvt. Ltd, New Delhi, Platelet counts of all the cases were recorded, irrespective of positive or negative result for the above test. The results were analyzed statistically using Chi square test. Of the 327 serum samples tested, a total of 126 (38.5%) specimens were positive for either one or more of the three serological markers (NS1, IgM and IgG) tested. Of the 126 positive serum samples, 54 (42.9%) specimens were positive for NS1 only, 6 (4.7%) positive for IgM only, while 30(23.9%) patients had only IgG positive. Platelet count less than 1, 00,000/ml was noticed in 76 (60.31 %) out of 126 cases Statistical analysis revealed a significant association of NS1 antigen positivity and thrombocytopenia. Rapid immunochromatography tests are helpful in initiating instant treatment and minimizing the serious complications and mortality of dengue infection.

Introduction

Dengue virus is a single stranded enveloped RNA virus belonging to the genus *flavivirus* in the family *Flaviviridae* and transmitted through the bites of *Aedes* mosquitoes (Gould EA *et al*, 2008). There are five serotypes of the virus distinguished on the basis of antigenicity; first four are referred to as DENV-1, DENV-2, DENV-3 and DENV-4 (Rodenhuis Zybert *et al*, 2010) while the fifth type was announced in 2013 (Normile D *et al*, 2013). Clinical manifestations of dengue range from undifferentiated viral fever to fatal forms like dengue hemorrhagic fever (DHF) and

dengue shock syndrome (DSS) (Malavige GN *et al*, 2013). As there is no effective antiviral treatment or a vaccine to prevent infection, careful fluid management and monitoring for complications is the only option available. It is estimated that more than 2.5 billion people are at risk of infection and more than 100 countries including India have endemic dengue virus transmission (Guzman MG *et al*, 2010). As a result of better fluid management regimes and early interventions, the case fatality rates have significantly dropped in many dengue endemic countries (Magpusao NS *et al*, 2003). Appropriate patient management is achieved when laboratory tests provide an accurate and rapid diagnosis of dengue infection. In many dengue endemic settings, laboratory diagnostic resources are limited and simple rapid diagnostic tests (RDTs) provide the opportunities for point-of-care diagnosis.

The dengue virus genome contains about 11,000 nucleotide bases, which code for the three different types of protein molecules (C, prM and E) that form the virus particle and seven other types of protein molecules (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) that are only found in infected host cells and are required for replication of the virus (Guzman MG *et al*, 2010, Rodenhuis-Zybert *et al*,2010).

NS1 antigen is present in the serum in the early phase of infection; however patients that present late in the course of infection may have undetectable levels of NS1 antigen. Dengue IgM antibodies are usually present following 2–5 days of infection and by combining the results of dengue NS1 antigen and IgM antibody testing, accurate diagnosis during acute presentation is achieved (Blacksell SD *et al*, 2008). NS1 antigen detection is reported to be sensitive as well as highly specific (Libraty DH *et al*, 2002). Ju et al. have suggested that platelet counts along with serum IL-10 levels were the most important variables associated with severe dengue (Ju H *et al*, 2013). Duyen *et al*. have shown that higher NS1 antigen levels on day 3 of infection were associated with lower platelet counts (Duyen HT *et al*, 2011). Apart from the dengue specific markers, platelet count is the only supportive laboratory test available in the peripheral areas that can be estimated by microscopy.

Considering the boundaries of healthcare system in the peripheral areas in a dengue endemic country, we tried to correlate the platelet counts and immunochromatography (ICT)-based dengue serology tests.

Materials and Methods

The present study was a retrospective study, conducted in the department of Microbiology, KIMS, Amalapuram, from July 2014 to December 2014. A total of 327 serum samples were collected from clinically suspected cases of dengue fever. The samples were tested immediately for NS1 antigen, IgM and IgG antibodies by using the immunochromatography test kit - Dengue NS1 Ag and Ab Combi Card supplied by J Mitra and Co. Pvt. Ltd, New Delhi, India. The tests were performed in accordance with the manufacturer's instructions. Platelet counts of all the cases were recorded, irrespective of positive or negative result for the above test.

Statistical analysis

The results were analyzed statistically using Chi square test (STATA version 10.1, software used for statistical analysis) to study correlation between NS1 antigen positivity and thrombocytopenia. P value of < 0.05 was taken as significant for interpretation.

Result and Discussion

Of the 327 serum samples tested, a total of 126 (38.5%) specimens were positive for either one or more of the three serological markers (NS1, IgM and IgG) tested. Of the 126 positive serum samples, 54 (42.9%) specimens were positive for NS1 only, 6 (4.7%) positive for IgM only, while 30(23.9%) patients had only IgG positive. A combination of more than one serological marker was detected in the remaining 36 samples (Table 1). Platelet count less than 1, 00,000/ml was noticed in 76 (60.31 %) out of 126 cases (Table 1).

Out of the remaining 201 suspected dengue cases that were negative for any of the above dengue specific parameters by the immunochromatography test, 18 (8.9%) cases were reported to have thrombocytopenia. Statistical analysis for correlation between NS1 antigen and platelet count by Chi square test, revealed a significant association of NS1 antigen positivity and thrombocytopenia in dengue infection, as shown in Table 2.

Efficient, rapid and accurate diagnosis of dengue is of prime importance for early detection of severe cases, case confirmation, differential diagnosis with other infectious diseases, outbreak control or for research activity. Dengue infection is common in the tropical countries where several other infections resembling dengue like malaria, enteric fever are endemic and diagnosis based merely on clinical symptoms is unreliable. Therefore dengue can be easily under diagnosed in the absence of adequate and quality laboratory diagnostic methods. Detection of dengue by virus isolation or molecular methods (RT-PCR) are considered as confirmatory tests with high sensitivity and specificity for the diagnosis of dengue infection (WHO, 2011). However, the need for necessary infrastructure, technical expertise and high cost of the test, make these methods limited in selected advanced laboratories. Many commercial assays are presently available for the detection of dengue NS1 antigen, which is a non structural protein of the dengue virus (Costa VG *et al*, 2014**).** NS1 Ag circulates uniformly in all serotypes of dengue virus at a high level during the initial days of illness therefore has a higher detection rate in acute phase sera (Bessof K, 2008; Alcon S, 2002).

In the present study, a total of 126 (38.5%) out of 327 specimens were positive for either one or more of the three serological markers (NS1, IgM and IgG) tested. Various studies have reported a seropositivity ranging from 15.2% (Kulkarni RD *et al*, 2011) to 39.41% (Santosh Tathe *et al*, 2013) for one or more serological markers of dengue. In the present study, 42.9% specimens were positive only for NS1antigen, by immunochromatography method. Several studies have reported a lesser values of 16% (Shrivastava A *et al*, 2011), 23.3% (Datta S *et al*, 2010) , 30% (Kulkarni RD *et al*, 2011) as well as higher value of even 60 % (Santosh Tathe *et al*, 2013) positivity exclusively for NS1 antigen for diagnosis of dengue. NS1 Ag assay is an effective tool for diagnosis of dengue infection, especially within the first four days of illness (Datta S *et al*, 2010). 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 (Zhang H *et al*, 2014).

In our study, 4.7% cases were positive for IgM only as well as equal numbers of samples were positive for NS1 and IgM antibodies. This clearly concludes that we would have missed a few positive cases if

only NS1 antigen or only IgM antibodies were detected. Similarly, various studies also report a significant increase in detection of dengue when both the assays were performed together in a single sample. (Schilling S *et al*, 2004, Ampaiwan C *et al*, 2008).

The revised 2009 WHO criteria classifies dengue according to the levels of severity: dengue without warning signs; dengue with warning signs (abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, increasing haematocrit with decreasing platelets); and severe dengue (dengue with severe plasma leakage, severe bleeding, or organ failure) (WHO, 2009). A rapid decrease in platelet count in parallel with a rising haematocrit compared to the baseline is suggestive of progress to the plasma leakage or critical phase of the disease (WHO, 2009). Dengue NS1 antigen levels have been shown to associate with disease severity. Cases having dengue fever with an elevated level of NS1 antigen have a higher risk to develop thrombocytopenia and consequently DHF (Libraty DH *et al*, 2002). In the present study, a significant correlation was observed between NS1 antigen and thrombocytopenia. Various studies also concluded that the NS1 alone and with IgM correlated well with thrombocytopenia (Kulkarni RD *et al*, 2011, Santosh Tathe *et al*, 2013).

The remarkable increase in the worldwide burden of dengue has gained increased awareness in developing improved diagnostics for dengue infections. The ELISA test shows greater sensitivity in detecting dengue-specific antibodies than the rapid tests, but rapid tests are quick, easy to perform, convenient, user friendly, with the results available in a short timeframe. 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. Considering the increasing load of dengue in our country, with minimal health care resources at primary level, introduction of rapid tests for speedy and accurate diagnosis of the clinically suspected cases would be the need of the hour.

Table.1 Comparison of platelet counts with various serological markers for Dengue infection

Serological marker for Dengue	**Total positive serum samples (%) n=126**	**Samples with platelet count < 1, 00,000 (%)**
NS1 Ag	54 (42.9%)	42 (77.7%)
IgM only	6 (4.7%)	4 (66.6%)
IgG only	30 (23.9%)	3 (10%)
NS1 + IgM	6 (4.7%)	5 (83.3%)
NS1 + IgG	24 (19.1%)	18(75 %)
NS1 + IgG+ IgM	6 (4.7%)	4(66.6 %)
Total positive	**126 (100%)**	**76 (60.31 %)**

Table.2 Association of platelet counts with NS1 antigen in dengue infection

Platelet range	**NS1 positive**	**NS1 negative**
Platelet count < 100,000/ml	69	25
Platelet count > 100,000/ml	21	212
Total	**90**	**237**

p value is < 0.0001, (p value < 0.05 is significant by Chi – Square Test)

References

Alcon S, Talarmin A, Debruyne M, Falconar A, Duebel V, Flamand M. 2002. Enzyme-Linked Immunosorbent Assay Specific to Dengue Virus Type 1 Nonstructural Protein NS1 Reveals Circulation of the Antigen in the Blood during the Acute Phase of Disease in Patients Experiencing Primary or Secondary Infections. J Clin Microbiol; 40:376-81.

Ampaiwan C, Wathanee C, Viroj P, Kanchana T, Sarapee L, Sutee Y. 2008. The use of Nonstructural protein 1 antigen for the early diagnosis during the febrile stage in patients with dengue infection. J Paed Infect Dis; 27:43-8.

Bessof K, Delorey M, Sun W, Hunsperger E. 2008. Comparison of Two Commercially Available Dengue Virus (DENV) NS1 Capture Enzyme-Linked Immunosorbent Assays Using a Single Clinical Sample for Diagnosis of Acute DENV Infection. Clin Vaccine Immunol; 15: 1513-18.

Blacksell SD, Mammen MP, Thongpaseuth S, et al. 2008. Evaluation of the Panbio dengue virus nonstructural 1 antigen detection and immunoglobulin M antibody enzyme-linked immunosorbent assays for the diagnosis of acute dengue infections in Laos. Diagnostic Microbiology and Infectious Disease; 60(1):43–49

Costa VG, Marques-Silva AC, Moreli ML. 2014. A meta-analysis of the diagnostic accuracy of two commercial NS1 antigen ELISA tests for early dengue virus detection. *PLoS One*, 9(4):e94655.

Datta S, Wattal C. 2010. Dengue NS1 antigen detection: A useful tool in early diagnosis of dengue virus infection. Indian J Med Microbiol; 28:107‑10.

Duyen HT, Ngoc TV, Ha do T, Hang VT, Kieu NT, Young PR, Farrar JJ, Simmons CP, Wolbers M, Wills BA. 2011. Kinetics of plasma viremia and soluble nonstructural protein 1 concentrations in dengue: differential effects according to serotype and immune status. *J Infect Dis*, 203(9)**:**1292-1300.

Gould EA, Solomon T. 2008. "Pathogenic flaviviruses". *The Lancet* 371 (9611): 500–9.

Guzman MG, Halstead SB, Artsob H, et al. 2010. Dengue: a continuing global threat. *Nature Reviews Microbiology*; 8(supplement 12):S7–S16

Ju H, Brasier AR. 2013. Variable selection methods for developing a biomarker panel for prediction of dengue

hemorrhagic fever. *BMC Res Notes*, 6:365.

Kulkarni RD, Patil SS, Ajantha GS, Upadhya AK, Kalabhavi AS, Shubhada RM, *et al.* 2011. *A*ssociation of platelet count and serological markers of dengue infection-importance of NS1antigen. Indian J Med Microbiol; 29:359-62.

Libraty DH, Young PR, Pickering D, Endy TP, Kalayanarooj S, Green S, *et al*. 2002.High Circulating Levels of the Dengue Virus Nonstructural Protein NS1 Early in Dengue Illness Correlate with the Development of Dengue Hemorrhagic Fever. J Infect Dis; 186:1165-8.

Magpusao NS, Monteclar A, Deen JL: 2003. Slow improvement of clinically-diagnosed dengue haemorrhagic fever case fatality rates. Trop Doct, 33(3):156–159.

Malavige GN, Ogg GS. 2013. T cell responses in dengue viral infections. *J Clin Virol*, 58(4):605-611.

Normile D.2013. "Surprising new dengue virus throws a spanner in disease control efforts". *Science* 342 (6157): 415.

Santosh Tathe, Chincholkar VV, Kulkarni DM, Nilekar SL, Ovhal RS, and Halgarkar CS. 2013. A study of NS1 antigen and platelet count for early diagnosis of dengue infection. *Int.J.Curr.Microbiol.App.Sci* 2(12): 40-44

Rodenhuis-Zybert IA, Wilschut J, Smit JM. August 2010. "Dengue virus life cycle: viral and host factors modulating infectivity". *Cell. Mol. Life Sci.* 67 (16): 2773–86.

Schilling S, Ludolfs D, Le VA, Schmitz H. 2004. Laboratory diagnosis of Primary and secondary dengue infections. J Clin Virol; 31:179-84.

Shrivastava A, Dash PK, Tripathi NK, Sahni AK, Gopalan N, Lakshmana Rao PV. 2011. Evaluation of a commercial dengue NS1 enzyme linked immunosorbent assay for early diagnosis of dengue infection. Indian J Med Microbiol; 29:51-5.

WHO. 2011. Comprehensive guidelines for prevention and control of dengue fever and dengue haemorrhagic fever. In Geneva, Switzerland: World Health Organization; 2011.

World Health Organization. Geneva, Switzerland: WHO; 2009. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control.

Zhang H, Li W, Wang J, Peng H, Che X, Chen X, Zhou Y. 2014. NS1-based tests with diagnostic utility for confirming dengue infection: a meta-analysis. Int J Infect Dis. Sep; 26:57-66.