



## Original Research Article

### A study of NS1 antigen and platelet count for early diagnosis of dengue infection

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#### A B S T R A C T

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Dengue fever is an acute febrile arboviral disease affecting tropical & subtropical regions of the world. Dengue infection produces a spectrum of clinical illness, ranging from an asymptomatic to its most severe form like dengue haemorrhagic fever and dengue shock syndrome. In view of high morbidity and mortality, it is imperative to have a rapid and sensitive laboratory assay for early detection of the dengue infection. The newer parameter NS1 antigen has gained a lot of interest for early diagnosis of the disease. Apart from dengue specific parameters, thrombocytopenia and haemoconcentration are constant findings. So we tried to correlate the platelet counts with immunochromatography based test for NS1 antigen & antibody detection. Samples from suspected cases of dengue fever were tested for NS1 antigen and Ig G/M antibody. Platelet counts of all these cases were noted and statistical analysis was done. Out of 236 serum samples tested, 93 were positive for either one or more of the three serological markers. Of these 93 samples, 56 were positive for NS1 antigen with or without antibody. Among the 56 NS1 positive samples, 34 were exclusively positive for NS1 only. Out of 93 seropositive cases thrombocytopenia was evident in 76 cases. As the NS1 antigen is detectable in blood from day one after onset of fever, its assay is an effective tool for early diagnosis of dengue infection so as to avoid complications.

#### Introduction

Dengue fever is an acute febrile arboviral disease caused by dengue virus belonging to family *Flaviviridae* and genus *Flavivirus* affecting tropical & subtropical regions of the world. Virus transmission in its simplest form involves ingestion of

viremic blood by mosquitoes and passage to a second susceptible host (Monath, 1994). Dengue virus infection with any one of four serotypes (DENV 1-4) produces a spectrum of clinical illness, ranging from an asymptomatic to its most

severe form like dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). DHF is a vasculopathy characterized by capillary leakage and haematological dysregulation; in severe case hypovolaemic shock (DSS) may develop (Vaughn *et al.*, 1997; Tricou *et al.*, 2010).

In view of high mortality and morbidity rates, it is imperative to have a rapid and sensitive laboratory assay for early detection of the disease. Several laboratory methods such as virus isolation, genomic RNA, antigen and antibody detection methods are available to diagnose the dengue infection (Datta *et al.*, (2010). However methods like virus isolation, genomic RNA detection by PCR, antigen and antibody detection by ELISA needs well trained staff and an expensive setup which is not feasible in peripheral hospital settings (Kassim *et al.*, 2011).

In most cases antibody (Ig G/M) detection by immunochromatographic (ICT) based tests are commonly used for diagnosis of dengue infection, but time required for appearance of Ig M antibody is approximately 4 – 6 days (World Health Organization , 1997). The newer parameter, DENV non-structural 1 antigen (NS1) has gained lot of interest as a new biomarker for early diagnosis of dengue infection. Dengue NS1 antigen, highly conserved glycoprotein produced in both membrane associated and secretory forms (Young *et al.*, 2000). ELISA directed against NS1 antigen have demonstrated its presence at high concentrations in sera of dengue virus infected patients during early clinical phase of disease. (Kumarasamy *et al.*, 2007). Apart from dengue specific parameters, thrombocytopenia and haemoconcentration are constant findings in DHF. A drop in platelet count below

1,00,000 per mm<sup>3</sup> is usually found between the third and eighth day of illness (World Health Organization 1997).

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. Keeping in mind of the available resources of healthcare system in the peripheral areas, we tried to correlate the platelet counts and ICT based serological tests for NS1 antigen & antibody detection for early diagnosis of dengue fever.

The main aim of this study to correlate the platelet count with various dengue specific serological markers (NS1 antigen and IgM/ G antibodies) by immunochromatography based test in a peripheral healthcare setup.

## **Materials and Methods**

The present study was conducted in a tertiary care rural hospital & medical college from June 2012 to May 2013. A total of 236 serum samples were processed from suspected cases of dengue fever by using *dengue day1test* for detection of NS1 antigen and IgG/ M antibodies according to manufacturer's instructions (J. Mitra). Platelet counts of all these cases were noted. Statistical analysis was done by using Chi-square test and Z- test.

## **Result and Discussion**

Out of 236 serum samples tested, 93 (39.41%) were positive for either one or more of the three serological markers. Of these 93 samples, 56 (60.22%) were positive for NS1 antigen with or without antibody and remaining 37 (39.78%) were

**Table.1** Association of platelet counts with seropositivity in dengue infection (n=236)

| <b>Platelet range</b>       | <b>Dengue seropositive</b> | <b>Dengue seronegative</b> |
|-----------------------------|----------------------------|----------------------------|
| Platelet count < 100,000/ml | 76 (81.72)                 | 40 (27.97%)                |
| Platelet count > 100,000/ml | 17(18.28%)                 | 103 (72.03%)               |
| <b>Total</b>                | <b>93 (100%)</b>           | <b>143 (100%)</b>          |

(Chi-square test,  $X^2=65.14$ ,  $P<0.001$ )

**Table.2** Comparison of platelet count with various dengue parameters (n=93)

| <b>Serological markers for Dengue</b> | <b>Total positive serum samples</b> | <b>Positive serum samples with platelet count &lt; 100,000/ml</b> | <b>Percentage</b> |
|---------------------------------------|-------------------------------------|---|-------------------|
| NS1 only                              | 34                                  | 30  | 88.23%            |
| NS1+IgM                               | 5                                   | 5   | 100%              |
| NS1+IgM+IgG                           | 17                                  | 15  | 88.23%            |
| IgG only                              | 20                                  | 14  | 70.00%            |
| IgM only                              | 16                                  | 11  | 68.75%            |
| IgG + IgM                             | 1                                   | 1   | 100%              |
| <b>Total</b>                          | <b>93</b>                           | <b>76</b>   | <b>81.73%</b>     |

**Table.3** Correlation of NS1 antigen with platelet counts. (n=236)

| <b>Platelet range</b>  | <b>NS1 antigen positive</b> | <b>NS1 antigen negative</b> |
|------------------------|-----------------------------|-----------------------------|
| Platelets < 100,000/ml | 50                          | 66                          |
| Platelets > 100,000/ml | 6                           | 114                         |
| <b>Total</b>           | <b>56</b>                   | <b>180</b>                  |

(Chi-square test,  $X^2=47.32$ ,  $P<0.001$ )

positive for either IgM or IgG or both. Among the 56 NS1 positive samples, 34 (60.71%) were exclusively positive for NS1 antigen, 5 (8.93%) were also positive for IgM antibody and 17 (30.36%) were

positive for all the three serological markers.

Among 93 seropositive cases thrombocytopenia was evident in 76

(81.72%) while out of 143 seronegative cases thrombocytopenia was seen in 40 (27.47%).

Out of the 56 NS1 positive cases, 50 (89.28%) had platelet count less than 100,000/ml while out of 180 NS1 negative cases, 66 (36.66%) had platelet count less than 100,000/ml. Of the 34 exclusively NS1 positive cases 30 (88.23%) had platelet count less than 100,000/ml.

As the initial symptoms of dengue mimic those of malaria, typhoid and leptospirosis which are endemic in the country, availability of a rapid and differential diagnosis at an early stage of infection is of utmost importance for better patient management (Kothari *et al.*, 2006; Dutta and Christopher, 2005). For long time detection of dengue specific antibody has been main stay of diagnosis of dengue infection. The role NS1 Ag for early detection of dengue infection is currently being evaluated by many investigators without requirement of paired sera. (Datta *et al.*, 2010).

In our study, among the 56 NS1 positive cases, 34(60.71%) were negative for antibodies and these would have otherwise been missed. They were suffering from infection and were also viremic i.e. they could transmit the virus if bitten by a mosquito. Similar observation was given by Subhash C Arya *et.al.*

Among 93 seropositive cases, 20 (21.50%) cases were positive for Ig G only. Such patients either presented in later stage of illness or may have immunity due to previous flavivirus infection or immunization (World Health Organization, 1997).

When we tried to correlate various dengue specific parameters with

thrombocytopenia, we found that out of 93 seropositive cases, 76 (81.72%) had platelet count less than 100,000/ml. This value was higher than as shown by RD Kulkarni *et al.*

Out of 56 NS1 positive cases, thrombocytopenia was evident in 50 (89.28%) and in 37 exclusive antibody positive cases, thrombocytopenia was noted in 26 (70.27%). This difference was statistically significant by using Z-test and the finding is in accordance with RD Kulkarni *et al.* Among 34 exclusively NS1 positive cases, thrombocytopenia was evident in 30 (88.24%) cases which was statistically significant by applying Chi-square test.

As the NS1 antigen is detectable in blood from day one after onset of fever, its assay is an effective tool for early diagnosis so as to avoid complications of dengue infection. The ease, speed and dependability of ICT tests make them an effective technique in addressing this potentially fatal, epidemic prone infection. Apart from these dengue specific parameters, platelet count is the only accessory laboratory test available in the remote areas that can support the diagnosis of dengue infection. Therefore studies like this will contribute significantly to the clinical management and can reduce morbidity and mortality in dengue infection.

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