



Original Research Article

The Study of Detection of Dengue NS1 Antigen and IgM Antibody by ELISA in and around Aurangabad, India

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ABSTRACT

Keywords

NS1 (nonstructural protein 1) Antigen, DENV (dengue virus), IgM (Immunoglobulin M), IgG (Immunoglobulin G), ELISA (enzyme linked immunosorbent assay), DHF (dengue haemorrhagic fever), DSS (dengue shock syndrome)

An early and accurate diagnosis of dengue in the acute phase of illness is important for initiation of therapy as well as for early enhancement of epidemic control measures. Patients suspecting of dengue infection were studied. The study includes detection of dengue NS1 antigen by ELISA in patients having 1 to 5 days fever, detection of dengue NS1 antigen and IgM antibody by ELISA in patients having fever of five to nine days and only detection of IgM antibody by ELISA in patients having fever of more than 9 days. Platelet count of the positive patients for dengue NS1 antigen and IgM antibody by ELISA were seen. The present study was done in department of Microbiology Government Medical College, Aurangabad during year Jan 2014 to Dec 2014. 872 serum samples from patients with a suspecting of dengue infection were collected. These patients were divided into three groups according to days of fever Group I - Patients having 1 to 5 days fever were tested for NS1 antigen ELISA. Out of 392 tested 141(35%) patients were positive for dengue NS1 antigen. 251 patients were negative for dengue NS1 antigen. Group II: Total patients having fever more than 5 days to 9 days were 380. These patients were tested both by dengue NS1 antigen and by dengue IgM by ELISA. Patients positive for dengue NS1 antigen by ELISA were 30 (8%) and dengue IgM ELISA were 50 (13%). Of these four (1%) patients shows both NS1 antigen and IgM positive for dengue. Remaining 300 patients were negative by both dengue NS1 antigen and dengue IgM. GROUP III: 100 patients were having fever of more than 9 days. These were tested for only Dengue IgM ELISA. 16(16%) patients were showing positive for dengue IgM by ELISA. Platelet counts of patients positive for dengue NS1 antigen and IgM were noted. Five patients having platelet counts less than 25000 with rash, petechiae and encephalitis died .Out of five one patient was positive for NS1 antigen and other three were positive for IgM antibody. Dengue NS1 antigen is used for rapid and accurate diagnosis in acute phase of illness. Combination of dengue NS1 antigen and IgM ELISA on single sample can improve the diagnosis of dengue without the requirement of paired sera. Correlations of positive samples with platelet counts were noted. Apart from dengue specific marker parameter platelet count is the only accessory laboratory test available that can support the diagnosis for dengue.

Introduction

Dengue virus belongs to family flaviviridae. Dengue virus causes dengue fever which is an acute febrile arboviral disease (Datta *et al.*, 2010). There are four distinct but antigenically related serotypes of dengue virus. The virus is transmission is by *Aedes aegypti* mosquito. The symptoms of dengue virus infection are insufficient for clinical differentiation from other viral illness; definitive diagnosis relies on laboratory tests. An early and accurate diagnosis of dengue in the acute phase of illness is important for early enhancement of epidemic control measures. There is high mortality and morbidity caused by dengue infection. Several laboratory methods such as virus isolation, RNA, Antigen and antibody detection methods are available to diagnose the dengue infection (Chakravarti *et al.*, 2006). However methods like virus isolation, RNA detection by PCR, need well trained staff and expensive set up which is not feasible in peripheral hospital setting (Dussart *et al.*, 2006). Viral isolation is costly, the results are usually available after 6 to 10 days and it is only obtainable in laboratories with the appropriate infrastructure for cell culture or mosquito colonies. The RT-PCR and other PCR-based techniques give results within 24 hours but they are also costly and they are not available for most clinicians. The recently introduced dengue rapid kits like rapid immunochromatography test (RICT) are available in Indian market. These are combination packs, which detect circulating non-structural protein (NS1) antigen, as well as IgM and IgG against dengue virus (WHO, 2009).

The dengue virus NS1 (Non-structural protein 1) is a highly conserved glycoprotein that is essential for the viability of dengue virus and is produced both in membrane-

associated and secretory forms by the virus. Enzyme-linked immunosorbent assays (ELISA) directed against NS1 antigen (NS1 Ag) have demonstrated its presence at high concentrations in the sera of dengue virus infected patients during the early clinical phase of the disease (Kumarasamy *et al.*, 2007). Antigen detection of non-structural dengue antigens may be of benefit for an early-stage rapid diagnosis of infection due to its long half life in the blood (Das *et al.*, 2009). The detection of secretory NS1 protein represents a new approach to the diagnosis of acute dengue virus infection (Dussart *et al.*, 2006).

Materials and Methods

The present study was conducted in department of Microbiology Government Medical. College Aurangabad during Jan 2014 to Dec 2014. Blood samples from both, out patients department (OPD) and Inpatients department (IPD) were collected in plain bulb. The blood samples comprised of both acute and early- convalescent phases depending on the reporting time of the patients. Blood samples collected from patients who reported within one to five days of fever were called acute samples. Samples collected from patients who came with history of fever for five to nine days were called early convalescent samples. Samples collected from patients who came with history of fever for more than nine days were called late convalescent samples. All patients were having the symptoms of fever, arthralgia, with or without rash and bleeding manifestations. Blood samples were centrifuged and serum was separated. The serum of patients was used for serological testing. These samples were negative for other illness like malaria and typhoid.

Total of 872 samples were tested for suspected dengue fever. These patients were

divided into three groups according to days of fever as follows.

Group I - Patients having fever of 1 to 5 days were 392. These were tested for NS1 antigen by ELISA. Out of 392 tested 141(35%) samples were positive for NS1 antigen. 251 were negative for NS1 antigen. As in first 5 days only NS1 antigen is present these patients were not tested for IgM Antibodies.

Among total 141(35%) positive patients for NS1 antigen 100 were males and 41 were females. More males were showing dengue infection than females. The age groups mostly affected were between 11 and 20 groups of age.

Group II - Patients having fever for 5 days to 9 days were 380. These patients were tested both for dengue NS1 antigen and dengue IgM by ELISA. Among these 380 patients dengue NS1 Antigen was positive in 30 (8%) and dengue IgM in 50 (13%) patients. Of these, four patients (1%) had both NS1 antigen and IgM antibodies against dengue. Remaining 300 patients were negative for both dengue NS1 antigen and dengue IgM.

Group III – Remaining 100 patients were having fever of more than 9 days. These were tested for only dengue IgM ELISA. 16(16%) patients were showing positive for dengue IgM. These patients were in the age groups of 11-20 years.

NS1 antigen of dengue was detected by ELISA kits from (J. Mitra & Co. Pvt. Ltd. Dengue NS1 Ag Microlisa. Microwell ELISA tests for the detection of dengue NS1 Antigen in Human Serum /Plasma). Tests were performed as per manufacturer guidelines.

Interpretation of result by J. Mitra ELISA test for detection of NS1 antigen of dengue. Once the test was found to valid, NS1 units were calculated as per manufacturer's guidelines.

1. The sample is negative if dengue NS1 antigen units are less than 9.
2. The sample is equivocal if dengue NS1 antigen units are between 9 -11.
3. The sample as positive if dengue NS1 antigen units are more than 11.

All NS1 antigen positive samples showed more than 11 dengue antigen units and samples were positive. None of sample were Equivocal. Remaining samples showed less than 9 and were negative for Dengue NS1 antigen.

Dengue IgM capture ELISA kits were obtained from National Institute of Virology Pune. Tests were performed as per manufacturer guidelines. Sample results were expressed in terms of units as per the recommendations of the manufacturer. Tests were performed as per manufacturer guidelines.

Interpretation of result for presence of dengue specific IgM antibodies by IgM capture ELISA in the patient's sera is.

1. Non-reactive for dengue IgM antibody if < 9 units
2. Equivocal in range of 9–11 units.
3. Reactive for a value of >11 units.

All samples tested shows dengue IgM antibody more than 11 units. No equivocal range seen. Platelet count of total 171 positive patients for NS1 dengue antigen (in group I and group II) and dengue IgM ELISA were reported. Platelet count of IgM positive patients in group II were above the 1 lakh.

Platelet count - 1, 50,000 to 4, 50,000 – Normal.

Less than 1 lakh – Dengue haemorrhagic fever (WHO cut off for platelet count for DHF).

In table 3 platelet counts were less than 1 lakh in 71(41%) patients of positive dengue NS1 antigen. These patients show thrombocytopenia. Out of 71 patients 14 showed platelet count less than 50 000. Five patients show platelet count less than 25000. These five patients showed rash and haemorrhagic patches. All the patients showing thrombocytopenia were admitted. They were given treatment. Five patients showing 25000 platelet counts were given platelets, and IV fluids. Follow up was done. They were admitted in ward for 15 days. One patient having dengue NS1 antigen positive and platelet count less than 25000 died on second day in hospital all the other patients recovered and were discharged from hospital.

Platelet counts in positive dengue IgM ELISA were less than 1 lakh in 25 patients. These patients show thrombocytopenia. Out of 25 patients four patients shows platelet count less than 20000 and have rash, encephalitis and Petechiae, Malena and Bleeding tendency. These patients were admitted in hospital. During treatment, four patients died. Other patients recovered from illness and were discharged.

In order to provide early public health control measures of dengue outbreaks, it is important to establish the diagnosis of acute dengue virus infection during the first few days after manifestation of clinical symptoms. Early diagnosis of dengue is essential to keep a watch on complication as DHF and DSS.

In our study IgM was not tested in patients of group one (fever less than five days), as IgM takes time to appear. In group three (fever more than nine days), NS1 was not tested as it is known to disappear after about five days.

We preferred dengue NS1 antigen and dengue IgM ELISA over rapid test for dengue as it has higher sensitivity and specificity. Among the serological markers, NS1 antigen appears to be a better and earlier marker appearing between 1 and 9 days. Dengue NS1 antigen is identified only till 5 days of fever after onset of symptoms, and rapidly disappear following the appearance of specific antibodies. NS1 is synthesized by all flaviviruses and is secreted from infected mammalian cells. The presence of secreted NS1 in the blood stream stimulates a strong humoral response. The availability of a commercial dengue NS1 antigen ELISA test kit has allowed early detection of dengue virus (Kao *et al.*, 2005). Following the bite of an infected *Aedes mosquito*, dengue virus replicates quickly before the development of signs and symptoms. NS1 Ag circulates uniformly in all serotypes of dengue virus and circulates at high level during the 1st few days of illness (Besoff *et al.*, 2008). This is the reason for high detection rate of NS1Ag in acute phase sera, there after decreases gradually and antibody detection becomes more prominent after 5 days. But practically this is not feasible as the patients never approach a clinician when there are no symptoms.

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).

Table.1 Group – I shows age and sex wise distribution of dengue NS1 antigen ELISA positive patients

Sr.no	Age groups	Male	Female	Total
1	1- 10 yrs	10	5	15(10.6%)
2	11 -20 yrs	50	10	60(42.5%)
3	21 -30 yrs	25	20	45(31.9%)
4	31 -40 yrs	10	5	15(10.6%)
5	41 -50 yrs	1	1	2(1.4%)
6	More than 50	3	0	3(2.1%)
		91	41	Total n=141

Table.2 Group II shows age and sex wise distribution of dengue NS1 antigen and IgM positive patients

Sr. no	Age groups	NS1antigen positive Patients	IgM Positive patients
1	1-10yrs	12	10
2	11-20 yrs	8	15
3	21-30 yrs	6	18
4	31-40 yrs	2	4
5	41-50 yrs	1	3
6	More than 50	1	0
	Total	n=30	n=50

In table 2 group II children were showing higher positivity of NS1 antigen. It also shows IgM Positivity higher in age group of 21–30 years.

Table.3 Comparison of platelet count and patients positive for total dengue NS1 antigen in group I, group II and dengue IgM ELISA

Platelet count	NS1 antigen positive patients	IgM positive patients
Platelet counts more than 1 lakh	100 (58%)	25 (50%)
Platelet counts less than 1 lakh	71 (41%)	25 (50%)
	Total n=171	Total n=50

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 1 (Datta *et al.*, 2010). Before fifth day of fever, NS1 antigen positivity was 35%. Similar studies were done by Shrivastava *et al.* (2011).

In our study we detected IgM against dengue in 13% cases from day 5 to 9. NS1 antigen was found in 8% cases in the patients having fever of 5–9 days. This indicates that NS1 antigen indeed declines after day five. The person having NS1 antigen for the longest period was till day seven. The persons positive for both simultaneously were four (1%). Similar

study showing both positivity was done by Shrivastava *et al.*, 2011.

16% persons having clinical features of dengue and fever of more than 9 days duration were positive for IgM dengue antibodies. Maximum duration of fever was 20 days in whom IgM was detected. This is perfectly possible as IgM antibodies remain in the body for a period of about 90 days before declining. However the detection of antibodies in a dengue infected person is only possible after 4-5 days of disease onset (World Health Organization (WHO, 2009). Apart from dengue specific marker parameter platelet count is the only accessory laboratory test available that can support the diagnosis for dengue (Kulkarni, *et al.*, 2011).

In conclusion, Dengue NS1 antigen is used for early and accurate diagnosis in acute phase of illness. Combination of dengue NS1 antigen and IgM ELISA on single sample can improve the diagnosis of dengue without the requirement of paired sera. Correlations of positive samples with platelet counts were noted.

Reference

- Bessoff, K., Delorey, M., Sun, W., Hunsperger, E. 2008. Comparison of two commercially available Dengue virus NS1 antigen capture ELISA using a single clinical sample for diagnosis of acute DEN V infection. *Clin. Vaccine Immunol.*, 15(10): 1513–18.
- Chakravarti, A., Kumaria, R., Batra, V., Verma, V. 2006. Improved detection of dengue virus serotypes from serum samples; evaluation of single tube multiplex RT-PCR with cell culture. *Dengue Bull.*, 30: 133–40.
- Das, D., Mongkolaunkoon, S., Suresh, M.M.R. 2009. Super induction of dengue virus NS1 protein in *E. coli*. *Protein Express. Purif.*, 66: 66–72.
- Datta, S., Wattal, C. 2010. Dengue NS1 antigen infection. an useful tool in early diagnosis of dengue virus infection. *Indian J. Med. Microbiol.*, 28(2): 107–10.
- Dussart, P., Labeau, B., Lagathu, G., Louis, P., Nunes, M.R.T., Rodrigues, S.G., *et al.* 2006. Evaluation of an enzyme immunoassay for detection of dengue virus NS1Antigen in human serum. *Clin. Vaccine Immunol.*, 13: 1185–89.
- Kao, C.L., King, C.C., Chao, D.Y., Wu, H.L., Chang, G.J. 2005. Laboratory diagnosis of dengue virus infection: current and future perspectives in clinical diagnosis and public health. *J. Microbiol. Immunol. Infect.*, 38: 5–16.
- Kothari, M.V., Karnad, D.R., Bichile, L.S. 2006. Tropical infection in ICU. *J. Assoc. Phys. India*, 54: 291–8.
- Kulkarni, R.D., Patil, S.S., Ajantha, G.S., Upadhya, A.K., Kalabhavi, A.S., Shubhada, R.M., Shetty, P.C., Jain, P.A. 2011. Association of platelet count and serological markers of dengue infection-importance of NS1 antigen. *Indian J. Med. Microbiol.*, 29: 359–62.
- Kumarasamy, V., Wahab, A.H.A., Chua, S.K., Hassan, Z., Chem, Y.K., Mohamed, M., Chua, K.B. 2007. Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection. *J. Virol. Methods*, 140: 75–9.
- Shrivastava, A., Dash, P.K., Tripathi, N.K., Sahni, A.K., Gopalan, N., Lakshmana Rao, P.V. 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, 2009World Health Organization (WHO) and the special programme for research and training in tropical diseases: Dengue: guidelines for diagnosis, treatment, prevention and control - New edition. France.