

TO EVALUATE THE ROLE OF NS1 ANTIGEN FOR EARLY DETECTION OF DENGUE FEVERN. S. Chithambaram¹, Kushal D. Shah²**HOW TO CITE THIS ARTICLE:**

N. S. Chithambaram, Kushal D. Shah. "To Evaluate the Role of NS1 Antigen for Early Detection of Dengue Fever". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 71, December 18; Page: 15063-15069, DOI: 10.14260/jemds/2014/4026

BACKGROUND: In India, Dengue epidemics are becoming more frequent and are straining the limited resources of the public health system. Diagnosing dengue early is challenging because the initial symptoms of dengue infection are often non-specific. It is for this reason diagnosing dengue infection early becomes important. **AIM:** To evaluate the role of NS1 antigen for early detection of dengue fever. **SETTING AND DESIGN:** Prospective Study done in a tertiary care hospital between January 2013 to September 2014. **MATERIAL AND METHODS:** Patients in the age group between 6 months to 14 yrs with onset of fever up to 7 days and patients fulfilling the WHO clinical criteria of diagnosis dengue fever. **STATISTICAL ANALYSIS:** sensitivity, specificity, PPV and NPV. **RESULTS:** Of the 143 patients enrolled, 100 were serologically proved to have dengue illness and the rest 43 were non dengue patients. Of the 100 dengue patients NS1, IgM and IgG was positive in 62, 53 and 15 patients respectively. The sensitivity, specificity, PPV and NPV of NS1 Ag were found to be 62%, 100%, 100% and 53% respectively. **CONCLUSION:** The present study showed that dengue serological tests have a significant role in the early diagnosis of dengue fever. Hence it is recommended to do the serological tests (NS1 and IgM) early in all suspected dengue cases so that we can diagnose early and initiate necessary treatment.

KEYWORDS: Dengue fever, NS1 Ag, IgM, IgG .

INTRODUCTION: In India, Dengue epidemics are becoming more frequent and are straining the limited resources of the public health system. Diagnosing dengue early is challenging because the initial symptoms of dengue infection are often non-specific. It is for this reason diagnosing dengue infection early becomes important. Dengue illness, caused by the dengue viruses, members of the Flaviviridae family of small enveloped viruses carry a single-stranded RNA genome of relatively simple organization. They encompasses four closely related serotypes: DV-1, DV-2, DV-3, and DV-4. All four DV serotypes are transmitted between humans in nature by mosquitoes of the genus *Aedes*, principally *Aedes aegypti*, which is highly domesticated and has a preference for biting humans. It is a day-biting mosquito and breeds in standing water.

Most of these infections are clinically in apparent. Among symptomatic cases, the majority of subjects experience uncomplicated dengue fever (DF), accompanied by headache, myalgia, and less often, a maculopapular rash. Headache and myalgia may be quite debilitating, which originated the name "break-bone fever. If left untreated, mortality from the complications of DF is as high as 20%, whereas if recognized early and managed properly, mortality is less than 1%.^[1]

Diagnosing dengue early is challenging because the initial symptoms of dengue infection are often non-specific and serological tests, which are the mainstay of current laboratory diagnosis, to confirm dengue late in the course of illness.^[2]

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The life threatening complications like hepatitis,^[3,4] encephalitis,^[5,6,7] glomerulonephritis^[8] and myocardial dysfunction^[9] usually starts between 3-7 days of dengue virus infection during the critical phase of illness. It is for this reason diagnosing dengue infection early becomes important.^[10]

The onset of dengue symptoms is marked by the presence of dengue NS1 (Non-structural 1) antigen in the patient's serum. NS1 is a glycoprotein that is common to all dengue serotypes and can be used to detect either primary or secondary infections in the earliest stages.^[11] It is detected on day 1 and can be found up to day 9 after the onset of fever.^[12,13,14] Dengue infections can be diagnosed early by virus isolation in cell culture or by detection of viral RNA by nucleic acid amplification tests (NAAT) which requires expertise, expensive equipment and reagents. If we do NS1 (Non-structural 1) antigen detection which is cost effective dengue can be detected at an early stage and untoward complications can be prevented. Since there are limited studies using NS1 antigen detection as a diagnostic tool in the early detection of dengue illness and found to be sensitive, this study was done to corroborate with the other studies.

MATERIAL AND METHODS: This prospective study was conducted in the paediatric intensive care unit (PICU) and paediatric ward of a tertiary care hospital in Bangalore. This was a prospective study done between January 2013 to September 2014. Sample size was calculated by universal sampling technique with the objective to enroll every consenting participant with a minimum number of 100. Patients in the age group between 6 months to 14 yrs, Onset of fever up to 7 days and patients fulfilling the WHO clinical criteria of diagnosis dengue fever were included in this study. The actual enrolment was 143 cases due to dengue surge during the study period. Those patients less than 6mths or > 14 yrs, fever for more than 7 days and children which do not fulfil the WHO criteria for the diagnosis of dengue illness were excluded. The patients were enrolled after obtaining written informed consent from the parents. At the time of enrolment, detailed history and examination was performed to diagnose dengue fever based on clinical criteria of WHO and findings were recorded on predesigned and pretested proforma. Investigations like Complete blood count, serum electrolytes, renal function test, liver function test and dengue serology were done in all patients. In these patients chest x-ray, USG abdomen, coagulation profile (PT, APPT, INR) were performed wherever indicated. Serological testing (NS1, Ig M and Ig G) was done using SD bioline dengue duo test kit. It is an in-vitro, one step assay designed to detect both dengue virus NS1 and differential IgM/IgG antibodies to dengue virus in human serum or plasma. All the cases diagnosed as Dengue were treated as per the WHO treatment protocol. All the patients were treated effectively and no mortality noted during the study period. The data obtained was tabulated and analysed using SPSS software for estimating the Sensitivity, Specificity, Positive predictive value and Negative predictive value of NS1 antigen for detecting Dengue illness early. Chi square test and Fischer exact test were also done.

RESULTS: A total number of 143 patients suspected clinically to have dengue fever were enrolled and analyzed. Of these 143, 100 (69.93%) were serologically proved to have dengue illness and the rest 43 (30.06%) were non-dengue patients. Of the 100 dengue patients NS1 was positive in 62 patients. The sensitivity, specificity, PPV and NPV of NS1 Ag were found to be 62%, 100%, 100% and 53% respectively.

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Age	No. of pts	%
<1 yr	2	1.39
1 to 5 yrs	37	25.87
6 to 10 yrs	63	44.05
>10 yrs	41	28.67
Total	143	100

Table 1: Age distribution of the patients under study

Table 1 shows the age distribution of the patients who were enrolled for the study. Majority of the patients were between the age group of 6 to 10 years, 63 out of the total 143(44.05%). Males were 53.14% and Females 46.85%.

Duration of fever	No. of pts	%
1-3 days	49	34.26
4-5 days	69	48.25
6-7 days	25	17.48
Total	143	100

Table 2: Duration of fever

Table 2 demonstrates the number of patients who presented with fever <7 days of duration. Maximum number of patients 69(48.25%) presented with the fever duration lasting for 4 to 5 days.

NS 1 Ag	No of pts	%
Only NS1 +	46	46%
NS1 + with other parameters	16	16%
NS1 –negative	38	38%

Table 3: NS1 Ag in dengue patients

This table 3 demonstrates that only NS1 antigen was positive for dengue in 46(46%) number of patients out of the 100 patients who were diagnosed as dengue fever using clinical and serological evaluation. About 16 (16%) number of patients were positive for NS1 antigen with either IgM or IgG antibodies and the rest 38(38%) were negative for NS1 antigen. Overall 62 number of patients were positive for NS1 antigen out of the 100 dengue patients.

Class of dengue fever	NO. OF PATIENTS	%
Class A	40	40
Class B	30	30
Class C	30	30
TOTAL	100	

Table 4: Class of dengue illness

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This table 4 demonstrates that the maximum number of dengue patients suffered from the classical dengue fever and required only conservative management with minimum intervention. Patients belonging to class C were all admitted to the PICU and were closely observed and all were successfully treated.

Day of positivity	NS1 positivity
1 – 3	11
4 – 7	51
Total	62

Table 5: Division of serological positive cases based on the day of positivity

Out of the total 100 patients NS1 was positive for about 62 number of patients with a maximum positivity on 4th and 7th day of illness. 11 (17.7%) number of patients were positive between 1-3 days; 38 (61.2%) number of patients were positive for NS1 between day 4-5; 13 (20.9%) number of patients were positive after 5 days. Though NS1 antigen production is on day 1 of illness up to 9 days in our study it was found to have maximum positivity between 4 – 7 days.

Test	Dengue	Dengue	TOTAL
	Yes	No	
Positive	62(a)	0(b)	62
Negative	38(c)	43(d)	81
Total	100	43	143

Table 6: NS1 Ag status for the statistical analysis

This demonstrates that although the NS1 antigen is highly specific has 100 % positive predictive value, they are fairly low sensitive with NS1 62% sensitive.

DISCUSSION: Dengue has become a major public health problem due to the human morbidity and mortality it causes. Controlling Dengue infection is challenging because it requires effective vector control. Morbidity and mortality can be prevented by early diagnosis and treatment.

Several laboratory methods like NS 1 Ag, IgM and IgG Ab, virus isolation, RNA detection are available to diagnose dengue infection. However methods such as virus isolation and RNA detection needs a specialized laboratory and trained personnel which are not widely available in our hospital settings. In this study the potential use and the role of NS1 antigen for the early diagnosis of dengue illness has been analyzed.

In our study of 143 samples, NS1 positivity was detected with a very low sensitivity 33.3% till day 3 of fever. In contrary, a study done by Singh M P et al., found NS1 positivity with good sensitivity (71-100%) till day 3 of fever, whereas IgM had a sensitivity of 0% to 50% at this time.^[15]

In our study of the 143 patient sample tested, Dengue NS1 antigen gave an overall positivity rate of 62 % (62/100) in dengue patients. Only NS1 antigen can be used to test during the second and the third day of illness. IgM positivity showed positivity by fourth day of illness and then gradually positivity increases. From day 4 to day 7, no significant difference in detection rates was seen

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between the NS1 antigen and IgM antibody. Thus NS1 antigen may be a useful tool for detecting dengue infection during the first three days of illness. As compared to another study done by Chakravarti A, et al., in 2011, of the 145 patient samples tested, 88 (60.7%) were positive for either NS1 antigen or IgM antibody. Dengue NS1 antigen-capture ELISA gave an overall positivity rate of 65.9% (58/88), and IgM gave an overall positivity rate of 60.2% (53/88) and the authors concluded that only NS1 antigen can be used to test during the first two days of fever.^[16]

In a study by S Dutta, et al., in 2010, NS1 Ag positivity varied from 71.42% to 28.4% in early convalescent and acute phase sera, conversely IgM detection rate was 93.61% and 6.38% in early convalescent and acute phase sera respectively ($P < 0.0001$).^[17]

In our study we have detected NS1 positive in about 62 patients (43.3%), In a similar study done by Fauziah Md, Kassim, et al., 208 dengue suspected fever cases it was shown that NS1 antigen was positive for about 67 (32.2%) and a total of 107 (51.4%) number of patients were positive for IgM and IgG antibodies positive while a combination of these tests would raise the detection of dengue fever in 129 cases out of 208 patients (62%). Therefore the dengue NS1 antigen test can be used to complement the current antibody detection test and the combination of these serological test would increase the diagnostic efficiency of early diagnosis of dengue illness.^[18]

In our study it was found that the sensitivity of NS1Ag was 62 % and a very high specificity (100%) and the positive predictive value of NS1 Ag was 100% as compared to the negative predictive value of 53%. In a similar study done by Keswadee Lapphra, et al., done on 235 patients suspecting to have dengue fever it was noted that NS1 antigen has a similar sensitivity of 63.2%, high specificity of 98.4%, positive predictive value of 99.0% and negative predictive value of 52.5%. Thus above studies suggest that NS1 antigen although has a low sensitivity, has a very high specificity and the positive predictive value.^[19]

The strength of our study was to include all suspected dengue patients as per WHO criteria and were meticulously screened for dengue infection using the dengue duo test kit for NS1 antigen, IgM and IgG antibodies irrespective of the other laboratory parameters.

The limitation of this study are that the gold standard tests for the detection of dengue illness like the virus isolation and the viral RNA PCR were not performed and the follow up with the dengue serological test were not done in the recovered patients for the persistence of IgM/IgG antibodies.

CONCLUSION: The present study showed that dengue serological tests have a very significant role in the early diagnosis of dengue fever and thus allowing for the early necessary intervention. The results revealed that NS1 has a very high specificity and positive predictive value. However the sensitivity is comparatively low for NS1 (63%).

Although NS1 is found to be positive on day 1 of illness up to day 9 of illness Our study revealed that NS1 antigen had a limited role in detection of dengue illness on first three days of illness. NS1 antigen detection is effective in detecting the illness between days 4 to day 7. After day 7 again the positivity of NS1 reduced significantly. Hence it is recommended to do the serological tests (NS1 and IgM) early in all suspected dengue cases so that we can diagnose early and initiate necessary treatment.

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