European Journal of Molecular & Clinical Medicine (EJMCM)
ISSN: 2515-8260 Volume 07, Issue 10, 2020

A Study On Ns1 Antigen As An Early Marker For The Detection Of Dengue Virus Infection

Dr. Harish Thummala, Dr. P. Vijayalakshmi

1 &2. Micro Lab, Guntur, Andhra Pradesh, India.

Corresponding author: Dr. Harish Thummala,

Abstract

Background and objectives: Dengue is a mosquito-borne disease affecting mainly tropical and subtropical regions of the world. The early diagnosis of dengue is required for identifying an epidemic and also for implementing effective vector control measures. This study was aimed to look at the utility of dengue NS1 (non structural protein 1) Ag as an early marker of dengue infection.

Methods:

The study was conducted on 200 clinically suspected cases of dengue, from the out- patients and in-patients attending various clinical departments of in and around Guntur. Patients presenting within the first week of fever were included in the study. Patients' serum sample was subjected to ELISA for NS1 Ag, IgM Ab and IgG Ab.

Results:

Of the study group, 36.33% and 44.33% of cases were positive by NS1 Ag test and IgM Ab test respectively; when both the tests were combined together, the detection rate increased to 62.33%, which was statistically significant. In the first 3 days of fever, a higher number of cases were positive for NS1 Ag only, with a peak positivity of 42% on day 3. By day 4, a combination of NS1 Ag and IgM Ab gave a positivity of 28.22%. Day 5 onwards, a higher number of cases were positive for IgM Ab only, with a peak positivity of 33% on day 5.

Conclusion:

NS1 Ag is a very useful tool in the diagnosis of dengue infection especially in the first 3 days of fever. When used in combination with IgM Ab assay, the diagnostic algorithm significantly improves on a single serum sample. NS1Ag is an early diagnostic marker that is feasible in a routine diagnostic laboratory.

Key words: Dengue; NS1 antigen; IgM antibody; Early diagnosis; Acute phase sera.

INTRODUCTION

Dengue is a mosquito-borne disease affecting humans mainly in tropical and subtropical regions of the world. [1] It is an increasing public health concern in urban and suburban areas causing morbidity and mortality [2-4]. Globally, WHO has estimated that around 3 billion people reside in areas where there are risks of exposure to dengue virus and nearly 50 million people are infected with dengue virus every year [5-7]. Dengue virus is a RNA virus, consisting of four serotypes [1, 2, 3] and [4] all of which cause infection. Infection with one serotype does not confer cross-protection against the other serotypes, instead can cause a severe form of infection [1]. Recently a fifth serotype was identified 8. Early diagnosis plays a crucial role in

detecting an epidemic or outbreak and in undertaking effective vector control measures [9].

Over the past three decades, there has been a dramatic global increase in the frequency of DF, DHF and DSS and their epidemics, with a concomitant increase in disease incidence. Dengue is found in tropical and subtropical regions around the world, predominantly in urban and semi-urban areas.[1] The major diagnostic methods currently available are- viral culture, viral ribonucleic acid (RNA) detection by reverse transcriptase polymerase chain reaction (RT-PCR) and serological tests such as immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (MAC-ELISA). However, early dengue diagnosis still remains a problem, as all these assays have their drawbacks. Viral culture though the gold standard, cannot be used as a routine diagnostic procedure due to its low sensitivity, laborious procedure and time consumption.

The molecular techniques give results within 24 hours but they are also costly and they are not available for most clinicians. The MAC-ELISA which is a commonly used assay has low sensitivity in the first four days of illness and does not provide early diagnosis, as the first detectable IgM appears only on days 4 to 5 of illness. The requirement of paired sera, which improves the accuracy of the diagnosis, further delays it.[2,4]

Non- Structural Protein 1(NS1) is a highly conserved glycoprotein that is essential for the viability of DENV and circulates uniformly in all serotypes of DENV. NS1 Ag levels varies from 0.04 – 2 µg/ml in acute-phase serum samples, to only 0.04 µg/ml or even less in convalescent phase serum.[4] ELISA directed against NS1 antigen have demonstrated its presence at high concentrations in the early clinical phase of DF, which represents a new approach to the diagnosis of acute DENV infection.[5,6]

OBJECTIVES OF THE STUDY

1. Detection of dengue NS1 antigen (NS1 Ag) by ELISA method in the acute phase sera of clinically suspected cases of and also detection of IgM antibody (IgM Ab) and IgG antibody (IgG Ab) by ELISA method in the same serum sample.

MATERIALS AND METHODS

SOURCE OF DATA

Clinically suspected cases of DF, from the in and around Guntur and analysis carried out at Micro Labs Guntur. A total of 200 samples, between May 2017 and April 2019, were included in the study.

INCLUSION CRITERIA

1) Blood samples of clinically suspected cases of DF[11] have been included. Cases of DF which progressed to DHF and DSS have also been included.
2) Blood samples of only the acute phase sera i.e within first 7 days of fever have been included.

EXCLUSION CRITERIA

Cases of fever other than clinically suspected DF were excluded from the study.

PROTOCOL

- Blood samples (one sample per patient) from the out-patients and in-patients of clinically suspected cases of DF, which were received in and around Guntur were included in the study.
- The selection of cases was based on convenience sampling.

- The clinical details of these selected cases were collected from the patients' medical records

according to a clinical proforma.

- Only those cases which fulfilled the inclusion criteria were included in the study.
- The blood samples received in a red capped vacutainer without any anticoagulant, were centrifuged to separate the serum.
- These serum samples were subjected to ELISA using commercially available kits (PANIBO KITS) to detect dengue NS1 Ag, IgM Ab and IgG Ab.

Dengue IgM and IgG capture ELISA

Dengue NS1 Ag capture ELISA

RESULTS

A total of 200 clinically suspected cases of DF, attending in and around Guntur at Micro Labs between May 2017 and April 2019, were included in the study.

The study group included 120 males and 80 females. Maximum number of patients belonged to the age group of 21-40 years (45%), followed by ≤10 years (22%). The mean age was 25 years with a standard deviation of 15.26 (minimum age was 1 year and maximum age was 70 years).

Of the 120 patients who were positive for either NS1 Ag or IgM Ab or both, 48.36% positivity was seen in the age group 21 – 40 years and 24.27% positivity in the age group ≤10 years; the male: female ratio was 1.67:1.

Among the cases included in the study, 85% were those who were admitted to the hospital and 15% were those who attended the outpatient departments. The number of cases attending the hospital increased from day 3 (20%) onwards, peaking on day 4 with an average of 17.2% per day between days 3-7. However the number of cases on day 6 off ever was only 7.22%.

Of the 200 cases, 40% (n=80) were positive for NS1 Ag, 50% (n=100) were positive for IgM Ab, and 70% (n=140) were positive for IgG Ab.

Of the 80 positive for NS1 Ag, maximum positivity was seen on day 3 (62.5%). Of the 100 positive for IgM Ab, maximum positivity was seen on day 5 (28%). Of the 140 positive for IgG Ab, 20% positivity was seen on day 4 and another 22.82% on day 7.

Out of the 33.5% (n=47) who were positive for both NS1 Ag and IgM Ab, maximum positivity was observed on day 4 (42.37%), with an average of 25.5% between day 3 and day 5.

Out of the 17% (n=39) who were positive for NS1 Ag but negative for IgM Ab, the highest positivity was seen on day 3 (63.8%).

Out of the 38.5% (n=54) who were positive for IgM Ab but negative for NS1 Ag, maximum positivity was observed on day 5 (45.7%).

TABLE 1: TEST RESULTS OF NS1 Ag AND IgM Ab WITH RESPECT TO NUMBER OF DAYS OF FEVER

Fever period	**NS1 Ag + IgM Ab + (%)**	**NS1 Ag + IgM Ab – (%)**	**NS1 Ag - IgM Ab + (%)**	**Total (%) n=140**
Days 1-3	12 (25.53)	**30(63.8)**	5(10.6)	47 (100)

Days 4-5	25 (42.37)	7 (11.86)	**27 (45.7)**	59 (100)
Days 6-7	10 (29.4)	2 (5.8)	**22 (64.7)**	34 (100)
Total (%)	47 (33.5)	39 (27.8)	54 (38.5)	140 (100)

In the table above, it is evident that in the first 3 days of fever, positivity for NS1 Ag was high (63.8%); after day 4 a higher number of cases were detected by IgM Ab (45.7% and 64.7% between days 4-5 and 6-7 respectively), which shows a statistically significant association between the number of days of fever and the test results.

TABLE 2: DETECTION RATE OF NS1 Ag AND IgM Ab

NS1 antigen	**IgM antibody**		**Total (%) n=140**
	Positive (%)	**Negative (%)**	
Positive	60 (60)	30 (40)	90(100)
Negative	50 (100)	0 (0.00)	50 (100)
Total (%)	110 (73.3)	32 (26.6)	140 (100)

There is a statistically significant association between NS1 Ag and IgM Ab by Fischer's Exact Test ($p< 0.001$)

Out of the total 200 cases, 40% (n=80) of cases were positive for NS1 Ag; 50 (n=100) of cases were positive for IgM Ab; when both the above were combined together, the detection rate increased to 140 (n=140). Since the above proportion of cases were not mutually exclusive, McNemar Test was used to look for an association between the above two parameters and, a statistically significant association was found ($p=0.04$).

TABLE 3: TEST RESULTS OF IgM Ab IN THE NS1 Ag POSITIVE GROUP WITH RESPECT TO NUMBER OF DAYS OF FEVER

	Fever period	**IgM Ab test**		**Total (%)**
		Positive (%)	**Negative (%)**	
NS1 Ag positive	1-3 days	15 (37.5)	**25(62.5)**	40 (100)
	4-5 days	**22 (68.75)**	10 (31.25)	32 (100)

	6-7 days	**6 (75)**	2(25)	8(100)
	Total (%)	43 (53.7)	37 (46.25)	80 (100)

From the above table is it evident that amongst the NS1 Ag positives - in the first 3 days of fever 62.5% of the cases were negative for IgM Ab; between days 4-5, 68.75% of the cases were positive for IgM Ab also and between days 6-7, 75% of the cases were positive for IgM Ab also, which shows a statistically significant association between IgM Ab and NS1 Ag positivity with respect to the number of days of fever.

TABLE 4: DETECTION RATE OF NS1 Ag AND IgG Ab

NS1 antigen	**IgG antibody**		**Total (%)**
	Positive (%)	**Negative (%)**	**n=180**
Positive	42 (52.5)	38 (47.5)	80 (100)
Negative	100 (100)	0 (00.00)	100 (100)
Total (%)	142 (78.8)	38 (21.1)	180 (100)

There is a statistically significant association between NS1 Ag and IgG Ab by Fischer's Exact Test ($p < 0.001$)

TABLE 5: TEST RESULTS OF IgG Ab IN THE NS1 Ag POSITIVE GROUP WITH RESPECT TO NUMBER OF DAYS OF FEVER

	Fever period	**IgG Ab test**		**Total (%)**
		Positive (%)	**Negative (%)**	**n=80**
NS1 Ag positive	1-3 days	**20 (52.6)**	18 (47.3)	38 (100)
	4-5 days	**25 (67.5)**	12 (32.4)	37 (100)
	6-7 days	1 (20)	**4 (80)**	5 (100)
	Total (%)	47 (58.7)	33 (41.2)	80 (100)

From the above table is it evident that amongst the NS1 Ag positives - in the first 3 days of fever 52.6% of the cases were negative for IgG Ab; between days 4-5, 67.5% of the cases were positive for IgM Ab also and between days 6-7, 80% of the cases were positive for IgM Ab also, which does not show a statistically significant association between IgG Ab and NS1 Ag positivity with respect to the number of days of fever. Out of the total study group, 180 cases (90%) presented with thrombocytopenia (platelet count <1,50,000/µl).

TABLE 6: THROMBOCYTOPENIA AS SEEN IN NS1 Ag AND/OR IgM Ab POSITIVES

TEST RESULTS	TOTAL NO. OF CASES	CASES WITH PLATELET COUNT <1,50,000/μl	PERCENTAGE(out of the total no. of cases)
NS1 positive	22	21	87.50
IgM positive	3	3	100.00
NS1 and IgM positive	20	19	95.45

A higher percentage of thrombocytopenia was seen in cases which were positive for IgM Ab. A comparatively lesser number of NS1 Ag positive cases were thrombocytopenic.

Out of the 39 patients who had normal platelet count, 8 tested positive for NS1 Ag, 3 for both NS1 Ag and IgM Ab, 4 for IgM Ab.

40 cases of thrombocytopenia which were negative for IgM Ab, tested positive for NS1 Ag.

DISCUSSION

Dengue is the most important arthropod-borne viral infection of humans. Worldwide, an estimated 2.5 billion people are at risk of infection.[2] The annual average number of DF and DHF cases reported to the World Health Organization (WHO) has increased dramatically in recent years.[2] Dengue epidemics are increasingly being recorded worldwide and can have a significant economic and health toll.

The geographical areas in which dengue transmission occurs have expanded in recent years, and all four dengue virus serotypes (DENV-1–4) are now circulating in Asia, Africa and the Americas.[2] In view of increasing mortality rate and disease burden, it is imperative to have a rapid and sensitive laboratory assay for early detection of the disease.

The aim of this study was to look for the utility of dengue NS1 Ag as an early marker of DENV infection.

The study was conducted on 200 clinically suspected cases of DF, in and around Guntur between May 2017 and April 2019.

The study group included 120 males and 80 females. Out of the 120 patients who were positive for either NS1 Ag or IgM Ab or both, 48% belonged to the age group 21 – 40 years (30.27% for the age group 21 – 30 years) and 22.27% to the age group ≤10 years; the male: female ratio was 1.67:1.

The results of our study are similar to that of a study done at New Delhi during an outbreak between September to November 2003 by Gupta et al.[91] which has reported IgM Ab positivity of 34.2% in age group of 21-30 years and 24.8% in the age group ≤10 years; male: female ratio of 2.1:1.

Gupta et al.[7] are of the opinion that quick control measures taken during 2003 outbreak which included intensive household mosquito elimination programs might have shifted the affected mosquito population towards non-residential areas and thereby infecting the mobile working population (21-30 yr).

In our study we have observed that the number of patients attending the hospital peaked from day 3 (20%)

onwards and 85% of the total study group were in-patients. Ours, being a tertiary care referral centre, the number of patients attending the hospital in the initial phase of infection would be lesser compared to those attending primary health centers and local health clinics. This is reflected well in the observations of our study.

Overall, 40% were positive for NS1 Ag, 50% were positive for IgM Ab. The results for NS1 Ag positivity (36.33%) is in concordance with a study done at Malaysia in 2011 by Kassim FM et al.[104] (32.2%). Certain other authors have reported a variable positivity between 23.3% (Datta et al at New Delhi in 2010)[2]

The results for IgM Ab positivity (50%) is similar to that of other studies (Srivastava et al [9]- 45.7%, Kassim FM et al.[8] - 40.9%,Kumarasamy et al.[11] - 39.1%). In a study by Gupta et al.[7] on suspected dengue cases with fever duration of ≥5 days, 52.3% positivity was reported for dengue specific IgM antibodies.

Of the 80 cases positive for NS1 Ag, a positivity of 62.5% was seen between days 1-4, and 68.75% after day 5, which is in concordance with a study by Datta et al.[2] who has reported a positivity of 71.42% and 28.4% respectively and with a study by M.P. Singh et al.[92] who has reported a positivity of 72.09% and 27.91% respectively. The lower sensitivity of NS1 Ag from day 4 onwards could possibly be due to formation of immune complexes.[5,92]

Of the 100 positive for IgM Ab, a positivity of 45.7% was seen between days 1-4 and 64.7% after day 5. These results closely resemble to that of a study by M.P. Singh et al.[92], where a positivity of 36.8% for IgM Ab was seen in patients with fever ≤ 5 days and 45.7% was seen in patients with fever of 6-13 days. Antidengue IgM antibody was detectable on the third day of onset of illness with a positive rate of 42.9%, according to a study by Kumarasamy et al [11], which is similar to our results.

In this study, we have observed that in the first 3 days of fever, a higher number of cases were positive for NS1 Ag only, with a peak positivity of 41.18% on day 3. By day 4, a combination of NS1 Ag and IgM Ab tests gave a positivity of 29.31% as compared to 24% positivity for IgM Ab only and 15.69% positivity for NS1 Ag only. Day 5 onwards, a higher number of cases were positive for IgM Ab only, with a peak positivity of 32% on day 5. These results are comparable to that of a study by Srivastava et al [9]

Out of 200, 20 (10%) were positive for NS1 Ag only, which would have otherwise been undetected with the Ab assays alone. Amongst these, 75% cases were detected in the first 3 days of fever. It can be inferred from the above observations, that, in the first 3 days of fever, NS1 Ag assay is a better indicator of dengue infection; however after this, a combination of NS1 Ag and IgM Ab assays help diagnose a higher number of cases upto the end of first week of fever.

Overall, 140 (70%) cases were positive for IgG Ab; of whom 50 (35.7%) were positive for IgG Ab alone.

When the test results of NS1 Ag and IgG Ab were compared, 35.33% and 64.67% were positive by NS1 Ag test and IgG Ab test respectively. When both the tests were combined together, the detection rate increased to 85.00%, which was statistically significant.

The results of IgG Ab alone (without considering the other two parameters) on a single serum sample may not be a reliable indicator of primary dengue infection unless one of the gold standard tests like viral culture, RNA detection by molecular methods or seroconversion in paired sera, are also done in addition. In our study, we were unable to perform any of the above tests because of the high cost, time factor and the intense labour associated with them.

In our study, 90% of patients presented with thrombocytopenia. Of these, 65% were positive for either NS1

Ag or IgM Ab or both, the remaining tested negative for both the parameters.

A higher number of cases with thrombocytopenia were seen in IgM Ab positives as compared to that of NS1 Ag positives. In our study, 40 patients were negative for all the three parameters tested, 12 of whom had laboratory evidence for other infections. Hence, it is important to consider these clinical conditions in the differential diagnosis of DENV infection, as all these cases initially presented with clinical features which were in favour of dengue.

In endemic areas most of the population would be seropositive for dengue due to subclinical infection. In such cases when a secondary infection occurs, there are high chances of increased disease severity and serious complications, unless diagnosed early and appropriate treatment initiated. Even in these cases of secondary dengue infection, NS1 Ag assay helps diagnose the infection at an early phase.

Certain studies[12,13] have even reported that NS1 Ag assay is a cost effective tool, which is as good as the RT-PCR for diagnosis in the acute phase of illness.

CONCLUSION

The present study substantiates that, in comparison to IgM Ab assay, NS1 Ag assay is an effective tool for aiding early diagnosis of DENV infection. NS1 Ag assay can be considered as the test of choice for patients presenting with a history of fever for up to 3 days. The study aimed at the utility of dengue NS1 Ag as a tool in the early diagnosis of DENV infection. Observations of this study suggest that NS1 Ag assay can be considered as the test of choice in the first 3 days of illness; combining NS1 Ag and IgM Ab assays improves the diagnosis after day 4 upto the end first week of fever. Co-circulation of multiple dengue virus serotypes has been reported from many parts of the world including India. Several studies have reported that specific DENV serotypes are associated with severe disease manifestations. Therefore serotype specific NS1 Ag ELISA not only helps in epidemiological studies but also in vigilant case management.

Acknowledgment

To all the Doctors who supported this work by giving permission to process samples , to P.Vijayalakshmi CEO, Micro Lab for supporting and to all the technical and non-technical team of Micro Lab, Guntur for participating in this work.

Conflict of Interest

None

Financial Support: P Nagabhushan Rao, Finance Manager, Micro Lab, Guntur.

REFERENCES

1. Chua KB, Mustafa B, Abdul Wahab AH, Chem YK, Khairul AH, Kumarasamy V, et al. A comparative evaluation of dengue diagnostic tests based on single-acute serum samples for laboratory confirmation of acute dengue. Malays J Pathol. 2011;33(1):13–20.
2. WHO | Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. 2nd edition. Geneva : World Health Organization. [Internet]. WHO [cited 2012 May 6];Available from:http://www.who.int/csr/resources/publications/dengue/ Denguepublication/en/.
3. Gibbons RV, Vaughn DW. Dengue: an escalating problem. BMJ. 2002;324(7353):1563–66.
4. Gubler DJ, Meltzer M. Impact of dengue/dengue hemorrhagic fever on the developing world. Adv. Virus Res. 1999;53:35–70.

5. Tang KF, Ooi EE. Diagnosis of dengue: an update. Expert Rev Anti Infect Ther. 2012;10(8):895-907.
6. WHO. Dengue Guidelines for diagnosis, treatment, prevention and control. 2009.
7. Gupta E, Dar L, Narang P, Srivastava VK, Broor S. Serodiagnosis of dengue during an outbreak at a tertiary care hospital in Delhi. Indian J Med Res 2005 Jan;121:36-8.
8. Kassim FM, Izati MN, TgRogayah TAR, Apandi YM, Saat Z. Use of dengue NS1 antigen for early diagnosis of dengue virus infection. The Southeast Asian Journal of Tropical Medicine and Public Health. 2011;42(3):562.
9. Shrivastava A, Dash PK, Tripathi NK, Sahni AK, Gopalan N, Lakshmana Rao PV. Evaluation of a commercial Dengue NS1 enzyme-linked immunosorbent assay for early diagnosis of dengue infection. Indian J Med Microbiol. 2011;29(1):51–55.
10. Halstead SB. Dengue. The Lancet. 2007;370(9599):1644–52.
11. Kumarasamy V, Chua SK, Hassan Z, Wahab AHA, Chem YK, Mohamad M, et al. Evaluating the sensitivity of a commercial dengue NS1 antigen-capture ELISA for early diagnosis of acute dengue virus infection. Singapore Med J. 2007;48(7):669–73.
12. Alcon S, Talarmin A, Debruyne M, Falconar A, Deubel V, Flamand M. Enzymelinked 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. 2002;40(2):376–81.
13. Young PR, Hilditch PA, Bletchly C, Halloran W. An Antigen Capture EnzymeLinked Immunosorbent Assay Reveals High Levels of the Dengue Virus Protein NS1 in the Sera of Infected Patients. J Clin Microbiol. 2000; 38(3):1053–57.