e ISSN 2277 - 3290
Print ISSN 2277 - 3282

Journal of Science

Microbiology

www.journalofscience.net

Research article

A STUDY ON EARLY SEROLOGICAL CONFIRMATION OF DENGUE INFECTION BY NS1 ELISA IN A SINGLE ACUTE PHASE SERUM SAMPLE

[1]Nandhitha A, [2] Shumaila Abdul Rehman, [3]Srikanth Sandanala, [4]Sasikala G

[1&2].Senior resident, Gandhi Medical College, Secunderabad, Telangana, India.
[3]. Assistant Professor, Department of Paediatrics, Gandhi Medical College, Secunderabad, Telangana, India.
4. Professor, Department of Microbiology, Osmania Medical College, Hyderabad, Telangana, India.

ABSTRACT

Introduction: Dengue fever is a major public health problem worldwide. The 2011 revised World Health Organization (WHO) guidelines have emphasized on early diagnosis and intervention to reduce the case fatality rate due to dengue fever. Rapid diagnostic tests like NS1 antigen assays have improved the detection of cases in early clinical phase of illness but its role as a predictor of severe dengue infection is not very clear. Aim: To evaluate the utility of NS1 Ag assay as an early diagnostic marker and predictor of severe dengue infection. Materials and Methods: All age groups (0-60) A total of 426 clinically suspected Dengue cases were screened for Serological markers NS1 a g diagnosed and confirmed with dengue fever at a tertiary care Osmania General hospital Hyderabad during the period March –October 2015, were reviewed retrospectively from hospital case records as per the revised WHO guidelines for dengue fever. The diagnosis was confirmed by NS1antigen-based ELISA test. The data was analyzed using SPSS 16.0 statistical software. After collecting all the data, all the variables were summarized by descriptive statistics. Results: Among the total study, 132 samples were screened for detection of NS1 Ag. Among them 35 (26.5%) cases were positive. In which more number of cases were shown only dengue fever 34 (25.7%) and only one case showed severe dengue fever. In the present study, A total of 144 patients belongs to age group 20-29 yrs. The present study showed more seropositive cases were identified in 20-29 yrs age group. In this study males (57.7%) are more commonly affected compared to females (42.3%). Conclusion: NS1 Ag assay is a useful early diagnostic marker for dengue fever but cannot be used as an early predictor of severe dengue infection. The criteria for admission in hospitals of cases of dengue fever should be based on clinical warning signs rather than positive NS1 Antigen test.

Keywords: Spinal NS1Ag, seropositive, ELISA, Antigen.

Access this article online

Home page: http://journalofscience.net//

DOI: http://dx.doi.org/10.21276/jos.2018.8.2.1

Quick Response code

Received:25.04.18 **Revised**:02.05.18 **Accepted**:15.05.18

Corresponding Author

Nandhitha. A

Department of Microbiology, Osmania Medical College, Hyderabad, Telangana, India.
Email:- nandiitaa.86@gmail.com

INTRODUCTION

Dengue is fast emerging Pandemic-Prone Viral disease in many parts of the world. It is an acute febrile arboviral disease caused by Dengue virus belonging to the family Flaviviridae and the genus Flavivirus [1]. It is transmitted by the bite of female mosquitoes mainly of Species *Aedes* aegypti and to a lesser extent, *A.albopictus* [2]. According to WHO estimates 390 million Dengue infections peryear, of which 96 million manifests clinically. The prevalence of Dengue, estimates that 3900 million people, in 128 countries, are at risk of infection with Dengue viruses [3].

The epidemiology of dengue fever in the Indian subcontinent has been very complex and has substantially

changed over almost past six decades in terms of prevalent strains, affected geographical locations and severity of disease[4]. The seasonal trend is observed during post monsoon season i.e, September-october[5]There are five serotypes of the virus distinguished on the basis of antigenicity. First four are DENV-1, DENV-2, DENV-3 and DENV-4 while the fifth type was announced in 2013 [6].

As the initial symptoms of Dengue mimic those of Malaria, Typhoid and Leptospirosis, which are endemic in our country, availability of a rapid and differential diagnosis at an early stage of infection is of utmost importance for better patient management. As there is no specific anti viral treatment and effective vaccine available , Laboratory diagnostic methods play an important role for early confirmation of infection that include virus isolation, detection of viral genomic sequence, detection of viral Antigen(NS1 Ag) and Antibodies IgM and IgG or a combination of these techniques [7,8].

For long time detection of dengue specific antibody has been main stay of diagnosis of dengue infection but time required for appearance of Ig M antibody is approximately 4 -6 days. The role NS1 Ag for early detection of dengue infection is currently being evaluated by many investigators without requirement of paired sera.As the NS1 antigen is detectable in blood from day one after onset of fever and has long half life in blood, its assay is an effective tool for early diagnosis so as to avoid complications of dengue [9].

Efficient and accurate diagnosis of dengue is of primary importance for clinical care (i.e. early detection of severe cases, case confirmation and differential diagnosis with other infectious diseases), surveillance activities, outbreak control, pathogenesis, academic research, vaccine development, and clinical trials.

Laboratory diagnosis methods for confirming dengue virus infection may involve detection of the virus, viral nucleic acid, antigens or antibodies, or a combination of these techniques. After the onset of illness, the virus can be detected in serum, plasma, circulating blood cells and other tissues for 4–5 days. During the early stages of the disease, virus isolation, nucleic acid or antigen detection can be used to diagnose the infection. At the end of the acute phase of infection, serology is the method of choice for diagnosis.

A range of laboratory diagnostic methods has been developed to support patient management and disease control. The choice of diagnostic method depends on the purpose for which the testing is done (e.g. clinical diagnosis, epidemiological survey, vaccine development), the type of laboratory facilities and technical expertise available, costs, and the time of sample collection.

ELISA detected NS1 antigen have demonstrated its presence at high concentrations in the sera of DV infected patients during the early clinical phase of the disease even before seroconversion. The detection of secretory NS1 protein represents a new approach to the diagnosis of acute DV infection [10].

NS1 Ag levels varies from 0.04 – 2 μg /ml in acute-phase serum samples, to only 0.04μg/ml or even less in convalescent phase serum [10]. It is detected from day 1 upto day 9 to 18 after the onset of fever. The dengue NS1antigen was not found in patients with Japanese encephalitis virus or yellow fever virus infections thereby implying that there is no cross-reaction of dengue NS1 protein with those of other related flaviviruses

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 NS1antigen. 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. NS1 antigen detection is reported to be sensitive as well as highly specific and have shown that higher NS1 antigen levels on day 3 of infection were associated with lower platelet counts [6].

The present study was conducted in the Department of Microbiology, Osmania General Hospital, Hyderabad.

Study design

It is Prospective cross sectional study and total size of the population is 426 and duration of this study is 8 months.

Nature of Study population

All Dengue fever suspicious cases defined according toWHO suggested case classification as Dengue fever, Dengue Hemorrhagic fever & Dengue shock syndrome were enrolled into the study.The serum samples comprised of both acute and early convalescent phase depending on the reporting time of the patients. Acute phase serum samples were collected from patients who reported < 5 days of fever in whom NS1 ag assay was done.

Inclusion criteria

Febrile patients admitted in medical and pediatric wards of Osmania General Hospital. Patients of all age groups clinically diagnosed as having DF.

Exclusion criteria

Febrile cases with definite source of infection (eg. respiratory, UTI, Meningitis) as per available clinical and Laboratory data. The following patients were excluded, History of bleeding tendency since birth, Patients with thrombocytopenia and no fever, patients with fever of more than 2 weeks duration and other conditions that cause TCP like Autoimmune connective tissue disorders, ITP, Malignancy.

Data collection

Approval of the Institute's Ethics Committee was obtained to carry out the study. Informed consent was obtained from each patient. Information on demographic features and symptoms of the patients were collected by a structured questionnaire.

Sample collection and storage

2-3 ml of venous blood was collected underaseptic precautions in vaccutainers with no additives and kept at room temperature for 30 min to clot. Serum was separated by Centrifugation and transferred to aliquots and stored at -20 until tested.

Laboratory methods

EDTA blood samples were collected and Hematological parameters were detected in all samples using automated cell count analyzer which were confirmed by peripheral blood smear examination. All the acute phase serum samples were detected for NS1 ag using the Commercially available kit (Panbio Dengue Early Elisa Kit).

RESULTS

A total of 426 clinically suspected Dengue cases were screened for Serological markers NS1 Ag at Osmania General Hospital, Hyderabad during the period March – October 2015.In the present study, total of 426 clinically suspected cases, Among them 147 (34.5%) case were seropositive cases (Table.1). In the present study, a total of 144 patients belonged to age group 20-29 out of 426 patients reported. The present study showed more number of cases was reported in males than woman. In the present study, more seropositives cases (36%) were identified in 20-29 yrs agegroup (Table 3). More no. of males (39.4%) was affected than females (27.7%) with ratio of 1.36:1 that belonged to age group 20-29 yrs. (Table 4). In the present study, DF was noticed in higher no. of patients (95.3%) than DHF which was observed in only 4.7 % of cases. (Table 6). Present study revealed that out of 132 samples that reported with fever < 5 days duration NS1 Ag Elisa was done and 35 samples were seropositive accounting to 26.5%. Among them 25.7 % of DF and 0.7 % of DHF were identified using NS1 ag assay (Table.7).

Table 1: Distribution of study population

Total no.of samples	No. of Seropositives	Percentage
426	147	34.5 %

Table 2: Age profile of study population (n= 426)

Age (yrs)	No. of cases	Percentage %
0-19	112	26%
20-29	144	34.2%
30-39	67	15.4%
40-49	49	11.5%
50-59	27	6.3%
>60yrs	27	6.3%

Table 3: Age groups of seropositive Dengue cases n= 147

Age groups (yrs)	No. of positives	percentage (%)
0-19	41	27.8
20-29	53	36.05
30-39	24	16.3
40-49	16	10.8
50-59	8	5.44
>60	5	3.4

Table 4: Total Sex wise distribution of study population.

Males	Females	Total
246(57.7%)	180(42.3%)	426

Table 5: Age with Sex wise distribution of Seropositives

Age gp (yrs)	Total males	Serppositives (%)	Total Females	Serppositives(%)
0-19	61	29 (47.5%)	51	12(23.5%)
20-29	85	36(42.3)	59	17 (28.8%)
30-39	44	15 (34)	23	9(39.1%)
40-49	27	9(33)	22	7(31.8%)
50-59	15	4(26.6%)	12	4(33.3%)
>60	14	4(28.5)	13	1(7.7%)
TOTAL	246	97(39.4)	180	50(27.7%)

Table 6: Dengue category classification of study population

Category	Total cases	Seropositives (%)
DF	410	140 (95.3)
DHF/DSS	16	7(4.7%)

Table 7: Detection by using NS1 ELISA

Duration of Fever	Samples Screened	Total NS1 positives	DF	DHF/DSS
<5 days	132	35 (26.5%)	34(25.7%)	1 (0.7%)

DISCUSSION

Dengue fever is a major public health problem with high morbidity and mortality. The revised World Health Organization dengue fever guidelines 2011 have emphasized the need for early diagnosis and treatment to reduce the mortality due to severe dengue infection [11]. The classical methods of confirmation of diagnosis are virus isolation, serotype identification, antibody detection tests (IgM and IgG MAC-ELISA), haemagglutination inhibition or neutralization tests but all these tests are time consuming and do not help in the confirmation of diagnosis at an early stage of illness [11]. The serological diagnosis by IgM/IgG MAC ELISA has been the most common method of confirmation of dengue fever and has sensitivity and specificity of approximately 90% and 98%, respectively but the problem is that it is only detected in the convalescent phase of illness. With the advent of NS1 antigen assay, there has been unprecedented rise in early diagnosis of dengue fever as it develops during the acute phase of illness (0-7 days) and is emerging as a suitable option for dengue diagnosis in the first week of illness with high sensitivity and specificity [12].In the present study about 36% of cases were affected with dengue fever who mostly belonged to younger age group 20-29 yrs. These studies correlated with previous studies [13,14]. In the present study Dengue fever cases were more common. Our results similar with previous studies [15,16]. No case with Dengue shock syndrome was noticed and no mortality was reported. Saraswathy MP et al.,has suggested that continuous scrutiny of warning signs can prevent development of DSS [17]. In the present study serum samples that grouped into 3 categories according to the days of fever, NS1 Ag detection was noticed in 26.5% of cases who reported during acute phase of illness i.,e < 5 days. Our results correlated with above studies, howeverDharitri Mahapatra et al 82 observed highest rate of positivity 81.2% when only IgM ab was detected. Further, study done by Anithachakravarthy et al 118 reported highest rate 39.7% among NS1ag seropositives. These results correlated with previous studies studies done by Shiran Ajith Paranavitane et al and Nishant Hussain et al recoreded highest NS1 detection rates of 53.5 % , 66.6% ,32.7 % respectively [18,19]. The total seropositivity rate in the present study was 34.6% which is in concordance with other studies done by Anagha G Kanikar et al, Basavaraju Janardhana Raju et al where 38.5%, 30.6%,37.5% seropositivity was recorded [20, 21].

In our study the diagnosis of dengue fever was confirmed by NS1 Ag test in 26.5% cases and among them majority of the cases was detected in the acute phase sera in the first week of illness. The NS1Ag test was a useful early diagnostic marker for dengue infection in acute phase illness in primary and secondary dengue infection in this study as has been reported with few previous studies [22-24]. The detection of specific IgM by MAC-ELISA is still used as the diagnostic technique but the main disadvantage being it is usually positive during the convalescent phase of illness and as a result a large number of cases go undetected during the acute phase of illness as suggested in the previous studies [25-28]. NS1 antigen assay is a useful tool in the diagnosis of dengue infection in the early phase of the disease but it is not clear whether they can be used as an early predictor of the severity of the disease [10,15]. Libraty et al., in their study showed a very high concentration of NS1 antigen during early phase of illness in patients with severe dengue infection and used as an early diagnostic marker for severe dengue infection [29]. However, in our study the predominant mode of presentation of different age groups who were NS1 Ag positive was non-severe dengue infection. The sensitivity of detection of NS1 Ag test in non-severe dengue infection was good. The demerit of NS1 Ag assays are that they are

unable to distinguish the serotype of the dengue virus causing infection. Real time and nested RT-PCR is fast becoming the method of choice as it enables rapid detection, serotype identification, as well as viral RNA quantification which are 80-90% sensitive and >95% specific [30].

In our study, there was an increase in early detection of dengue infection with the help of NS1Ag rapid diagnostic test, but we also witnessed traumatized and panic-struck parents putting unnecessary pressure on the treating physicians for hospitalization in different age group patients who were NS1 Ag test positive. Hence, the real need of the hour especially for the treating physicians is to recognize the early warning signs in children with dengue fever and it should remain the only indicator for admission rather than positive results in rapid diagnostic tests like NS1 Ag assay. It is also important for the treating physicians to counsel the panic struck parents and educate them regarding home care treatment and identification of symptoms and danger signs and bring the child to immediate medical attention should the need arise especially during endemics.

Limitations

There are several limitations to the present study. The study is retrospective analysis of dengue fever cases from a single centre and included only those cases which were admitted to the hospital. The diagnosis was confirmed by either NS1Ag test or dengue serology. Virus isolation and serotype identification was not done in the present study. There were cases which behaved like dengue fever but remained undiagnosed due to lack facilities for other confirmatory tests.

CONCLUSION

NS1 Ag assay is useful, sensitive and specific for the diagnosis of dengue infection, especially during the acute phase when antibodies are not detectable and the dengue serology was negative. Early diagnosis and timely intervention can reduce the mortality due to severe dengue infection. However from our experience it cannot be used as an early predictor of sever dengue infection and the criteria for admission in hospitals of cases of dengue fever should be based only on clinical warning signs rather than NS1 Ag positive reports.

Acknowledgement: The author thankful to Department of Microbiology for providing research facilities to carry out research work.

Conflict of interest: None.

REFERENCES

1. Kulkarni RD, PatilSS, Ajantha GS et al. (2011). Association of platelet count and serological markers of dengue infection -importance of NS1 antigen. *Indian Journal of Medical Microbiology*, 29(4), 359-62.
2. Chandy S, Ramanathan K et al. (2013). Assessing effect of climate on the incidence of dengue in Tamil Nadu. Indian Journal of Medical Microbiology, 31(3), 283- 286.
3. World Health Organization (2015). Dengue and Severe Dengue. Fact sheets 2015. Available http://www.who.int/mediacentre/factsheets/fs117/en/. Accessed 26 July 2015.
4. Nivedita Gupta, Sakshi Srivastava, Amita Jain, Umesh C. Chaturvedi. (2012). Dengue in *India. Indian J Med Res,* 136, 373-390.
5. Preeti Bharaj, Harendra S Chahar et al., (2008). Concurrent infections by all four dengue virus serotypes during an outbreak of dengue in 2006 in Delhi, India. *Bio Med central Virology journal,* 5(1), 1-5.
6. Gitanjali K, Badave. P, Sai Swaroop and Nageswara Rao.P. (2015). Importance of NS1 antigen detection and its association with platelet count for early diagnosis of dengue virus infection. *Int.J.Curr.Microbiol.App.Sci,* 4(3), 779-784.
7. Santosh ShivajiraoTathe, Chincholkar V V, 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 and App.Sci,* 2(12), 40-44.
8. www.who.int/tdr/publications/documents/dengue-diagnosis.pdf
9. Parameswarappa Jyothi, Basavaraj C. Metri. (2015). Correlation of serological markers and platelet count in the diagnosis of Dengue virus infection. *Advanced Biomedical Research 4,* 26, 1-4.
10. S Datta, C Wattal. (2010). Dengue NS1 antigen detection: A useful tool in early diagnosis of dengue virus infection. *Indian journal of Medical Microbiology*, 28(2), 107-10.
11. Guzman MG, KouriG.(2002). Dengue: an update. *Lancet Infect Dis,* 2, 33-42.
12. Chakravarthi A, Kumaria R, Batra VV, Verma V. (2006). Improved detection of dengue virus serotypes from serum samples-Evaluation of single-tube multiplex RT-PCR with cell culture. *Dengue Bulletin*, 30, 133-40.
13. Ekta Gupta, Lalit Dar, Geetanjali Kapoor and Shobha (2006). The changing epidemiology of dengue in Delhi, India. *Virology Journal*, 3, 92.
14. R. N. Makroo, V. Raina, P. Kumar and R. K. Kanth (2007). Role of platelet transfusion in the management of dengue patients in a tertiary care hospital. *Asian J Transfus Sci*, 1(1), 4–7.
15. P.V. Barde, M.K. Shukla, B.K. Kori, G. Chand, L. Jain, B.M. Varun, D. Dutta, K. Baruah &Neeru Singh (2015). Emergence of dengue in tribal villages of Mandla district, Madhya Pradesh, India.*Indian J Med Res,* 141,584-590.

16. Sharma Y, Kaur M, Singh S, Pant L, Kudesia M, Jain S (2012). Seroprevalence and trend of dengue cases admitted to a Government hospital, Delhi- 5-year Study (2006-2010): A look into the age shift. *Int J Prev Med*, 3, 537-43.
17. Saraswathy M P et al., (2013). Incidence of Dengue Haemmorhagic fever in children: A report from Melmaruvathur, Tamilnadu, India. *J of Pharmaceutical and Scientific Innovation* 2(1), 34-36.
18. Shiran Ajith Paranavitane, Laksiri Gomes, AchalaKamaladasa, Thiruni N Adikari, NilankaWickramasinghe, ChandimaJeewandara (2014). Dengue NS1 antigen as a marker of severe clinical disease. BMC Infectious Diseases, 14, 570, 1-7.
19. Nishat Hussain Ahmed & Shobha Broor (2014). Comparison of NS1 antigen detection ELISA, real time RT-PCR and virus isolation for rapid diagnosisof dengue infection in acute phase. *J Vector Borne Dis*, 51, 194– 199.
20. Basavaraju Janardhana Raju, Gandikota Rajaram. Prevalence of dengue fever and dengue hemorrhagic fever in government general hospital Tirupati (2013). *International Journal of Research in Health Sciences*, 1(1), 23-27.
21. Guidelines for Clinical Management of Dengue fever, Dengue Haemorrhagic fever and Dengue shock syndrome. Government of India.2008.
22. Xu H, Pan YX, Qiu LW, Wang YD, Hao W, He LJ, et al (2006). Serotype 1-specific monoclonal antibody-based antigen capture immunoassay for detection of circulating nonstructural protein NS1: implications for early diagnosis and serotyping of dengue virus infections. *J ClinMicrobiol*, 44, 2872-78.
23. Thomas L, Najioullah F, Verlaeten O, Martial J, Brichler S, Kaidomar S, et al (2010). Relationship between nonstructural protein 1 detection and plasma viral load in dengue patients. *Am J Trop Med Hyg*, 83, 696-99.
24. Sekaran SD, Lan EC, Mahesawarappa KB, Appanna R, Subramanian G. (2007). Evaluation of a dengue NS1 capture ELISA assay for rapid detection of dengue. *J Infect Developing Countries*, 1, 182-88.
25. Shu PY, Huang JH (2004). Current advances in dengue diagnosis. *Clinical and Diagnostic Laboratory immunology*, 11, 642-50.
26. Alcon S, Talarmin A, Debruyne M, Falconar A, Duebel V, Flammand M (2002). Enzyme linked immunosorbent assay specific to dengue virus type 1 nonstructural protein ns1 reveals circulation of the antigen in the blood during acute phase of the disease in patients experiencing primary or secondary infections. *J ClinMicrobiol*, 40, 376-81.
27. Dussart P, Labeau B, Lagathu G, Louis P, Nunes MRT, Rodrigues SG, et al (2006). Evaluation of an Enzyme Immunoassay for detection of dengue virus NS1 antigen in human serum. *Clin Vaccine Immunol*, 13, 1185-89.
28. Datta W, Wattal C (2010). Dengue NS1 antigen detection test: A useful tool in early diagnosis of dengue virus infection. *Indian J Med Microbiol*, 28, 107-10.
29. 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.
30. Kumarasamy V, Wahab AHA, Chua SK, Hassan Z, Chem YK, Mohamed M, et al. (2007). Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection. *J Virol Methods*, 140, 75-9.

Cite this article:
Nandhitha A, Srikanth Sandanala, Sasikala G. A Study on Early Serological Confirmation of Dengue Infection by NS1 Elisa in a Single Acute Phase Serum Sample. *Journal of Science*, 2018;8(2):57-62.
DOI: **http://dx.doi.org/10.21276/jos.2018.8.2.1**