

ORIGINAL ARTICLE

Utility of NS1 Antigen for Diagnosis of Dengue Virus Infection*Pramod S. Manthalkar^{1*}, B.V. Peerapur²**¹Department of Microbiology, BLDE University's, Shri B.M Patil Medical College Vijayapura-586103 (Karnataka) India, ²Department of Microbiology, RIMS, Raichur-584102 (Karnataka) India***Abstract:**

Background: Dengue has become a major global public health problem in the developing countries. *Aim and Objectives:* This study was carried out to evaluate the utility of NS1 antigen assay in early diagnosis of dengue infection. *Material and Methods:* The performance, detection rate of NS1 antigen assay in comparison to IgM Antibody Capture Enzyme Linked Immunosorbent Assay (MAC-ELISA) was evaluated in a single sample constituted Group I. One thousand acute/ early convalescent sera were screened by both the assays. NS1 antigen assay was used to evaluate the efficacy of single assay in 30 acute phase sera of paediatric outpatient department, patients constituted Group II. Specificity of NS1 assay in comparison to MAC- ELISA was evaluated on 40 samples used as controls constituted group III. *Results:* Out of 1000 samples in Group I, 247 (24.7%) and 184 (18.4%) samples were positive by NS1 assay and MAC –ELISA respectively. Increase in the detection rate to 431 (43.1%) was seen when both the assays were used together on a single sample. NS 1 Ag positivity varied from 97.57% to 2.42% in acute and early convalescent sera, conversely IgM detection rate was 94.56% and 5.43% in early convalescent and acute phase sera respectively (P<0.0001). Twenty (66.66%) samples were positive by NS 1 assay in Group II. All 40 samples in Group III were negative showing 100% specificity of both the assays. *Conclusion:* NS-1 Ag assay is a useful tool for early diagnosis of dengue virus infection. When used in combination with MAC-ELISA on a single sample it significantly improves the diagnosis algorithm without the requirement of paired sera.

Keywords: Dengue diagnosis, comparison, NS1 antigen assay, MAC- ELISA.

Introduction:

Dengue has become a major global public health problem in the developing countries. The estimated risk of acquiring Dengue Virus (DV) infection is approximately 2.5 billion people living mainly in urban areas [1]. DV causes various clinical symptoms ranging from asymptomatic or undifferentiated fever, known as Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF), Dengue Shock Syndrome (DSS), leading to death, especially among the children [2].

Viral isolation by culture or viral RNA detection by Polymerase Chain Reaction (PCR) helps in the diagnosis of recent dengue infection. But this viral isolation is very time consuming and requires specialized laboratory equipment [3]. Newer PCR types like nested PCR and real time PCR have significantly reduced processing time but are expensive and technically exacting [4]. As a result, dengue culture and PCR have limited utility in routine clinical use.

Rapid newer test for presumptive diagnosis of dengue is the detection of Non Structural Protein Antigen (NS1). This antigen is a highly conserved glycoprotein that is essential for the viability of dengue virus [5]. The first immunoglobulin isotype to appear is IgM antibody, suggesting recent infection. One of the most recent advances for routine dengue diagnosis is IgM Antibody Capture Enzyme Linked Immunosorbent Assay (MAC-ELISA) [6]. To confirm dengue during both early and late infection, combined usage of

NS1 antigen and IgM antibody ELISA are promising [7].

In this study, we have performed both types of immunoassays; NS1 and IgM, on the samples received in our laboratory and the results of the combined tests have been compared individually with each test separately.

Materials and Methods:

One thousand samples were collected from the patients suspected of DV infection, attending the Bidar Institute of Medical Sciences and Teaching Hospital, Bidar. Demographic details of the patients were collected. Depending on the reporting time of the patients, sera comprised of both acute and early convalescent phases. Samples were divided into three groups I, II, III.

Group I: consisted of 1000 samples obtained from suspected cases of DF, classified as undifferentiated fever/ Dengue fever (DF)/ DHF (WHO classification). Adult samples from Out-Patients Department (OPD) and In-Patients Department (IPD) and paediatric IPD patients were included in this group. Single blood sample was screened by NS1 Ag assay and MAC ELISA and then compared.

Group II: consisted of 30 samples obtained from paediatric OPD patients classified as undifferentiated fever/ Dengue fever (DF). Acute phase sera (presenting within 7 days of fever) from this group were screened only by NS1 Ag assay, to evaluate the cost effectiveness of this assay in the acute phase. Since, this group patient attended OPD at regular intervals; paired samples of the patients who were negative by NS1 Ag assay were screened for antibodies in the early convalescent phase.

Group III: consisted of 40 samples, 25 were obtained from patients with fever due to known etiology other than dengue (enteric fever [18], bacterial meningitis [3], UTI [4]) and 15 were from healthy blood donors. All these 40 were screened by both the assays.

The samples were screened for the presence of dengue specific IgM antibodies by Mac ELISA, using a kit prepared by National Institute of Virology, Pune, India, (as an integral part of National Vector Borne Disease Control Programme), strictly following the manufacturer's protocol [8]. NS1 Ag was detected in the sera by Pan Bio NS1 Ag ELISA kit. Haematological parameters (leukocyte count, Hb and platelet count) of dengue positive patients were also observed and correlated. Fisher's exact test was used to find out the P values.

Results:

In Group I, of the 1000 samples, 462 (46.2%) were positive either for NS1 or IgM antibody or both. 247 (24.7%) samples were positive for NS1 Ag and 184 (18.4%) were positive for IgM antibody including those that were positive by both (Table 1). When single sample was tested by both the assays, the detection rate increased to 431.

Out of 247 NS1 Ag positive samples, 97.57% (241) were from acute phase sera and 2.42% (6) were from early convalescent phase. NS1 Ag detection rate decreased from 97.57% in acute phase sera to 2.42% in early convalescent sera ($P < 0.0001$). All the 183 samples that were positive for NS1 Ag alone in this study, Group I belonged to acute phase sera (Table 2).

Table 1: Detection rate of NS1 Ag Assay and MAC-ELISA in Dengue Positive Samples of Group 1 (n=462)

Ns1 Ag	IgM Antibody		Total
	Negative	Positive	
Negative	95 (9.5%)*	120 (12%)	215 (21.5%)
Positive	183 (18.3%)	64 (6.4%)	247 (24.7%)
Total	278 (27.8%)	184 (18.4%)	462 (46.2%)

(*95 samples were negative by both NS1 Ag assay and MAC ELISA but positive by IgG ELISA)

Table 2: Positivity of NS1 and IgM in (1-7 days) and (8-14 Days)

Day post onset of illness	NS1	IgM
1 to 7 days	241/247(97.57%)	10/184 (5.43%)
8 to 14 days	06/24 (2.42%)	174/184 (94.56%)

IgM antibodies were positive in 10 (5.43%) cases during 1-7 days period of illness and 174 (94.56%) were positive during 8-14 days of illness. All the 120 samples that were only IgM positive belonged to convalescent phase (Table 2). IgM antibody detection rate increased from 5.43% to 94.56% ($P < 0.0001$). NS1 Ag was positive in 241(97.57%) samples during 1-7 days period and 06(2.42%) in 8-14 days period

All the 30 samples from study Group II were screened only for NS1 Ag and 20 (66.66%) were positive. All these 20 patients were classified as DF. All had normal platelet count. The 10 NS1 Ag negative samples from patients with undifferentiated fever remained seronegative on subsequent screening at early convalescent stage. All the 40 samples from Group III were negative by both the assays.

Discussion:

The IgM capture ELISA is most commonly used in India due to its low cost and ease of handling. But here it is important to understand that, NS1 antigen detection assay has an advantage of detecting infection very early, however it disappears early also and is of little use in the early convalescence phase when IgM is useful [7]. To confirm a case of acute dengue infection by serology, IgM sero-conversion or a fourfold increase of IgG antibody titer in paired sera must be demonstrated [9].

In this study, NS1 Ag was only positive in 183 (18.3%) samples and IgM was positive in 120 (12%) but both the assays performed on a single sample could statistically increase the number of positive to 431. NS1 antigen is detectable by most of the commercial kits in first 7 to 9 days of infection while IgM antibodies are detectable only after 8 to 14 days of infection, the reason why NS1 antigen capture ELISA could detect more cases

compared to IgM capture ELISA alone [9-11]. NS1 Ag detection decreased from 97.57% in acute phase sera to 2.42% in early convalescent sera and detection rate of IgM increased from 5.43% in acute phase sera to 94.56% in convalescent sera. Similar findings were seen in other studies along with an increase in sensitivity of detection when both the assays were used together in a single sample [12, 13].

Since it is difficult to judge accurately which post infection day the sample is being tested, it is ideal to use both assays, not to miss the diagnosis.

Conclusion:

Although, there is no specific treatment available for dengue, early diagnosis has a role in individual case management as well as planning and implementing control strategies. Of the two different tests used to diagnose dengue, NS1 antigen detection had the highest sensitivity in the early stages while IgM detection was more sensitive in the latter half of the illness. Because of these characteristics of the assays, it is recommended to use both assays simultaneously to ensure that the diagnosis is not missed.

References

- Halstead SB. Dengue. *Lancet* 2007; 370:1644-52.
- Gubler DJ, Melzer M. Impact of dengue/ dengue hemorrhagic fever on the developing world. *Adv virus Res* 1999; 53:35-70.
- Satish N, Vijayakumar TS, Abraham P, Sridharan G. Dengue fever: its laboratory diagnosis, with special emphasis on IgM detection. *Dengue Bull* 2003; 116-21.
- Bessof K, Delorey M, Sun W, Hunsperger E. Comparison of two commercially available dengue Virus (DENV) NS1 capture ELISA using a single clinical sample for diagnosis of acute DENV infection. *Clin Vaccine Immunol* 2008; 15:1513-8.
- Dussart P, Labeau B, Lagathu G, Louis P, Nunes MRT, Rodrigues SG, et al. Evaluation of Enzyme immunoassay for detection of dengue virus NS1 antigen in human serum. *Clin Vaccine Immunol* 2006; 13: 1185-90.
- Vaughn DW, S Green, S Kalayanarooj et al. Dengue viremia, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* 2000; 181(1): 2-9.
- Andries AC, Duong V, Nagan C, Ong S, Huy R, Sroin KK et al. Field evaluation and impact on clinical management of a rapid diagnostic kit that detects dengue NS1, IgM and IgG. *PLoS Negl Trop Dis* 2012; 6(12): e1993.
- Cecilia D, Kakade MB, Bhagat AB et al. Detection of dengue-4 virus in pune, western India after an absence of 30 years- its association with two severe cases, *Virology* 2011; 8, article 46.
- Tricou V, Vu HT, Quynh NV Nguyen C, Tran HT, Farrar J et al. comparison of two dengue NS1 rapid tests for sensitivity, specificity and relationship to viraemia and antibody responses. *BMC Infect Dis* 2010; 10:142.
- Duong V, Ly S, Lom Try P, Tuiskunen A, Ong S, Chroeueng N et al. clinical and virological factors influencing the performance of a NS1 antigen capture assay and potential use as a marker of dengue disease severity. *PLoS Negl Trop Dis* 2011; 5(7): e1244.
- Fry SR, Meyer M, Semple MG, Simmons CP, Sekaran SD, Huang JX et al. The diagnostic sensitivity of dengue rapid test assays is significantly enhanced by using a combined antigen and antibody testing approach. *PLoS Negl Trop Dis* 2011; 5(6): e1244.
- Schilling S, Ludolfs D, Le VA, Schmitz H. Laboratory diagnosis of primary and secondary dengue infections, *J Clin Virol* 2004; 31:179-84.
- Ampaiwan C, Wathanee C, Viroj P, Kanchana T, Sarapee L, Sutee Y. The use of Nonstructural protein 1 antigen for the early diagnosis during the febrile stage in patients with dengue infection. *J Paed infect Dis*: 2008; 27: 43-8.

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