Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 07, pp.54826-54831, July, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

USEFULNESS OF DENGUE- NS1 ANTIGEN AS AN EARLY MARKER OF DENGUE VIRUS INFECTION IN A TERTIARY CARE HOSPITAL

[1,*]Mamatha, V., [1]Muralidharan, S., [1]Ranjani Shamsundar and [2]Seena Thomas

[1]Department of Microbiology, St. John's Medical College, Bangalore, India
[2]Department of Community Medicine, St. John's Medical College, Bangalore, India

ARTICLE INFO

Article History:
Received 25th April, 2017
Received in revised form
20th May, 2017
Accepted 22nd June, 2017
Published online 31st July, 2017

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

ABSTRACT

Dengue is one of the most important mosquito borne viral infections of man. Dengue epidemics have taken a significant economic and health toll. The study was aimed to look at the utility of dengue NS1 (non structural protein 1) antigen as an early marker of dengue infection. The study group included 300 clinically suspected cases of dengue presenting within the first week of fever. Patients' serum sample was subjected to ELISA for NS1 Ag, IgM Ab and IgG Ab. 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 61.33%, which was statistically significant (p=0.04). In the first 3 days of fever, a higher number of cases were positive for NS1 Ag only. By day 4, a combination of NS1 Ag and IgM Ab gave a positivity of 29.31%. Day 5 onwards, a higher number of cases were positive for IgM Ab only. The results show that NS1 Ag is a very useful tool in the diagnosis of dengue infection especially in the first 3 days of fever even before IgM Ab becomes detectable; when used in combination with IgM Ab assay, the diagnostic algorithm significantly improves on a single serum sample.

Citation: Mamatha, V., Muralidharan, S., Ranjani Shamsundar and Seena Thomas, 2017. "Usefulness of dengue- ns1 antigen as an early marker of dengue virus infection in a tertiary care hospital", *International Journal of Current Research*, 9, (07), 54826-54831.

INTRODUCTION

Dengue is the most rapidly spreading mosquito-borne viral disease in the world. Dengue fever (DF) and its severe forms – dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), have become major international public health concerns. 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 (WHO 2011). Many clinical conditions like leptospirosis, rickettsial fever, malaria, chikungunya, mimic DF in their early clinical phase. It is important to differentiate DF from these conditions to choose the appropriate management, including avoidance of misuse of antibiotics or antimalarials. Early diagnosis of DF becomes imperative not only for the above reason, but also due to its increasing complications like DHF and DSS. Some 2.5 billion people – two fifths of the world's population in tropical and subtropical countries – are at risk. An estimated 50 million dengue infections occur worldwide annually. An estimated 500000 people with DHF require hospitalization each year. A very large proportion (approximately 90%) of them are children aged less than five years, and about 2.5% of those affected die (WHO, 2011). 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. Non-Structural Protein 1(NS1) is a highly conserved glycoprotein that is essential for the viability of dengue virus (DENV) and circulates uniformly in all serotypes of DENV. It is conserved across all the four dengue serotypes. NS1 Ag level 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 (Alcon *et al,* 2002). ELISA directed against NS1 Ag 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 (Datta *et al,* 2010).

The objectives of the present study were:

- Detection of dengue NS1 antigen (NS1 Ag) by ELISA method in the acute phase sera of clinically suspected cases of DF.

***Corresponding author:* Mamatha, V.**
Department of Microbiology, St. John's Medical College, Bangalore, India.

- Simultaneous detection of IgM antibody (IgM Ab) and IgG antibody (IgG Ab) by ELISA method in the same serum sample.

MATERIALS AND METHODS

Clinically suspected cases of DF, from the out-patients and in-patients attending various clinical departments of a tertiary care hospital, formed the study group. A total of 300 samples, between May 2011 and April 2012, were included in the study.

Inclusion criteria

- Blood samples of clinically suspected cases of DF (CDC – Case Definition - Dengue) and cases of DF which progressed to DHF and DSS were included.
- Blood samples of only the acute phase sera i.e within first 7 days of fever were included.

Exclusion criteria

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

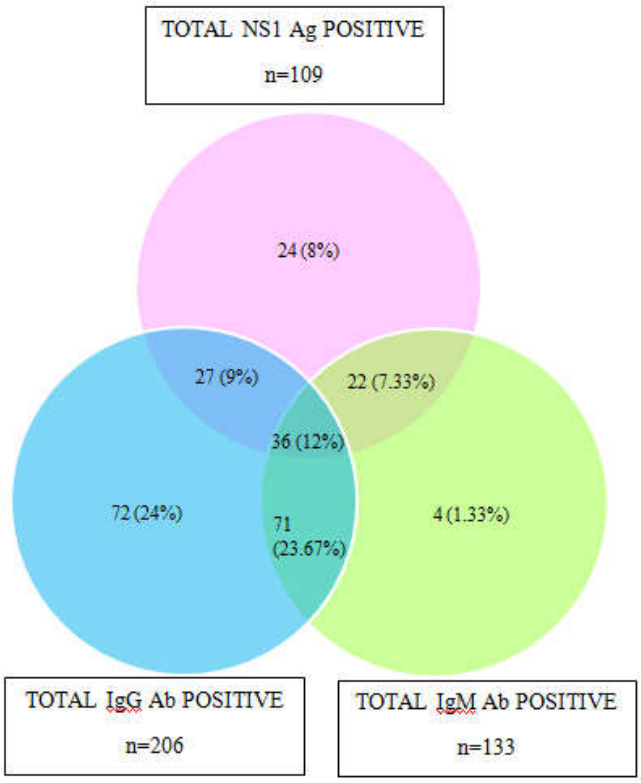
Study protocol

- Blood samples (one sample per patient) from the out-patients and in-patients of clinically suspected cases of DF, which were received at the Microbiology laboratory of a tertiary care hospital, 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 (5 ml) 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 (Standard Diagnostics, Inc. Korea) to detect dengue NS1 Ag, IgM Ab and IgG Ab.

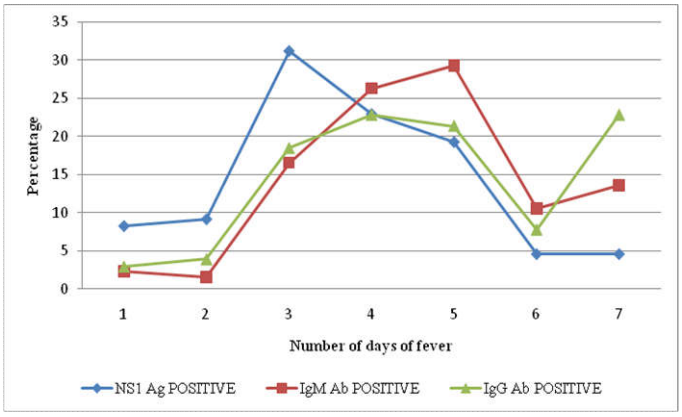
RESULTS

The study group included 182 males and 118 females with a male: female ratio of 1.54:1. Maximum number of patients belonged to the age group of 21-40 years (46.33%), followed by ≤10 years (21.67%). The mean age was 26 years with a standard deviation of 16.26 (minimum age was 1 year and maximum age was 70 years). Of the 184 patients who were positive for either NS1 Ag or IgM Ab or both, 49.46% positivity was seen in the age group 21 – 40 years and 23.37% positivity in the age group ≤10 years.

Of the 300 cases, 36.33% (n=109) were positive for NS1 Ag, 44.33% (n=133) were positive for IgM Ab and 68.67% (n=206) were positive for IgG Ab. Graph 1



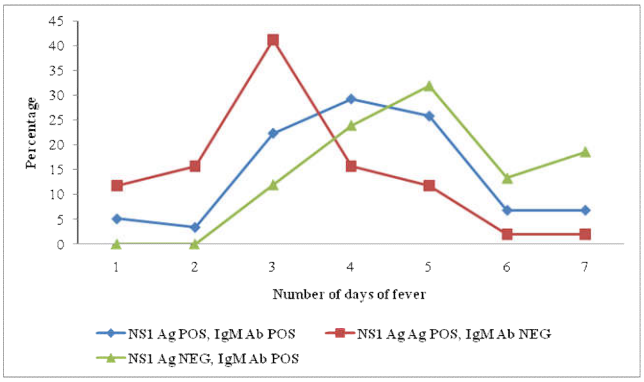
Graph 1. Combined test results NS1 Ag, IgM Ab and IgG Ab



Graph 2. Comparison of overall time to positivity of NS1 Ag, IgM Ab and IgG Ab

Of the 109 positive for NS1 Ag, maximum positivity was seen on day 3 (31.19%). Of the 133 positive for IgM Ab, maximum positivity was seen on day 5 (29.32%). Of the 206 positive for IgG Ab, 22.82% positivity was seen on day 4 and another 22.82% on day 7. Graph 2

When the test results of NS1 Ag and /or IgM Ab were considered, 19.33% (n=58) were positive for both the parameters. 17% (n=51) were positive for NS1 Ag but negative for IgM Ab, 25% (n=75) were positive for IgM Ab but negative for NS1 Ag, and 38.67% (n=116) were negative for both the parameters. Out of the 19.33% (n=58) who were positive for both NS1 Ag and IgM Ab, maximum positivity was observed on day 4 (29.31%. Out of the 17% (n=51) who were positive for NS1 Ag but negative for IgM Ab, maximum positivity was seen on day 3 (41.18%). Out of the 25% (n=75) who were positive for IgM Ab but negative for NS1 Ag, maximum positivity was observed on day 5 (32%). Graph 3



Graph 3. Time to positivity of NS1 Ag in combination with IgM Ab

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=184
Days 1-3	18 (29.03)	35(56.45)	9(14.52)	62 (100)
Days 4-5	32 (36.36)	14 (15.91)	42 (47.73)	88 (100)
Days 6-7	8 (23.53)	2 (5.88)	24 (70.59)	34 (100)
Total (%)	58 (31.52)	51 (27.72)	75 (40.76)	184 (100)

Positive and negative test results are represented as + and – respectively.

In the table above, it is evident that in the first 3 days of fever, positivity for NS1 Ag was high (56.45%); after day 4 a higher number of cases were detected by IgM Ab (47.73% and 70.59% 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 ($\chi^2 = 49.16$, $p < 0.001$)

Table 2. Detection rate of NS1 Ag and IgM Ab

NS1 antigen	IgM antibody		Total (%) n=184
	Positive (%)	Negative (%)	
Positive	58 (53.21)	51 (46.79)	109 (100)
Negative	75 (100)	0 (0.00)	75 (100)
Total (%)	133 (72.28)	51 (27.72)	184 (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 300 cases, 36.33% (n=109) of cases were positive for NS1 Ag; 44.33% (n=133) of cases were positive for IgM Ab; when both the above were combined together, the detection rate increased to 61.33% (n=184). 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 2

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	18 (33.96)	35(66.04)	53(100)
	4-5 days	32 (69.57)	14 (30.43)	46 (100)
	6-7 days	8 (80.00)	2(20.00)	10(100)
	Total (%)	58 (53.21)	51 (46.79)	109 (100)

There is a statistically significant association between IgM Ab and NS1 Ag positivity with respect to the number of days of fever ($\chi^2 = 15.71$, $p< 0.001$). Table 3

There is a statistically significant association between NS1 Ag and IgG Ab by Fischer's Exact Test ($p< 0.001$). Out of the total 300 cases, 36.33% (n=109) of cases were positive for NS1 Ag; 68.67% (n=206) of cases were positive by IgG Ab test; when both the above were combined together, the detection rate increased to 84.00%(n=252). 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 ($p< 0.001$). Table 4

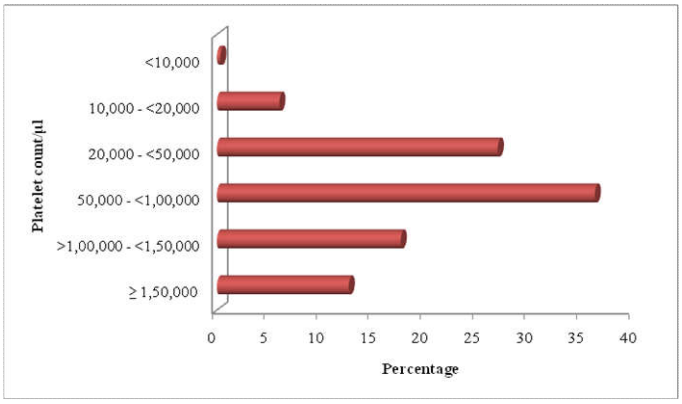
Table 4. Detection rate of NS1 Ag and IgG Ab

NS1 antigen	IgG antibody		Total (%) n=252
	Positive (%)	Negative (%)	
Positive	63 (57.80)	46 (42.20)	109 (100)
Negative	143 (100)	0 (00.00)	143 (100)
Total (%)	206 (81.75)	46 (18.25)	252 (100)

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 (%) n=109
		Positive (%)	Negative (%)	
NS1 Ag positive	1-3 days	31 (58.49)	22 (41.51)	53 (100)
	4-5 days	29 (63.04)	17 (36.96)	46 (100)
	6-7 days	3 (30.00)	7 (70.00)	10 (100)
	Total (%)	63 (57.80)	46 (42.20)	109 (100)

There is no statistically significant association between IgG Ab and NS1 Ag positivity with respect to the number of days of fever ($\chi^2 = 3.70$, $p< 0.157$). Table 5



Graph 3. Range of platelet count

Out of the total study group, 262 cases (87.33%) presented with thrombocytopenia (platelet count <1,50,000/µl). Graph 3 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. Table 6. Out of the 38 patients who had normal platelet count, 8 tested positive for NS1 Ag, 3 for both NS1 Ag and IgM Ab, 4 for IgM Ab. 43 cases of thrombocytopenia which were negative for IgM Ab, tested positive for NS1 Ag.

Out of the total 44 patients who were negative for all 3 tests, 10 were diagnosed as other diseases:

- 2 for Plasmodium vivax malaria by peripheral smear
- 1 for Plasmodium falciparum malaria by peripheral smear
- 2 for rickettsial infection by Weil Felix test

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	24	21	87.50
IgM positive	4	4	100.00
NS1 and IgM positive	22	21	95.45

- 3 for leptospirosis by anti-leptospira IgM ELISA
- 1 for enteric fever by Widal test
- 1 for enteric fever by blood culture (Salmonella Paratyphi 'A' positive)

Out of the total 109 patients who were positive for NS1 Ag, 7 were positive for other tests:

- 2 for Plasmodium vivax malaria by peripheral smear
- 3 for rickettsial infection by Weil Felix test
- 2 for leptospirosis by ELISA for anti-leptospira IgM

DISCUSSION

Out of the 184 patients who were positive for either NS1 Ag or IgM Ab or both, 49.46% belonged to the age group 21 – 40 years and 23.37% to the age group ≤10 years. The results of our study are similar to that of a study done at New Delhi (Gupta E *et al*, 2005). The same group of authors have reported maximum number of seropositive cases in 5-20 years age group in 1996; 40% culture positive cases in 5-12 yr age group in 1999. Studies from Singapore, Indonesia and Thailand have shown that in areas where dengue infection is either endemic or epidemics are more frequent, a shift occurs in the predominant age group involved and this shift in the affected age group may be attributable to changes in locations where disease transmission takes place (Patumanond *et al*, 2003; Goh *et al*, 1997; Corwin *et al*, 2001). Gupta *et al* 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). Overall, 36.33% were positive for NS1 Ag, 44.33% were positive for IgM Ab. The results for NS1 Ag positivity (36.33%) is in concordance with a study done at Malaysia (Kassim *et al*, 2011) which has reported 32.2% positivity. Certain other authors have reported a variable positivity between 23.3% (Datta *et al*, 2010) and 49.43% (Singh MP *et al*, 2010). In a study at China (Hu *et al*, 2011), 89% positivity for NS1Ag was observed in patients within first 7 days of fever; but all these patients had been laboratory-confirmed previously as being infected with DENV1 by virus isolation and/or viral RNA detection by RT-PCR and/or serological diagnosis by MAC-ELISA. The results for IgM Ab positivity (44.33%) is similar to that of other studies (Singh *et al*, 2010- 45.7%, Kassim *et al*, 2011 - 40.9%, Datta *et al*, 2010 - 39.1%). Of the 109 cases positive for NS1 Ag, a positivity of 71.56% was seen between days 1-4, and 28.45% after day 5, which is in concordance with other studies (Datta *et al*, 2010- 71.42% and 28.4% respectively; Singh *et al*, 2010- 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 (Young *et al*, 2000; Singh MP *et al*, 2010). Of the 133 positive for IgM Ab, a positivity of 46.62% was seen between days 1-4 and 53.38% after day 5. Various other studies have reported different rates of IgM positivity (Singh *et al*, 2010; Datta *et al*, 2010; Hu *et al*, 2011). However it is difficult to explain the differences in the IgM Ab results; one of the probable factors being differences in the immune responses of the individuals studied.

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 Singh MP *et al*, 2010. Overall, 36.33% and 44.33% were positive by NS1 Ag test and IgM Ab test respectively. When both the tests were combined together, the detection rate increased to 61.33%, which is statistically significant (p=0.04). These results are similar to that of a study by Datta S *et al*, 2010. Out of 300, 24 (8%) 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 up to the end of first week of fever.
Overall, 206 (68.67%) cases were positive for IgG Ab; of whom 72 (24%) were positive for IgG Ab alone. When the test results of NS1 Ag and IgG Ab were compared, 36.33% and 68.67% were positive by NS1 Ag test and IgG Ab test respectively. When both the tests were combined together, the detection rate increased to 84.00%, which was statistically significant ($p< 0.001$). 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; viral culture being time consuming, PCR being expensive, demonstration of seroconversion being difficult due to inability in obtaining 2nd sample from the patients, as some were outpatients and even if inpatients, they would get discharged before a week.

Various studies have shown that in secondary dengue infection IgG rises within 1-2 days after onset of symptoms, simultaneously with or 1-2 days prior to IgM antibodies. Therefore, patients with secondary infections will have a positive IgG result, usually, but not always a positive IgM result (WHO 2011; Shu PY *et al*, 2004). Utility of IgG Ab in the diagnosis of infections relies mainly on demonstration of rising titres, especially in the endemic areas. However, repeat testing for the same infection, when the first test is negative or sending samples for determination of rise in titre is 'almost never' utilized in clinical practice (Kulkarni RD *et al* 2011). When NS1 Ag is positive, there is no need of repeat testing as it is a highly specific marker for DENV infection (Peeling RW *et al*, 2010). Of the total 300 sera tested in our study, we have found 9% positive for both NS1Ag and IgG Ab, but negative for IgM Ab. Suspecting that these could probably be cases of secondary dengue infection, IgM/IgG ratio of >1.32 for

primary infection and ≤ 1.32 for secondary infection was applied (Prince HE *et al*, 2011); all the above positives turned out to be cases of secondary infection by this ratio. If not for the simultaneous detection of NS1 Ag in these cases, they would not have been diagnosed as cases of acute dengue. Various studies have shown that NS1 Ag detection in cases of secondary dengue is low compared to that of primary dengue, due to formation of immune complexes with preexisting antibodies (Singapore study and Hu D *et al*, 2011), whereas some other studies have demonstrated that NS1 concentrations did not differ significantly in serum specimens obtained from patients experiencing primary or secondary dengue virus infections (Alcon S *et al*, 2002). 24% (n=72) of the total study group were positive for IgG ab only, all of which were cases of secondary dengue infection using the IgM/IgG ratio described above. Furthermore, 90.28% (n=65) of these cases presented with thrombocytopenia; hence, in the absence of NS1 Ag or IgM Ab positivity, IgG Ab positivity along with thrombocytopenia could probably point towards the diagnosis of DENV infection. 12% of the total was positive for all the three parameters (triple positives). It was thought that, the presence of an early IgG Ab (as early as day 1) in these cases could probably be due to dengue infection in the past. When these cases were further categorized using the IgM/ IgG ratio described above, majority of the cases turned out to be secondary infections (34 out of 36). A study at Delhi (Arya SC *et al*, 2011) has reported 5.83% triple positives.

In our study, 87.33% of patients presented with thrombocytopenia. Of these, 64.5% 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. A study at Dharwad (Kulkarni RD *et al*, 2011), has reported that thrombocytopenia had excellent association when both NS1 Ag and IgM Ab were positive compared to NS1 Ag alone. Although the detection of dengue specific Ag is diagnostic of acute dengue infection, 7 out of the 109 NS1 Ag positive cases were also positive for other diseases; 2 for malaria, 3 for rickettsial infection, 2 for leptospirosis. Since the specific gold standard tests for these infections could not be performed for confirmation in any of the above disease conditions, the possibility of co-infection with acute dengue cannot be ruled out. In our study, 44 patients were negative for all the three parameters tested, 10 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.

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. A combination of NS1 Ag and IgM Ab assays would improve the diagnosis after day 4 upto the end of first week of fever. However, a negative NS1 Ag result does not rule out the possibility of DENV infection. NS1 Ag assay, if used in combination with IgM Ab assay on a single serum sample of a clinically suspected case of dengue, has the ability to improve the diagnostic algorithm contributing significantly to the clinical treatment and control of DENV infections. Since it needs the same instruments as for any ELISA, which is normally carried out in most diagnostic laboratories, NS1 Ag assay has the prospect for a much wider usage in resource poor endemic countries. 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 (Singh MP *et al*, 2010; Wang SM *et al*, 2010) 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.

REFERANCES

Alcon, S., Talarmin, A., Debruyne, M., Falconar, A., Deubel, V., Flamand, M. 2002. Enzyme linked immunosorbent assay specific to dengue virus type-1 non structural 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.,* Feb;40(2):376-81.

Arya, S.C., Agarwal, N., Parikh, S.C., Agarwal, S. 2011. Simultaneous Detection of Dengue NS1 Antigen, IgM plus IgG and Platelet Enumeration during an Outbreak. *Sultan Qaboos Univ Med J.,* Nov;11(4):470-6.

Corwin, A.L., Larasati, R.P., Bangs, M.J., Wuryadi, S., Arjoso, S., Sukri, N., et al. 2001. Epidemic dengue transmission in southern Sumatra, Indonesia. *Trans R Soc Trop Med Hyg.,* May-Jun;95(3):257-65.

Datta, S., Wattal, C. 2010. Dengue NS1 Ag detection: a useful tool in early diagnosis of dengue virus infection. *Indian J Med Microbiol.,* Apr-Jun;28(2):107-10.

Goh, K.T. 1997. Dengue--a re-emerging infectious disease in Singapore. Ann Acad Med Singapore, Sep; 26(5):664-70.

Gupta, E., Dar, L., Narang, P., Srivastava, V.K., Broor, S. 2005. Serodiagnosis of dengue during an outbreak at a tertiary care hospital in Delhi. *Indian J Med Res.,* Jan;121:36-8.

Hu, D., Di, B., Ding, X., Wang, Y., Chen, Y., Pan, Y., et al. 2011. Kinetics of non-structural protein 1, IgM and IgG antibodies in dengue type 1 primary infection. *Virol J.,* Feb 2;8:47.

Kassim, F.M., Izati, M.N., TgRogayah, T.A., Apandi, Y.M., Saat, Z. 2011. Use of dengue NS1 antigen for early diagnosis of dengue virus infection. *Southeast Asian J Trop Med Public Health,* May;42(3):562-9.

Kulkarni, R.D., Patil, S.S., Ajantha, G.S., Upadhya, A.K., Kalabhavi,A.S., Shubhada, R.M., et al. 2011. Association of platelet count and serological markers of dengue infection- importance of NS1 antigen. *Indian J Med Microbiol.,* Oct-Dec;29(4):359-62.

Patumanond, J., Tawichasri, C., Nopparat, S. 2003. Dengue hemorrhagic fever, Uttaradit, Thailand. *Emerg Infect Dis.,* Oct;9(10):1348-50.

Peeling R.W., Artsob, H., Pelegrino, J.L., Buchy, P., Cardosa, M.J., Devi, S., et al. 2010. Evaluation of diagnostic tests: dengue. *Nat Rev Microbiol.,* Dec;8(12):S30-8.

Prince, H.E., Yeh, C., Lapé-Nixon, M. 2011. Utility of IgM/IgG ratio and IgG avidity for distinguishing primary and secondary dengue virus infections using sera collected more than 30 days after disease onset. *Clin Vaccine Immunol.,* Nov;18(11):1951-6.

Shu, P.Y., Huang, J.H. 2004. Current advances in dengue diagnosis. *Clin Diagn Lab Immunol.,* Jul;11(4):642-50.

Singh, M.P., Majumdar, M., Singh, G., Goyal, K., Preet, K., Sarwal, A., et al. 2010. NS1 antigen as an early diagnostic marker in dengue: report from India. Diagn Microbiol Infect Dis., Sep;68(1):50-4.

Wang, S.M., Sekaran, S.D. 2010. Early diagnosis of Dengue infection using a commercial Dengue Duo rapid test kit for the detection of NS1, IGM, and IGG. *Am J Trop Med Hyg.,* Sep;83(3):690-5.

World Health Organisation, 2011. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. WHO Regional publication, SEARO No 60.

Young, P.R., Hilditch, P.A., Bletchly, C., Halloran, W. 2000. An antigen capture enzyme-linked immunosorbent assay reveals high levels of the Dengue virus protein NS1 in the sera of infected patients. *J Clin Microbiol.,* March; 38(3):1053–1057.

****