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Research Article

Effectiveness of serological tests for early detection of Dengue fever

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Abstract: Dengue fever is one of the emerging infectious diseases which cause significant morbidity and mortality in children and adults especially in developing countries. The mortality from the complications of DF is as high as 20%, whereas if recognized early and managed properly, mortality is less than 1%. Diagnosing dengue fever in early phase is challenging because the initial symptoms are often non-specific like any other viral infections. The aim of the study was to evaluate the effectiveness of serological tests for early detection of Dengue fever. It was a prospective study. The study included patients in the age group between 6 months to 14 yrs with fever up to 7 days fulfilling the WHO clinical criteria of dengue fever. Statistical analysis included sensitivity, specificity, PPV, NPV Chi square test and Fischer exact test. 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. NS1 antigen and IgM antibody high specificity and positive predictive value (both 100 %), they have low sensitivity with NS1 62% and IgM 53 %. This study shows that by doing both NS1 antigen detection and IgM and IgG antibodies we can diagnose dengue fever early and hence they do have a significant role in the early diagnosis of dengue fever.
Keywords: Dengue fever, NS1 Ag, IgM, IgG.

INTRODUCTION

Dengue fever is one of the emerging infectious diseases which cause significant morbidity and mortality in children and adults especially in developing countries. Dengue fever is caused by the dengue viruses, an Arbo virus from the *Flaviviridae family of small enveloped RNA viruses* with four serotypes namely DV-1, DV-2, DV-3, and DV-4. These viruses are transmitted to humans by *Aedes group* of mosquitoes *especially Aedes aegypti* which is a day-biting mosquito and breeds in standing water. Dengue epidemics are becoming more frequent especially during rainy and post rainy season. It may be difficult to diagnose dengue fever in the initial stages of the disease because the clinical presentations are almost similiar to any other viral illness. The 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 fever in early phase is challenging because the initial symptoms 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].

The major life threatening complications involving liver (hepatitis) [3, 4], brain (encephalitis) [5-7], kidneys (glomerulonephritis) [8] and heart (myocardial dysfunction) [9] usually start between the initial 3-7 days of dengue infection. Hence it is important to diagnose Dengue fever early to prevent the above complications [10].

Since the clinical presentation is almost similiar to any other viral illness, accurate diagnosis may not be possible only with the clinical presentation. In order to detect Dengue fever, there are various tests available like antigen detection tests (Non structural 1 antigen - NS1 antigen) , Antibody detection tests like Dengue IgM and Dengue IgG, virus isolation in cell culture or by detection of viral RNA by nucleic acid amplification tests (NAAT). Out of these, virus isolation and nucleic acid amplification tests require expertise, expensive equipments and reagents and time delay. NS1 antigen detection and Dengue IgM and Dengue IgG detection which detects dengue infection are easy to do and cheap. Moreover they detect the disease early so that early and prompt treatment can be given. NS 1,a glycoprotein that is common to all

dengue viruses appears as early as day 1 of fever and can be found up to day 9 of fever , IgM approximately 5 days after infection and IgG 2-4 weeks after the onset of fever [11-14].

Hence we can diagnose Dengue fever early by detecting NS1 antigen and doing serological tests like Ig M and IgG and initiate effective treatment and prevent life threatening complications. Since the serological tests are used for diagnosing Dengue fever early only in the past few years and since there are only few studies are done in India, this study was done to find out the effectiveness for early diagnosis of Dengue fever.

MATERIAL AND METHODS

This study is a prospective study conducted, in the paediatric department of a tertiary care hospital for a period from January 2013 to September 2014. The study patients are those children in the age group from 6 month to 14 years who present with fever upto 7 days who fulfill the WHO clinical criteria for diagnosing dengue fever. After obtaining the informed written consent from the care takers, a detailed history, thorough clinical examination was done to diagnose patients as Dengue like illness. Further laboratory tests like haemoglobin, hematocrit, platelet count, White blood counts were done. Serological testing (NS1, Ig M and Ig G) was done using SD BIOLINE dengue duo test kit, which 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. This contains two test devices (left side: dengue NS1 antigen test and right side: dengue IgM/IgG test). If the patient is having Dengue fever, other investigations like liver function tests, renal function tests, serum electrolytes, chest x ray, coagulation profile (PT, APPT, INR) and ultrasonogram of abdomen were done to find out the life threatening complications. The patients were managed based on the WHO protocol for treating Dengue Fever. The data obtained was analysed using SPSS software for estimating the sensitivity, specificity, positive predictive value and negative predictive value of NS1 antigen and IgM antibody for detecting Dengue illness early. Chi square test and Fischer exact test were also done.

RESULTS

A total of 143 who fulfilled the study criteria for Dengue fever were entrolled in the study. The following Table 1 gives the age distribution of study subjects.

Table 1: Age distribution of the patients under study

Age	No. of patients	%
<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

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% .

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.

Table 2: Duration of fever

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

Table 3: Dengue and Dengue like illness

Illness	No. of patients	%
Dengue	100	69.93
Non dengue	43	30.06
Total	143	100

As shown in Table 3, out of 143 subjects, 100 were found to have dengue fever, diagnosed serologically using NS1, IgM and IgG antibodies and the rest 43 were serologically negative for dengue.

Table 4: NS1 Ag in dengue patients (n=100)

NS 1 Ag	No. of patients	%
Only NS1 +	46	46%
NS1 + with IgM and IgG	16	16%
NS1 –negative	38	38%

Out of 100 Dengue patients,only NS1 antigen was positive for about 46 patients (46%) who were diagnosed as dengue fever using clinical and serological evaluation. About 16 patients (16%) were positive for NS1 antigen with either IgM or IgG antibodies and the rest 38(38%) were negative for NS1 antigen. Overall 62 patients were positive for NS1 antigen out of the 100 dengue patients.

Table 5: IgM antibodies in dengue patients (n=100)

IgM antibody	No. of patients	%
Only IgM +	27	27%
IgM with NS1 or IgG	26	26%
IgM negative	47	47%

The above table demonstrates that only IgM was positive for about 27 patients out of the 100 dengue patients where as 26 patients were positive for IgM along with either NS1 antigen or IgG antibody. Overall 53 patients were positive for IgM antibody out of the 100 dengue patients.

Table 6: IgG antibodies in dengue patients (n=100)

IgG antibody	No. of patients	%
Only IgG +	0	0%
IgG with NS1 or IgM	15	15%
IgG negative	85	85%

This table demonstrates that IgG antibody was positive for only 15 patients out of the total 100 dengue patients. The presence of IgG represents secondary infection in the patient. Thus out of the 100 dengue patients, 85 patients were having primary dengue and 15 were secondary dengue.

Table 7: Class of dengue illness

Class of dengue fever	No. of patients	%
Class A	40	40
Class B	30	30
Class C	30	30
Total	100	100

This table 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. There was no mortality during the study period.

Table 8: Division of serological positive cases based on the day of positivity (n=100)

Day of positivity	NS1 + No of pts	IgM	IgG
1	0	0	0
2	1	0	0
3	10	0	0
4	19	2	0
5	19	21	4
6	10	14	5
7	3	13	6
8	0	2	0
9	0	1	0
Total	62	53	15

Out of the total 100 patients, NS1 was positive in 62 patients with a maximum positivity on 4^{th} and 5^{th} day of illness. 11 patients (17.7%) were positive between 1-3 days ; 38 patients (61.2%) were positive

between day 4-5 ; 13 patients(20.9%) were positive between 6 - 7days . Remaining 38 Dengue cases were negaive for NS1after 7 days of illness. Though NS1 antigen production is from day 1 of illness up to 9 days, in our study it was found to be positive only up to 7 days of illness, maximum positivity between 4 – 5 day.

Out of total 100 dengue patients, IgM was positive in 53 patients with a maximum positivity was on the 5th day. IgM was positive with only 2 patients positive on day 4. Once serology was positive, the test was not repeated and patients were not followed up, thus presence of IgM up to few weeks after illness couldn't be elicited.

Out of the 100 dengue patients IgG was positive in 15 patients. IgG antibody occurs only after few weeks of illness, it represents secondary dengue infection. Out of the 43 non dengue patients IgG was positive in only 1 patient. However NS1 antigen and IgM antibody was negative for the same patients thereby representing dengue infection in the past.

Table 9: Comparison of NS1Ag and IgM Ab in dengue pts (n=100)

Day of positivity	NS1 positivity	IgM positivity
1 – 3	11	0
4 – 7	51	50
8 – 9th day	0	3
Total	62	53

Both NS1 antigen and IgM antibody were mostly positive between 4 – 7 days of illness.

Table 10: Statistical analysis: sensitivity, specificity, PPV, NPV

	NS1 antigen	IgM antibody
Sensitivity	62%	53%
Specificity	100%	100%
Positive predictive value	100%	100%
Negative predictive value	53%	47.7%

This demonstrates that although the NS1 antigen and IgM antibody high specificity and positive predictive value (both 100 %), they have low sensitivity with NS1 62% and IgM 53 % .

Table 11: Statistical analysis: Chi square test and Fischer exact test table

	IgM/IgG positive	IgM/IgG negative	Total
NS1 positive	16	46	62
NS1 negative	39	42	81
Total	55	88	143

The chi square value is 7.4063 (p value 0.0065) and The Fischer exact value is 0.009051.On comparison of NS1 antigen and / or IgM, IgG antibodies, and on using both chi square and Fischer exact test, p value is <0.01 which is statistically significant.

DISCUSSION

Dengue fever, an acute febrile arbo-viral disease has become a major public health problem in tropical and subtropical regions of the world especially in India, due to the 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 in comparison with the IgM antibody for the early diagnosis of dengue illness has been analyzed.

In our study of 143 samples, only NS1 positivity was detected with a very low sensitivity 33.3% till day 3 of fever, whereas IgM had a sensitivity of 0% during this time. Singh M P et al., compared IgM antibody detection with NS1 antigen for the diagnosis of acute dengue in 87 samples. Only NS1 was detected with good sensitivity (71-100%) till day 3 of fever, whereas IgM had a sensitivity of 0% to 50% at this time [15].

Of the 143 patient sample tested in our study, 101 (70.6%) were positive for NS1 antigen, IgM and IgG antibodies. Of the 100 Dengue fever cases in our study, NS1 antigen gave an overall positivity rate of 62 % (62 patients), IgM 53 %(53 patients). Only NS1 antigen can be used to detect Dengue fever upto the third day of illness. IgM showed positivity by fourth

day of illness. From day 4 to day 7, no significant difference in detection rates was seen between the NS1 antigen and IgM antibody. Thus NS1 antigen will be an useful tool for detecting dengue infection early during the first three days of illness. These findings in our study were corroborated by another study by Chakravarti A *et al*. 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). He also concluded that NS1 antigen assay may be a useful tool for detecting dengue infection during first few days of fever [16].

We had detected NS1 positivity in about 62 patients (43.3%) , IgM and/or IgG positivity in about 55 patients (38.46%) while a combination of NS1 antigen with or without IgM/IgG positive in 101 patients (70.6%) in our study thus implying that the combination of these serological tests would increase the rate of detection of dengue fever. These findings were similar to a study done by Fauziah Md *et al*. They found that on 208 dengue suspected fever cases, NS1 antigen was positive in 67 patients (32.2%) and a total of 107 patients (51.4%) 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 tests and the combination of these serological tests would increase the diagnostic efficiency of early diagnosis of dengue illness [17].

We found that the sensitivity of NS1Ag and IgM Ab was 62 % and 53 % respectively however both were found to be highly specific (100%). The positive predictive value of both NS1 Ag and IgM Ab was 100% as compared to the negative predictive value of 53 %, and 47.7% respectively. These findings are similar to a study by Keswadee Lapphra *et al*. Of the 235 patients suspecting to have dengue fever they found that NS1 antigen had sensitivity of 63.2%, specificity of 98.4%, positive predictive value of 99.0% and negative predictive value of 52.5%. These findings are similar to our study. Thus above studies suggest that NS1 antigen although has a low sensitivity, has a very high specificity and the positive predictive value [18].

The positive aspect of our study was that we included all patients who presented with fever who were suspected to be dengue patients as per WHO criteria and were screened for dengue infection by detecting NS1 antigen, IgM and IgG antibodies irrespective of the other laboratory parameters. The main limitation was that we did not perform virus isolation and the viral RNA PCR which are considered the gold standard tests for the detection of dengue illness. We also did not perform the persistence of IgM/IgG antibodies in convalescent serum.

CONCLUSION

Dengue fever, common in developing countries like India, causes significant morbidity and mortality, presents like any other viral illness. Hence these patients should be diagnosed early for prompt treatment. The present study showed that NS1 antigen detection along with IgM and IgG antibodies tests have a very significant role in the early diagnosis of dengue fever thereby early necessary intervention can be started. The results revealed that both NS1 and IgM have a very high specificity and positive predictive value. However, the sensitivity is comparatively low for both NS1 (63%) and IgM (53%). As shown by this study, the combination of the antigen and antibody testing would increase the case detection up to 70%.

Our study revealed that doing only NS1 antigen had a limited role in detection of dengue illness on first three days of illness eventhough NS1 is found to be positive from day 1 of illness up to day 9 of illness. Both NS1 antigen and IgM antibody were both equally effective in detecting the illness between days 4 to day 7. After day 7 again the positivity of NS1 reduced significantly. Hence if we do both NS1 antigen detection test and IgM /IgG antibody detection tests, we can diagnose dengue fever early so that the morbidity and mortality can be reduced and hence we conclude that the serological tests do have a significant role in the early diagnosis of dengue fever.

REFERENCES

1. World health organization; WHO report on global surveillance of epidemic prone infectious diseases. Available from http://www.who.int/emc-documents/surveillance/docs/whocdscsrisr2001.htm
2. Ramos MM, Tomashek KM, Arguello DF, Luxemburger C, Qunones L, Lang J *et al.*; Early clinical features of dengue infection in Puerto Rico. Transactions of the Royal Society of Tropical Medicine and Hygiene, 2009; 103(9): 878-884.
3. Guzman MG, Kouri G; Dengue: an update. Lancet Infect Dis., 2001; 2: 33-42.
4. Poovorawan Y, Hutagalung Y, Chongrisawat V, Boudville I, Bock HL; Dengue virus infection: a major cause of acute hepatic failure in Thai children. Ann Trop Paediatr., 2006; 26: 17-23.
5. Solomon T, Dung NM, Vaughn DW, Kneen R, Thao LT, Raengsakulrach B *et al.*; Neurological manifestations of dengue infection. Lancet, 2000; 355: 1053-1059.
6. Soares CN, Faria LC, Peralta JM, de Freitas MR, Puccioni-Sohler M; Dengue infection: neurological manifestations and cerebrospinal fluid (CSF) analysis. J Neurol Sci., 2006; 249: 19-24.
7. Kalita J, Misra UK, Mahadevan A, Shankar SK; Acute pure motor quadriplegia: is it dengue myositis? Electromyogr Clin Neurophysiol., 2005; 6: 357-361.

8. Lizarraga KJ, Nayer A; Dengue associated kidney disease. Journal of Nephropathology, 2014; 3(2): 57-62.
9. Kabra SK, Juneja R, Madhulika, Jain Y, Singhal T, Dar L *et al*.; Myocardial dysfunction in children with dengue hemorrhagic fever. Natl Med J India, 1998; 11: 59-61.
10. WHO; Dengue and dengue haemorrhagic fever. Factsheet No 117, revised May 2008. Geneva, World Health Organization, 2008; 2: 25-28.
11. Chuang YC, Wang SY, Lin YS, Chen HR, Yeh TM; Re-evaluation of the pathogenic roles of nonstructural protein 1 and its antibodies during dengue virus infection. J Biomed Sci., 2013; 20: 42.
12. Wang SM; Sekaran SD; Early diagnosis of dengue infection using a commercial dengue duo rapid test kit for the detection of NS1, IgM, IgG. Am J Trop Med Hyg., 2010; 83(3): 690-695.
13. Gowri Sankar S, Dhananjeyan KJ, Paramasivan R, Thenmozhi V, Tyagi BK, John Vennison S; Evaluation and use of NS1 IgM antibody detection for acute dengue virus diagnosis: report from an outbreak investigation. Clin Microbiol Infect., 2012; 18(1): E8-10.
14. Moi ML, Omatsu T, Tajima S, Lim CK, Kotaki A, Ikeda M *et al*.; Detection of dengue virus nonstructural protein 1 (NS1) by using ELISA as a useful laboratory diagnostic method for dengue virus infection of international travelers. J Travel Med., 2013; 20(3):185-93.
15. Singh M P, Majumdar M, Singh G, Goyal K, Preet K, Sarwal A *et al*., NS1 antigen as an early diagnostic marker in dengue: report from India. Diagn Microbiol Infect Dis., 2010; 68(1): 50-54.
16. Chakravarti A, Kumar A, Malik S; Detection of dengue infection by combining the use of an NS1 antigen based assay with antibody detection. Southeast Asian J Trop Med Public Health, 2011; 42(2): 297-302.
17. Kassim FM, Izati MN, Apandi YM, Saat Z; Use of dengue NS1 antigen for early diagnosis of dengue virus infection Southeast Asian J Trop Med Public Health, 2011; 42(3): 562-569.
18. Lapphra K, Sangcharaswichai A, Chokephaibulkit K, Tiengrim S, Piriyakarnsakul W, Chakorn T *et al*.; Evaluation of an NS1 antigen detection for diagnosis of acute dengue infection in patients with acute febrile illness. Diagn Microbiol Infect Dis., 2008; 60(4): 387-391.