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Research Article

Detection of NS1 antigen, IgM antibody for the diagnosis of dengue infection in patients with acute febrile illness

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ABSTRACT

Background: Dengue is an endemic viral disease affecting tropical and subtropical regions around the world. Infection with any 1 of 4 dengue viruses produces with spectrum of clinical illness ranging from a mild undifferentiated febrile illness to dengue fever (DF) to dengue haemorrhagic fever (DHF), a potentially life threatening disease. The mortality and morbidity of DHF can be reduced by early diagnosis, hospitalisation and careful supportive care. Detection of non-structural antigen (NS1 Ag), IgM and IgG antibody may help in the early diagnosis.
Methods: The present study was taken from the Pt J N M Medical College, Raipur (CG.) department of Microbiology. The one year study from August 2014 to July 2015 detection of NS1 Ag and IgM antibody. Elisa were tested a penal compared of 1637 serum specimens collected from acute febrile patients. Out of 1637 patients 538_were found to have acute dengue infection by detection of NS1Ag and anti-dengue IgM Elisa.
Results: Out of 1637 patients 538- were found to acute dengue infection. Out of 538– NS1 161—IgM 294 positive. Males are more affected than females and 21 to 30 year age group were more infected. Dengue illness were more in rainy and post rainy season.
Conclusions: The present study showed that dengue serological tests have a significant role in the early diagnosis of dengue fever, Hence, it is recommended to do the serological tests (NS1 Ag, IgM, IgG Ab) early in all suspected dengue cases so that, we can diagnosis early and initiate necessary treatment.

Keywords: Dengue fever, Dengue haemorrhagic fever, NS1, IgM/IgG antibody

INTRODUCTION

Dengue virus infection has emerged as a notable public health problem in recent decades in term of the mortality and morbidity associated with it.[1,2] Dengue is endemic in many parts of India and epidemics are frequently reported from various parts of India and abroad.[3,4]

Dengue fever is a sever flu like illness that affects the infants, children's, adolescents, and adults.[5] Dengue is one of the most serious and the most common mosquito-borne viral infections of the man affecting mainly the tropical and subtropical countries in the world and caused by the bite of Aedes group of mosquitoes especially *Aedes aegypti* which is a day biting mosquito and breeds in standing water.[6,7] Dengue is an acute viral disease caused by a virus belonging to the broad group of Arbo-viruses, family Flaviviridae, subfamily Flavivirinae and genus Flaviviruses. Dengue virus has a positive sense, ss RNA viral genome.[8] Dengue epidemics are becoming more frequent especially during rainy season and post rainy season. It may be difficult to diagnose dengue fever in the initial stage of the disease because the clinical presentations are almost similar to any other viral illness.[9]

There are four serotype of dengue viruses DEN 1, DEN 2, DEN 3, and DEN 4. Infection with dengue virus can cause three clinical syndromes with undifferentiated febrile illness or viral syndrome, classical dengue fever (DF), dengue haemorrhagic fever (DHF) which may occur with shock or as dengue shock syndrome (DSS).Classical dengue fever (DF) is generally self-limited and is characterized by fever and a variety of non-specific signs and symptoms such as headache, malaise, weakness, rash and body aches. The DHF is distinguished from DF by the onset of plasma leakage, marked thrombocytopenia, and a bleeding diathesis.[5,9-11]

With the increasing incidence of dengue infection, the early diagnostic confirmation of dengue infection in patients allows for timely clinical intervention, etiological investigation, and disease control, hence, diagnosis of dengue disease during the acute phase should be a priority and is a public health concern. 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, immunoflurocence or by detection of viral RNA by nucleic acid amplification tests (NAAT).[10] Out of these, virus isolation and nucleic acid amplification tests require expertise, expensive equipments & 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 and prompt treatment can be given.[8,11]

The NS1 a glycoprotein and NS1 antigen capture ELISA, first developed in 2000 for DENV was based on the premise it would act as a surrogate marker of viremia12, 13. NS1 is present at high concentration in sera of dengue infected patients during the early clinical phase of disease, and is found from day 1 and up to day 9 of fever, IgM approximately 3 to 5 days of infection and persist for 2 to 3 months, and IgG appear by 2-4 weeks after the onset of fever and persist for life.[8,11]

The aim of this study was to a evaluate the use of NS1 test against IgM assay as the most suitable test for the laboratory confirmation of dengue at primary health care settings where prompt diagnosis is required. In which designed for the detection of dengue NS1 antigen and IgM/IgG antibody was evaluated for it potential application foe early diagnosis of acute dengue virus infection. And also detection of NS1 Ag and IgM Ab by using ELISA kit. Hence, we can diagnose dengue fever early to initiate effective treatment and prevent life threatening complications.

METHODS

The present study was conducted in the department of Microbiology, Pt. J. N. M. Medical College, and Raipur (C.G.) for a period from August 2014 to July 2015. A total number of 1637 blood sample from clinically suspected cases of dengue fever, according to the WHO criteria, where obtained from both outdoor and hospitalized patients from the Medical college hospital, Raipur.

Serum sample collection

A total of 1637 sera used in this study. These blood samples were collected from outdoor and hospitalized patients, for the acute viral infection. Serum was separated by centrifuging samples at 3000 rpm for 5 min and tested immediately; in case of delay in processing they were stored in a refrigerator at a temperature of 2-8^{0}C.

Detection of NS1Ag and IgM Ab by the ELISA (Enzyme-Linked Immunosorbent Assay) test using Dengue NS1 Ag and IgM Ab Microlisa kit by J. Mitra co. pvt. Ltd, New Delhi was performed in selected 1637 samples out of the 538 are dengue positive. The PC and NC from the kit were put up with the test samples as per the kit literature provided. Two kits from the same manufacture containing 96 microwells used for testing a total of 1637 samples. The test was a solid phase ELISA based on 'Direct Sandwich' principle. The micro wells coated with monoclonal Anti-dengue NS1 antibodies. A positive reaction was indicated by a yellow colour which was finely read at 450nm spectrophotometrically by an ELISA reader the cut- off value (COF) was calculated using the formula. Data was compiled in MS-Excel and checked for its completeness and correctness. Then it was analyzed.

RESULTS

A total of 1637 samples are tested and detected for Dengue infections. Of these 1637, 538(32.86%) were serologically proved to have dengue illness and rest 1099(67.13%) were non dengue patients. Of these 161(29.92%) samples were NS1 positive, 83(15.42%) were both NS1 +IgM Ab positive And 294 (54.64%) were IgM Ab positive.

Table 1: Age distribution of the dengue illness (n-1637).

Age	No. of Patient /NS-1and IgM Positive.	%/Percent of NS1 and IgM positive.
0 – 10	96/15	5.86/2.7
11 – 20	155/25	9.4/4.64
21 – 30	495/223	30.23/41.44
31 – 40	398/133	24.31/24.72
41 – 50	151/30	9.22/5.57
51 – 60	191/62	11.66/11.52
>60	151/50	9.22/9.29
Total	1637	100%

Table 1 gives the age distribution of the dengue infection. Majority of the dengue illness were between the age group of 21 to 30 years (41.44%) and followed age group of 31 to 40 (24.72%).

Table 2: Sex distribution of the patients under study.

Sex	Total No.	NS1 and IgM	
Male	958(58.52%)	340	63.19
Female	679(41.47%)	198	36.80
	1637	538	32.86

Table 2 shows the Male 340 (63.19%) and Female 198 (36.80%), the males are more affected than females.

Table 3: NS1 Ag in dengue patients (n=538).

NS 1 Ag	No. of patients	%
Only NS 1	161	29.92
NS1+ with IgM	83	15.42
NS1 negative	294	54.64

Out of 538 dengue illness only NS1 antigen was positive for about 161 (29.92%) samples were serologically evaluation (Table 3).

Table 4 shows 294 (54.64%) samples were IgM Ab positive.

Table 4: IgM antibodies in dengue patients (n =538).

IgM antibody	No. of patients	%
Only IgM+	294	54.64
IgM with NS1	83	15.42
IgM negative	161	29.92

DISCUSSION

Dengue fever, an acute febrile arbo-viral disease has become a major public health Problem in tropical and subtropical region 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 NS1 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 and the IgM antibody for the early diagnosis of dengue illness.[8,14]

In this study, total 1637 samples tested for NS1 Ag and IgM Ab. In our present study, the dengue cases occurred during the rainy and post monsoon season i.e. from September to November only, which is similar to most of the previous outbreaks in India 15, 16. It may because this season is very favourable for high breeding of the vector, i.e., Aedes aegypti. The difference between serologically positive cases as compared to serologically negative ones in post monsoon was significantly higher15, 16. The peak incidence of dengue cases in Ekta et al, (2006) study was in the 2nd and 3rd week of October.[17] This seasonal outbreak of disease transmission is very important at local level for effective control measure.

The present study has shown a male preponderance (63.19%) as compared to the females (36.80%) with the male: female ratio being 2:1 in the clinically suspected dengue patients. Similar results were observed by Atul Garg et al who gave the male: female ratio as being G, 2:1 and Tank Arun G, Jain Mannu R who have given a sex ratio for dengue sero-positive patients for male to female as 2.54:1.[18,19]

We found that the mean age group affected was 21-30 years. This was consistent with the other studies on dengue in India R. N. Makroo et al.[20] In their study have reported the mean age of dengue patients as 27 years and the most belonged to the 21-30 year age group (32.44%). Ekta Gupta et al 17 have also gave an age group preponderance of 21-30 years in their study.

We had detected NS1 positivity in about 161 patients (29.92%), IgM positivity in about 294(54.64%). Implying that the combination of these serology tests would increase the rate of detection of dengue fever .These finding 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%). There for 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 diagnosis efficiency of early diagnosis of dengue illness.[14]

Elisa based on immunoglobulin IgM and IgG antibodies to more efficient and more popular serological test due to its simplicity, high specificity and great sensitivity. However, such antibody can persist in peripheral circulation for extended which periods which may lead to error in interpretation of diagnosis.[7,21]

Dengue NS1 antigen has gained considerable interest as new biomarker for early diagnosis of dengue infection. NS1 antigen is abundant in serum of patients during the early stage of infection. It can be detected in peripheral blood before the formation of antibodies, from the first day after onset of fever up to day 9. Studies revealed that the detection rate of NS1 antigen is higher in acute primary infection than in acute secondary infection.[13]

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 tests have can started. The result revealed that both NS1 and IgM have a very high specificity.

Our finding suggest that the NS1 antigen –capture ELISA is very useful and specific tool for the diagnosis of acute dengue infection .However ,the sensitivity of the NS1 assay is depended on the level of viremia and host humoral immune response Therefore ,a combine use of NS1 antigen with dengue IgM test could significantly improve diagnostic sensitivity of dengue infection . Hence if we do both NS1 antigen detection test and IgM/IgG antibody detection test, 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 significant role in the early diagnosis of dengue fever.

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