THE STUDY OF DETECTION OF DENGUE CASES BY NS1 ANTIGEN AND IGM ANTIBODY IN RIMS, ADILABAD, INDIA.

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Abstract

Dengue fever is an acute febrile arboviral disease affecting tropical & subtropical regions of the world. Dengue infection produces a spectrum of clinical illness, ranging from an asymptomatic to its most severe form like dengue haemorrhagic fever and dengue shock syndrome. In view of high morbidity and mortality, it is imperative to have a rapid and sensitive laboratory assay for early detection of the dengue infection. The newer parameter NS1 antigen has gained a lot of interest for early diagnosis of the disease. Detection of non-structural antigen (NS1 Ag), IgM and IgG antibody may help in the early diagnosis. The present study was conducted in a RIMS Adilabad, tertiary care hospital & medical college in the Department of General Medicine. A total of 100 serum samples were processed from suspected cases of dengue fever by using *dengue test* for detection of NS1 antigen and IgG antibodies. Platelet counts of all these cases were noted. . Of these 100 subjects 85 were serologically proved to have dengue illness, 57 patients were NS1 antigen positive, 28 patients were IgM antibody positive patients. As the NS1 antigen is detectable in blood from day one after onset of fever, its assay is an effective tool for early diagnosis of dengue infection so as to avoid complications.

Key words: Dengue, NS1 Antigen, IgM antibody, Platelet

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 severe 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.[10,11]

In view of high mortality and morbidity rates, it is imperative to have a rapid and sensitive laboratory assay for early detection of the disease. Several laboratory methods such as virus isolation, genomic RNA, antigen and antibody detection methods are available to diagnose the dengue infection (Datta *et al.*, (2010).

However methods like virus isolation, genomic RNA detection by PCR, antigen and antibody detection by ELISA needs well trained staff and an expensive setup which is not feasible in peripheral hospital settings . In most

cases antibody (Ig G/M) detection by immunochromatographic (ICT) based tests are commonly used for diagnosis of dengue infection, but time required for appearance of Ig M antibody are approximately 4- 6 days (World Health Organization, 1997). The newer parameter, DENV non-structural 1 antigen (NS1) has gained lot of interest as a new biomarker for early diagnosis of dengue infection. Dengue NS1 antigen, highly conserved glycoprotein produced in both membrane associated and secretory forms . ELISA directed against NS1 antigen have demonstrated its presence at high concentrations in sera of dengue virus infected patients during early clinical phase of disease. (Kumarasamy *et al.*, 2007). Apart from dengue specific parameters, thrombocytopenia and haemoconcentration are constant findings in DHF. A drop in platelet count below 1,00,000 per mm³ is usually found between the third and eighth day of illness (World Health Organization 1997). 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 viremia[12, 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. The aim of this study was to evaluate the use of NS1 test and IgM assay as the most suitable test for the laboratory confirmation of dengue at tertiary 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 its potential application for 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.

Materials and Methods:

The present study was conducted in a RIMS Adilabad, tertiary care hospital & medical college in the Department of General Medicine. All patients were having the symptoms of fever, arthralgia, with or without rash and bleeding manifestations. Blood samples were centrifuged and serum was separated. The serum of patients was used for serological testing. These samples were negative for other illness like malaria and typhoid. A total of 100 serum samples were processed from suspected cases of dengue fever by using *dengue test* for detection of NS1 antigen and IgG antibodies according to manufacturer's instructions. Platelet counts of all these cases were noted. Both male and female patients of all age group were included in study. Data was compiled in MS-Excel and checked for its completeness and correctness. Then it was analyzed.

Results:

A total of 100 samples are tested and detected for Dengue infections. Of these 100 subjects 85 were serologically proved to have dengue illness and rest 15 were non dengue patients.

Table 1: Group shows age and sex wise distribution of dengue patients.

Age groups	Male	Female	Total
< 1 year	1	0	1
1-10 years	17	12	29
11-20 years	14	10	24
21-40 years	15	6	21
41-60 years	8	2	10
	55	30	Total n=85

Table 1 shows out of 85 dengue patients 55 were male and 30 were female patients.

Table 2: Age and sex wise distribution of dengue NS1 antigen and IgM positive patients

Age groups	NS1 antigen positive Patients	IgM Positive patients
<1 year	1	0
1-10 years	28	1
11-20 years	15	9
21-40 years	9	12
41-60 years	4	6
Total n=85	n =57	n =28

Table 2 shows 57 patients were NS1 antigen positive , 28 patients were IgM antibody positive patients. Maximum NS1 antigen positive patients were in age group of 1 to 10 years.

Table 3: Comparison of platelet count and patients positive for dengue NS1 antigen and dengue IgM .

Platelet count	NS1 antigen positive patients	IgM positive patients	Total
Platelet counts< 1 lakh	31	15	46
Platelet counts> 1 lakh	26	13	39
	n = 57	n = 28	n =85

Table 3 shows out of 57 NS1 antigen positive patients 31 patients had platelet count below 1 lakh and 26 patients had platelet count above 1 lakh. Out of 28 IgM positive patients 15 had platelet count below 1 lakh and 13 patients had platelet count above 1 lakh. Out of 85 dengue patients 46 patients had platelet count below 1 lakh and 39 patients had platelet count above 1 lakh.

Graph 1: Platelet counts in Dengue patients.

Graph shows number of dengue patients in relation to platelet count above and below 1 lakh. NS1 antigen positive patients were more than IgM antibody positive patients.

Pie Diagram 1: Percentage of NS1 And IgM Positive patients.

Above diagram shows 67 % patients were NS1 antigen positive and 33% patients were IgM positive .

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 was considered.[14]. out of 85 dengue patients 55 were male and 30 were female patients. 57 patients were NS1 antigen positive , 28 patients were IgM antibody positive patients. Maximum NS1 antigen positive patients were in age group of 1 to 10 years. out of 57 NS1 antigen positive patients 31 patients had platelet count below 1 lakh and 26 patients had platelet count above 1 lakh. Out of 28 IgM positive patients 15 had platelet count below 1 lakh and 13 patients had platelet count above 1 lakh. Out of 85 dengue patients 46 patients had platelet count below 1 lakh and 39 patients had platelet count above 1 lakh. As the NS1 antigen is detectable in blood from day one after onset of fever, its assay is an effective tool for early diagnosis so as to avoid complications of dengue infection. The ease, speed and dependability of NS1 antigen and IgM antibody tests make them an effective technique in addressing this potentially fatal, epidemic prone infection. Apart from these dengue specific parameters, platelet count is the only accessory laboratory test available in the remote areas that can support the diagnosis of dengue infection. Therefore studies like this will contribute significantly to the clinical management and can

reduce morbidity and mortality in dengue infection. 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. It may be because this season is very favourable for high breeding of the vector, i.e., *Aedes aegypti*. 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.[15]

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.

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 result revealed that both NS1 and IgM have a very high specificity. In conclusion, Dengue NS1 antigen is used for early and accurate diagnosis in acute phase of illness. Combination of dengue NS1 antigen and IgM ELISA on single sample can improve the diagnosis of dengue without the requirement of paired sera. Correlations of positive samples with platelet counts were noted.

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