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Original Research Article

To estimate the prevailing Serotype of dengue virus and IgM antibody in suspected dengue fever cases

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ABSTRACT

Introduction: Dengue virus infection a vector borne disease affecting more than 50 million people worldwide annually. It is distributed in tropical and subtropical regions of the world, abundant in urban and semi-urban areas. It is the disease that spreads by bite of Aedes aegypti and Aedes albopictus mosquitoes. The virus being a RNA virus belongs to Flaviviridae family. The four serotypes of Dengue virus are DENV-1, DENV-2, DENV-3, and DENV-4 which have distinct antigenicity complex.
Materials and Methods: This study was conducted in Microbiology Department, Government Stanley Medical College, Chennai, during the period Jan 2018 to Dec 2018 with 120 blood samples from patients of suspected dengue fever. Samples were selected by stratified random sampling method into three strata and each strata consisting of 40 samples based on duration of fever. All the samples were subjected to procedure for both NS1 antigen and IgM antibodies.15 NS1 positives samples with duration of fever less than 5 days were serotyped by PCR.
Results: A total of 18 cases had NS1 antigen, 26 cases had IgM antibodies and 27 cases had both. A combination of the two tests detected a total of 44 cases. The chance of detection of NS1antigen decreased with duration of fever and IgM antibodies increased with duration of fever. The serotyping detected were DENV1 and DENV3**.**
Conclusion: NS1 antigen detection by ELISA is useful in early phase of infection. A combination of NS1 ELISA and IgM ELISA have high sensitivity depending upon the duration of illness. Detection of DENV1 and DENV3 by PCR procedure helps to know the presence of prevailing serotype in this particular region which would be a good guide to predict the recurrence of outbreak and for efficient prevention and management.

1. Introduction

Dengue virus infection affects more than 50 million people worldwide annually.[1] It is distributed in tropical and subtropical regions of the world, abundant in urban and semi-urban areas.[1] It is vector borne disease the spread by bite of Aedes aegypti and Aedes albopictus mosquitoes.[2] The virus being a positive sense RNA virus belongs to Flaviviridae family.[1] The four serotypes of Dengue virus are DENV-1, DENV-2, DENV-3, and DENV-4[3]. Dengue fever can be caused by any of four genetically related but antigenicaly distinct dengue virus DENV serotypes.[3]

Dengue virus three structural proteins, seven non-structural proteins are produced the glycoproteins NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5.[3] The nonstructural protein(NS1) is a glycoprotein of molecular weight 46-50kD and this is not the part of virion but is expressed in infected mammalian cells and exist in two

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forms namely membrane associated mNS1 and secreted sNS1 forms.[4] Early diagnosis is at most essential for detecting an epidemic or outbreak and in taking necessary steps in effective vector control measures.[5,6] DENV NS1 detection coincides with detectable. Viraemia before an antibody response is mounted.[7,8]

The Infection is transmitted from one human to human via a mosquito vector but can also be transmitted through blood donation.[9] The median Incubation period is about 5 days. Dengue fever presents with fever, headache, muscle and joint pains, and petechial rash.[10] The bone and muscle pain is generally severe hence the disease is known as 'break-bone fever'.[10] Clinical manifestations of dengue Fever ranges from self-limiting fever to severe dengue characterized by plasma leakage with or without haemorrhage.[11] The clinical manifestational and severity depends on the type of serotype and DENV-2 is associated lower platelet count and complications.[11]

Laboratory diagnosis includes detection of the viral RNA, antigens antibodies, or a combination of these procedures.[12] During the acute stage of the disease, virus isolation, antigen detection can be used to diagnose the infection.[13] At the end of the acute phase of infection, serology is the main stay of diagnosis.[14–17] This study aims at detecting dengue antigen, antibodies and serotyping of viral RNA.

2. Materials and Methods

This study was done in department of Microbiology, Government Stanley Medical College, Chennai, during year Jan 2018 to Dec 2018 with 120 serum samples from patients of suspected dengue fever. The Aims and objectives of this study are to serotype antigen and detect IgM antibodies in suspected dengue fever cases. This study was approved by the institute research and ethics committees. Samples were selected by stratified random method consisting of three strata and each stratum consisting of 40 samples based on duration of fever. The first strata had samples of patients with history of fever for 1-5 days, the second strata had samples of 6-10 days duration of fever and third one had samples of fever cases more than 10 days. All cases were tested for NS1 antigen and IgM antibodies by ELISA procedures. NS1 antigen ELISA positives samples with duration of fever less than 5 days were serotyped by PCR.

Blood samples about 2-5ml were collected after obtaining written informed consent from the patients or guardians. Serum was separated and stored at -80^{o}C till the tests were performed. The detection of Non Structural antigen 1 was done using Panbio Dengue ELISA kit. The detection of IgM antibodies was done using Dengue IgM capture ELISA kit. 15 NS1 positives samples with duration of fever less than 5 days were serotyped by PCR. RNA extraction and dengue serotyping was done as per manufacturer's kit instructions.

3. Results and Discussion

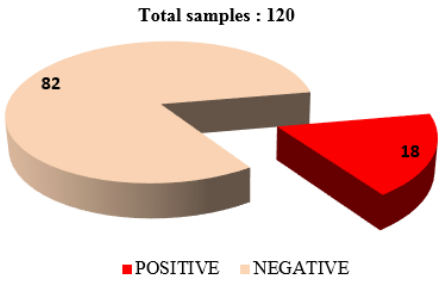

Chart 1: Detection of NS1 antigen

Out of 120 patients, 18 cases were tested positive for NS1 antigen as shown in Chart 1.

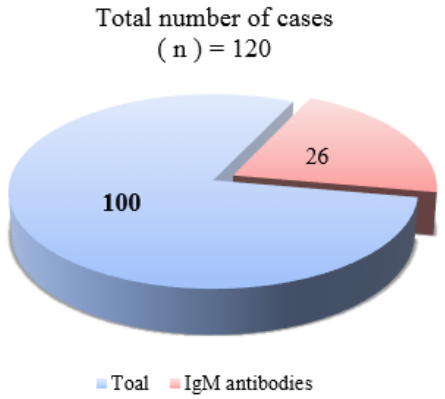

Chart 2: Detection of dengue - IgM antibodies

Chart 2 shows among 120 samples, 26 cases were tested positive for IgM antibodies

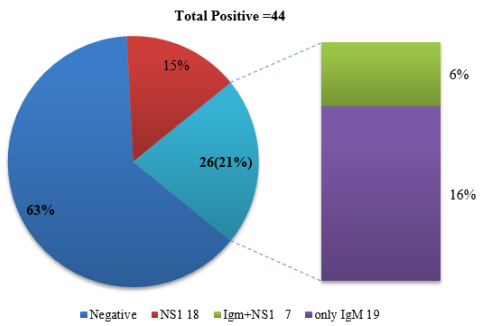

Chart 3: Total dengue positive

Pie chart showing total positives= 44, NS1 positives= 18, IGM =26 and both positives =7 cases.

Percentage positivity of NS1 antigen and IGM antibodies among the cases with same of duration of fever.

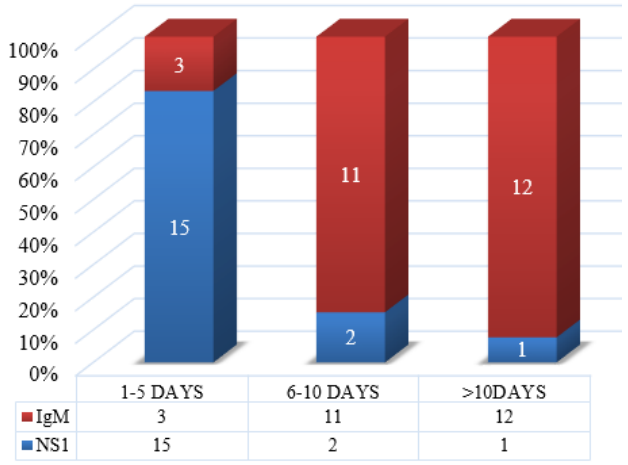

Chart 4: Proportional bar chart showing positivity of NS1and IgM.

The positive percentage of NS1antigen and IgM antibody with respect to the same duration of fever among study population are as follows 37.5% (15/40), 5% (2/40), 2.5% (1/40) –Decreasing tendency. Positive percentage for IgM is 7.5% (3/40), 27.5% (11/40), 30% (12/40) - An increasing tendency of detection. Chart 4

NS1 positivity and duration of fever

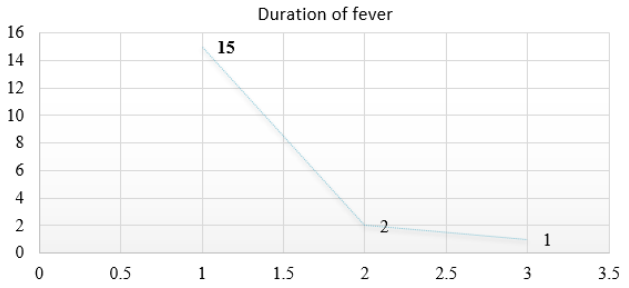

Chart 5: Line chart showing NS1 positivity and duration of fever.

Chart 5 shows that 15 cases were detected in1-5 days, 2 cases were detected in 6-10 days and only one case was detected after 10 days. These results were similar to the study by Seok Mui Wang et al.

As detection of NS1 decreases with duration of fever, the study population cases were sorted based on duration of fever into 3 groups. A chi - square test is applied with help of (2*3) contingency table.

Chi-square (X^2) is 23.9216. The p value is < 0.00001 with degree of freedom 2. The result is significant at $p < 0.05$. So, there is a strong association.

Table 1: Contingency table for testing association between duration and NS1 positivity

Duration of fever	Number of cases positive	Number of cases negative	Total
1-5 days	15	25	40
6-10 days	2	38	40
10-15 days	1	39	40
Total	18	102	120

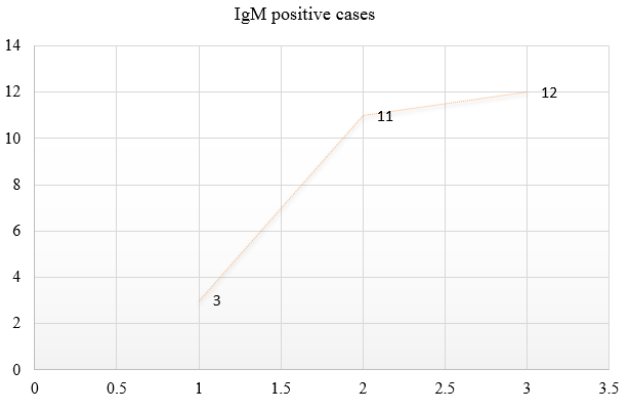

Chart 6: Line chart showing IgM positivity and duration of illness.

IgM Positivity and duration of fever

There were only 3 cases detected by IgM ELISA in 1-5days of fever, 11 cases in 6-10 days of fever and 12 cases in 11-15 days of fever as shown in Chart 6. An increase in duration of illness increases the IgM detection rate. This association is tested with chi-square by comparing with the cases of same duration of illness in the study population.

Testing of association between IgM positivity and duration of fever by chi - square test

Table 2: Contingency table for testing association between duration and IgM positivity

Duration of fever	Number of cases Positive	Number of cases Negative	Total
1-5 days	3	37	40
6-10 days	11	29	40
10-15 days	12	28	40
Total	26	94	120

The chi square value X^2 = is 7.168. The p valve is < 0.02776 with degree freedom 2. The result is significant at $p < 0.05$. So, there is a definite association.

3.1. Serotyping of dengue

Among the NS1 positive cases 15 samples with period of illness less than 5 days of fever were serotyped by PCR procedure to find the circulating serotype. The identified serotypes were DENV1 and DENV3. Out of 15 samples,

5 were DENV1, 6 were DENV3. The negative results do not prelude the disease. Severe dengue was common in infections with DENV-3 consistent with previous study by Rishi Gowtham Racherla et al. [18]

4. Results of RT PCR in Gel Electrophoresis

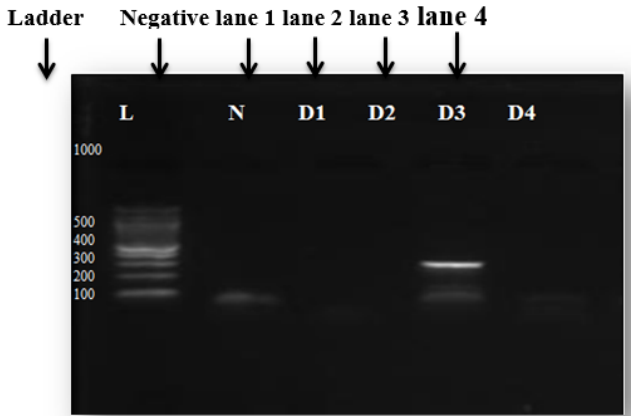

Fig. 1: Shows the presence of dengue 3 serotype

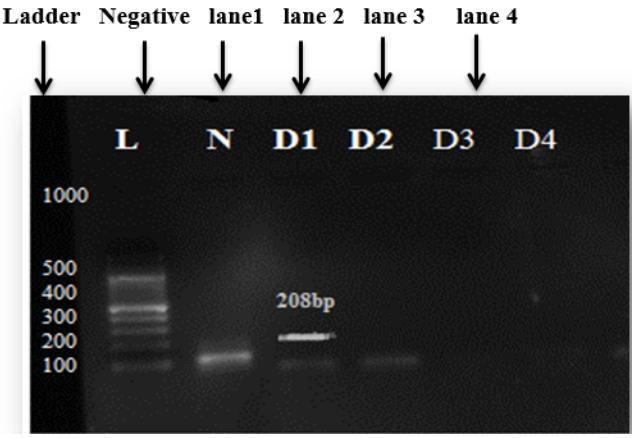

Fig. 2: Shows the presence of Dengue 1 serotype

5. Conclusion

NS1 antigen detection by ELISA is useful in early phase of infection. A combination of NS1 ELISA and IgM ELISA has high sensitivity depending upon the duration of illness. Detection of DENV1 and DENV3 by PCR procedure helps to know the presence of prevailing serotype in this particular region which would be a good guide to predict the recurrence of outbreak and for efficient prevention and management.

6. Source of Funding

None.

7. Conflicts of Interest

There is no conflict of interest.

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