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ROLE OF IGM IN IMMUNE-DIAGNOSIS OF DENGUE VIRAL INFECTION

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ABSTRACT

Dengue fever is a benign syndrome caused by several arthropod borne viruses. Recently Dengue Virus caused outbreaks within significant morbidity mortality. In our study, twenty seven blood samples were collected from patients suspected for dengue in Nilgiri district. Considering the advantages of cell culture, an attempt was made to culture or propagate the Dengue virus in susceptible cell line. The analyses of the serum by the IgM capture ELISA, IgG capture ELISA, Dengue Early capture ELISA (NS1). Among them the anti-Dengue IgM antibodies in 3 of 27 serums sample. The selected cells were sub cultured successfully in the laboratory. The Characteristic cytopathic effect on the cells in response to Dengue virus was observed. Further the cultured virus has been detected by IFA (Immunoflourescent antibody). Based on the above result it could be concluding that the presence of IgM antibodies paves a way to detect the presence of Dengue infection by various tests. These various tests have become an invaluable tool for surveillance of Dengue virus.

Key words: Dengue, IFA, ELISA

INTRODUCTION

The mosquito *Aedes aegypti*, *Aedes albopictus* is the main dengue vector. The distribution of the vector borne diseases is grossly disproportionate with impact in developing countries located in tropical and subtropical areas and is relatively rare in temperate zones (Ciesin 1994). The World wide resurgence of vector borne diseases since 1970s is malaria, dengue and yellow fever (Gubler 1964). A Pandemic of Dengue began in South East Asia after World War 2, and has spread around 1952 the globe since then (Sabin). Dengue is an arbor virus caused by one of four closely related but antigenically

distinct, virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4). Dengue is an acute fever which is caused by a virus occurs in two forms namely Dengue fever and dengue haemorrhagic fever. Dengue virus which belongs to the group four positive single stranded RNA virus, family flavi viridae and genus flavi virus. This illness is currently endemic in additional than one hundred countries. Some 2.5billion people, two fifths of the world populations, are now at risk from dengue and estimates that there may be 50 million cases of dengue infection worldwide every year (WHO and CDC) and epidemic transmission in tropical and sub topical countries. The first reported epidemics of DF occurred in 1779-1780 in Asia continent and North America. Dengue virus infection is the most common arthropod borne disease worldwide and is transmitted by the bite of infected Aedes mosquitoes viz.., *Aedes aegypti* and *Aedes albopictus* that breed in the peridomestic environment. The average life expectancy of 4-6 weeks, include four developmental stages: egg, larva, pupa and adult .The first three stages of life requires still and relatively clean water. Mosquitoes bite during the last four stage of life adult for reproduction. Only the female mosquito will feed on blood to use the proteins and lipids for egg development. The second species, the Asian tiger mosquito, *Aedes albopictus* can be common in the per domestic environment, particularly in urban areas with abundant vegetation. Recently Dengue Virus caused outbreaks within significant morbidity mortality. One of the fastest emerging vectors borne virus infection worldwide. It is necessary to understand various factor such as virus pathogenesis, virus-human, virus-vector interactions and vaccine development in order to face the possible future challenges. To study the above said various parameters, the Dengue virus needs to be cultured in susceptible cell lines or animal systems. Considering the advantages of cell culture, an attempt was made to culture or propagate the DNV in susceptible cell line.

MATERIALS AND METHODS

27 Members blood samples were collected at Nilgiri District. The collected sample was transported to CRME lab and it was stored in a vial and kept at 4^0C till it reaches lab. The serum was prepared from the blood. The serum was analyzed by the IgM capture ELISA, IgG capture ELISA technique. The selected culture was subcultured shown in fig 1 and confirmed by IFA.

RESULTS AND DISCUSSION

The clinical specimens collected from 27 Dengue patients were examined for Dengue infection. The analyses of the serum by the IgM capture ELISA, IgG capture Dengue Early capture ELISA (NS1), IgG capture ELISA was shown in fig2, 3. The positive result of anti-Dengue IgM antibodies in 3 of 27 serum sample was shown in table 1. Dengue IgG, Early capture ELISA (NS1) antibodies was shown in table 2 and 3.

Sub culturing or passaging:

The selected cells were sub cultured using L15 medium supplemented with 10% of FCS Disaggregation of cells occurs due to the trypsination process.

Propagation of dengue virus cells:

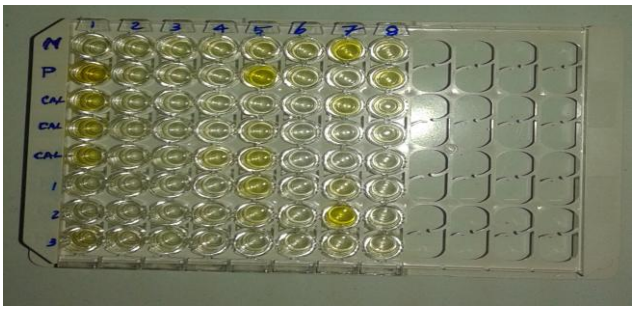
The sub cultured cells were infected with Dengue virus and kept for incubation for 4-6 days in a co_2 incubator to observe the cytopathic effect. (Morphological changes were observed in the host cell due to virus infection). Cytopathic effect may consist of cell rounding, disorientation, swelling or shrinking, death and detachment.

Indirect immunofluorescence antibody test:

DNV monoclonal antibodies and Mouse Fluoro Immuno Thio Cyante were added to the smear. We observed fluorescent apple green colour when observed under the fluorescent microscope was shown in fig 4.

Fig 1: Cell line inoculation in 24 well plates

Fig2: IgM Capture ELISA



NC-Negative control ; Positive control ; CAL-Calibrator

Fig 3: Dengue Early ELISA (NS1)

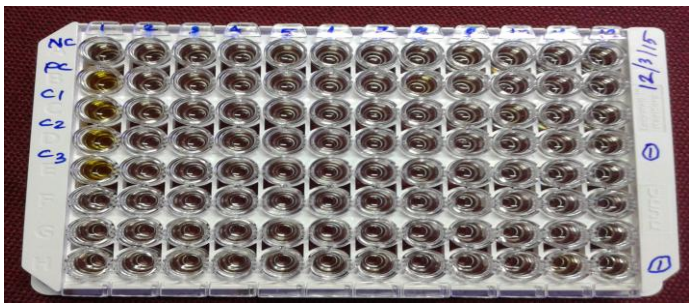


Fig4: Detection of Dengue virus by Indirect immunofluorescence antibody (IFA)test

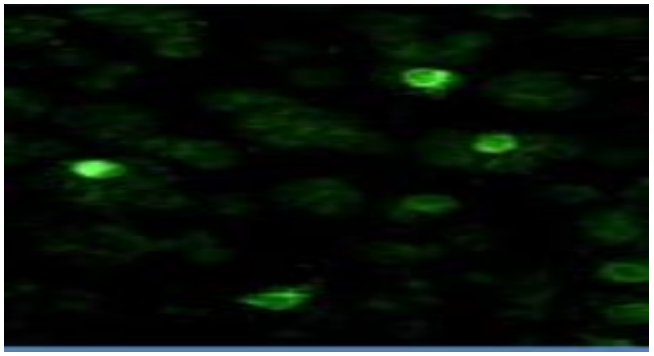


Table 1: Dengue IgM Caputre ELISA

Date:11.3.15 **Tested by: M.Divyapraba**

	1	2	3	4
A	NC 0.139	NG-4 0.225	NG-12 0.049	NG-20 0.060
B	PC 3.091	NG-5 0.421	NG-13 0.037	NG-21 0.051
C	CAL 1 0.535	NG-6 0.365	NG-14 0.071	NG-22 0.204
D	CAL 2 0.475	NG-7 0.211	NG-15 0.085	NG-23* 0.903
E	CAL 3 0.485	NG-8 0.076	NG-16 0.104	NG-24 0.139
F	NG- 1 0.231	NG-9 0.215	NG-17 * 0.726	NG-25 0.194
G	NG-2* 1.042	NG-10 0.212	NG-18 0.095	NG-26a 0.353
H	NG-3 0.093	NG-11 0.069	NG-19 0.304	NG-27 0.166

*-positive ; NG-Nilgiri; PC-positive Control; NC-Negative Control; CAL-Calibrator

Table 2: Dengue IgG Caputre ELISA

Date: 13.3.15 Tested by: M.Divyapraba

	1	2	3	4
A	NC 0.060	NG-4 0.108	NG-12 0.118	NG-20 0.0108
B	PC 2.255	NG-5 0.079	NG-13 0.070	NG-21 0.115
C	CAL 1 0.649	NG-6 0.075	NG-14 0.095	NG-22 0.061
D	CAL 2 0.664	NG-7 0.072	NG-15 0.078	NG-23 0.338
E	CAL 3 0.672	NG-8 0.072	NG-16 0.056	NG-24 0.083
F	NG- 1 0.073	NG-9 0.076	NG-17 0.079	NG-25 0.094
G	NG-2 0.085	NG-10 0.080	NG-18 0.078	NG-26 0.073
H	NG-3 0.104	NG-11 0.081	NG-19 0.092	NG-27 0.121

*-positive ; NG-Nilgiri; PC-positive Control; NC-Negative Control; CAL-Calibrator

Table 3: Dengue Early ELISA (NS1)

DATE: 13.3.15 Tested by: M.Divyapraba

	1	2	3	4
A	NC 0.138	NG-4 0.081	NG-12 0.079	NG-20 0.078
B	PC 1.607	NG-5 0.079	NG-13 0.052	NG-21 0.066
C	CAL 1 1.025	NG-6 0.061	NG-14 0.047	NG-22 0.077
D	CAL 2 1.037	NG-7 0.058	NG-15 0.039	NG-23 0.027
E	CAL 3 1.112	NG-8 0.052	NG-16 0.04	NG-24 0.047
F	NG- 1 0.053	NG-9 0.058	NG-17 0.054	NG-25 0.057
G	NG-2 0.056	NG-10 0.061	NG-18 0.06	NG-26 0.076
H	NG-3 0.089	NG-11 0.084	NG-19 0.078	NG-27 0.065

*-positive; NG-Nilgiri; PC-positive Control; NC-Negative Control; CAL-Calibrator

CONCLUSION

In the study, low percentage of Dengue positive cases using IgM MAC-ELISA. A characteristic cytopathic effect on the cells in response to Dengue virus was

observed. The presence of Dengue virus into the cells was detected by IFA. This technique is very useful to detect the Dengue virus.

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