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SEROPOSITIVITY OF DENGUE VIRUS AND COMPARISON OF DENGUE IGM RAPID CARD SEROREACTIVE TEST WITH DENGUE IgM ELISA: A STUDY AT A TERTIARY CARE HOSPITAL, UDAIPUR, RAJASTHAN

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ABSTRACT

Background: Dengue virus infection is one of the major public health problems and a major constituent of emerging infectious diseases worldwide. **Objectives**: To study the Seropositivity of Dengue Virus and comparison of Dengue IgM Rapid Card Test Seroreactive Test with Dengue IgM ELISA. **Materials and Methods:** 23820serum samples from patients with febrile illness attending to the inpatient and outpatient department were processed by Dengue Rapid card test and then 312 IgM seroreactive sample processed by Dengue IgM Capture ELISA for confirming. **Results:** Out of 23820 serum samples, seropositivity for DEN V was found in 312 (1.30%). In which 230 (0.96%) were IgM Positive and 82 (0.34%) were IgM,+ IgG Positive and 23506 (98.69%) were found sero negative for DEN V by Dengue IgM and IgM+IgG Rapid Card Test, period out of 312 total serum positive samples 195 (62.50%) were males and 117 (37.50%) were females. Most affected age group from febrile illness is 11-20 years (27.05%), followed by age group 21-30 years (24.03%. Maximum seropositive samples were in the month of October 69(39.42%), and then in November 44(25.14%) and in September 40(22.85%) were come out. Out of 312 Positive Rapid card test, only 136 [43.59%] was positive for DEN IgM Capture ELISA which is a gold standard test for Dengue disease. **Conclusion:** Early diagnosis and treatment of Dengue virus infection is important for patient and community. It is need of continuous surveillance for Dengue virus disease using multiple diagnostic tests. In Indian setting screening of Dengue virus by Rapid Card test is necessary.

KEYWORDS: Dengue Virus, ELISA.

INTRODUCTION

Dengue virus belongs to the genus Flavivirus and family Flaviviridae. The name Dengue is derived from the Swahili word 'Ki denga pepo', which means sudden seizure by the demon. Following the Philadelphia epidemic in 1780, it was called as the 'Break bone fever' or 'Bone crush disease' by Benjamin Rush. **(1)** The first recognized dengue epidemic occurred almost simultaneously in Asia, Africa, and North America in 1780. The first confirmed case report from 1789. Dengue virus is round and enveloped virus with a single-stranded, positive-sense RNA. The virus has structural protein C, prM and E and nonstructural protein [NS1, NS2A, NS2B, NS, NS4A, NS4B, and NS5]. **(2)** Serologically Dengue virus can be classified into 5 immunologically related but genetically and

antigenically distinct serotypes [DEN V 1-5]. **(3, 4)** Dengue is transmitted by a mosquito vector, primarily by Aedes aegypti and sometimes by Aedes albopictus. **(5, 6)** Dengue fever is an acute febrile disease affecting the tropical and subtropical region in the world. Two type of serological infection occur in patience-**(1)** Primary response- patience is not immune to flavivirus. **(2)** Secondary response- patience is previously infected by flavivirus with same or different serotype. Primary Dengue infection present as either a non-specific illness or DF. Secondary infection may lead to DHF and DSS. The incubation period is 4-7 days [ranging from 3-14 days].

Serological tests detect anti-DEN antibodies using immunochromatographic Rapid card test, IgM capture enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescent method, hemagglutination inhibition, neutralization techniques. **(7, 8)** Molecular diagnosis of dengue by Polymerase chain reaction (PCR), RT-PCR and Reverse transcription loop-mediated isothermal amplification (RT-LAMP).

MATERIALS AND METHODS:

This study was approved by Institutional Ethics Committee R.N.T. Medical College and Controller and Attached Hospitals, Udaipur, Rajasthan. No.RNT/STAT/IEC/2016/2098 Date-10/03/2016

This study was conducted from Jan. 2015 to Dec. 2016, in the Serology Laboratory, Department of Microbiology, R.N.T. Medical College, Udaipur, Rajasthan. During the study period a total of 23820 samples were collected from patients suffering from fever, joint pain, headache, and rashes, who presented in various (IPD and OPD) Departments of M.B. Govt. Hospital and Pannadhay Hospital, Udaipur, Rajasthan.

Serum sampling- About 3-5 ml of whole blood collected into the collection tube without anticoagulant, by vein puncture, leave to settle for 30 minutes for blood coagulation and then centrifuged blood to get serum specimen of the supernatant.

Test performed – The samples were tested for DEN IgM antibody using SD BIO LINE Dengue IgG/IgM Rapid immunochromatographic card test kit. Then the IgM positive samples were confirmed by IgM antibody Capture ELISA kit produced by NIV (Arbovirus Diagnostic NIV, Pune, India). The sensitivity and specificity for the Dengue IgM antibody capture ELISA is 98.53% and 98.84% respectively. The tests were carried out following the manufacturer instruction. The principle of IgM Capture ELISA: IgM antibodies in the patient`s serum are capture by anti-human IgM (μ chain specific) that are coated on to the solid surface (wells). In the next step, DEN antigen is added, which binds to capture IgM, if the IgM and antigen are homologous. The unbound antigen is removed during the washing step. In the subsequent steps, the Biotinylated anti-DEN monoclonal antibody is added followed by Avidin-Histidine rich protein (HRP). Subsequently, substrate\ chromogen (TMB/H2O2) is added and monitored for development of color. The reaction is stopped by 1N H2SO4. The intensity of color/ optical density (OD) is monitored at 450nm. OD values are directly proportional to the amount of DENGUE virus-specific IgM antibodies present in the serum sample. The sample considered positive for IgM antibody if the OD of the sample exceeds OD of negative control by a factor 4.0 (sample OD= negative OD×4.0). Both positive and negative controls used to validate the test.

RESULTS:

Out of 23820 serum samples, seropositivity for DEN V was found in 312 (1.30%). In which 230 (0.96%) were IgM Positive and 82 (0.34%) were IgM,+ IgG Positive and 23506 (98.69%) were found seronegative for DEN V by Dengue IgM and IgM+IgG Rapid Card Test. (Table 1) (figure 1) Out of 312 total serum positive samples, 195 (62.50%) were males and 117 (37.50%) were females. Most affected age group from febrile illness is 11-20 years (27.05%), followed by age group 21-30 years (24.03%. Maximum seropositive samples were in the month of October 69(39.42%), and then in November 44(25.14%) and in September 40(22.85%) were come out. Out of 312 Positive Rapid card test, only 136

[43.59%] was positive for DEN IgM Capture ELISA which is a gold standard test for Dengue disease. (Table 3) (figure 2)

DISCUSSION

Area-specific monitoring studies which are aimed to gain knowledge about the type of infectious agents which are responsible for the infection, help the clinicians to choose the right empirical treatment. This is important not only to provide an appropriate therapy but also for the prevention of the infection.

In this study, a total of 23820 serum samples were tested for Dengue virus by Rapid ICT kit. Of the total samples were tested, only 312 (1.30%) were found to be positive for DEN V. While Smita Sood et al **(9)** reported 18.99% in Jaipur (Rajasthan) and D Turbadkar et al **(10)** reported 13.67% in Mumbai Maharashtra and Mahesh Ku et al **(11)** reported 3.55% in Ajmer Rajasthan. (Table 2) In the present study, there is a significant difference in findings because previous studies were carried out at the time of Dengue epidemic. In present situation a little number of Dengue Seropositivity was present in the population, in spite of the fact, there is no epidemic. Hence this study gives about local endemically present Dengue Seropositivity. In this study Males (62.50%) were affected more than Females (37.50%), these findings were similar to study carried out by Smita Sood et al **(9)**, and Mahesh ku.et al **(11)** reported. The most affected age group is 11-20 years in this study, similar result was also noted in a study conducted by Seema et al **(12)** in Rohtak, Haryana, while in the study of Manisha et al **(13)** 21-30 years age group was most affected. The maximum case was present in October month; similar results were also noted in a study conducted by Manisha et al **(13)** and Seema et al **(12)**. Out of 312 only 136 (43.59%) was seropositive for Dengue IgM Capture ELISA while in Seema et al **(12)** was 59.86% and D Turbadkar et al **(10)** 26.41%. (Table 4)

CONCLUSION

The present study highlights the fact that Dengue virus infection is an important cause of febrile illness and emphasizes the need for continuous surveillance for Dengue virus disease using multiple diagnostic tests. In Indian setting, low socio-economic conditions; overcrowding, poor sanitary conditions facilitated by the presence of Aedes vector species contribute to the spread of Dengue V. Therefore screening of DEN V and other arboviruses is necessary because through the clinical features are similar the outcomes may vary. Rapid card test seems to less predictive value but can be used for screening purpose because it is rapid and easy to perform but not for confirm. Confirmation should be done by Dengue IgM Capture ELISA test.

Limitations

1. Samples declared as equivocal by reference assay were excluded. This design of the study prevented us from obtaining a repeat sample for testing.

2. Only one assay IgM Capture ELISA was used to classify DEN V. It is likely that some antibody negative samples may have been positive by antigen detection, culture, and RT-PCR.

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TABLE 1: Seropositivity of Dengue Virus by Dengue IgM and IgG+IgM Rapid Card Test

S. No.	DEN V IgM and IgG+IgM Rapid Card Test	Total N = 23820	%
1	IgM Positive	230	0.97
2	IgM+ IgG Positive	82	0.34
3	Negative	23508	98.69

TABLE 2: Comparison of Sero-positivity of Dengue Virus by Dengue IgM and IgG+IgM Rapid Card Test with Other Studies

S. No.	Studies	Year	Seropositivity of DEN V
1	Smita Sood	2008-2011	18.99%
2	D Turbadkar et al	2004-2007	13.67%
3	Mahesh Ku et al	2014-2015	3.55%
4	Present Study	2015 -2016	1.30%

TABLE 3 : Seropositivity of Dengue virus by DEN IgM capture ELISA in seropositive Dengue cases by Dengue rapid card test

S. No.	DEN IgM CAPTURE ELISA	Total No = 312	Percentage
1	Positive	136	43.59
2	Equivocal	35	11.22
3	Negative	141	45.19

TABLE 4: Comparison of sero-reactive rapid card test to IgM Capture ELISA with other studies

S.No.	Studies	Year	Rapid IgM card positivity	IgM Capture ELISA test Positivity (%)
1	Seema et al	2007 -2012	720	431(59.86%)
2	D. Turbadkar et al	2004-2007	212	56 (26.41%)
3	Present studies	2015-2016	312	136 (43.59%)

Figure 1: Seropositivity of Dengue Virus by Dengue IgM and IgG+IgM Rapid Card Test

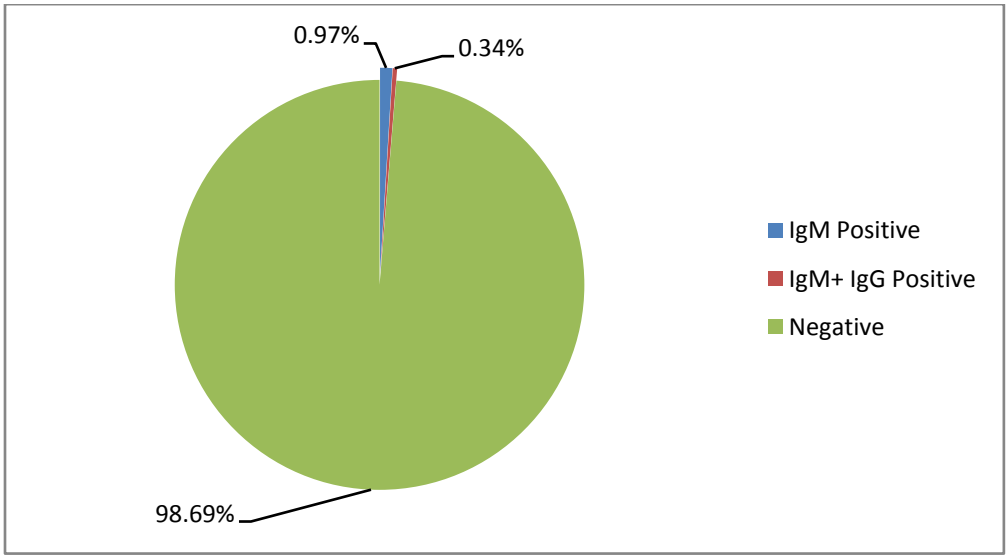

Figure 2: Comparison between rapid IgM card test and DEN IgM Capture ELISA