International Journal of Medical Science and Education

An official Publication of Association for Scientific and Medical Education (ASME)

Original research Article

ANTIBODY DETECTION OF DENGUE INFECTION IN CLINICALLY SYMPTOMATIC PATIENTS BY MAC-ELISA DURING POST MONSOON SEASON AT A TERTIARY CARE HOSPITAL AT JAIPUR, RAJASTHAN

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Received: 25/02/2017 ***Revised: 25/08/2017*** ***Accepted*** **: *01/09/2017***

ABSTRACT

Objectives: Cyclic epidemics of dengue infection are increasing with time in India. The disease shows a wide spectrum of clinical manifestations ranging from mild self-limiting illness to severe fatal haemorrhagic condition. The present study was conducted to detect dengue infection in its peak season in Jaipur, Rajasthan using Dengue MAC-ELISA. **Materials and Methods:** Serum samples from 3730 patients clinically suspected of having dengue infection visiting a tertiary care hospital during the period of two months from October to November 2015 were screened for the presence of Dengue IgM and IgG antibodies using one-step immuno-chromatographic assay (Dengue Rapid IgG/IgM Test by SD BIOLINE. Positive samples were subjected to Dengue IgM ELISA. **Results:** Out of to 3730 samples 413(11.07%) were found positive by rapid test. Of these positive samples 318/413 (76.99%) were found positive, 56/413 (13.55%) were equivocal and 39/413 (9.4%) were negative by Dengue MAC-ELISA. **Conclusion:** Rapid immuno-chromatographic tests may offer a convenient method to screen samples for dengue infection in field during epidemic threats but confirmatory tests should be performed for the confirmation of Dengue infection as accuracy of available rapid tests has yet to be verified.

Key words: Dengue MAC-ELISA, Immuno-chromatographic, IgM, Dengue virus

INTRODUCTION:

Dengue has emerged as a major infectious disease in recent times **(1, 2)**. The disease shows a wide spectrum of clinical manifestations ranging from mild asymptomatic illness to severe fatal Dengue haemorrhagic fever/Dengue shock syndrome (DHF/DSS). Dengue fever is an acute febrile illness of 2-7 days with two or more of the following manifestations: Headache, retro-orbital pain, myalgia, arthralgia, rash and haemorrhagic manifestations. Severe Dengue is characterized by plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment. Critical stage starts from 3–7 days after the onset of symptoms accompanied with a decrease in temperature (below 38°C/100°F). Severe abdominal pain, persistent vomiting, rapid breathing, bleeding gums, fatigue, restlessness and blood in vomit have also been observed. The next 24–48 hours can be critical for the patient. Rapid diagnosis

and proper medical care lower the fatality of severe Dengue.

Around 2.5 billion people are at risk of Dengue infection around the world with about 100 million new cases each year. In India where the disease is hyper-endemic, it presents highly complex patho-physiological, economic and ecologic problems. The Indian subcontinent is reported to hyper-endemic to Dengue with circulation of all the four serotypes **(3)** and virus is prevalent for the last 50 years **(4)**. Dengue is caused by Dengue virus. The Virus has four serotypes and each serotype is capable to cause the disease. Dengue is transmitted mainly by *Aedes aegypti*. Other species such as *Aedes albopictus, Aedes polynesiensis and Aedes scutellaris* have also been reported to transmit the infection. Each year increased number of Dengue infection is reported in India.**(5)** Rapid urbanization, changes in life style and improper storage of water, deficient water management during rainy seasons are the factors resulting in increase in the mosquito breeding sites and their proliferation. Since there is no specific medicine or antibiotic to treat Dengue and close monitoring of the vital signs are critical for the maintenance, epidemiological surveillance plays the key role to control the damage caused by epidemics. This retrospective study was conducted to the see the sero-prevalence of Dengue infection in symptomatic patients at a tertiary care hospital in Jaipur, Rajasthan.

MATERIALS AND METHODS

In this retrospective study a total of 3730 clinically suspected Dengue cases attending medicine outdoor unit at Govt. RDBP Jaipuria Hospital, Jaipur from October to November 2015 were included. Age, gender and clinical history of the patients were noted. Samples were first screened for Dengue infection by using one-step immuno-chromatographic assay (Dengue Rapid IgG/IgM Test by SD BIOLINE) as per manufacturer's protocol. Briefly 10 µL serum was added to the prescribed sample well (S) followed by the addition of 4 drops (90–120 µL) of assay diluents to the round shaped assay diluents well. The test device is coated with anti-human IgG in the IgG line region and anti-human IgM in the IgM region. When the sample mixture passes through these regions coloured lines appear according to the presence of the antibodies of the serum. The appearance of coloured lines at both regions shows the presence of both IgG and IgM antibodies in the sera. Results were noted within 20 minutes after the addition of the buffer. The control line was observed for the validity of the assay. Positive samples were further tested for the presence of Dengue IgM antibodies by Dengue MAC-ELISA Kits provided by NIV-Pune as per manufacturer's protocol. In brief Dengue NIV IgM Capture ELISA was performed by diluting patient's serum (1:1000) in sample diluents and adding to the plate with controls (incubated for 1 hour). IgM antibodies in the patient's blood were captured by Anti-human IgM coated on to the solid surface (wells). In the next step, after washing the plate 5 times DEN antigen was added (1 hour incubation) which bound to capture IgM, if the IgM and antigen were homologous. Unbound antigen was removed during the next washing step (5 times). In the subsequent Biotinylated Flavivirus cross-reactive monoclonal antibody was added (1 hour incubation) followed by 5 times washing and adding Avidin-HRP(30 mins incubation). Subsequently, substrate/Chromogen was added and watched for development of colour (10 mins). The reaction was stopped by 1N H_2SO_4. The intensity of colour was monitored at 450nm. OD readings are directly proportional to the amount of Dengue virus specific IgM antibodies in the samples.

RESULTS

Total 3730 cases enrolled in the study were tested by rapid immuno-chromatographic test for the presence of Dengue IgM antibodies and 413 were found positive. Positive samples were then tested by Dengue NIV MAC-ELISA. Out of these 413 samples 318/413 (76.99%) were found positive, 56/413 (13.55%) were equivocal and 39/413 (9.4%) were negative.

Table 1 shows the gender-wise distribution of total samples testes and Dengue positive cases. Of all the 3730 patients tested, 2376 were males and 1354 females. From the total Dengue positives by rapid test, 75.54% (n=312) were males and 24.45% (n=101) females while among Dengue MAC-ELISA positive cases 226 were males and 92 females. So, it was observed that Dengue affected males and more than females.

Table 1: Gender wise distribution of Dengue positive Patients

	Male	female
Total samples (3730)	2376 (63.69%)	1354 (36.3%)
Positive by Rapid (413)	312 (75.54%)	101 (24.45%)
Positive by MAC-ELISA (318)	226 (71.06%)	92 (28.93%)

Of 3730 cases, 2752 (73.78 %) were received in the month of October and 344(83.29 %) in November. Table 2 shows the number of Dengue positive cases by rapid test in the two months.

Table 2: Monthly distribution of Dengue positive cases by rapid test

	Total	Positive
Oct-15	2752 (73.78%)	344 (83.29%)
Nov-15	978 (26.21%)	69 (16.70%)
Total	3730	413

Table 3 shows the distribution of Dengue positive cases in various age groups. The most affected age group was of young adults ranging from 16-30 years (n=224, 54.23%) followed by the age group of 31 to 45 years (n=102, 26.69%).

Table 3: Distribution of Dengue positive cases in various age groups

Age Groups	Dengue positive cases	Percentage
0-15 years	21	5.08
16-30 years	224	54.23
31-45 years	102	26.69
46-60 years and above	66	15.98
Total	**413**	**100**

Table 4 and Figure 1 show the common clinical symptoms in Dengue positive cases. Fever (98%) was observed to be the most common symptom followed by retro-orbital pain (90.31%), arthralgia (70.94%), thrombocytopenia (46%), vomiting (30%), abdominal pain (25.42%) and rash (5.42%). Hemorrhagic manifestations were also observed in a significant number of patients (2.17%).

Table 4: Common clinical symptoms in Dengue positive cases

Symptoms	Dengue positive cases	%
Fever	**405**	**98**
Retro-orbital pain	**373**	**90.31**
Arthralgia	**293**	**70.94**
Thrombocytopenia	**190**	**46**
Vomitting	**124**	**30.02**
Abdominal pain	**105**	**25.42**
Rash	**22**	**5.42**
Hemorrhagic manifestations	**9**	**2.17**

DISCUSSION

Dengue is the most rapidly spreading mosquito borne viral infection. It causes severe morbidity and mortality. It can present with a wide spectrum of clinical manifestations which can be self-limiting febrile illness to a severe life threatening hemorrhagic condition characterized by multiple organ failure and plasma leakage. The treatment of Dengue is symptomatic requiring close monitoring and the development of vaccines is under process. This makes the early diagnosis even more important. In hospital settings where the laboratory set up and diagnostic facilities are less developed Dengue MAC-ELISA serves as the main diagnostic tool. In India where outbreaks of dengue are reported, almost every year from many parts of the country epidemiological studies are a need. The present study was carried out to analyse the sero-prevalence of Dengue infection in symptomatic patients attending a tertiary care hospital in Jaipur, Rajasthan in the post-monsoon season in 2015. In this study 413 cases were found positive for Dengue IgM antibodies by a rapid immunochromatographic test out of total 3730 cases included. Further when tested by Dengue NIV MAC-ELISA 318/413 (76.99%) were found positive, 56/413 (13.55%) were equivocal and 39/413 (9.4%) were negative. The majority of Dengue positive cases were males as reported earlier in other studies also by Gupta *et al* 2005, Ahmed *et al* 2008 and Mahesh *et al* 2015.**(6, 7, 8)** Higher prevalence of males was seen probably due to more outdoor activities by males as compared to females due to more exposure of day biting mosquitoes in their surrounding.

More number of Dengue cases was observed during the month of October than November which in agreement to other reports reflecting the gradual increase from August, peak during September and October and then gradually decrease in Dengue positivity as seen in studies of Vajpeyi *et al* 1999, Gupta *et al* 2005, Ukey *et al* 2010.**(6,9,10)** Therefore, effective control measures are to be applied at local level for prevention of epidemic due to seasonal outbreak of disease transmission that should come into full swing during water stagnation periods that help in vector breeding after the initial bouts of rainfall and at the end of monsoon.

The maximum positivity (224/413, 54.23%) was found in young adults between the ages of 16–30 years as compared to other age groups. Gupta *et al* 2006, Garg *et al* 2011 and Mehta *et al* 2014 were also show similar type of study.**(11,12,13)** The high number of cases in the young adult age group implies that the disease is endemic in these regions as adults manifest with disease less because they were immune to the virus due to more subclinical infections and also due to more outdoor activity. However high numbers of cases were seen in the adult age group by a study done by Neerja et al, this indicates that the virus had been introduced to a non-exposed population in non-endemic region.**(14)**

Fever was observed to be the most common symptom followed by retro-orbital pain, arthralgia, vomiting and abdominal pain as found in other studies as of and Ahmed *et al* 2008, Mahesh *et al* 2015 and Chairulfatah *et al* 1995. **(7,8,15)**

CONCLUSION

Rapid immunochromatographic tests may offer a convenient method to screen samples for dengue infection in field during epidemic threats but confirmatory tests should be performed for the confirmation of Dengue infection as accuracy of available rapid tests has yet to be verified. Therefore, health authorities and people of the region should make efforts to prevent further increase in dengue cases which can be diagnosed by available methods.

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