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Original Research

To evaluate the seroprevalence of dengue viral infection using IgM antibody capture ELISA for the early diagnosis

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ABSTRACT:

Aims: Aim of the present study to evaluate the seroprevalence of dengue viral infection using IgM antibody capture ELISA for the early diagnosis. **Methods:** This was a prospective observational study conducted in the Department of Microbiology. A total of 520 serum samples from suspected dengue cases attending OPD or admitted in the hospital were tested for the confirmation of Dengue. We have received blood samples in our microbiology laboratory, the blood samples were allowed to clot at room temperature and then we centrifuged the samples and serum samples were separated. From the serum samples we have done NS1 Ag and IgM Ab testing by ELISA. **Results:** Out Of 520, 90 samples were positive for dengue. Seroprevalence of Dengue was 17.31%. All dengue positive patients in our study had fever of 2 to 7 days. The most common presenting symptoms of dengue were fever with body ache (47.78%), headache (35.56%), nausea (33.33%) and vomiting (22.22%). Out of 90 dengue cases fever with rash was observed in 7 cases (7.78%). Out of 90 dengue cases, NS1/NS1+IgM/IgM were positive for 81(90%) patients, suggesting primary infection. IgM and IgG positive was seen in 5(5.56%) patients, suggesting late primary or early secondary infection. IgG was positive in 4(4.44%) cases, suggesting secondary or past infection. **Conclusion:** The present results revealed that the study region is epidemic for dengue viral infection and there is an urgent need for the constant monitoring to control further spreading of the infection in the community, hence serological test have important role in the early diagnosis.

Keywords: IgM ELISA, Dengue viral infection, Aedes aegypti.

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INTRODUCTION

Dengue is the most prevalent arthropod-borne viral disease in the world. It is caused by a single-stranded RNA arbovirus of the genus *Flavivirus* with 5 distinct antigen serotypes (DENV1-5) that are transmitted to humans through the bites of infected mosquitoes, namely, *Aedes aegypti* and *Aedes albopictus*.[1,2] According to the World Health Organization (WHO), the current worldwide burden of dengue is about 2.5 billion infected people in more than 100 countries with approximately 20 000 fatal cases per year, and its global incidence is predicted to grow dramatically to affect about half of the world's populations.[3,4] The Infection with any one serotype confers an individual life-long immunity to that same serotype but it has cross reactivity to the other serotype. Secondary infection with another serotype or multiple infections with different serotypes leads to severe form of dengue dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) due to this cross-reactivity.The incidence of dengue has increased over last 50 years with 2.5 billion people living in areas where dengue is endemic.[4] It affects 100 million people each year with 500,000 cases of DHF and DSS with around 30,000 deaths mostly among children.[1] It is known that early and specific diagnosis of DHF and DSS followed by supportive therapy reduces mortality and morbidity.[5] Viral Isolation by cell culture and subsequent detection by immunofluorescence, though the gold standard tests for identification of dengue infection are not within the reach of peripheral and even most tertiary care laboratories.[6] For a long time, detection of dengue specific IgM/IgG has been the main stay of diagnosis of dengue infection. Antibody detection is an indirect method of diagnosis and therefore is prone to false positive as well as false negative results.[7] NS1 antigen is detectable from day 1 of fever both in primary and secondary infections.

NS1 is shown to be highly specific viral marker making it extremely reliable parameter for diagnosis of dengue infection from day 1 of fever.[8]

A small percentage of persons who have previously been infected by one dengue serotype develop bleeding and endothelial leak upon infection with another dengue serotype. This syndrome is termed severe dengue (reclassified in 2009 by the WHO, previously referred to as dengue hemorrhagic fever and dengue shock syndrome). Severe dengue has also been termed dengue vasculopathy. Vascular leakage in these patients results in hemo concentration and serous effusions and can lead to circulatory collapse. This, in conjunction with severe hemorrhagic complications, can lead to a shock syndrome, which poses a greater fatality risk than bleeding per se.[9]

Dengue is endemic to the Indian sub-continent. Dengue is associated with explosive urban epidemics and has become a major public health problem in India.[10]

MATERIAL AND METHODS

This was a prospective observational study conducted in the Department of Microbiology, after taking the approval of the protocol review committee and institutional ethics committee. A total of 520 serum samples from suspected dengue cases attending OPD or admitted in the hospital were tested for the confirmation of Dengue. All the age group patients were include in this study. A suspected case of dengue was considered a patient with signs and symptoms like headache, retro-orbital pain, myalgia, arthralgia, rash and haemorrhagic manifestation, etc.

Serum samples from these patients were tested for Dengue NS1 antigen using dengue NS1 antigen capture ELISA (PanBio Diagnostics) and dengue IgM antibody by dengue IgM capture ELISA (PanBio Diagnostics) for the confirmation of dengue cases. ELISA tests were performed as per the manufacturer's instructions. We have received blood samples in our microbiology laboratory, the blood samples were allowed to clot at room temperature and then we centrifuged the samples and serum samples were separated. From the serum samples we have done NS1 Ag and IgM Ab testing by ELISA.

RESULTS

Table 1-Seroprevalence of Dengue

Total no of patients	Dengue positive patients	%
520	90	17.31

Out Of 520, 90 samples were positive for dengue. Seroprevalence of Dengue was 17.31%. Table 1

Table 2-Demographic profile of patients

Gender	N=90	%
Male	65	72.22
Female	25	27.78
Age years		
Below 10	8	8.89
10-20	21	23.33
20-30	28	31.11
30-40	19	21.11
40-50	8	8.89
Above 50	6	6.67
Area		
Urban	68	75.56
Rural	22	24.44

Out of 90 dengue patients 65(72.22 %) were male patients and 25(27.78%) were female patients. Out of 90 dengue patients, 68(75.56%) patients were from urban area and 22(24.44%) from rural area. In our study dengue infection was observed more (31.11%) in the age group 20 to 30 years followed by 10 to 20 years (23.33%) and 30 to 40 years (21.11%).

Table 3-Clinical profile of dengue patients

Clinical presentation	No of Patients	%
Fever + myalgia	10	11.11
Fever + rash	7	7.78
Fever + headache	32	35.56
Fever+ nausea	30	33.33
Fever + vomiting	20	22.22
Fever + arthralgia	14	15.56
Fever + bodyache	43	47.78
Fever + itching	11	12.22

All dengue positive patients in our study had fever of 2 to 7 days. The most common presenting symptoms of dengue were fever with body ache (47.78%), headache (35.56%), nausea (33.33%) and vomiting (22.22%). Out of 90 dengue cases fever with rash was observed in 7 cases (7.78%).Table 3.

Table 4-Serology results of rapid dengue tests

Test results	No. of patients	%
NS1/NS1+IgM/IgM Positive	81	90
IgG Positive	5	5.56
IgG + IgM Positive	4	4.44
Total	90	100

Out of 90 dengue cases, NS1/NS1+IgM/IgM were positive for 81(90%) patients, suggesting primary infection. IgM and IgG positive was seen in 5(5.56%) patients, suggesting late primary or early secondary infection. IgG was positive in 4(4.44%) cases, suggesting secondary or past infection. Out of all dengue cases thrombocytopenia (<1, 00,000/mm^3) was observed in 35 cases. In 4 patients platelet count was< 20,000/mm^3

DISCUSSION

Securing the safety of blood transfusion and blood products for the recipients is a mandatory medical demand. In that respect, the risk of blood transfusion-transmission of DENV and/or its antibodies from donors to recipients has recently emerged and become an important clinical fact. Total 520 blood samples of the patients suspected of having dengue infection were tested in the laboratory by rapid immunochromatography tests for NS1 Ag, IgG and IgM. Out of these 90 samples were positive for dengue. Seroprevalence of Dengue was 17.31%. 11.92% prevalence was reported by P. Jyoti and B Metri.[11]18.99% prevalence was observed over period of 2008 to2011 by Smita Sood in Rajasthan.[12] Low prevalence 3.55% was reported by Mahesh kumar et al.[13] A study from central; India reported 31.3% prevalence rate.[14]

Out of 90 dengue patients 65(72.22 %) were male patients and 25(27.78%) were female patients. Similar result was observed by Mahesh kumar et al, in their study out of total positive dengue cases, 62.63% were males and 37.37% females.[13] Many studies have observed higher prevalence of dengue infection among males than females.[11,12,15,16] S. Fayaz Ahammad et al reported 46.6%male &53.4 female dengue patients.16Study by Kale A V et al reported 63.33%were males &36.66% were females.[15]

In our study, Out of 90 dengue patients, 68(75.56%) patients were from urban area and 22(24.44%) from rural area. similar results was by S. Fayaz Ahammad et al. (2016), 109 cases (75%) were from rural area where as 25 cases (25%) were from urban area.[16] According to their report the rural broaden of dengue infection is comparatively a recent phenomenon which is supposed to be linked with the shortage of water in rural areas, designing of schemes for water supply to the rural areas and development of newer water transport system in the rural places.

In our study dengue infection was observed more (31.11%) in the age group 20 to 30 years followed by 10 to 20 years (23.33%) and 30 to 40 years (21.11%). Mahesh Kumar et al in their study observed maximum dengue cases in age group 10-20 years (31.58%) and 21to30yrs. (15.78%).[13] Kale et al,observed commonest age group affected was (34%) was between11-15 years.[15] Some Indian studies have reported that dengue infection is more common in children.[17,18]

All dengue positive patients in our study had fever of 2 to 7 days. The most common presenting symptoms of dengue were fever with body ache (47.78%), headache (35.56%), nausea (33.33%) and vomiting (22.22%). Out of 90 dengue cases fever with rash was observed in 7 cases (7.78%). Similar clinical presentation was observed by Mahesh Kumar et al, fever was present in almost all cases (n=380) followed by, headache (n=274), joint pain (n=2432), myalgia (n=144), retro-orbital pain (n=141), backache (n=95), skin rash (n=80).[13]

Out of 90 dengue cases, NS1/NS1+IgM/IgM were positive for 81(90%) patients, suggesting primary infection. IgM and IgG positive was seen in 5(5.56%) patients, suggesting late primary or early secondary infection. IgG was positive in 4(4.44%) cases, suggesting secondary or past infection. Mahesh kumar et al reported that, Out of the 380 dengue positive cases, 136(35.79%) were NS-1 positive, 117(30.79%) were IgM positive, 38(10%) were IgG positive, 71(18.68%) were IgG/IgM positive, 14(3.68%) were IgG NS- 1/IgMNS-1 positive and 4(1.05%) were IgGIgMNS-1 positive.[13]

Though among methods used for diagnosis of dengue the virus isolation, molecular methods are more specific tests, facilities are not available in all institutes. Serological tests are most commonly used in most of the laboratories. Dengue virus specific IgM antibodies tend to appear as early as 3 days after infection and remains in circulation for 30 to 60 days. IgG antibodies arise at about 7 days, they reach a peak at 2-3 weeks and persists for life long.[18] NS1 detection has been a promising test to diagnose dengue in its early febrile stage. The NS1 protein was found to be highly conserved in all dengue serotypes, circulating in high levels during the first few days of illness. It correlates with the development of Dengue Fever. There is no cross reaction of the dengue NS1 protein with those of other related *flavi viruses.*[19,20] Out of all dengue cases thrombocytopenia (<1, 00,000/mm^3) was observed in 35 cases. In 4 patients platelet count

was< 20,000/mm^3. One of the WHO diagnostic criteria for DHF is Thrombocytopenia: <1 lakh/mm^3. P Jyoti and Basawaraj reported thrombocytopenia in 51.5% patient.[11] Kale A V et al observed thrombocytopenia in 56% patients, platelet count <40,000 in 33.33% cases.[15] Platelet count less than 1, 00,000/ml was noticed in 220 cases (68.75%), report published by R D Kulkarni et al.[21]

CONCLUSION

The present results revealed that the study region is epidemic for dengue viral infection and there is an urgent need for the constant monitoring to control further spreading of the infection in the community, hence serological test have important role in the early diagnosis. Therefore IgM ELISA is recommended in all the suspected dengue patients so as to instigate essential treatment and assessment of morbidity and mortality rate during an outbreak.

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