

Detection of anti dengue IgM antibodies in clinically suspected dengue cases at a tertiary care hospital, Mumbai

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Abstract

Introduction: Dengue is an emerging mosquito borne viral infection affecting tropical and subtropical countries. It can lead to life threatening complications like dengue hemorrhagic fever and dengue shock syndrome. In India, the epidemiology of dengue virus infection is very complex and ever changing. The infection has expanded over the last few decades to all regions of the country.

Aims and Objectives: The present study was conducted to determine the seroprevalence of dengue virus infection among patients attending a tertiary care hospital and to study demographic features and seasonal variation of positive cases.

Materials and Method: Patients attending the outpatient and inpatient departments with signs and symptoms suggestive of dengue infection over a period of 7 months were included in the study. IgM antibody against dengue virus was detected by anti dengue IgM antibody capture enzyme linked immunosorbent assay (ELISA).

Results: Amongst 7280 clinically suspected cases of dengue virus infection, 561 were positive for anti-dengue IgM antibodies. Infection was predominant in the age group of 15-30 years (46.7%) and 63.6% of the male population was affected. Seasonal trend showed a peak level of infection in the month of October (post-monsoon).

Conclusion: Epidemiological surveillance of dengue infection is necessary to monitor the spread of dengue virus and for implementation of effective prevention and control strategies.

Keywords: Dengue, Seroprevalence, IgM antibody, ELISA

Introduction

Dengue is the most important arthropod-borne viral infection of humans. Dengue viruses belong to the genus *Flavivirus* and family *Flaviviridae*. Four serotypes of the dengue virus, DEN-1, DEN-2, DEN-3 and DEN-4 are responsible for the disease. The virus is transmitted from one human to another by mosquito vectors mainly through *Aedes aegypti* and sporadically by *Aedes albopictus*.⁽¹⁾

The spectrum of clinical illness in dengue virus infection ranges from asymptomatic or mild febrile illness, dengue fever (DF) to a severe form of disease such as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Classic dengue fever (DF) is characterized by a rapid onset of high grade fever, headache, retro-orbital pain, diffuse body pain (both muscle and bone), weakness, vomiting, sore throat, altered taste sensations and a centrifugal maculopapular rash². Primary infection in nonimmune persons usually causes DF.⁽¹⁾ Dengue haemorrhagic fever is characterized by high fever, haemorrhagic manifestations, hepatomegaly and in severe cases circulatory failure. These patients may develop hypovolaemic shock called dengue shock syndrome. These are fatal complications of dengue infection and mostly associated with secondary dengue virus infection.^(3,4)

Worldwide, approximately 2.5 billion people are at risk of infection, of which, around 975 million people live in urban areas in tropical and subtropical countries

in Southeast Asia, the Pacific and the Americas. An estimated 5,00,000 people with severe dengue require hospitalization each year and about 2.5% of those affected die.⁽⁴⁾ The epidemiology of dengue fever in India has been very complex and has significantly changed over the last six decades in terms of geographical area, prevalent strains and severity of disease. The first confirmed outbreak occurred in Kolkata in 1963-1964. Eventually, there was spread of dengue virus throughout the entire country. The first major nationwide outbreak occurred in 1996. The serotypes of the virus kept changing year after year and between 1996-2003 all the four serotypes (DEN1, DEN2, DEN3, and DEN4) were reported.⁽¹⁾

Laboratory diagnosis of dengue virus infection is based mainly on serological tests. They are relatively inexpensive and easy to perform as compared to culture and nucleic acid based methods. In the primary infection, anti dengue IgM antibodies are produced from fifth day of infection and remain in circulation for 60-90 days. Anti dengue IgG antibodies are produced after one week, attains maxima after 2-3 weeks and remain lifelong in circulation. In secondary infection, IgG antibodies are detected at high levels in acute stage while IgM antibodies are present at low levels.⁽⁵⁾

Standard serological tests are haemagglutination inhibition, neutralization test, indirect immunofluorescence antibody test, enzyme linked immunosorbent assay (ELISA), complement fixation and rapid immunochromatography test. Out of these

ELISA is the most widely used methods for routine diagnosis of dengue infection due to its simplicity and high specificity and sensitivity.⁽⁶⁾

Detection of NS1 (Nonstructural protein 1) antigen is a new approach for diagnosis of acute dengue infection. NS1 protein is found circulating in the blood of patients from the first to ninth day of dengue fever.^(5,6)

As the dengue virus infection has a wide spectrum ranging from mild febrile illness to severe syndrome, accurate clinical diagnosis is difficult and therefore, laboratory confirmation can help in accurate diagnosis and timely interventions such as supportive therapy, thus, assisting in preventing progression of disease and its life threatening complications.

The present study was carried out to determine the seroprevalence of Dengue virus infection by detecting IgM antibodies against dengue virus in all clinically suspected cases of Dengue infection attending the outpatient and inpatient departments at a tertiary care hospital in Mumbai, India.

Materials and Method

The present study was conducted from June to December 2016 at the Department of Microbiology of a tertiary care hospital in Mumbai, India.

Inclusion criteria: All clinically suspected cases of Dengue virus infection attending the outpatient department (OPD) and inpatient department (IPD) having fever and signs and symptoms of Dengue infection such as retroorbital pain, muscle and joint pain, vomiting, diarrhoea, loss of appetite, thrombocytopenia and leucopenia were included in the study.

Collection of sample: A single blood sample approximately 2-3 ml was collected from each patient in a plain vacutainer tube with all aseptic precautions. Sample was allowed to clot at room temperature for about 30 min for clot retraction. Serum was separated by centrifugation at a speed of 3000 g (RCF) for 10 min & stored upto 72 h at 2^o-8^oC

Detection of anti-dengue IgM antibodies: Detection of anti-dengue IgM antibodies was done by IgM capture ELISA, using a in house kit prepared by the National Institute of Virology, Pune, India. Test results were interpreted as either positive or negative as per manufacturer's instructions.

Results

The study period included 7280 clinically suspected dengue cases. Anti-dengue IgM antibodies were positive in 561 (7.7%) of these cases.

Of the total 561 positive cases, majority, 262 (46.7%), belonged to the 15-30 years age group (Table 1).

Table 1: Age wise distribution of Anti dengue IgM antibody positive cases

Age groups	Number of positive cases	%
<15 Years	131	23.3
15-30 Years	262	46.7
31-45 Years	106	18.8
46-60 Years	36	6.4
>60 Years	26	4.6
Total	561	

Infection was most common in males, 63.6% (358/561) as compared to females, 36.18% (203/561) (Table 2).

Table 2: Sex wise distribution of anti dengue IgM antibody positive cases

	Number of positive cases	%
Males	358	63.6
Females	203	36.18
Total	561	

Seasonal trend showed that there was gradual increase in positive cases from July, that is, the beginning of the monsoon season with a peak in October and a gradual decrease from the month of November onwards. (Table 3)

Table 3: Month wise distribution of anti dengue IgM antibody positive cases

Month	No. of positive cases	%
June	17	3
July	82	14.6
August	78	14
September	144	25.6
October	168	30
November	47	8.3
December	25	4.4
Total	561	

Discussion

Dengue is an emerging disease of tropical and subtropical regions. In India, the infection has been seen significantly over the last few decades with rapidly changing epidemiology.⁽¹⁾ A study was conducted at the University of Oxford by Bhatt et al., to estimate the global distribution of dengue cases. Worldwide, 96 million people suffered each year from apparent infections. Asia contributed 70% of this burden due to large areas of densely populated regions which favor transmission of infection. India had the largest number of dengue cases with about 33 million apparent and another 100 million asymptomatic infections occurring annually.⁽⁷⁾

In the present study, 7.7% of the total patients had serologically confirmed dengue infection. Other studies by Turbadkaret al⁽⁸⁾ and Ghosh et al⁽⁹⁾ from Mumbai reported prevalence of dengue infection as 13.6% and

17.9%, respectively. Table 4 shows seroprevalence of dengue in different regions of India.

Table 4: Seroprevalence of dengue infection in different regions of India

Region	Year	Authors	Seroprevalence (%)
Jaipur ⁽¹⁰⁾	2008-2011	Sood S	18.99%
Puducherry ⁽¹¹⁾	2015-2016	Balamurugan R	13.9%
Kanpur ⁽¹²⁾	2006-2010	Garg A	19.7%
Gujarat ⁽¹³⁾	2014-2015	Ahir HR	12.91%

We included only detection of IgM antibodies against dengue virus in our study and other parameters like IgG antibodies and NS1 antigen detection were not studied. This could be the reason for comparatively lower seroprevalence rate of dengue infection in our study.

The prevalence of infection was higher in males (63.6%) as compared to females (36.18%) with male to female ratio being 1.76: 1. Similar findings are reported by Nisarata A,⁽¹⁴⁾ Nepal HP et al⁽¹⁵⁾ and Balamurugan R et al.⁽¹¹⁾ This high prevalence in males as compared to females is probably due to social, cultural (women being covered) and exposure differences.

Majority of infections occurred in age group of 15-30 years (46.7%) followed by paediatric age group (23.3%). Predominant infection rate in adult population was also noted by Kumar A et al⁽¹⁶⁾ and Gupta E et al.⁽¹⁷⁾ However, Garg et al,⁽¹²⁾ Gunasekaran P et al⁽¹⁸⁾ and Martin JLS et al⁽¹⁹⁾ reported higher prevalence of dengue infection in paediatric age group.

We also studied seasonal variation of infection by analyzing the data on a monthly basis. There was a gradual increase in cases from July with a peak in the month of October, followed by successive decrease in number of cases by November. This increase in cases of dengue infection during post monsoon period is also reported by other studies in India.^(20,21,22) Seasonal humidity and temperature variations may have a role in vector survival that ultimately causes emergence of dengue epidemics.

Conclusion

The seroprevalence rate of Dengue virus infection amongst patients attending a tertiary care hospital in Mumbai is 7.7%. Dengue affected predominantly males and young adults. Maximum cases occurred during post monsoon period which may be due to increased breeding of mosquitoes during these seasons. So, vector control measures should be started before setting in of the monsoon season to prevent outbreak situations.

Early diagnosis of infection can prevent life threatening complications and immunological assays

such as the IgM capture ELISA is a reliable method for diagnosis of dengue virus infection.

Also, periodic surveillance should be carried out to monitor the infection rate in a particular geographical area for timely initiation of preventive and control measures.

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