Original Research Article

A study on seroprevalence of dengue viral infection using IgM antibody capture ELISA for the Early diagnosis in Kalaburagi district, North-Eastern part of Karnataka, India

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Abstract

Introduction: Dengue is an acute viral illness caused by RNA virus of the family Flaviviridae and spread by Aedes mosquitoes which occur mainly in four closely related serotypes (DENV1- 4). It has developed into emergent threat in the India and Karnataka state now. The most challenging difficulty associated with patient administration in dengue infection is early diagnosis.

Objective: To determine the Prevalence of Dengue infection in the Kalaburagi district by serological test IgM ELISA. The detection of Immunoglobulin IgM is the core method for the laboratory diagnosis of dengue. The present study considered the relevance of a screening test for dengue pertaining to new cases and assessment of the epidemic attack rate.

Materials and Methods: This evocative study was conducted at our Gulbarga Institute of Medical Sciences hospital, Kalaburagi for the period between January to December 2018. Serum samples were collected from suspected dengue patients attended in and out patient department, Sera were analysed by ELISA test for the presence of dengue IgM antibodies.

Results: Over the study period, out of 1,807 samples, 123 were showed positive for dengue virus infection. Both male 61(49.59%) and female 62 (50.40%) patients predominantly affected. The positive peak values were noticed between April to December 2018. The most positivity was observed between the age group of 1-20 years.

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.

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

Introduction

Dengue is a mosquito-borne viral disease that has quickly spread in all regions of World Health Organization (WHO) in recent years. Female mosquito mainly of the species *Aedes aegypti* and to a lesser point, *A. Albopictus,* transmit dengue virus.[1] Dengue infection is a major health problem in our country. Worldwide the occurrence of dengue has increased in the recent years. The WHO approximates that presently about two-fifth of the population is at risk for this viral infection. In recent years dengue in Asian and Latin American countries are common, where it has become the leading cause of hospitalization among both adults and children in these regions, India is one of the recognized countries in South-East Asian region frequently reporting occurrence of dengue fever/dengue hemorrhagic fever (DF/DHF) outbreaks and transforming into surrounding regions and nearby niches for dengue infection[2]

Dengue is caused by dengue virus, a Flavivirus in the family of Togaviridae. During acute dengue infections when IgM is not readily detectable, NS1 antigen based ELISA shall be considered as an important diagnostic tool. There are four known virus serotypes (DEN 1, DEN 2, DEN 3, and DEN 4).[1,3] All these four serotypes share common geographical and ecological niche. All the four serotypes are now spreading in Asia, Africa and American continents. Their infection, transmission, different symptoms and pathogenesis are causing severe challenge to public health.

The epidemiology of dengue in Indian subcontinent is very composite and distorted over time. Dengue was previously supposed to be an urban disease as most cases were reported from bigger cities. But from last decade there are many outbreaks from rural areas of southern and western India.[4,5] In 2012, outbreak of dengue epidemic in our country was about three times higher than the previous years. Twelve states of the country recorded a large number of cases and Karnataka state ranked second in total number of dengue positive cases.[4,6]

Viral infection in early stage is misleading, 90% of dengue cases will be asymptomatic following infection and such people play an important role in spreading of disease.[7] Dengue virus infects mid gut of *Aedes aegypti* female mosquito and spreads to salivary glands over a period of 8-12days. Dengue fever symptoms include high-degree fever, headache, muscle and joint pains, retro-orbital pain and skin rash similar to measles. Severe dengue characterized by plasma leakage, haemoconcentration, hemorrhagic shock and multiple organ failure leading to patient death.[7]

Mortality rate in Karnataka due to Dengue is 0.8-1% from reported cases from medical record. To tackle increasing dengue cases in urban, semi-urban and rural areas because of expanding urbanization, deficient water and solid waste management, the emphasis is on avoidance of mosquito breeding conditions in homes, workplaces and minimizing the man-mosquito contact. Looking at the cost-effectiveness of the preventive measures over the treatment charges for Dengue fever in private hospitals, there is an urgent need to bring about awareness in people regarding the preventive measures in controlling dengue fever. The current study was undertaken to know the pattern and nature of dengue prevalence in Kalaburagi district and surrounding areas of Karnataka. Also measure the different clinical manifestations as well as early recognition and rapid management to reduce the morbidity and mortality associated with dengue patients.

Materials and Methods
Study region and population
Kalaburagi district of Karnataka, India lies in North-Eastern part of Karnataka (Fig. 1). District has 56.03% literacy rate and a population of 2564892 distributed in seven talukas: Kalaburagi, Jewargi, Afzalpur, Aland, Chincholi, Chittapur and Sedam. This present study was conducted at Viral Research and Diagnostic Laboratory, Department of Microbiology, Gulbarga Institute of Medical Sciences, Kalaburagi, Karnataka for a period from January 2018 to December 2018. Recently, Kalaburagi district has seen a rapid increase in population size in recent years. The insufficient, deficient and poor infrastructure for waste disposal provides the sympathetic environment for the spread and outbreak of these viral diseases.

Study design
Samples were collected from clinically suspected cases of Dengue fever with the following complaints – fever, myalgia, arthralgia, headache and rashes etc, according to the WHO criteria, where obtained from both outdoor and hospitalized patients from Gulbarga Institute of Medical Sciences hospital, Kalaburagi. This study protocol was approved by Institutional Ethical Committee. A proforma with detailed epidemiological, clinical and laboratory parameters recorded during the hospital. District health department records section, Gulbarga Institute of Medical Sciences Medical Record section, were used as a tool for the data collection.

Sample size
One Thousand Eight Hundred Seven (1807) blood samples were screened from clinically suspected cases of Dengue fever reporting at our hospital during the studies. All adult samples both from out patients department (OPD) and in-patients department (IPD) and only paediatric IPD patients were included in this study.

Sample collection and storage
Blood samples were collected from outdoor patients and hospitalized patients with suspected dengue fever. Clinical diagnoses of dengue were considered with forewarning signs or severe dengue, according to the WHO guidelines.[8]

The serum samples consisted of both acute and early convalescent phases depending on the reporting time of the patients. Acute phase serum samples were collected from patients who reported within one to four days of fever and early convalescent phase serum were collected from patients who came with history of fever for > 5 years. Serum was separated by centrifuging samples at 3000 rpm for 5 min and tested immediately; in case of delay in processing they were stored in a refrigerator at a temperature of 2- 8°C until analysis.

Seroassays
IgM Capture ELISA
Detection of IgM Antibodies by the ELISA (Enzyme-Linked Immunosorbent Assay) test using Dengue IgM ELISA kit by National Institute of Virology (NIV), Pune was performed for 1807 samples. The Positive control and Negative control from the kit were put up with the test samples as per the kit literature provided. The test was a solid phase ELISA based on 'Direct Sandwich' principle.

A positive reaction was indicated by a yellow colour which was precisely read at 450nm spectrophotometrically by an ELISA reader the cut- off value (COF) was calculated using the formula as per the recommendation of the manufacturer. Further samples were interpreted as Non-reactive, Equivocal and Reactive for dengue IgM antibody. Data was compiled in MS-Excel and checked for its completeness and rectifications. Then it was evaluated.

Results
Over the study period from January to December 2018, a total of 1,807 samples were investigated for suspected dengue infection from out patients department (OPD) and in-patients department (IPD) and only paediatric IPD patients, admitted in a respective ward, with a record of dengue warning signs or severe dengue. Out of these 1,807 samples, 123(6.80%) were positive for IgM antibodies, both male and female patients were predominantly affected, 61(49.59%) and 62(50.40%) (Table 1 and Fig. 3).

Age group-wise assessment for dengue infection
Among the serologically confirmed cases of dengue, 123(6.80%) were total positive; 03(2.43%) patients were under the age of 1 month to 1 year, 29(23.57%) patients were under the age of 1-10 years, 30 (24.39%) patients were under the age of 11-20 years, 14(11.38%) patients were from 21-30 years, 16(13.08%) patients were from 31-40 years, 19(15.44%) patients were from the age of 41-50 years, 8(6.50%) patients were from 51-60 years and 4 (3.25%) patients were 60 years above (Table 2).

Table 1: Prevalance and Sex distribution of dengue infected patients

Type of Seroassay	No. of Patients	No. of Positive Patients	Sex determination of the positive patients	
			Male	Female
IgM ELISA	1807	123(6.80%	61(49.59 %)	62(50.40 %)

Table 2: Age-wise distribution of 123 positive cases of dengue infection

Age Group (in Years)	No. of Positive patients	Percentage (%) of Positive patients
< 1 Year	03	2.43
1-10	29	23.57
11-20	30	24.39
21-30	14	11.38
31-40	16	13.08
41-50	19	15.44
51-60	08	6.50
> 60	04	3.25
Total	123	100 %

**Periodic variance of dengue infected patients**

Month wise assortment of the dengue infected patients showed wide infection rate during April to December over the period of this study. During 2018, a narrow infection was also observed during the months of January to March (Fig. 2).

Fig. 1: Map of the Karnataka with study site-Kalaburagi district
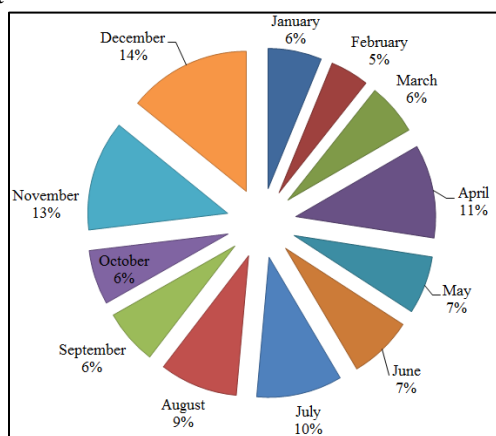
Fig. 2: Seasonal Variance of Dengue infected patients during this study
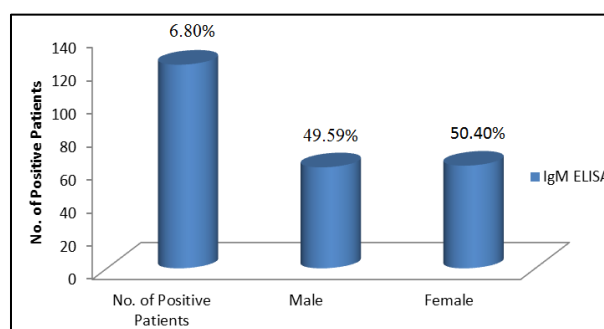
Fig. 3: Distribution of dengue positive patients from male and females

Discussion

Dengue is the utmost wide spread arboviral disease worldwide commanding a heavy economic and health burden.[9] Persistent dengue epidemics with increased occurrence in last few years in Karnataka instigated us to occupy a thorough investigation of several aspects associated to dengue infection, prevalence, transmission and gravity in Kalaburagi district of Karnataka. Dengue infection has developed into a major public health problem in tropical and subtropical region of the India especially Karnataka, due to the morbidity and mortality basis. Controlling dengue infection is difficult because it involves efficient vector control.[10]

The detection of IgM antibody to dengue virus by ELISA has become one of the laboratory's essential methods for the diagnosis of dengue virus infection.[11,12] Anti-dengue IgM antibody is produced rapidly during primary and secondary infections.[13] In this study, the possible use and the role of IgM antibody for the early diagnosis of dengue infection.[13]

In this study, total 1807 samples tested for IgM Ab, in our study, the dengue cases occurred during the rainy and post monsoon season, which is comparable to most of the earlier outbreaks in India,[14,15] it is due to this season is very favourable for high procreation of the vector. The present study has shown a both male (49.59%) and female (50.40%) equally predominantly effected. Similar results were observed by Atul Garg et al who gave the male: female ratio

and Tank Arun G, Jain Mannu R who have given a sex ratio for dengue sero-positive patients for male to female.[16,17]

We found that the mean age group affected was 11-30 years. This was reliable with the other studies on dengue in India R.N. Markoo et al.[18] in this study have been reported the mean age of dengue patients as 27 years and most of them were under the age of 21-30 years. Ekta Gupta et al[19] revealed the age group preponderance of 21-30 years in their study.

Conclusion

Dengue fever is most common and important public health problem in developing countries like India, it can present with various acute clinical symptoms and causes major morbidity and mortality compared to any other viral infections. Therefore, a high index of interpretation is required for these patients to be diagnosed. Our results propose that, IgM-capture ELISA is very useful and effective method for the diagnosis of acute dengue infection. Therefore this IgM antibody detection test will be helpful in diagnosis of dengue infection early so that the morbidity and mortality can be monitored and thus we conclude that this serological test (IgM ELISA) have crucial part in the early diagnosis of dengue infection. This study also provided the proper evidence of diagnosis, early association of treatment or therapy, public consciousness and vector control are vital factors to be occupied into deliberation in the prevention and management of dengue infection.

Ethical Approval

This study protocol was approved by the Institutional Ethics and Research Committee (GIMS/GLB/IEC/02/2017-18), Gulbarga Institute of Medical Sciences (GIMS), Kalaburagi.

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