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RESEARCH ARTICLE

A Study on Seroprevalence among Clinically Suspected Dengue Viral Infection using IgM Antibody Capture ELISA for the Early Diagnosis

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ABSTRACT

Background: Dengue is an acute viral illness caused by RNA virus of the family Flaviviridae and spread by *Aedes* mosquitoes occur mainly in four closely related serotypes (DENV1-4). It has developed into emergent threat in the India and Karnataka state now. Karnataka state now and also challenges associated with patient administration in dengue infection is early diagnosis. **Objective:** The objective of the study was to determine the prevalence of dengue infection in the Kalaburagi district by serological test IgM enzyme-linked immunosorbent assay (ELISA). The detection of immunoglobulin (Ig)M is the core method for the laboratory diagnosis of dengue. The present study considered the relevance of a rapid test for dengue pertaining to new cases and assessment of the epidemic attack rate. **Methods:** This evocative study was conducted at our Gulbarga Institute of Medical Sciences Hospital, Kalaburagi, for the period between January and December 2018. Serum samples were collected from suspected dengue patients attended in- and out-patient department, a detailed history and full clinical assessment was done. Sera were analyzed by capture ELISA for the presence of dengue IgM antibodies. **Results:** Over the study period, out of 1807 samples, 123 were showed positive for dengue virus infection (14.69%). Both male 61 (49.59%) and female 62 (50.40%) patients predominantly affected equally. The positive peak values were noticed between the month of April and December 2018. The most positivity was observed in the age group of 1–20 years. Among these tests, the National Institute of Virology (NIV, ICMR) kit has shown to have an overall sensitivity of 81.6% and a specificity of 97.9–99%. **Conclusion:** The present results revealed that the study region is both epidemic and endemic 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 has an important role in the early diagnosis. Therefore, IgM ELISA is recommended in all the suspected dengue patients so as to diagnose early, instigate essential treatment, and assessment of morbidity and mortality rate during an outbreak.

Keywords: Dengue viral infection, Dengue, IgM ELISA, Kalaburagi, Prevalence

INTRODUCTION

Dengue is a mosquito-borne viral disease that has quickly spread in all regions of the World Health

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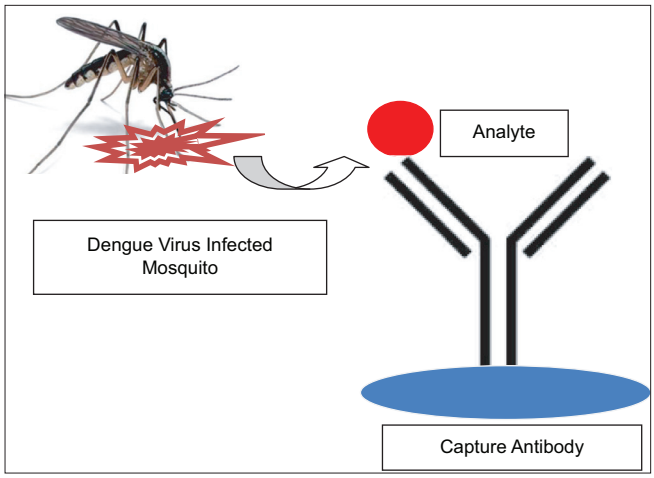
GRAPHICAL ABSTRACT



Organization (WHO) in recent years. Female mosquito mainly of the species *Aedes aegypti* and, to a lesser point, *A. albopictus,* transmits 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 is common in Asian and Latin American countries, 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 the infection of dengue virus, a Flavivirus in the family of Togaviridae. 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 posturing severe challenge to public health.

The epidemiology of dengue in Indian subcontinent is very composite and changed overtime. Dengue was previously supposed to be an urban disease as most cases were reported from bigger cities. However, from the past decade, there are many outbreaks from rural areas of South and West India.[4,5] In 2012, outbreak of dengue epidemic in a country was about 3 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 midgut of *Aedes aegypti* female mosquito and spreads to salivary glands over a period of 8–12 days. Then, it is ready to transmit to human host during consequent nourishment. 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, hemoconcentration, hemorrhagic shock, and multiple organ failure leading to patient death.[8]

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 present study was undertaken to know the pattern and nature of dengue prevalence in Kalaburagi district and surrounding areas of Karnataka.

MATERIALS AND METHODS

Study region and population

Kalaburagi district of Karnataka, India, lies in North-Eastern part of Karnataka [Figure 1]. District has 56.03% literacy rate and a population of 2,564,892 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

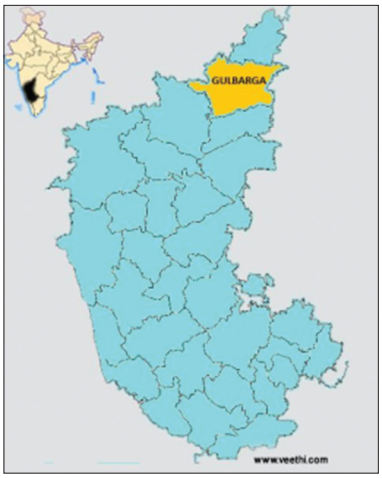


Figure 1: Map of the Karnataka with study site – Kalaburagi district

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 the Institutional Ethical Committee. A pro forma with detailed epidemiological, clinical, and laboratory parameters recorded during the hospital stay, district health department records section, Gulbarga Institute of Medical Sciences Medical Record section, was used as a tool for the data collection.

Sample size

A total of 1807 blood samples were screened from clinically suspected cases of dengue fever reporting at our hospital during the studies. All adult samples both from outpatients department (OPD) and inpatients department (IPD) and only pediatric IPD patients were included in this study.

Sample collection and storage

Blood serum samples were collected from patients of outdoor and hospitalized patients with suspected dengue fever. Clinical diagnoses of dengue were considered as dengue, dengue 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 1–4 days of fever and early convalescent phase serum was collected from patients who came with a history of fever for >5 days. 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 enzyme-linked immunosorbent assay (ELISA)

Detection of IgM Ab by the ELISA test using dengue IgM ELISA kit by National Institute of Virology (NIV), Pune, was performed for 1807 samples. The PC and NC 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 color which was precisely read at 450 nm spectrophotometrically by an ELISA reader the cutoff 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 were 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 1807 samples were investigated for suspected dengue infection from outpatients department (OPD) and inpatients department

(IPD) and only pediatric IPD patients, admitted in a respective ward, with a record of dengue warning signs or severe dengue. Out of these 1807 samples, 123 (14.69%) were positive for IgM Ab, both male and female patients were predominantly affected, 61 (49.59%) and 62 (50.40%) [Table 1 and Figures 2 and 3].

Age group-wise assessment for dengue infection

Among the serologically confirmed cases of dengue, 123 (6.80%) were total positive; 3 (2.43%) patients were under the age of 1 month–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 to 30 years, 16 (13.08%) patients were from 31 to 40 years, 19 (15.44%) patients were from the age of 41 to 50 years, 8 (6.50%) patients were from 51 to 60 years, and 4 (3.25%) patients were 60 years above [Table 2].

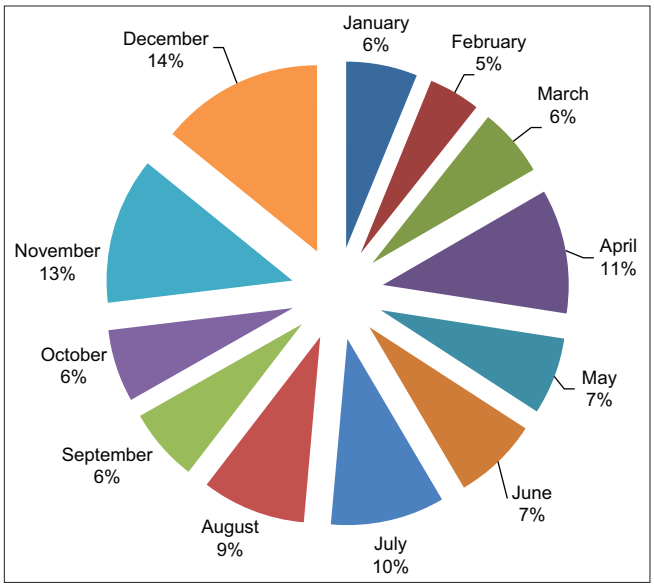


Figure 2: Seasonal variance of dengue-infected patients during this study

Table 1: Prevalence and sex distribution of dengue-infected patients

Type of seroassays	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%)

Periodic variance of dengue-infected patients

Month-wise assortment of the dengue-infected patients showed wide infection rate during April–December over the period of this study. During 2018, a narrow infection was also observed during the months of January–March [Figure 1].

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 the past few years in Karnataka instigated us to occupy a thorough investigation of several aspects associated to dengue infection, prevalence, transmission, and severity 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 necessitates efficient vector control.[10]

Detection of dengue virus by virus isolation or by nucleic acid detection methods by polymerase chain reaction is considered as confirmatory tests for confirming the diagnosis of dengue infection. Although, due to the need for advanced laboratory facilities, these two methods may not be accurate for routine diagnosis of dengue virus infection early in the disease in resource poor communities. Many commercial assays are presently available for the detection of dengue virus. Thus, detection of IgM antibody to dengue virus by ELISA has become

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	3	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	8	6.50
>60	4	3.25
Total	123	100

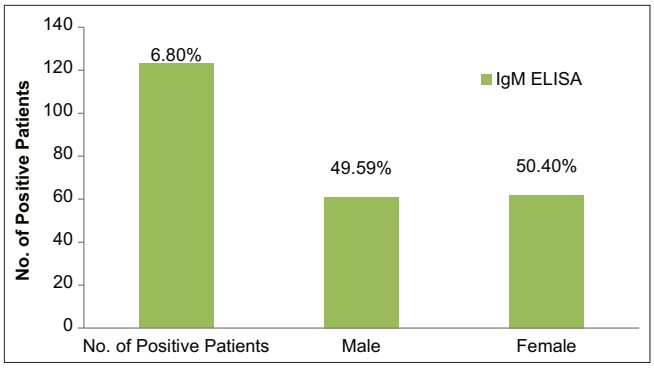


Figure 3: Distribution of dengue-positive patients from male and females

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] Secondary infections and possible use of IgM antibody for the early diagnosis of dengue infection.[13]

In this study, a total of 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 favorable for high procreation of the vector. The present study has shown a both male (49.59%) and female (50.40%) equally predominantly affected. Similar results were observed by Garg *et al*. who gave the male: female ratio as being G, 2:1 and Tank *et al*. who have given a sex ratio for dengue seropositive 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. 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 the 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 perception 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) has crucial part in the early diagnosis of dengue infection. This study also provided that 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.

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST STATEMENT

We declare no conflicts of interest.

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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