

Original Research Article

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The Seroprevalence of Dengue in a Tertiary Care Hospital

S. Dhivya Lakshmi, P. Nanthini Devi* and Chitralkha Saikumar

Department of Microbiology, Sree Balaji Medical College and hospital, Chennai,
Tamil Nadu, India

*Corresponding author

ABSTRACT

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Dengue or break bone fever is a deadly disease caused by the Flavivirus. There are approximately 390 million dengue infections every year in the tropical and sub-tropical regions of the world. It is caused by the day biting Aedes mosquitoes which has expanded its geographical boundaries recently. Early diagnosis of Dengue is essential to prevent the complications. The study was done in a tertiary care hospital to have an insight about the seroprevalence during the dry months from March to August. The seropositivity was found to be 33.64% with the maximum number of cases in the 21-30 years age group (37.57%). The number of cases recorded was the highest in the month of August. 62.98% of cases were due to primary Dengue. The study highlights the importance of mosquito control measures all the year round and not just during the monsoon season.

Introduction

Dengue fever is a deadly arboviral disease caused by any of the five serotypes DENV1, DENV2, DENV 3, DENV 4 and DENV 5 which are placed in the Family Flaviviridae and genus Flavivirus (Murray *et al.*, 2013; Mustafa *et al.*, 2015). Infection with any one serotype provides lifelong immunity to the particular serotype but are at risk of infection to the other serotypes (Mustafa *et al.*, 2015). Approximately 390 million dengue infections occur every year, of which 96 million manifests clinically with any severity of disease and 128 countries are at risk of infection with dengue viruses (Bhatt *et al.*, 2013; Brady *et al.*, 2012). The fever is caused by the day biting Aedes mosquitoes *Aedes aegyptius* and *Aedes albopictus* (Gyawali *et*

al., 2016). Dengue can present as classic Dengue fever, Dengue hemorrhagic fever (DHF) and Dengue shock syndrome (DSS). Classic Dengue fever is characterized by sudden onset of high fever, retro orbital pain, myalgia, arthralgia and rash and occurs mostly in adults. DHF occurs mostly in children less than 15 years of age and caused by increased capillary permeability and plasma leakage leading to thrombocytopenia, bleeding and hemoconcentration and shock (termed Dengue shock syndrome) (Hadinegoro, 2012). To prevent the untoward complications of Dengue, it is imperative that early diagnosis is established. It can be done by the detection of NS1 (nonstructural protein) by RT-PCR which is not available in all hospitals and health centers. Serologic diagnosis relies on the testing of fourfold rise in antibodies by ELISA

(Enzyme linked immunosorbent assay). Both IgM and IgG ELISA testing can be used for the differentiation of primary and secondary infections (CDC Dengue-Laboratory Guidance and Diagnostic Testing). The present study was done in a tertiary care hospital in South India and gives an insight into the seroprevalence of dengue fever and its relation to seasonal variations.

Materials and Methods

Study design and period

This is a cross sectional study done at the Central Microbiology laboratory of a tertiary care hospital between March 2017 and August 2017 for a period of six months. The blood collection was performed under strict aseptic precautions. Sera were separated after centrifuging and were subjected to ELISA testing of NS1 antigen, IgM and IgG antibodies by the JMitra kit.

Inclusion criteria

All patients in any age group presenting with fever, headache, rash, retroorbital pain, myalgia and other symptoms suspicious of Dengue fever from various outpatient, inpatient departments and emergency services.

Exclusion criteria

Fever confirmed due to non-infectious causes or other causes such as *Leptospira*, Hepatitis and Typhoid.

Blood collection and processing

About 5 ml of blood was collected under strict aseptic precautions. Sera were separated after centrifuging at 1000 rpm for 2 minutes and were subjected to ELISA testing of NS1 antigen, IgM and IgG antibodies by the JMitra kit (Microwell ELISA Test).

Precautions

Specimens should be free of microbial contamination and may be stored at 2-8⁰C for one week, or frozen at -20⁰C or lower. Repeated freezing and thawing should be avoided. Hemolyzed and hyperlipemic samples may give erroneous results.

Procedure

DENGUE NS1 Ag MICROLISA is a screening test designed for the qualitative detection of Dengue NS1 antigen in serum or plasma. The kit detects all four dengue subtypes; DEN1, DEN2, DEN3 and DEN4 of Dengue Virus. It is a solid phase enzyme linked immunosorbent assay (ELISA) based on the "Direct Sandwich" principle. The samples are added in the microwells which are coated with Anti-dengue NS1antibodies followed by addition of enzyme conjugate (monoclonal anti-dengue NS1antibodies linked to Horseradish Peroxidase enzyme (HRPO)). A sandwich complex is formed, where the dengue NS1 from patient's serum is trapped between the antibody and antibody HRPO conjugate. The unbound conjugate is washed off with wash buffer. Upon addition of the substrate buffer and chromogen, a blue color develops. The intensity of development is proportional to the concentration of dengue NS1 antigen in sample. A stop solution is added to limit the enzyme-substrate reaction and a yellow colour develops which is read at 450nm spectrophotometrically.

The sensitivity of Dengue NS1 Ag Microlisa kit was found to be 98.28%.

IgM/IgG CAPTURE ELISA

Dengue IgM/IgG Microlisa test is an enzyme immunoassay based on "CAPTURE ELISA". Anti-human IgM/IgG antibodies are coated onto microtiter wells. Serum samples and

controls are added to the microtiter wells and incubated. Antibodies to Dengue, will bind to the anti- human IgM/IgG antibodies adsorbed onto the surface of the wells followed by washing to remove unbound material.

Horseradish peroxidase (HRPO) conjugated Dengue antigen (DEN-4) is added to each well. This dengue antigen conjugate will bind to dengue specific IgM/IgG antibodies which is complex with anti-human IgM antibodies.

Finally substrate solution containing chromogen and hydrogen peroxide is added to the wells. A blue colour develops according to the amount of dengue antibodies present in the sample. The reaction is stopped by a stop solution. The enzyme substrate reaction is ready by EIA reader for absorbance at a wavelength of 450 nm. The sensitivity was found to be 99.13% and specificity was found to be 99.84%.

Results and Discussion

The total number of patients whose serum was sent to the Central Microbiology laboratory for the six months study period was 538 out of which 276 (51.3%) were males and 262 (48.7%) were females (Fig. 1).

The seropositivity for Dengue NS1, IgM, IgG by ELISA was 181 (33.64%) out of which 92 (50.83%) were males and 89 (49.17%) were females (Fig. 2).

Dengue is an important and life threatening arboviral infection in tropical countries with an estimated 390 million infection and 96 million symptomatic infections occurring annually (Bhatt *et al.*, 2013). The early diagnosis of Dengue is of great importance to arrest the progression of Dengue related complications. The total number of patients whose serum was sent to the Central Microbiology laboratory on suspicion of

Dengue, for the six months study period was 538 out of which 276 (51.3%) were males and 262 (48.7%) were females.

The seropositivity of Dengue in our study was 33.64% out of which 92 (50.83%) were males and 89 (49.17%) were females. Gopal *et al.*, (2016) reported a seroprevalence of 50%, Gupta *et al.*, reported a positivity of 29.09%, Kalaivani *et al.*, (2016) reported a 62% seroprevalence which is very higher compared to our study.

The table 1 shows increased prevalence in the male gender in many studies, but a study by Kalaivani *et al.*, (2016) reports equal prevalence. Our study also supports this finding showing an increased prevalence towards the male gender. This is due to the fact that males are involved in more outdoor activities compared to females.

Madan *et al.*, (2018) reported 26.47% in the 21-30 years age group followed by 21.56% in 11-20 years. Mahesh Kumar *et al.*, (2015) reported high prevalence in the 10-20 years age group (31.58%). Sujatha *et al.*, (2016) recorded maximum prevalence in the age group 21- 30 years (55.09%) followed by 11-20 years (27.54%). Bhat *et al.*, (2013) reported an increased prevalence in adults > 15 years (Mishra *et al.*, 2016) (Fig. 3).

Patankar *et al.*, (2014) reported 51% in the age group of 18-35 years followed the age group 5-17 years age group (20%). But our study showed high prevalence of seropositivity in the 21-30 years age group-37.57% followed by 18.58 % in the 11-20 years age group.

The high incidence of seropositivity in the 11-30 years shows that the children and young adults were exposed to mosquito bites due to their habits of involving in brisk outdoor activities.

Fig.1 Shows distribution of male and female among the study group

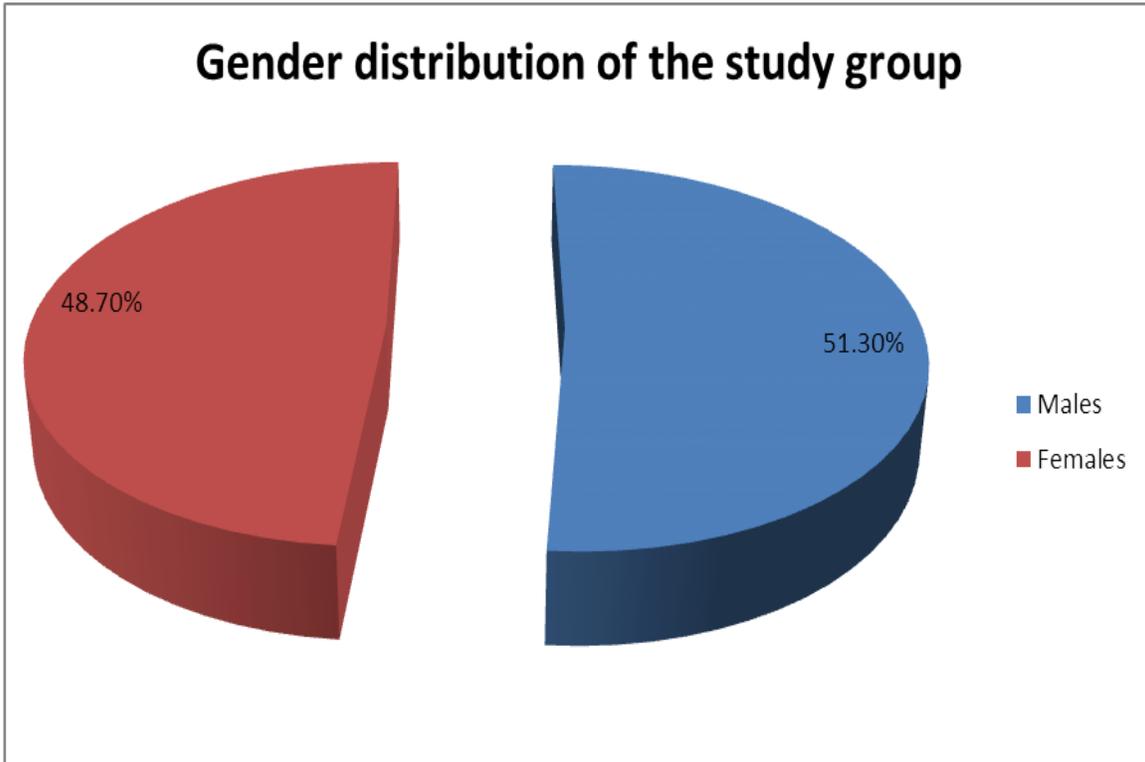


Fig.2 Shows distribution of male and female among dengue positive cases

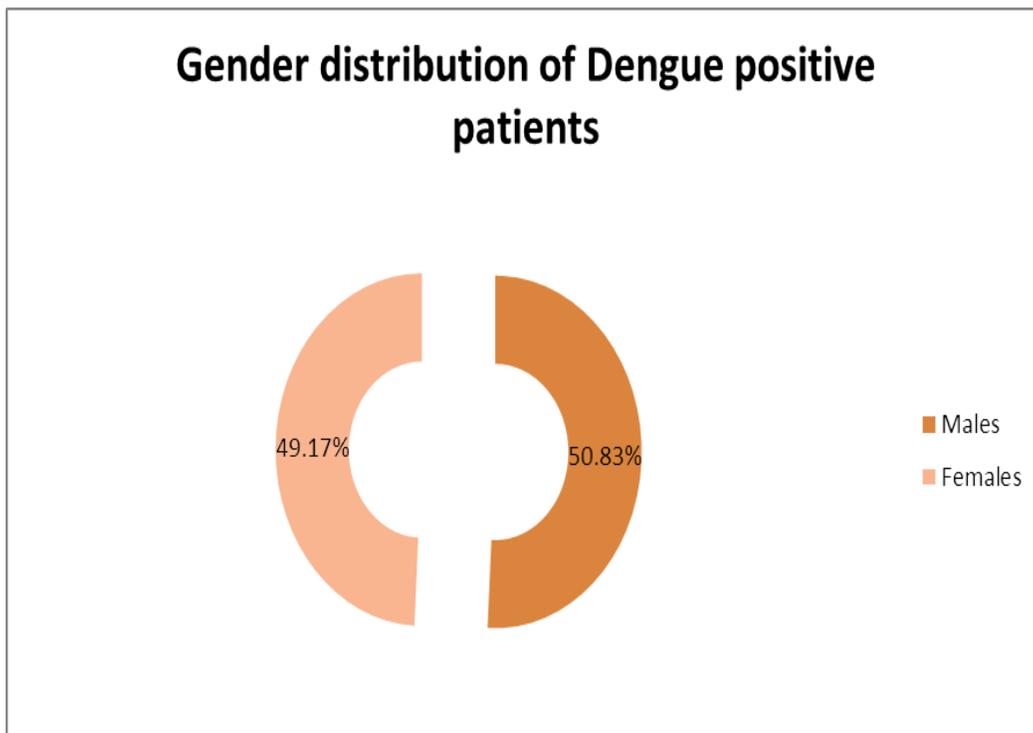


Fig.3 Age wise distribution of dengue positive patients

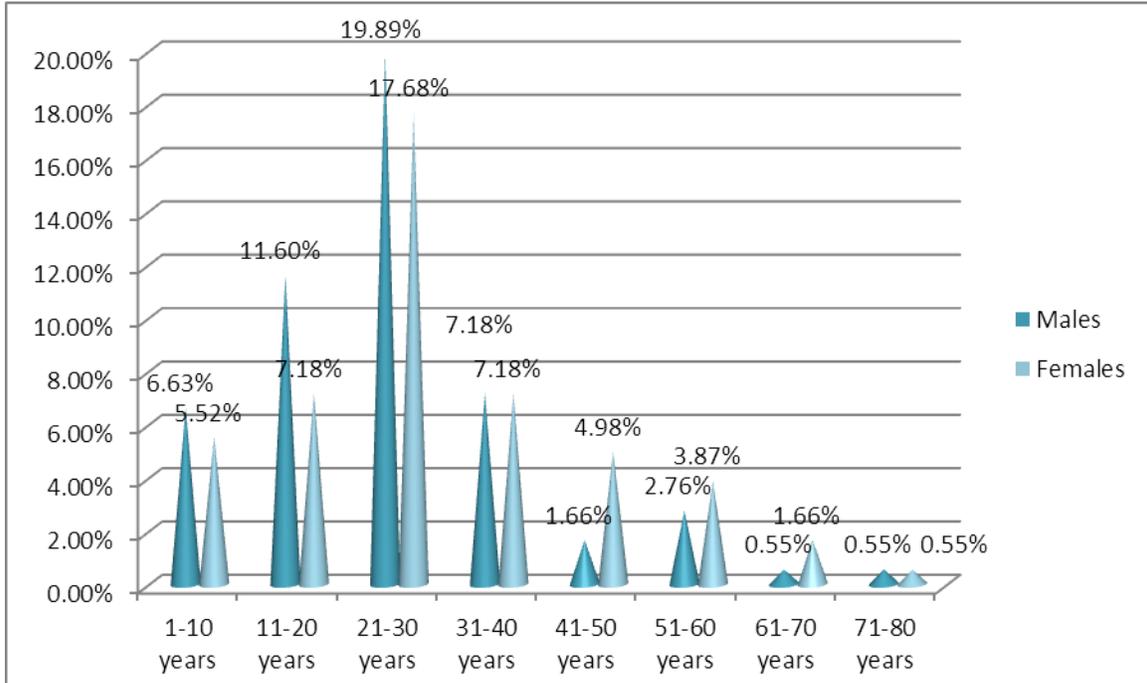


Fig.4 A graph that shows the prevalence of dengue in relation to months of the year

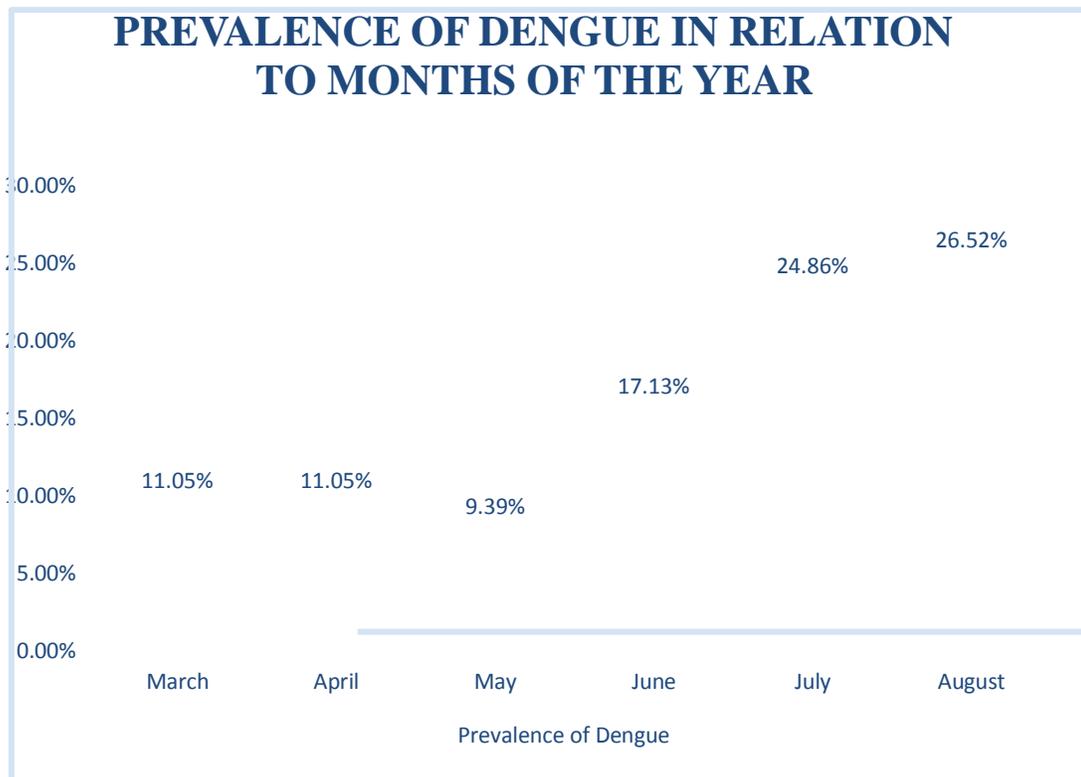


Table.1 Dengue Elisa NS1, IgM, IgG positivity with respect to gender

Positivity of antigen/antibody	Males		Females	
	N	%	N	%
NS1	39	21.55%	52	28.73%
IgM	11	6.08%	3	1.66%
IgG	16	8.84%	17	9.39%
IgM & IgG	8	4.42%	7	3.87%
NS1 & IgM	7	3.87%	2	1.1%
NS1 & IgG	5	2.76%	4	2.21%
NS1, IgM & IgG	6	3.31%	4	2.21%

Table.2 Types of infection in dengue

Type of infection	Number	Percent
Primary infection	114	62.98%
Late primary /Early secondary infection	15	8.29%
Secondary infection	19	10.5%
Past infection	33	18.23%

Table.3 Shows prevalence of dengue in various studies

S.NO	PREVALENCE	REFERENCE
1.	50%	Gopal <i>et al.</i> , (2016)
2.	29.09%	Gupta <i>et al.</i> ,
3.	62%	Kalaivani <i>et al.</i> , (2016)
4.	20%	Goswami <i>et al.</i> ,(2018)
5.	53.2%	Srinivas Rao <i>et al.</i> , (2013)
6.	21.65%	Madan <i>et al.</i> , (2018)
7.	3.55%	Mahesh kumar <i>et al.</i> , (2015)
8.	37.38%	Sujatha <i>et al.</i> , (2016)
9.	33.64%	Present study

Table.4

S. No	Males	Females	Reference
1.	50%	50%	Kalaivani <i>et al.</i> , (2016)
2.	77.3%	23.7%	Mishra <i>et al.</i> , (2016)
3.	61.76%	38.23%	Madan <i>et al.</i> , (2018)
4.	62.63%	37.37%	Mahesh kumar <i>et al.</i> , (2015)
5.	61%	39%	Sujatha <i>et al.</i> , (2016)
6.	65%	35%	Patankar <i>et al.</i> , (2014)
7.	50.83%	48.17%	Present study

The highest number of cases was recorded in November followed by October and December according to Mahesh Kumar *et al.*, (2015). Sujatha *et al.*, (2016) recorded maximum cases in October and November. Bhat *et al.*, (2013) also reported increased prevalence of dengue in the season after rainfall (KSB *et al.*, 2014). Patankar *et al.*, (2014) reported increased positivity in October. But Nissi Mathew *et al.*, (2017) reported increased prevalence in August which is different from other studies.

Though these studies highlight the fact that the prevalence of dengue increases in the after monsoon, our study shows that there is an increased prevalence even in the dry seasons (March-11.05%, April-11.05%, May-9.39%, June-17.13%, July-24.86%, August-26.52%), which shows the year round transmission of Dengue. So there is a dire need to take precautionary measures to control mosquitoes throughout the year and not just in the season after monsoon (Fig. 4).

The antibody response to dengue viral infection varies. When there is a primary infection with dengue, IgM antibody is the first antibody to rise by 3-5 days after the illness, peaks by the 10th day and declines by 2-3 months. IgG antibodies rise by the end of the first week of illness, rises slowly and remains detectable in serum for many months or even for life. In contrast when there is secondary infection due to dengue virus or any other flavivirus infection or vaccination, IgG antibodies rise rapidly and the level of IgM antibodies is lower compared to IgG antibody (Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control, 2009) (Table 2).

In a study by Gopal *et al.*, (2016) 26% was primary infection and 30 % was due to secondary infection. Srinivasa Rao *et al.*, (2013) reported 17.7% of primary infection,

28.05% of past infection, 54.25% of patients in the convalescent phase. Madan *et al.*, (2018) reported 87.25% of primary infection, 5.88% patients of late primary or early secondary infection, 6.86% cases, suggesting secondary or past infection. Kalaivani *et al.*, (2016) recorded 43.57% of primary dengue and 56.43% of secondary dengue. But in the present study, 62.98% of cases were due to primary infection, 10.5% due to secondary infection, 8.29% due to early secondary/late primary infection and 18.23% due to past infection (Table 3 and 4).

The chief drawback of the study was that paired sera samples were not used for testing which will differentiate the primary and secondary infection clearly. Confirmatory testing also was also not done by RT-PCR to determine the serotype.

Dengue has become a threat even in the current era. There are a lot of challenges in the control of Dengue. The vector *Aedes* mosquito has invaded new territories due to climatic changes and there is lack of suitable diagnostic facilities. The serologic testing of Dengue is not reliable as there is the problem of cross reactivity. Though Dengue is a notifiable disease, the number of cases reported is underestimated due to the lack of proper diagnostic tools.

There are no licensed vaccines for Dengue. Even when a vaccine is available it should cover all the serotypes of Dengue virus.

Vector control is the only measure to control the spread of Dengue but there is the problem of insecticide resistance. Personal protective measures to avoid mosquito bites should be undertaken to break the transmission of Dengue.

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