



## Original Article

# Study on Prevalence of Dengue Fever in a Tertiary Care Hospital, South Kerala

Authors

Nissi Mathew<sup>1</sup>, Dr Jyothi Rajahamsan<sup>2</sup>, Dr Sahira.H<sup>3</sup>, Biju Rani<sup>4</sup>,  
Dr Ramani Bai. J.T<sup>5</sup>

<sup>1</sup>M.Sc MLT Lab Technician, Govt. Medical College, Trivandrum

<sup>2</sup>Associate Professor, Dept. of Microbiology, Govt. Medical College, Trivandrum

<sup>3</sup>Assistant professor, Dept. of Microbiology, Govt. Medical College, Trivandrum

<sup>4</sup>Assistant Professor, Dept. Of MLT Govt. Medical College, Trivandrum

<sup>5</sup>Professor, Dept. of Microbiology, S R. Medical College, Trivandrum

## Abstract

**Background:-** Dengue virus causes acute febrile illness in tropical and sub tropical settings and its clinical manifestations ranges from mild form of dengue fever (DF) to the more severe forms of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). A range of diagnostic methods has been developed to support patient management and disease control.

**Objective:-** To find out the serological test that helps in early detection of dengue cases, prevalence of primary and secondary dengue cases and also to find out the prevalent serotype.

**Study Design:-** This cross sectional study was conducted in Govt. Medical College, Thiruvananthapuram from July 2014 to December 2014. Serum samples from 433 patients were screened for dengue NS1 Antigen, IgM and IgG enzyme-linked immunosorbent assay (ELISA) which helped in early detection and to differentiate primary and secondary dengue cases. PCR was done to find out the serotype

**Results:-** 84 (19.4%) cases were positive for dengue infection, out of which 23(11.6% of patients) were positive for NS1 antigen showing early detection. 37(44% of patients) had secondary dengue virus infection and the rest 47 (56% of patients) had primary infection. Serotype 2 was prevalent in the study population.

**Conclusion:-** The study provide strong evidence of the value of combining dengue virus antigen and antibody for the diagnosis of the acute dengue infection. NS1 antigen detection is valuable as it allows detection of infection prior to seroconversion.

**Keywords:** Dengue fever, nonstructural proteins NS1; ELISA.

## Introduction

*Arboviruses*, a diverse group of viruses, survive in nature by transmission from infected to susceptible hosts by certain species of mosquitoes, ticks, sand flies or biting midges. The viral multiplication within the tissues of the arthropod, produce a high level of viraemia which is then

passed on to human or other vertebrates by the bites of the insect. *Arboviruses* mostly causes zoonotic infections, however a number of viruses can cause incidental infection in humans. Two major exceptions to this are *O'nyong-nyong* (ONNV) and *dengue viruses*, whose known vertebrate host is human<sup>1</sup>.

The global incidence of dengue fever is on the rise since the year 2000 due to factors like rapid urbanization, expanding human population, their activities, increased global travel and geographical expansion of the primary vector, *Aedes aegypti*.

Dengue fever (DF) is a self limiting viral disease distributed throughout the tropical areas of the world<sup>2,3</sup>. Currently the morbidity and mortality of dengue fever is more than any other arboviral disease. Amplified mosquito population due to deterioration in the public health infrastructure and changing climatic conditions have an important role in the increasing incidence of Dengue fever<sup>4,5</sup>. Therefore, dengue is believed to pose a mounting challenge to the tropical and subtropical regions throughout the world. Occurrence of classical dengue fever has been in India only since 1988<sup>6</sup>. Dengue affects up to 100 million people each year, with 500,000 cases of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) and around 30,000 deaths, mostly amongst children<sup>7,8</sup>.

Diagnosis of acute dengue infection by clinical signs and symptoms is complicated by a wide range of possibilities for differential diagnosis, hence laboratory assays are relied upon to make diagnosis. Dengue infections may be asymptomatic or give rise to undifferentiated fever, dengue fever, DHF, or dengue shock syndrome. DHF usually follows infection with another serotype (secondary dengue), but may sometimes follow primary infections, especially in infants in whom maternally acquired dengue antibodies are presumed to enhance primary infections<sup>9,10</sup>. Such a phenomenon has not been described in human infections other than dengue<sup>10</sup>.

This study provides strong evidence of the value of combining dengue virus antigen and antibody for the diagnosis of the acute dengue infection. Hence NS1 antigen ELISA, IgM and IgG antibody ELISA were used for early detection of dengue fever and to differentiate primary and secondary dengue cases.

## Materials and Methods

**Study Population:** This study was done over a period of 6 months in the Dept. of Microbiology, Govt. Medical College Thiruvananthapuram in patients clinically suspected to have Dengue fever.

### Inclusion Criteria

Samples received from patients suspecting Dengue fever with two or more of the following symptoms like headache, rash, retroorbital pain, myalgia, arthralgia hemorrhagic manifestations and leucopenia.

### Exclusion Criteria

Unlabeled, haemolysed, and lipaemic blood samples were excluded. All cases of fever for which another definitive diagnosis has been established (Enteric fever, Leptospirosis, Malaria, Chikungunya and Hepatitis) were also excluded.

## Methodology

Blood samples were collected from patients with suspected dengue fever in Medical College, Thiruvananthapuram. Three samples were collected for doing NS1 antigen, IgM and IgG antibody. The first sample was collected at the time of admission, the second sample on the 3<sup>rd</sup> to 5<sup>th</sup> day of admission and the third sample from patients those who were tested negative at the time of discharge. PCR was done with samples from patients having fever, within four days.

Serum samples were screened for serological detection of dengue fever by NS1 Antigen, IgM and IgG enzyme-linked immunosorbent assay (ELISA ). RT-PCR was done to find out the serotype .Clinical history of patients were also collected. PCR was done for 9 NS1 positive samples.

## Results

A total of 433 samples were collected from patients with suspected dengue fever and processed for NS1 antigen, IgM and IgG antibody.

**Table No 1** Gender distribution of IgM Dengue positive cases

Gender	Number tested	IgM Positive
Male	256	44
Female	177	40
Total	433	84

Out of the 433 cases, 256 (59.1%) were males, and 177(40.9%) females. The total number of patients tested positive for IgM Antibody were

84, out of the which 44 (52%) were males and 40 (48%) were females.

**Table 2** Age wise and Gender wise analysis of IgM Dengue positive cases

Age in years	Gender				Total	
	Male		Female		Positive	%
	Positive	%	Positive	%		
<1	2	4.5	0	0	2	2.4
1-10	13	29.5	10	25	23	27.4
11-20	9	20.5	9	22.5	18	21.4
21-30	4	9.1	6	15	10	11.9
31-40	6	13.6	3	7.5	9	10.7
41-50	3	6.8	3	7.5	6	7.1
51-60	4	9.1	8	20	12	14.3
61-70	3	6.8	1	2.5	4	4.8
Total	44	100	40	100	84	100

The largest age group admitted belonged to the pediatric, ranging from 1-10years (27.4%)

followed by the teenagers 18 (21.4%) and then adults 12 (14.3) %.

**Table 3** Month wise Analysis of IgM dengue cases

IgM	July	August	September	October	November	December
Positive	10	24	12	18	12	7

Highest number of positive cases were seen in the month of August and lowest in December.

**Table 4** Distribution of cases with thrombocytopenia

Thrombocytopenia	NS1 Positive	IgM Positive	IgG Positive
Nil	3	14	Nil
Mild	10	34	7
Moderate	5	20	10
Severe	5	16	20
Total	23	84	37

In the present study, 1 lakh - 1.5 lakhs (mild thrombocytopenia) was observed in 34 (40% of primary) and 7 (18.9% of secondary) cases, a count between 50,000-1lakhs (moderate thrombocytopenia) was observed in 20 (23.8% of primary) and 10 (27.0% of secondary) cases, a count below 50,000 (severe thrombocytopenia) in 16 (19% of primary) and 20 (54% of secondary) of the patients. 14 (16.6% of primary) patients showed no thrombocytopenia.

In this study, out of 84 IgM dengue antibody positive cases 47 (56% of patients) had primary infection and 37(44% of patients) had secondary dengue.

**Table 5** Serotyping of 9 NS1 Positive cases

NS1 Positive	serotype	
	DEN 2	DEN 4
9	7	2

A total of 23 samples were NS1 antigen positive, out of which 9 samples were sent to Rajiv Gandhi Centre for Biotechnology for doing PCR. 7 samples were type 2 Dengue virus and two were type 4.

NS1 Antigen helps early detection, even from the first day of fever. Out of the 84 cases, 23 cases were NS1 antigen positive. This was confirmed by IgM dengue and PCR. Eight NS1 positive cases showed indeterminate IgM, which became positive on repeating after two days.

### Discussion

Efficient and accurate diagnosis of dengue is of primary importance for clinical care (i.e. early detection of severe cases, case confirmation and differential diagnosis with other infectious diseases). Laboratory diagnostic methods for confirming dengue virus infection may involve detection of the virus, viral nucleic acid, antigens or antibodies, or a combination of these techniques. During the early stages of the disease, virus isolation, nucleic acid or antigen detection can be used to diagnose the infection.

Antibody response to infection differs according to the immune status of the host<sup>11</sup>. IgM antibodies are the first immunoglobulin to appear. These antibodies are detectable in 50% of patients by days 3-5 after onset of illness, increasing to 80% by day 5 and 99% by day 10. IgM levels peak about two weeks after the onset of symptoms and then decline generally to undetectable levels over 2-3 months. IgG antibody is generally detectable at low titres at the end of the first week of illness, increasing slowly thereafter, with serum IgG still detectable after several months, and probably even for life<sup>12</sup>.

In the present study, a total of 433 samples were collected from patients with suspected dengue fever and processed for NS1 antigen, IgM and IgG antibody. Out of the 433 cases 256 (59.1%) were

males, and 177(40.9%) females. The total number of patients tested positive for IgM Antibody were 84, out of the 84 positive samples, 44 (52%) were males and 40 (48%) were females. Maximum number of cases belongs to the age 1-10 years (27.4%) . Highest number of positive cases were seen in the month of August and lowest in December. In a study conducted in Medical college, Thiruvananthapuram, by Dr Sheeba P M, among the 685 positive samples, 373 (54.46%) were males and 312 (45.54%) were females<sup>13</sup>. In a similar study conducted in KEM hospital, Mumbai by Anil Pardesh *et al*, out of the 420 patients 236 (56%) were males and 184 (44%) were females<sup>14</sup>. The average age of dengue patients was reported to be 22 years and 3 months. They also had 130 (30.95%) pediatric cases.

All of the patients with secondary dengue 20 (54%) had rashes and required transfusion with platelet rich or fresh frozen plasma. In a previous study conducted at Medical College, Thiruvananthapuram, mild thrombocytopenia was recorded in 23 (15.2% of primary) and 16 (10.5% of secondary) cases, moderate thrombocytopenia. in 60 (39.7% of primary) and 47 (30.9% of secondary), and severe thrombocytopenia in 13 (8.6% of primary) and 47 (30.9% of secondary) cases. Very low levels (< 20,000 was seen in 2 (1.3% of primary) and 14 (9.2% of secondary) cases. Low levels were associated more with secondary cases only<sup>14</sup>. In a study from Srilanka conducted by Jayaratne S D *et al* platelet counts less than, 20,000 cells/mm<sup>3</sup> was associated with severe disease<sup>15</sup>. A study by Rames S S & *et al* in Mysore had reported that 11% had mild, 35% had moderate, and 32% had severe thrombocytopenia<sup>16</sup>.

In early convalescent stage IgM levels were significantly lower in secondary infections than in primary ones and may be undetectable in some cases, depending on the test used<sup>17</sup> To distinguish

primary and secondary dengue infections, IgM/IgG antibody ratios are now more commonly used<sup>18</sup>.

### Summary

Out of 84 positive samples, 23 were NS1 antigen positive. Nine samples which were NS1 positive were analyzed by PCR and it was found that 7 were serotype 2 Dengue virus and two were type 4. Out of 23 cases only 15 were IgM positive, and 8 were indeterminate, repeated after 2 days and were found to be positive. NS1 antigen detection is a valuable procedure, as it allows detection of infection prior to seroconversion and can be detected in serum from day 1 after onset of fever compared to IgM antibodies that are detectable only after 3-5 days.

A range of laboratory diagnostic methods has been developed to support patient management and disease control. The choice of diagnostic method depends on the purpose for which the testing is done (e.g. clinical diagnosis, epidemiological survey, vaccine development), the type of laboratory facilities and technical expertise available, costs, and the time of sample collection.

Detection of the dengue NS1 antigen during the symptomatic phase of illness represents an important advance in the diagnosis of dengue fever and is useful for the detection of dengue viral infections early in the course of the infection, especially in nonendemic countries.

**Funding:** Nil

**Competing Interest:** None declared

Ethical approval: The study had the approval of Human Ethics Committee under registration number IEC.No.05/20/2014/MCT

### Acknowledgment

The authors would like to thank Dr. Radhakrishnapillai, Director molecular Microbiology, Rajiv Gandhi centre for biotechnology for helping with the molecular diagnosis part of the study.

### References

1. Gordon C Cook and Ali Muddin I Zumla. Mansons tropical diseases, 2003; 22<sup>nd</sup> edition; 715
2. Halstead SB. Dengue hemorrhagic fever public health problem and field for research. Bull WHO1980;58:1-21.
3. Smith AW, Chen LH, Massad E, Wilson ME. Threat of dengue to blood safety in Dengue - endemic countries. Emerg Infect Dis 2009;15:8-11.
4. World Health Organization. Geneva: World Health Organization; 2009. [Aug 31; 2009]. Dengue and dengue haemorrhagic fever; fact sheet no. 117, revised March 2009
5. Kyle JL, Harris E: Global spread and persistence of dengue. Annu Rev Microbiol 2008, 62:71-92.
6. Indian Council of Medical Research. Arboviruses in India- Part II. Division of Public Information, ICMR, New Delhi, ICMR Bulletin.1980.
7. WHO. Dengue and severe dengue. WHO Media centre. 2012. Fact sheet N°117
8. World Health Organization. 2000. Dengue/dengue haemorrhagic fever. Wkly. Epidemiol. Rec. 75:193-200
9. Martinez E, Guzman MG, Valdes M, et al. Dengue fever and hemorrhagic dengue in infants with a primary infection. Rev Cubana Med Trop1993;45:97-101.
10. Halstead SB, Lan NT, Myint TT, et al. Dengue hemorrhagic fever in infants: research opportunities ignored. Emerg Infect Dis 2002;8:1474-9.
11. Vorndam V, Kuno G. Laboratory diagnosis of dengue virus infections. In: Gubler DJ, Kuno G, eds. Dengue and dengue hemorrhagic fever. New York, CAB International,1997:313-333.
12. WHO. Dengue haemorrhagic fever: diagnosis, treatment, prevention and control, 2nd ed. Geneva, World Health Organization, 1997

13. Sheeba P M. A study on clinicolaboratory profile of Dengue Fever. Govt Medical College, Thiruvananthapuram. 2008
14. Anil Pardeshi, Ratendra Shinde, Abhijeet Jagtap, Ravindra Kembhavi, Mayur Giri, Snehal Kavathekar Retrospective Cross-sectional Study of Dengue Cases in IPD with Reference to Treatment- Monitoring & Outcome in KEM Hospital, Mumbai American Journal of epidemiology and infectious disease(2014):97-100
15. SD Jayaratne, Vajini Atukorale, Laksiri Gomes, Thashi Chan, Tharindu Wijesinghe, Sachie Fernando ,Graham S Ogg and Gathsaurie Neelika Malavige. Evaluation of the WHO revised criteria for classification of clinical disease severity in acute adult dengue infection. BMC Research Notes 2012,5:645.
16. Ramesh S. S, Basavaraju M. M, Sandeep R. Sharma, Shetty Shivakumar, Srinivasa M, Surakshith T. K, Ravichethan Kumar. Study of bradycardia in dengue fever. 2014; March Volume; vol 3 Issue ; Issue 9 Page : 2378-2388.
17. WHO. Dengue haemorrhagic fever: diagnosis, treatment, prevention and control, 2nd ed. Geneva, World Health Organization, 1997.
18. Falconar AK, de Plata E, Romero-Vivas CM. Altered enzyme-linked immunosorbent assay immunoglobulin M (IgM)/IgG optical density ratios can correctly classify all primary or secondary dengue virus infections 1 day after the onset of symptoms, when all of the viruses can be isolated. Clinical and Vaccine Immunology, 2006, 13:1044–1051.