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Research Article

Laboratory diagnosis and incidence of Dengue virus infection: A hospital based study, Perambalur

M. Anuradha*[1], Dandekar R. H[2] and Sageera Banoo[1]

[1]*Department of Microbiology, Dhanalalshimi Srinivasan Medical College and Hospital, Siruvachur, Perambalur- 621212 Tamilnadu, India.*
[2]*Department of Community Medicine, Dhanalalshimi Srinivasan Medical College and Hospital, Siruvachur, Perambalur- 621212 Tamilnadu, India.*

***Correspondence Info:**
Dr. M. Anuradha
Professor and head,
Department of Microbiology,
Address: A11, Staff quarters,
Dhanalalshimi Srinivasan Medical College and Hospital, Siruvachur, Perambalur- 621212 Tamilnadu, India
Email: mavilla.anuradha@gmail.com

Abstract

Background: Tamil Nadu recorded more than one fourth of all dengue cases and deaths in the country the state recorded 9,249 dengue cases and 60 deaths in 2012. There is a need for specific, inexpensive dengue diagnostic tests that can be used for clinical management, surveillance and outbreak investigations and would permit early intervention to treat patients and prevent or control epidemics.
Material and methods: This cross sectional study was done by using MAC capture ELISA for IgM, IgG capture ELISA and NS1 antigen detection by sandwich ELISA during 2013 to 2014 for one year.
Results: Out of 151 suspected cases of dengue 60 were found to positive by using dengue ELISA kit. The incidence of dengue viral infection was 39.74%, whereas during 2012 to 2013 out of 162 samples 27 (incidence 16.66%) were positive in our hospital based on statistics as mentioned above.
Conclusion: The laboratory diagnosis of dengue virus infection by three ELISA methods along with clinical correlation with proper case definition had improved case detection as well as proper treatment in our hospital. Based on this study we can conclude that September, October, November, and December are the months of higher incidence of dengue virus infection.
Keywords: incidence, NS1 antigen

1. Introduction

Dengue has been known to be endemic in India for over two centuries.[1] Among 18 endemic states, the most affected regions are Delhi, West Bengal, Kerala, Tamil Nadu, Karnataka, Maharashtra, Rajasthan, Gujarat and Haryana.[2] Tamil Nadu recorded more than one fourth of all dengue cases and deaths in the country the state recorded 9,249 dengue cases and 60 deaths in 2012.[3] During 2014, there was an increase in dengue virus infections in Perambalur, Tamilnadu compared to 2013.

Clinical manifestations from Dengue viral infections include asymptomatic infection to dengue fever (DF) and the severe disease dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Usually dengue infections are asymptomatic or may include mild symptoms in the form of undifferentiated fever with or without rash. Typical DF is characterized by high fever, severe headache, myalgia, arthralgia, retro-orbital pain and maculopapular rash.[4] Some patients show petechiae, bruising or thrombocytopenia. The clinical presentation of acute dengue infection is non-specific. However 5–10% of patients progress to severe DHF/DSS that can result in death if unmanaged appropriately.[5,6] Approximately 3–4 days after the onset of fever, patients can present with petechiae, rash, epistaxis, and gingival and gastrointestinal bleeding. Other common manifestations include Pleural effusion and ascitis. Some patients develop circulatory failure (DSS), presenting with a weak and fast pulse, narrowing of pulse pressure or hypotension, cold and moist skin and altered mental state.[7]

Thus there is a need for specific, inexpensive dengue diagnostic tests that can be used for clinical management, surveillance and outbreak investigations and would permit early intervention to treat patients and prevent or control epidemics.[8] Present study was under taken to know the incidence of dengue virus infection by ELISA methods (IgM capture ELISA, IgG capture ELISA, NS1 Ag direct sandwich ELISA) along with clinical correlation that are used in our tertiary hospital for laboratory diagnosis of dengue.

2. Materials and Methods

This was a crosssectional hospital based study conducted during one year from March 2013 to February 2014 in a tertiary hospital, Perambalur. The laboratory diagnosis of each case of dengue virus infection was done by all three ELISA methods along with clinical correlation. The brief description of procedure of MAC capture ELISA for IgM, IgG capture ELISA and NS1 antigen detection by sandwich ELISA.[9, 10]In this study NS1 and IgM positive cases were taken as primary infection and both IgG and IgM positive cases were taken as secondary infection, and early clinical phase as detection of NS1 antigen upto 9 days of clinical onset of the disease.[11, 12]

2.1 NS1 Ag micro ELISA (J. Mitra & Co. Pvt. LTD. LOT: EDA061013)

A solid phase enzyme linked immunosorbant assay ELISA based on direct sandwich principle. The microwells are coated with anti dengue NS1 antibodies with high reactivity for NS1 antigen. The samples are added to the wells followed by addition of enzyme conjugate (monoclonal anti dengue NS1 antibodies linked to horse radish peroxidase. A sandwich complex is formed in the wells. Dengue NS1 from serum sample is sandwiched between the

antibody and antibody HRPO conjugate. Unbound conjugate is washed then washed off with buffer. The amount of bound peroxidase is proportional to the concentration of dengue NS1 antigen in sample. To limit the enzyme and substrate reaction, stop solution is added and a yellow colour develops which is finally read at 450 nm spectrophotometrically. Cut off value was calculated as mean O.D of calibrator multiplied by calibration factor. Interpretation of results was done as if NS1 antigen units was less than 9 negative, if 9-11 equivocal, and more than 11positive.

2.2 MAC capture ELISA for IgG / IgM. (J. Mitra & Co. Pvt. LTD. LOT: EDA061013)

Anti human IgM/IgG antibodies were coated on to microtiter wells. Specimens and controls are added and incubated.[13] Antibodies to Dengue if present in the specimen, will bind to the Antihuman IgM/IgG antibodies adsorbed onto the surface of the wells. The plate is then washed to remove unbound material. HRPO conjugated to conjugated Dengue antigen is added to each well. This Dengue antigen conjugate will bind to dengue specific IgM /IgG antibodies that were complexed with anti human IgM/IgG antibodies. Finally substrate solution containing chromogen and hydrogen peroxide is added to wells and incubated. The colour reaction is stopped by a stop solution. The enzyme substrate reaction is read by EIA reader for absorbance at a wavelength of 450nm.If the sample does not contain Dengue IgM/IgG antibodies then the enzyme conjugate will not bind. The solution in the wells may be colourless or only a faint background colour develops. The cut of value and interpretation were same as NS1 Ag micro ELISA.

This study obtained the information of age, sex, seasonal distribution and incidence of Dengue virus infection.

3. Results

In our present study out of 151 samples received 60 were reported as positive in the laboratory from clinically suspected cases of dengue. The incidence of dengue viral infection was 39.74%, whereas during 2012 to 2013 out of 162 samples 27 (incidence 16.66%) were positive in our tertiary hospital based on statistics as mentioned above.

Figure No 1. Month wise incidence of dengue

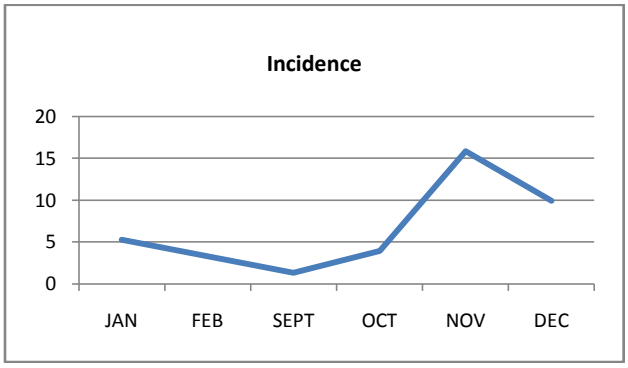


Figure no 2. Month wise dengue positive cases

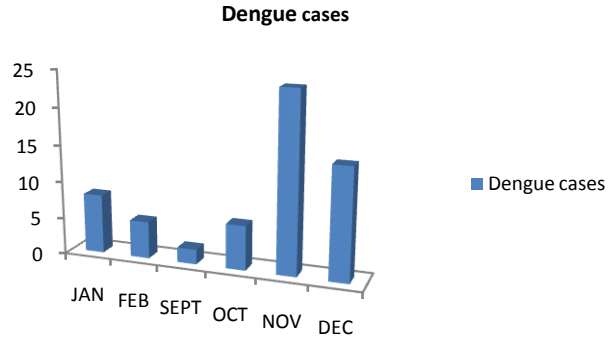


Table No 1. Distribution of dengue cases by diagnostic ELISA tests

Month	NS1 Positive	NS1 Total	IgM Positive	IgM Total	IgG Positive	IgG Total
Jan	5	13	6	13	2	13
Feb	4	10	1	10	2	10
Sept	0	10	2	10	2	10
Oct	4	17	4	17	4	17
Nov	18	53	8	53	9	53
Dec	12	48	2	48	3	48
Total	43	151	23	151	22	151

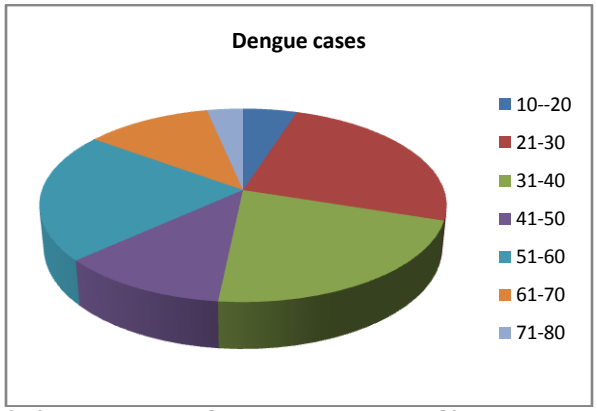
NS1 only positive dengue cases were 28 and 43 total NS1 positives. 6 only IgG positive and total IgG 22 positives. 4 were only IgM Positive and IgM total positives were 23. Both IgM and IgG positive were 12. All three tests positives were 4.

Table No 2. Clinical classification of dengue cases based on ELISA tests

Diagnostic tests	Primary dengue cases (IgM and NS1 positive)	Secondary dengue case. secondary dengue cases (both IgM andIgG positive)
Total NS1 positive	43	-
Only IgM positive	4	-
Only IgM &IgG positive	-	12

There were 47 primary dengue cases and 12 secondary dengue cases in our study.

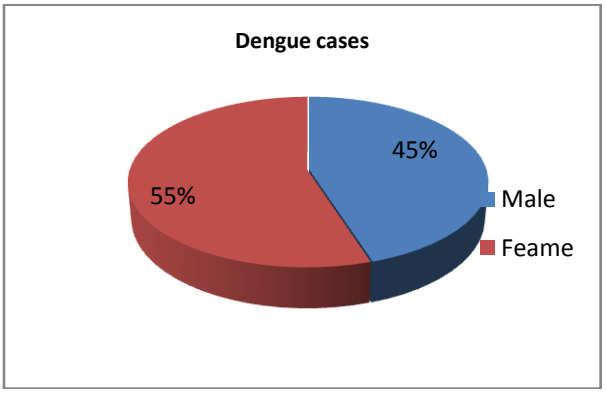
Figure no 3. Distribution of dengue cases according to age group in years.



Age wise distribution showed in 10-20 years age group, 3 cases were positive, 21-30 years 15 cases were positive, 13 cases were found to be positive between 31 – 40 years, 7 cases between 41 – 50 years, 13 cases were found to be positive between 51 -60 years, 7 cases were positive between 61 - 70 and only 2 cases were reported to be positive in 71-80years of age group.

Seasonal distribution shows that there is higher incidence of a Dengue during September, October, November, December months. No cases were tested for dengue during March, April, may, June, July, August. There were 27 (45%) males and 33(55%) females affected with dengue infection in the present study.

Figure no 3. Distribution of dengue cases according to sex



4. Discussion

Among available serological assays, capture IgM and/or IgG ELISA test are the most commonly used serological techniques for the diagnosis of dengue virus infection.[14] Capture IgM and IgG ELISA has many advantages, including that (i) a correct diagnosis is often possible for single serum sample (ii) the assay is able to measure IgM and IgG antibodies (IgM antibody is highly specific in the differentiation of flavivirus infection) (iii) the cross-reactivity of flavivirus-specific IgG antibodies measured by capture IgG ELISA is much less than that in the HI test, and (iv) full automation is possible. Thus, ELISA has become the most powerful assay for serodiagnosis due to its high sensitivity (99.5%) and specificity (100%) in the case of NS1 Ag MICROLISA and sensitivity (99.13%) and specificity (99.84%) for IgM/ IgG MICROLISA.[8]

The NS1 antigen was found circulating from the first day after the onset of fever up to day 9. The NS1 gene product is a glycoprotein produced by all flavi viruses and is essential for viral replication and viability. It is secreted by viral infected mammalian cells but not by insect cells. [15] A correlation has been made between disease severity and quantity of NS1 antigen in the serum. In present study along with clinical severity, NS1 sandwich ELISA positive and thrombocytopenia (platelet counts $\leq 30 \times 10^9$/L) taken for case identification.[16]

During the 2010 spurt in the incidence of dengue in New Delhi, simultaneous screening for NS1, IgM and IgG, platelet enumeration, was launched at the Sant Parmanand Hospital, a 140-bed tertiary care, multi-disciplinary, private hospital in Delhi which caters to populations in the national capital and adjoining townships.[11] As per our study the incidence of dengue infection is increased from 16.66% in 2013 to 39.74% in 2014. Clinical definition as Dengue positive cases with NS1 and IgM capture ELISA positive primary dengue infection case and positive cases with both IgM and IgG positive secondary dengue infection case further simplifies the case identification.[1]

Incidence of dengue infection increased from 16.66% in 2013 to 39.74% during 2014. It is correlating with Saini *et al*.[17] This can be taken as indirect suggestion to employ necessary vector control measures to prevent such an epidemic in future. Even though cross reactions occur with other Flavivirus IgM capture ELISA and IgG capture ELISA and NSI sandwich ELISA are sensitive and specific for diagnosis. In present study many positive cases diagnosed by NS1sandwich ELISA (cases 43), next being by IgM capture ELISA23, then by IgG ELISA 22. NS1 only positive were found to be 28. Primary dengue infection cases being (IgM and NS1 positive) 47. Secondary dengue infection (both IgM and IgG posive) 12 cases. Thus present study shows many of the cases were from 9 days after the onset of clinical signs and symptoms. Present study also shows that there is increase in case detection when all three tests along with platelet count and clinical correlation are made. Age distribution suggests 20-40 year aged males are most commonly infected.[17] There is also seasonal distribution most commonly during September, October, November and December many studies correlates this fact.

5. Conclusions

The laboratory diagnosis of dengue virus infection by three ELISA methods along with clinical correlation with proper case definition had improved case detection as well as proper treatment in our hospital. Based on this study we can conclude that September, October, November, and December are the months of higher incidence of dengue virus infection. Thereby there is need to include proper vector control measures during these months in local area by public health officials.

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