

Prevalence of Dengue Fever and Comparative Analysis of IgM and IgG Antibodies in Dengue Fever in Thoothukudi-Southern Coastal City, Tamil Nadu.

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ABSTRACT

Background: The most challenging problem associated with patient management in Dengue infection is early diagnosis. Secondary infection with dengue virus is the most accepted risk factor for the development of dengue haemorrhagic fever. Serologic diagnosis of dengue virus infection using ELISA of both IgM and IgG distinguishes primary and secondary infections. Aim: To determine the prevalence of Dengue Fever in Thoothukudi, the coastal district of Tamilnadu by serological tests IgM ELISA and IgG ELISA, to compare the IgM and IgG status of the dengue cases to differentiate between the primary and secondary dengue cases. **Methods:** A cross sectional comparative study in patients with fever suspicious of dengue, fevers with other causes excluded. Detailed history is obtained and complete clinical examination done. IgM and IgG Elisa test were performed. **Results:** Out of 50 patients, 28 (56%) were positive for dengue and 22 (44%) were negative. 13 cases had primary Dengue (IgM positive) and 15 had secondary Dengue (IgM and IgG positive and IgG positive). Among the 28 positive cases 16 are paediatric, 10 cases had hemorrhagic manifestations, all were secondary dengue. **Conclusion:** As this study was conducted during an outbreak in Thoothukudi, unusually high prevalence is seen particularly among the pediatric ages. Higher morbidity is seen in secondary dengue cases. Thus, early discrimination of primary and secondary dengue helps to reduce the morbidity and mortality.

Keywords: Dengue, Secondary dengue, IgG and IgM Elisa, Thoothukudi.

INTRODUCTION

The global prevalence of dengue has grown dramatically in recent decades. An estimated 50-million dengue infections occur annually and approximately 2.5 billion people live in dengue endemic countries. Some 1.8 billion (more than 70%) of the population at risk for dengue worldwide live in member states of the WHO South-East Asia Region and Western Pacific Region, which bear nearly 75% of the current global disease burden due to dengue.^[1] Among these infections, approximately 250,000–500,000 cases are dengue haemorrhagic fever (DHF), with 24,000 deaths that mostly occurred in children.^[2,3]

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One of the most important reasons for the increase in cases is most likely caused by rapid development and urbanization, which provide breeding sites for *Aedes aegypti*. Therefore, the emerging pattern and

the increasing trend in the incidence of dengue infection are of great concern as there is no specific treatment of dengue, and most forms of therapy are supportive in nature. Furthermore, a licensed vaccine is not available yet.

Dengue virus (DEN) belong to the genus Flavivirus is a small single-stranded RNA virus comprising four distinct serotypes (DEN-1 to 4), which can cause illnesses in humans ranging from the self-limiting to the life-threatening dengue haemorrhagic fever and dengue shock syndrome (DHF/DSS). Severe plasma leakage can lead to shock, with the mortality rate for untreated patients varying between 10% and 20% but can reach as high as 40% with the involvement of shock.^[4] Therefore, early diagnosis of dengue can reduce the morbidity and mortality. "Asian" genotypes of DEN-2 and DEN-3 are frequently associated with severe disease accompanying secondary dengue infections.^[5-7] Although secondary infection with dengue virus is the most widely accepted risk factor for the development of dengue hemorrhagic fever, there is no simple and reliable method that can routinely be used to discriminate between primary and secondary infections in the early days of an infection. The most challenging problem associated with patient

management in Dengue infection is early diagnosis. The IgM capture ELISA available will show a positive report only when tested after 5-7 days. Though parallel IgG testing can help in diagnosing secondary infections. Hence, serologic diagnosis of dengue virus infection using a commercial capture ELISA of both IgM and IgG distinguishes primary and secondary infections is preferred. ELISA is a simple, reliable and cost effective method in the diagnosis of dengue infection. The disease has seen a recent outbreak in the southern coastal regions of Tamilnadu. There are only a few studies in Tamilnadu. Government medical college hospital, Thoothukudi, being a tertiary care hospital and the district headquarters hospital provides the ideal setting for the conduction of this study.

Aim

To determine the prevalence of Dengue Fever in Thoothukudi, the coastal district of Tamilnadu by serological tests IgM ELISA and IgG ELISA, to compare the IgM and IgG status of the dengue cases to differentiate between the primary and secondary dengue cases.

MATERIALS AND METHODS

Clearance and permission of institutional ethical. This is a Cross-sectional study comparative study performed in June 2013 and July 2013 in a tertiary care hospital, Thoothukudi Government Medical College Hospital, Thoothukudi.

A total sample size of 50 patients with fever suspicious of dengue both out patients and inpatients who are attending the Government medical college hospital, Thoothukudi are included in this study. The subjects included in this study were patients with fever more than 5 days and other associated symptoms like headache, joint pains, purpuri crashes and reduction in platelet count. Patients with fever due to other causes like malignancy and autoimmune diseases were excluded from this study. Detailed history is obtained from all the patients and complete clinical examination is done. Microbiological test IgM Elisa and IgG Elisa were done.

RESULTS

A total sample size of 50 patients with fever suspicious of dengue both outpatients and inpatients who are attending the Government medical college hospital, Thoothukudi are included in this study. The age of the patients attended was from 0-70 years. Patients with fever and other associated symptoms like headache, joint pains, purpuric rashes and reduction in platelet count were noted. During the study period, the total number of samples screened was 50, 28 (56%) were positive for dengue and 22 (44%) were negative. [Figure 1]

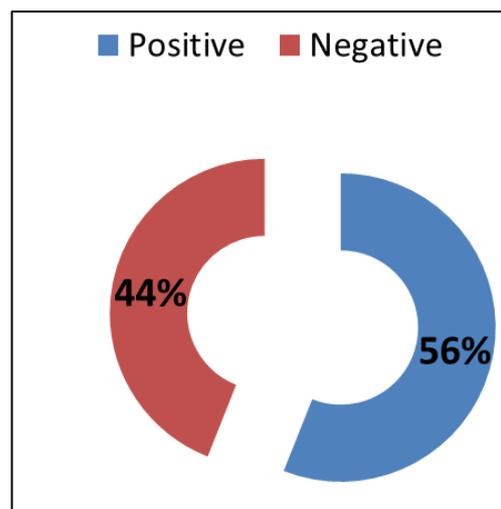


Figure 1: Prevalence of Dengue.

Of the 50 cases screened 27 (54%) were males, 23 (46%) were females showing mild difference in the sex distribution. Out of the dengue positive cases 13 (46%) were males and 15 (54%) of the positive were females. Among the cases tested for dengue by IgM and IgG Capture ELISA, 13 (26%) positive for IgM antibodies only, 3 (6%) were positive for only IgG antibodies and 12 (24%) were positive for both IgM and IgG antibodies [Table 1].

Table 1: Comparative Analysis of IgM and IgG Antibodies in Dengue Fever.

Clinical cases	Dengue positive		
	IgM	IgG	IgM+IgG
Number of cases			
50	13(26%)	3 (6%)	12(24%)

From the comparative Analysis of the serological tests it was found that 13 cases had primary Dengue (IgM positive) and 15 had secondary Dengue (IgM and IgG both positive 12 and IgG only positive 3). [Figure 2].

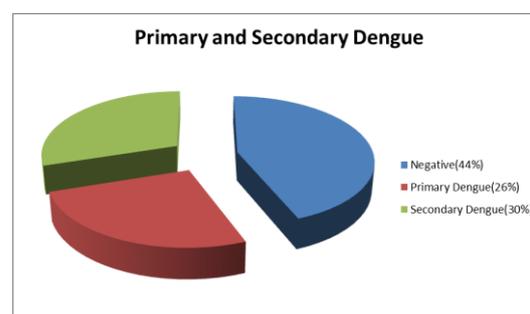


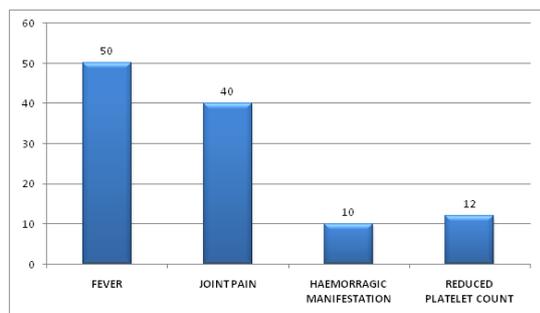
Figure 2: Distribution of study patients in dengue.

Among the age group, positivity was significantly high in 0-10 year age group. All other age group has more or less equal distribution [Table 2].

By clinical evaluation, ten cases had haemorrhagic manifestations including petechiae, gum bleeding and epistaxis. A point to be noted is all the 10 cases are found out to be secondary dengue [Figure 3].

Table 2: Age distribution of Dengue positive cases.

Age group	IgM Elisa Positive	IgG Elisa Positive	IgM+ IgG Elisa Positive	Total
0-10	11	1	4	16
11-20	-	1	2	3
21-30	-	-	2	2
31-40	-	-	3	3
41-50	1	1	-	2
Above 50	1	-	1	2
Total	13	3	12	28

**Figure 3: Signs and Symptoms observed in dengue cases.**

DISCUSSION

Of the 50 cases screened 27 (54%) were males, 23 (46%) were females showing mild difference in the sex distribution. Out of the dengue positive cases 13 (46%) were males and 15 (54%) of the positive were females. Among the age group, positivity was significantly high in 0-10 year age group in our study [Table 2]. This is comparable with Krishnasamy K et al^[13] that among the paediatric age group, positivity was significantly high in 6-12 year age group followed by 13-18 year age group. By clinical evaluation, out of 28 positive cases, ten cases had haemorrhagic manifestations in our study. Krishnasamy K et al^[9] showed in her study that Dengue fever was seen in 43 cases, eighteen cases had haemorrhagic manifestations.

Khoa T.D. et al^[10] analysed epidemiological information in Southern Vietnam, i.e., age-specific seroprevalence and a community-wide longitudinal study of clinical dengue attack. Using the seroprevalence data, the force of infection was estimated to be 11.7% per year. As our study was conducted during an outbreak in Thoothukudi, an unusually high prevalence is seen. Out of the 50 suspected cases, 28 cases were found to be dengue positive, indicating about 56% were positive. This can also be attributed to the fact that the cases were carefully selected based on clinical evaluation.

According to Ashwini Kumar et al^[11] 2010a record-based descriptive study was undertaken to determine the clinical profile and outcome of all patients admitted to a tertiary care teaching hospital at Manipal, Karnataka (Kasturba Hospital) with a diagnosis of dengue, DHF and DSS according to WHO protocol from January 2002 to December

2008. All standardized dengue enzyme linked immunosorbent assay (ELISA) IgM antibody positive (Pan Bio Kit - Australia) cases were included in the study. Haemorrhagic manifestations included petechiae 84 (67.2%), ecchymosis 29 (6.2%), gum bleeding 24 (5.2%), hematuria 23 (4.9%), melena 22 (4.7%), hematemesis 15 (3%), and epistaxis 12 (2.6%). In our study, among the 50 cases which were tested, 10 cases had haemorrhagic manifestations including petechiae, gum bleeding and epistaxis. A point to be noted is all the 10 cases are found out to be secondary dengue.

Detection of specifically elevated IgG antibodies to dengue virus by ELISA is a valuable diagnostic tool, for identification of secondary dengue infections where the occurrence of complications in patients is higher. According to WHO publication 1997 secondary dengue infection is characterized by high IgG, which may be accompanied by elevated IgM levels. The Panbio Dengue IgG antibodies to Dengue infection above this threshold. The assay does not detect low level IgG antibodies from past exposure typically present in many individuals from endemic regions. According to Hawkes et al early convalescent stage IgM levels are significantly lower in secondary infections than in primary ones and may be undetectable in some cases. In our study from the IgM and IgG ELISA, it was found that 13 cases had primary Dengue (IgM positive) and 15 had secondary Dengue (IgM and IgG both positive 12 and IgG only positive 3).

Similar studies were done by Ratho et al^[12] 2005, an outbreak of dengue fever was investigated in a peri-urban slum area of Chandigarh, India, during September to December, 2002. Of 218 acute phase sera tested, 58 samples were positive for dengue specific IgM antibody. Thus, the presence of dengue specific IgM antibodies in acute phase serum samples comprised the total of 76 (35%; 76/218) acute dengue confirmed cases. Amongst them, the presence of dengue specific IgG antibodies in 55 patients (72.4%) further categorized the cases in to secondary dengue infection.

Sankari et al 2012 worked at Chandel district in Manipur State, India, where the epidemic started in September 2007 with the appearance of suspected cases and reached a peak by December 2007, followed by another peak in June 2008. The minimum and maximum ages of the suspected cases were 7 and 77 yr, respectively. *Antibody detection and serotyping*: Of the 42 and 16 samples tested for IgM anti-dengue antibodies, 25 and 2 were positive, respectively.^[13]

Since our study was conducted during June-July 2013 during an outbreak, out of 50 samples tested 13 were only IgM positive, 12 were both IgM and IgG positive. According to Pei- Yun Shu et al, capture IgM and IgG ELISA has become the most powerful assay for serodiagnosis due to its high sensitivity, specificity and simplicity. The results shown in this

study of the comparison of the HI test with the capture IgM/IgG ratio support the current trend towards using capture IgM and IgG ELISA to differentiate primary and secondary infections .A total of 103 serum samples collected between days 3 and 30 after the onset of symptoms were analyzed for HI and capture IgM/IgG ratio. Good correlation was found, with a result concordance of 89.4%.^[14]

CONCLUSION

As this study was conducted during an outbreak in Thoothukudi, an unusually high prevalence is seen. This can also be attributed to the fact that the cases were carefully selected based on the clinical evaluation. Thus study indicates a slightly higher prevalence among the female section of the population. Early discrimination of primary and secondary dengue helps to reduce the morbidity and mortality.

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