Original Research Article

Seroprevalence of Dengue Virus in a Tertiary Care Hospital

S.Shazia Parveen*, S.Madhavi and V.Sharada

Department of Microbiology, Bhaskar Medical College and Hospital, Yenkapally, Moinabad,
R.R.District, Telangana State-500075 India
*Corresponding author

A B S T R A C T

K e y w o r d s	Dengue fever, also known as break bone fever, is a serious mosquito-borne viral infection affecting tropical and subtropical countries. It is caused by dengue virus belonging to family Flaviviridae, having four serotypes that spread by the bite of infected Aedes mosquitoes**.** It causes a wide spectrum of illness from mild asymptomatic illness to severe fatal dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) The present study was conducted to diagnose the dengue infection by detecting dengue NS-1 antigen and differential detection of dengue specific IgM and IgG antibodies and to know the seroprevalence of Dengue virus in a tertiary care hospital, R.R.dst. Dengue NS1 and IgM and IgG testing was done by immunochromatography and all positives were confirmed by ELISA. A total number of 164 samples were processed in the laboratory during a period of one year and 36 samples tested positive for dengue infection. Among them 10 were positive for NS1 antigen only, 4 tested positive for both NS1 and IgM, 10 tested positive for IgM, 6 for only IgG and 6 were positive for both IgM and IgG. As dengue related mortality and morbidity is more common, this study was conducted to assess the magnitude of dengue virus infection.
Dengue fever, NS - 1 protein, IgM and IgG antibodies – seroprevalence Rapid diagnostic test	

Introduction

Dengue virus infection is one of the most important human arboviral infection (World Health Organization). The worldwide incidence of dengue fever (DF), dengue haemorrhagic fever (DHF) and Dengue shock syndrome (DSS) has increased dramatically in recent decades (Geneva (2005) WHO report). Globally, it has been estimated that atleast 2.5 billion people are at risk of Dengue, and approximately 975 million of these live in urban areas of the tropical and subtropical countries of southeast Asia (WHO & Guzman MC et al).

Dengue is an acute mosquito-borne viral infection with potential fatal complications. It is transmitted mainly by *Aedes aegypti* mosquito and also by *Aedes albopictus* (Lambrechts Let al and Reiter P).

It is also called as "break bone fever" because of the symptoms of myalgia and arthralgia. Dengue viruses belong to family *Flaviviridae* and there are four antigenically distinct serotypes of the Dengue viruses referred to as (DENV-1-4) that can cause human infections (Gulber DJ).

All four serotypes can cause the full spectrum of disease from a subclinical infection to a mild self limiting disease, the dengue fever (DF) and a severe disease that may be fatal, the dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS).

The largest Dengue outbreak in India which occurred in 1996, was due to DENV-2. (Dar L et al) This was later replaced by DENV-3, as the dominant serotype in 2003.(Dash PK et al) The subsequent outbreaks witnessed a mixed type of infection with DENV-1, 2 and 3.(Dar L et al Himani Kukreti et al.).

Dengue virus is an enveloped, positive stranded RNA virus, and is composed of three structural protein genes that encode for nucleocapsid or core protein(C), a membrane – associated protein(M) an envelope protein (E), and seven non structural (NS) protein genes including NS 1protein. Among the non-structural proteins, NS 1 is highly conserved glycoprotein which appears essential for virus replication; no precise function has yet been assigned to it (Lata R. Patel).

Although NS1 antigen can be detected from as early as one day post onset syndrome, it is positive only upto 18 days (CDC).

Two patterns of immune response are distinguished : primary and secondary. Persons never previously infected with a flavivirus, nor immunized with a flavivirus vaccine, mount a primary antibody response when infected with dengue virus. The dominant immunoglobulin isotype is IgM. Anti-dengue IgM detectable by IgM capture ELISA appears in half of the patients with a primary infection while they are still febrile; in the other half, it appears within 2-3 days of defervescence. Anti-dengue IgG appears shortly afterwards (WHO).

The physiological definition of a primary infection is the one characterized by a high molar fraction of anti-dengue IgM and a low molar fraction of anti-dengue IgG. In contrast to primary infection, secondary infection with dengue virus results in the appearance of high levels of anti-dengue IgG before, or simultaneously with, the IgM response. Once detected IgG levels rise quickly, peak about 2 weeks after the onset of symptoms and then decline slowly over 3-6 months. The physiological definition of a secondary infection one characterized by a low molar fraction of anti-dengue IgM and a high molar fraction of IgG.(WHO).

This study was conducted to diagnose the Dengue virus infection and to know the seroprevalence of infection in and around Rangareddy district, so that early diagnosis of dengue virus infection is important for treatment and prevention of complications like dengue shock syndrome (DSS) and dengue haemorrhagic fever (DHF).

Materials and Methods

This study was carried out in the department of microbiology, Bhaskar Medical College and Hospital, Yenkapally, Moinabad for a period of one year (from october 2014 to october 2015) among clinically suspected dengue patients irrespective of their age and sex. The clinical diagnosis of dengue virus infection was based on the WHO definitions.(WHO) Probable dengue fever (DF) is defined as acute febrile illness with two or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations, and leucopenia.

A total of 164 serum samples were obtained and tested by a rapid qualitative immunochromatographic assay (J.Mitra and Co Pvt Ltd, Dengue Day 1 test) for

differential detection of dengue specific IgM and IgG antibodies and NS1 antigen.The dengue NS 1 antigen and antibody rapid test is an in-vitro immunochromatographic one-step assay designed for the qualitative determination of dengue virus NS1 antigen and IgM and IgG antibodies in human serum or plasma for the diagnosis of dengue infection. This test is intended as an aid in the earlier diagnosis of Dengue infection and presumptive diagnosis between primary and secondary dengue infection.

The procedure and interpretation of test results of the rapid Immunochromatographic assays were carried out according to the manufacture's literature guidelines.(J.Mitra Co Pvt. Ltd) The results were graded as reactive (visible band) or non reactive (no band). All the positive samples were confirmed by ELISA.

Results and Discussion

A total of 164 samples were tested for Dengue NS1 and IgM and IgG antibodies. Among them 36 samples tested positive. 10 (27.77%) for only NS1, 4(11.11%) samples were positive for NS1 and IgM, and 10(27.77%) samples were positive for IgM, 6(16.66%) positive for both IgM and IgG, 6(16.66%) positive for only IgG. Among 164 patients, 96 were males and 68 were females. More number of seropositive cases were observed in the age group below 15 years.(Table1).

24 (67%) of the patients had primary infection and 12(33%) had secondary infection out of the 36 cases. Among these 36 cases, NS1 alone was detected in two patients (6%) who came within 1-2 days of illness and IgM in seven patients (19%) who came on 3-5 days of illness (Table 2). In the present study out of 164 samples 27.77%

were NS1 and IgM positive separately which indicated recent infection. Only 16.66% were positive for IgG which indicated past infection with dengue. These findings are in accordance with the study of Sanghamitra Padhi et al(12).

In this study NS1 antigen detection was highest during days 3 to 5 which can be comparable with the other studies.(LR Patel, Hitesh Bhabhor et al)

Males (58.53%) were more affected than females and this was correlated with the studies of Hitesh Bhabhor et al, B. Arunasree et al.

Most of the patients affected in the present study were children (<15Years) as comparable to the study of P. Gunasekhar et al from Chennai. Another study conducted in Kanpur also showed 0-15 year's age group to be commonly affected.(Garg A et al) 24(67%) of the patients had primary infection and 12(33%) had secondary infection out of the 36 cases. Differentiation of primary and secondary dengue infection is essential to reduce morbidity and mortality due to DHF/DSS, particularly in children and elderly. Hence, this distinction is useful in prognostic and epidemiological purpose. Shu PY et al., Of the 36 cases, NS1 alone was detected in two patients (6%) who came on 1-2 days of illness. Datta *et al.*, also reported that NS1 is detectable from day 1 of fever both in primary and secondary infections.

So in the present study out of 164 cases with clinical symptoms of dengue fever, dengue IgM positivity was seen in 27.77% of cases which indicates active dengue virus activity. Males were more affected (58.53%). Age group most commonly affected were children between 1-15 years (55.55%).

Table.1 Age Wise Distribution of NS1 and IgM and IgG Positive Cases

Age(years)	NS1 positive cases	IgM positive cases	IgGpositive cases	NS1 and IgM positive cases	IgM and IgG positive cases	Total (%)
1 - 15	5	5	3	3	4	20 (55.55)
16 - 30	3	3	1	0	1	8 (22)
31 - 50	2	1	1	1	0	5 (13)
Above 50	0	1	1	0	1	3 (8)
Total	10	10	6	4	6	36

Table.2 Primary and Secondary Dengue Based on Markers

Markers	Days				Total	Primary infection(%)	Secondary infection(%)
	<2	3-5	6-7	>7			
NS1	2	7	1	-	10	10(28)	-
IgM	-	5	4	1	10	10(28)	
IgG	-	-	3	3	6		6(16.5)
NS1 and IgM	-	2	2	-	4	4(11)	
IgM and IgG	-	-	2	4	6		6(16.5)
Total	2	14	12	8	36	24(67)	12(33)

This study reported seroprevalence more in children so early diagnosis and timely intervention may reduce the risk of complications such as DHF and DSS. Differentiation of primary and secondary dengue infection is essential to reduce morbidity and mortality due to DHF/DSS, particularly in children and elderly. This study thus emphasizes the need for continuous sero epidemiological surveillance for the timely formulation and implementation of effective dengue control programme.

References

Arunasree, B., Prasad Uma, B. Rajsekhar. "Epidemiology of Dengue Fever in Srikakulam District, Andhra Pradesh". Journal of Evolution of Medical and Dental Sciences 2015; Vol. 4, Issue 27, April 02; Page: 4599-4604,

CDC Dengue Guidelines and Documents

Dar L, Broor S, Sengupta S, Xess I, Seth P. The first major outbreak of DHF in Delhi, India. Emerg Infect Dis. 1999 Jul-Aug; 5 (4):589–90.

Dash PK, Saxena P, Abhyankar A, Bhargava R, Jana AM. Emergence of Dengue virus type-3 in northern India. Southeast Asian J Trop Med Public Health. 2005Mar; 36(2): 370–77.

Dar L, Gupta E, Narang P, Broor S. Cocirculation of Dengue sero-types, Delhi, India, 2003. Emerging Infectious Diseases. 2006 Feb; 12: 352–53.

Datta S, Wattal C. Dengue NS1 antigen detection: A useful tool in early diagnosis of dengue infection. Indian J Med Microbiol 2010;28:107-10.

Guzman MC, Halstead SB, Artsob H, Buchy P,Farrar J, et al. Dengue: a continuing global threat.Nature Reviews. 2010:S8–16.

Gulber DJ. Dengue and Dengue hemorrhagic fever. Clin Microbiol Rev.1998 Jul; 11(3):480–96.

Gunasekaran P, Kaverik, Mohana S, Arunagiri K, Babu BVS, Priya PP et al. Dengue disease status in Chennai (2006-2008).A retrospective analysis. Indian J Med Res 2011; 133: 322-5.

Garg A, Garg V. Rao YK. Upadhyay GC, Sakhuja S. Prevalence of dengue among clinically suspected febrile episodes at a teaching hospital in North India.J.Infest.Dis.Immun.2011; 3: 85-9.

Himani Kukreti, Paban K Dash, Manmohan Parida, Artee Chaudhary, Parag Saxena, RS Rautela, et al. Phylogenetic studies reveal existence of multiple lineages of a single genotype of DENV-1 (genotype III) in India during 1956–2007. Virology Journal. 2009;6:1.

Hitesh Bhabhor, Nirmal Brahmbhatt, Ullas Machhar, Anoop Singh. Profile of dengue cases admitted to a Medical College hospital in Western India.2014; 4 (3): 235.

J.Mitra and Co Pvt. Ltd. Dengue Day 1 Test.

Lambrechts L, Scott TW, Gubler DJ. Consequences of expanding global distribution of *Aedes albopictus* for dengue virus transmission. *PLoS Negl Trop Dis* 2010; *4* : e646.

Laboratory Guidance and Diagnostic Testing. Centers for Disease Control and Prevention. Available from: *http://www.cdc.gov/dengue/clinicallab/laboratory.html*, accessed on July 20, 2013

LR Patel Sero prevalence of Dengue NS-1 Antigen in Tertiary care hospital, Ahmedabad Indian Journal of Basic & Applied Medical Research; June 2013: Issue-7, Vol.-2, P. 694-701

Reiter P.. *Aedes albopictus* and the world trade in used tires, 1988-1995: the shape of things to come? *J Am Mosq Control Assoc* 1998; *14* : 83-94.

Sang CT, Hoon LS, Cuzzubbo A, Devine P.Clinical Evaluation of a rapid immunochromatographic test for the diagnosis of Dengue Virus Infection. Clinical and Diagnostic Laboratory Immunology, May 1998; 5(3): 407-409.

Sanghamitra Panda, Muktikash Dasu, Pritilata Panda, Banojini Panda, Indrani Mohanty, Susmita Sahu and M. V. Narasimham. A three year retrospective study on increasing trend in seroprevalence of dengue infection from Southern Odisha, India. Indian J Med Res.2014; 140: 660-664.

Shu PY, Huang JH. Current advances in dengue diagnosis. Clin Diagn Lab Immunol 2004;11:642-50.

Shu PY, Chen IK, Chang SF, Yuch YY, Chow L, Chien LJ, *et al*. Comparison of capture immunoglobulin M (IgM) and IgG enzyme-linked immunosorbent assay (ELISA) and nonstructural protein NS1 serotype-specific IgG ELISA for differentiation of primary and secondary dengue virus infections. Clin Diag Lab Immunol 2003;10:622-30.

World Health Organization. Dengue Haemorrhagic Fever: Diagnosis, Treatment, Prevention and control. 2nd ed. Geneva: World Health Organization; 1997.

World Health Organization, Geneva (2005) WHO report on global surveillance of epidemic prone infectious diseases.