Original Research Paper Microbiology

A COMPARATIVE STUDY ON NS1 ANTIGEN DETECTION IN ACUTE DENGUE INFECTION BY RAPID DIAGNOSTIC TEST AND ELISA IN A TERTIARY CARE HOSPITAL IN KANCHIPURAM.

Dr.Geetha*	Post Graduate, Department of Microbiology, Meenakshi Medical College and Research institute , Kanchipuram, Tamil Nadu, India. *Corresponding Author
Dr. K.Muthulakshmi	Professor and Head, Department of Microbiology, Meenakshi Medical College and Research institute , Kanchipuram.
Dr. Nagajothi	Post Graduate, Department of Microbiology, Meenakshi Medical College and Research institute , Kanchipuram, Tamil Nadu, India.
Dr. Nisha Tiwari	Post Graduate, Department of Microbiology, Meenakshi Medical College and Research institute , Kanchipuram, Tamil Nadu, India.

ABSTRACT

Background and objectives: Dengue cases in India are increasing in epidemic proportions. Diagnosis of acute Dengue infection is important for clinical care , implementing control measures, surveillance and research. Virus isolation and viral nucleic acid detection requires specialized laboratory equipments and expertise. For resource limited labs test like rapid Immuno chromatography test (ICT), based or ELISA based tests for detection of NS_1 antigen can be used.

Methods: The acute phase serum samples of 100 patients presenting with Dengue fever were subjected to NS_1 antigen detection by ICT and ELISA simultaneously.

Results: Among the 100 patients, 58 patients positive by ELISA and 28 patients positive by ICT. The sensitivity, specificity, positive predictive value and negative predictive value of the rapid ICT tests for NS_1 antigen were 48.28%,100%,100% and 58.33% respectively .

Interpretation and conclusion: NS_1 antigen detection by ICT is less efficient than ELISA due to its less sensitivity and less negative predictive value. So its better to do ELISA directly in diagnosing Dengue infection within three days of onset of fever.

KEYWORDS : Dengue, NS_1 antigen, ELISA, Immunochromatography test.

INTRODUCTION:

Dengue fever is a life threatening infection and mimics various diseases such as Chikungunya, Malaria, viral infection, urinary tract infections, Typhoid, Leptospirosis, Scrub typhus etc. Hence prompt LAB diagnosis in the early management of cases is necessary. Since Dengue virus was first isolated in India in the year 1945 and is endemic in both urban and semi-urban areas. Dengue fever has struck again in India and cases of dengue fever (DF) / dengue haemorrhagic fever (DHF) have been reported from various parts of the country during the last 4 decades[4]. It has high mortality and morbidity, early and accurate diagnosis is needed. The first evidence of occurrence of Dengue fever was reported in 1956 from vellore district in Tamilnadu. Over the last five years , 22,584 Dengue cases were reported from Tamilnadu and the number of cases varied from year to year. Dengue virus, belonging to the genus Flavivirus and Family Flaviviridae, are mosquito borne viruses and the principal vector, Aedes aegypti is a day-biting mosquito of public importance that breeds in natural or artificial waters[8].

Dengue illnesses are caused by any one of the four serologically related viruses designated as DENV-1, DENV-2, DENV-3 and DENV-4[1]. Infection with any one of these serotypes mostly causes a mild, self-limiting febrile illness (Classical Dengue fever), however, a few cases develop severe life threatening Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS)[1]. Recently, an Rapid Diagnostic Test (RDT) test for early diagnosis of dengue infection is Dengue NS1 antigen detection. NS1 is a glycoprotein produced by all flaviviruses and is essential for viral replication and viability[9]. Because this protein is secreted into the blood stream, many tests have been developed to diagnose DENV infections using NS1. These tests include antigen-capture ELISA, lateral flow antigen detection and measurement of NS1-specific IgM and IgG responses[10]. In the present study, we evaluated two diagnostic tools for acute Dengue virus infection. An Immunochromatography lateral flow card test (RDT), an Enzyme Linked Immunoassay(ELISA) for detecting Dengue virus NS1 antigen in human serum

MATERIALS AND METHODS:

The present study was carried out in the Department of Microbiology of MMCH&RI, Kanchipuram.

STUDY DESIGN: This Cross-sectional study.

STUDY PERIOD: MARCH 2016 _ MARCH 2017

SAMPLE SIZE: Blood samples from 100 patients with clinical features suggestive of Dengue infection as per CASE DEFINITION were included in this study.

INCLUSION CRITERIA: All patients with clinical diagnosis suggestive of DF/DHF/DSS (CASE DEFINITION) of all groups were included in the study.

EXCLUSION CRITERIA: Patients with clinical evidence of urinary tract infection, pneumonia, abscess or any other apparent cause of fever due to long term illness (TB) were excluded.

SOURCE OF SAMPLE: The patient's Blood samples were collected aseptically from fever clinic and from in-patients with features suggestive of Dengue infection. The serum was separated by centrifugation technique. The ICT ,ELISA was done in the samples and was stored at -70 C in the laboratory.

RESULTS: A total of 100 patients presenting with clinical features of dengue infection were selected for the study.

Table-1: sex distribution

Sex	Distribution – Total 100 numbers	
	Number	Percentage
Male	58	58%
Female	42	42%
Total	100	100

TABLE.2. AGE DISTRIBUTION

AGE	Distribution – Total 100 numbers	
	Number	Percentage
< 30 Years	56	56%
31–40Years	16	16%

41-50 Years	11	11%
51-60 Years	13	13%
>60 Years	4	4%
Total	100	

TABLE-3: CLINICAL PROFILE

Particulars	Presenting Complaints – Total 100 numbers	
	Number	Percentage
Fever	100	100%
Headache	87	87%
Myalgia	77	77%
RetroorbitalPain	66	66%
Pain Abdomen	52	52%
Rash	20	20%
Arthralgia	37	37%
Bleeding Manifestation	5	5%
Vomiting	22	22%
Diarrhea	13	13%

Ns1 Antigen by RDT (Immuno chromatography Card Method

Findings	Distribution – Total 100 numbers	
	Number	Percentage
NS1 Positive	28	28%
NS1 Negative	72	72%
Total	100	100%

Ns1 ANTIGEN DETECTION BY ELISA METHOD

Findings	Distribution – Total 100 Samples	
	Number	Percentage
NS1 Positive	58	58%
NS1 Negative	42	42%
Total	100	100%

COMPARING RDT WITH ELISA

	RDT	ELISA
Cost	Rs.150	Rs. 600
Equipment	No equipment needed	ELISA, washer, reader, fridge
Technical expertise	No	Needed
Turnaround time	30mn- 2hrs	6hrs-24hrs
Batching	Not required	Required
Efficiency	50-60% sensitive 90-100% specific.	90-100% sensitive & specific.

DISCUSSION:

Dengue has been increasingly recognized as an emerging infectious disease for the last four decades. The global burden of Dengue has grown dramatically in recent years. The high prevalence of Dengue cases at Kanchipuram in the recent years, makes it necessary to evaluate the incidence of Dengue and to find out the serology positivity of Dengue cases. This study was done with 100 serum samples from patients with clinical symptoms suggestive of Dengue. In the present study, we looked into the worth of Dengue virus NS1 antigen detection and Dengue group specific RT-PCR for diagnosing Dengue cases in acute phase of illness.

The incidence of Dengue fever in this study, over a period of one year (from March 2016 to March 2017) was 58% (Table-5), among fever cases attending the fever clinic and those admitted in our hospital.In this study, an increased incidence of Dengue cases was found among male patients 58(58%), as compared to females 42 (42%) (Table-2). In the study done by Nadeem Sajjad Raja et al in 2006, they observed an incidence of 51.55% in males and 48% in females58, and in the study by Kurukumbi et al in 2001 it was observed that the incidence was 61.5% in males and 38.5% in females.

A higher distribution of Dengue cases in the present study was seen in the (Table-2) <30 year age group 56(56%), followed by 31-40 year age group 16(16%), the age between 41 – 50 years age group 11(11%) and above age 50 years age group (13%). This was similar to the study conducted by Preeti Bharaj et al in 2008, in which the common age group involved was 20-40 years (35.4%), followed by 0-20 years group (20.8%). Ekta gupta et al, in 2006, in her work also showed that the maximum number of cases in a 3 year study period was seen in the 21-40 year age group.Acute Dengue infection is a major health problem in India. It has risen to epidemic proportions and is endemic to many areas, both urban and rural. In our study highest prevalence was seen in the age groups between 11-50 years and with male preponderance which is seen in other studies also (Gupta et al., 2006; Chakravathi et al., 2005; sarkar et al 2012). In comparison with RDT and ELISA our study show 100% specificity and 48% sensitivity with Confidence Interval 95% (34-60%). Even though the test has various advantages like lesser turn over time, less technical support, does not require batching, this test has a main disadvantage of lesser sensitivity – according to our study[6].

REFERENCES:

1. Bailey and Scott's Diagnostic Microbiology, Twelfth Edition 2007, Betty A Forbes, Daniel F.Sahm, Alice S.Weissfeld. Chapter 8, p 120-146.
2. BIOLINE Dengue SD Duo Dengue NS1 Ag + Ab Combo Kit. Available from: URL:http://e-dok.rm.dk/e-dok/e_702615.nsf /$CXIV/ATTACH-RMAP-8Q5G79/$FILE/Dengue%20Diagnosis-EN%20%28R1010%29.pdf. Access Date: 24.06.2013.
3. Datta S, Wattal C. Dengue NS1 antigen detection: A useful tool in early diagnosis of dengue virus infection. Indian J Med Microbiol 2010;28:107- 10.
4. Dengue Diagnostics: proceedings of a joint TDR/WHO and PDVI workshop, 4-6 October 2004, Geneva, Switzerland.
5. Gurukumar KR, Priyadarshini D, Patil JA, Bhagat A, Singh A, Shah PS, et al. Development of real time PCR for detection and quantitation of dengue viruses. Virol J 2009; 6: 10.
6. Kabra SK,Juneja R, Madhulika, et al. Myocardial dysfunction in children with dengue haemorrhagic fever. Natl Med J India. 1998;11:59.
7. National guidelines for clinicl manifestation of Dengue fever:2014,government of India.
8. Park's Textbook of Preventive & Social Medicine, K Park, 18th Edition, Jan 2005,Epidemiology of communicable diseases; p-198-201
9. Textbook of Clinical virology, II edition. Douglas D. Richman, Richard J. Whitley & Frederick G.Hayden: Chapter: 51, Flaviviruses;p1097-1150,Chapter:9,Viral Haemorrhagic fevers: a comparative appraisal;p135-144.
10. Topley & Wilson Microbiology & Microbial infections, X edition, Chapter 46: p993-1009.
11. World Health Organisation.1997.Dengue haemmorhagic fever: diagnosis, treatment, prevention and control, World Health Organisation, Geneva, Switzerland.
12. World Health Organisation. First report on neglected tropical disease:working to overcome the global impact of neglected tropical disease. Geneva: world health organization:2010.