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Original Research Paper

A DIAGNOSIS OF DENGUE BY NS1 ANTIGEN, ELISA AND REVERSE TRANSCRIPTASE PCR

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ABSTRACT

Background: Dengue fever is the further most common developing tropical viral illness affecting humans today. Dengue is a mosquito-borne disease spread largely by Aedes aegypti, however, Aedes albopictus has also been identified as a vector. Dengue NS1, lgM, and lgG ELISA assays are commonly used in ordinary laboratories to diagnose dengue infection. NS1 canister is found from the first to the seventh day after the start of symptoms, while anti-DENV lgM and lgG antibodies take 4-5 and 1-14 days, respectively, depending on whether the patient has a primary or secondary infection. **Aim:** the study is to assess role of NS1 antigen determination in diagnosis of dengue. **Materials and Method: ICT (Immunochromatography Test)** ExDXTM Dengue Combo (Ag+Ab) is a rapid qualitative immune chromatographic test for Dengue detection of NS1antigen and variance detection of IgM and IgG antibodies against dengue virus in human serum. **Result** All 130 serum samples were tested for NS1 antigen using NS1 antigen ELISA kit and 63 (48.46%) were found to be positive and 67 (51.53%) for NS1 antigen. The NS1 antigen was detected from day 1 to day 7 of illness with maximum positivity 73.33 % on day 4 of illness. **Conclusion:** NS1 antigen detection and dengue group specific reverse transcriptase RT-PCR are valuable techniques for the rapid and early microbiological diagnosis of dengue.

Kew words: Dengue Fever, ELISA, ICT , NS1

INTRODUCTION

Dengue fever is the most common developing tropical viral illness affecting humans today[1]. Dengue fever affects an estimated 40 percent of the world's population, with 50-100 million cases occurring each year[2]. Dengue fever causes an estimated 500 000 hospitalizations per year, 2.5 percent of which are deadly. Dengue fever is endemic in Southeast Asia and South Asia, and it has now spread to Europe. More than 100 nations in the WHO's African, Americas, Eastern Mediterranean, South-East Asia, and Western Pacific regions are currently infected with the disease; the Americas, South-East Asia, and Western Pacific regions are the most severely afflicted (WHO). Dengue

fever has surpassed diarrheal disease and ARDS infection as the top cause of hospitalization and death among children in the WHO's South-East Asia region[3]. DENV is a flavivirus that belongs to the Flaviviride family and has four antigenically different serotytypes: DEN1, DEN2, DEN3, and DEN4. It's a single-strand positive sense RNA virus with a single-strand positive sense RNA envelope. The genome of DNEV comprises ten genes in an open reading frame (ORF), which are translated into a poly protein by a single-peptidase in the host cell, which is then processed into three structural proteins (C, E, and prM) and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5)[4]. Dengue fever is a mosquito-borne disease spread largely by Aedes aegypti, however Aedes albopictus has also been identified as a vector[5]. Dengue fever is derived from two words: "break-bone fever" and "walk of a dandie." The term 'break bone fever' was coined by Benjamin Rush to describe the disease's symptoms[6]. Asymptomatic and symptomatic infection, including dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome, which is usually lethal due to aberrant capillary permeability and plasma leakage, are all part of the clinical spectrum of disease. Myocardiopathy, hepatic failure, and neurological problems have all been recorded as unusual manifestations[7,8]. Dengue fever has no specific cure at this time, and vector control is the only way to prevent it. Dengue fever was first documented in India in 1946, but it was not until 1964 that it had a significant impact in Kolkata. Epidemics are becoming increasingly common in our country, with the disease swiftly spreading across India, particularly in the northern and southern regions[9]. One of the most severe outbreaks of DF/DHF in our country happened in 1996, with a total of 16,517 cases and 545 deaths reported, with 10,252 cases and 423 deaths reported from Delhi. DENV-2 serotype was found to be the source of the outbreak[10]. It was the most common circulating serotype in our country, according to studies from central and southern India[11]. In 2006, the country saw another DF/DHF pandemic, with 12,317 cases and 184 deaths. This outbreak saw all four DENV serotypes in circulation for the first time, but the DENV-3 serotype dominated the outbreak[12]. Dengue fever was recorded in 28.292 cases across the country in 2010. By 2012, the number had risen to 50.222, and by 2013, it had risen to 75808[13]. Dengue fever is a major public health concern around the world. As a result, for proper patient treatment and disease control, rapid and reliable diagnostic tools are essential. In order to build molecular epidemiology and understand the viral pathogen's evolving pattern, regular surveillance of the virus has become a need in our country. Only a laboratory can provide a conclusive diagnosis of dengue infection, which requires isolating the virus, finding viral antigen or RNA in serum or tissues, or detecting specific antibodies in the patient's serum. Dengue virus (DENV) infection is presently diagnosed by reverse transcription polymerase chain reaction (RT-PCR) or immunoglobulin M (lgM) capture enzyme-linked immunosorbent assay (ELISA), immunoglobulin G (lgG) ELISA, and NSI (non-structural antigen 1) antigen detection.

Dengue NS1, lgM, and lgG ELISA assays are commonly used in ordinary laboratories to diagnose dengue infection. NS1 can be found from the first to the seventh day after the onset of symptoms, while anti DENV lgM and lgG antibodies take 4-5 and 1-14 days, respectively, depending on whether the patient has primary or secondary infection[14]. RT-PCR detection of viral RNA enables for early diagnosis during the febrile period. The reverse transcriptase polymerase chain reaction (RT-PCR) has the ability to detect dengue viruses quickly, sensitively, and specifically[15]. The advantage of RT – PCR is that it can serotype viruses. NS1 antigen detection, on the other hand,

gives an advantage for early diagnosis of dengue fever in peripheral laboratories and primary health care institutions when PCR equipment are not available.

MATERIAL AND MATHODS

Study Design

All the clinically suspected cases of dengue during the study period will included in this study. About 130 blood samples was randomly collected from the Microbiology department of Index hospital. The clinical information was collected from the Medicine department. The study was approved by the Institutional Ethics Committee (1EC) of Index Medical College and University.

ICT

Ex DXTM Dengue Combo (Ag+Ab) is a rapid qualitative immunochromatographic test for detection of Dengue NS1 antigen and differential detection of IgM and IgG antibodies to dengue virus in human serum.

NS1-ELISA

Inbios DENV Detect TM NS1 ELISA is used in this study

MAC-ELISA

DENV Detect TM IgM Capture ELISA (Inbios, USA) is used in this study.

Real time RT- PCR

The procedures were performed according to the manufacturer's instruction.

RESULT AND OBSERVATION:

Table 1: Detection Nonstructural protein-1 antigen by ELISA:

	Positive		Negative		Total
NS1 by ELISA	N	%	N	%	
	63	48.46	67	51.53	130

All 130 serum samples were tested for NS1 antigen using NS1 antigen ELISA kit and 63 (48.46%) were found to be positive and 67 (51.53%) for NS1 antigen.

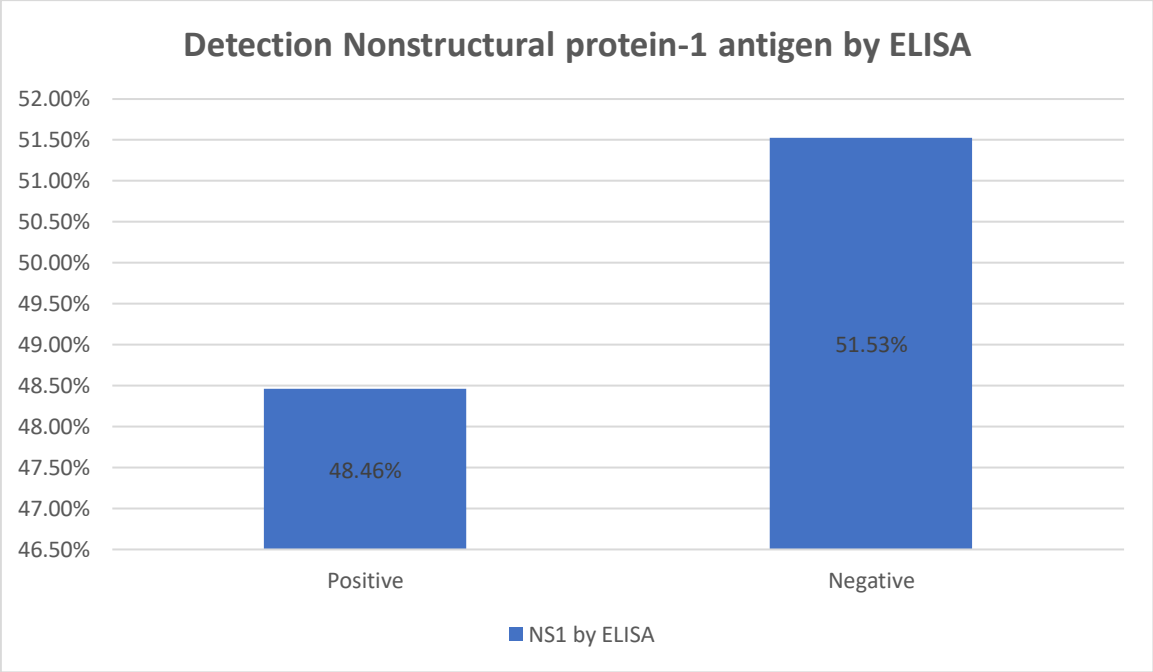

Figure 1: Detection Nonstructural protein-1 antigen by ELISA

Table 2: Comparison of NS1 antigen immune- chromatographic test and NS1 antigen ELISA.

NS1 antigen ELISA (gold standard)	NS1 antigen 1CT Positive	NS1 antigen 1CT Negative	Total	Sensitivity	Specificity
Positive	51	3	54	95.8%	75.6%
Negative	21	55	76		
Total	72	58	130		

On comparison of NS1 antigen by 1CT has a sensitivity of 95.8% as compared to ELISA but a low specificity of 75.6% as compared to ELISA.

Table 3: Positive samples by 1CT, ELISA and RT-PCR on different days of illness.

Day of illness	Positive by PCR	Positive by 1CT	Positive by ELISA	Total
1	2	2	2	2
2	12	16	12	27
3	8	9	7	23
4	6	4	5	9
5	4	7	4	12
6	1	3	1	7
7	0	0	0	2
Total	33	41	31	82

The maximum number of samples 12 which maximum positive by all the 3 assay uses on day 2- On day four 6 Samples were positive by PCR but only 5 of them were positive NS1 antigen by ELLSA and 4 by 1CT. on fifth day 4 samples positive out or 12 by PCR and NS1 antigen by ELISA. By 1CT 3 additional samples were positive for NS1 antigen. On day 7th none of 2 samples were positive in any of 3 tests. A total of 82 samples were tested by RT-PCR. From these samples 33 samples were positive by RT-PCR and 31 samples were positive for NS1 antigen by ELISA and 41 for NSI antigen by 1CT.

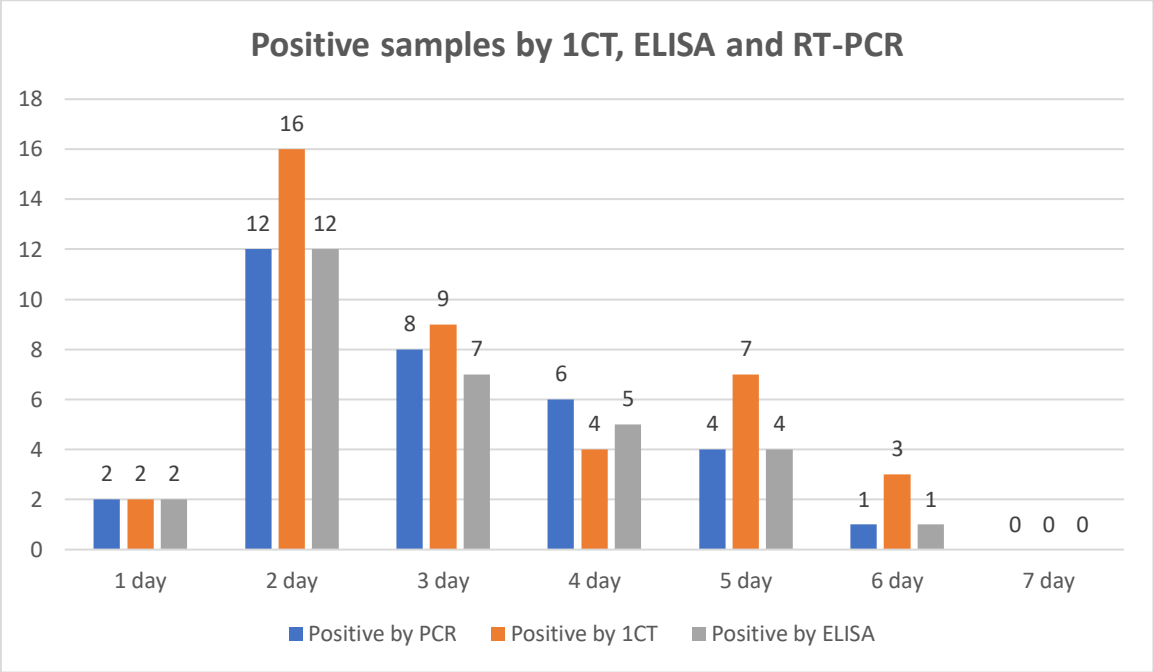

Figure 2: Positive samples by 1CT, ELISA and RT-PCR on different days of illness

Table 4: Comparison of nonstructural protein- 1 antigen by immunochromatographic test (1CT) and RT polymerase chain reaction (gold standard) on different days of illness

PCR	NS1 antigen Positive	NS1 antigen Negative	Total	Sensitivity	Specificity	PPV	NPV	Concordance
Positive	28	5	33	84.8%	73.5%	68.3%	87.8%	78.0%
Negative	13	36	49					
Total	41	41	82					

In the present study out of 82 samples 33 samples were positive for RT-PCR. Of these 41 were positive for NS1 rapid test. The sensitivity of NS1 antigen by ICT is 84.8% and specificity 73.5% with 68.3% positive predictive value and 87.8% negative predictive value.

Table 5: Comparison of dengue NS1 antigen ELISA assay and reverse transcriptase polymerase chain reaction (gold standard)

PCR	NS1 antigen ELISA Positive	NS1 antigen ELISA Negative	Total	Sensitivity	Specificity	PPV	NPV	concordance
Positive	31	2	33	93.9%	100%	100 %	96.0%	97.6%
Negative	0	49	49					
Total	31	51	82					

Out of 130 serum samples collected. RT-PCR Were carried out on 82 samples out of which 33 were tested positive for dengue by PCR, ELISA was positive in 31 samples out of the 33 samples subjected to PCR. The sensitivity of ELISA is 93.9% and specificity 100% with a positive predictive value of 100% and 96% negative predictive value.

DISCUSSION

On comparison of NS1 antigen by 1CT has a sensitivity of 95.8% as compared to ELISA but a low specificity of 75.6% as compared to ELISA. Ahmed et al., (2014)[16] suggested that the efficiency, sensitivity, specificity, positive and negative predictive values of NS1 Ag detection ELISA were 83.6, 73.5, 100, 100, and 70%, respectively. Out of 130 serum samples collected. RT-PCR Were carried out on 82 samples out of which 33 were tested positive for dengue by PCR, ELISA was positive in 31 samples out of the 33 samples subjected to PCR. The sensitivity of ELISA is 93.9% and specificity 100% with a positive predictive value of 100% and 96% negative predictive value. Gaikwad et al., (2017)[17] suggested that the sensitivity, specificity, positive, and negative predictive value of the rapid dengue NS1 antigen test were 81.5 percent, 66.7 percent, 78.2 percent, and 71.1 percent, respectively, whereas the NS1 ELISA had 89.9 percent, 100 percent, 100 percent, and 94 percent. Rapid NS1 and NS1 ELISA had 75.5 percent and 94 percent PCR concordance, respectively, and they discovered that the NS1 antigen ELISA may be used in diagnostic laboratories to diagnose dengue fever in the acute phase of disease. The test also has a lot of promise for application in epidemic conditions, since it could help with early patient screening and limiting disease spread.

Strength and Limitations of the Present Study

There are a few limitations of the study. In the present study, 10-60 years ages subjects participated in the research. Hence, in the feature, we would like to include an increase in the number of participants to reach a concrete conclusion. The Present study was given an impact on understanding the RT-PCR valuable technique for diagnosis of Dengue.

CONCLUSION

With the expansion of the geographic range of dengue fever and the increasing number and severity of reported cases, the use of NS1 antigen detection and reverse transcriptase RT-PCR allowed clinical diagnostic laboratories to identify dengue virus infections early enough to modify patient management, reducing the time between detection of the first cases, and the notification of public health authorities. NS1 antigen detection and dengue group specific reverse transcriptase RT-PCR are valuable techniques for the rapid and early microbiological diagnosis of dengue. NS1 antigen ELISA can be implemented in diagnostic laboratories for diagnosis of dengue in the acute phase of illness and the diagnosis can be made as early as within three days of onset of fever. The test also has great potential value for use in epidemic situations, as it facilitates the early screening of patients. However, NS1 antigen detection by 1CT may give false positive and false negative results as compared to ELISA and thus Microbiology laboratories should confirm all NSl antigen positive results by 1CT with ELISA. RT-PCR is more sensitive and specific but requires well equipped laboratory with trained staff. In comparison with other laboratory diagnostic tests such as MAC ELISA for anti-DENV IgM antibody detection NS1 antigen becomes positive earlier and thus helps in diagnosis of early infection, whereas IgM antibodies appear by 6–7-day post fever and lasts for about 3 months- This study thus showed the usefulness of NS1 antigen detection in the early diagnosis of Dengue.

REFERENCES

1. Guzman MG & Kouri G. Dengue: an update. Lancet Infect Dis 2002; 2:33-42.
2. Gubler DJ. Dengue. Urbanization and Globalization: The Unholy Trinity of the 21st Century. Tropical Medicine and Health 2011; 39(4)3-11.
3. Viera Ds, Bifano G,Honda ER,Tada MS, Batista WC. Isolation and identification of dengue virus serotype 3 in rondonia, Brazil.Virus Rev Res 2008; 13:1-6.
4. Rice C. M. Flaviviridae: the viruses and their replication. In Fields Virology, 3rd edn, pp. 931-959 Philadelphia: Lippincott-Raven 1996.
5. World health organization. Dengue: Guidelines for diagnosis, treatment, prevention and control, 2009 World Health Organization. Geneva, Switzerland.
6. Tantawichien. Terapong. "Dengue Fever and Dengue Haemorrhagic Fever in Adolescents and Adults. - Paediatrics and International child Health 2012; 32:22-7.
7. Key issues in dengue vector control towards the operationalization of a global strategy: Report of consultation. Geneva: world health organization 1995 ICTD/F1L(Den)/IC.96.1
8. Gabler DJ. Dengue and dengue hemorrhugic fever. Clin Microbiol, Rev 1998;1(1):480-96.
9. Bhatt S. Gething PW, Brady OJ, et al. The global distribution and burden of dengue. Nature 2013; 496(7446):504-7.
10. Dar, L., Broor, S., Sengupta, S., Xess, & Seth. P. (1999). The first major outbreak of dengue hemorrhagic fever in Delhi, India. Emerging Infectious Diseases 1999; 5(4):589-90.
11. Gupta, N., Srivastava, S., Jain, A., & Chaturvedi, U. C. (2012). Dengue in India. *The* Indian Journal of Medical Research 2012; 136(3):373-90.
12. Gupta E., Dar, L., Kapoor, G., & Broor, S. The changing epidemiology of dengue in Delhi. India. Virology Journal 2006; 3:92.
13. World health organization. Dengue: National Guidelines for Clinical Management of Dengue Fever. 2014 World Health Organization, India. Dengue national guidelines. 2014.
14. Pal, S., Dauner, A. L... Mitra, 1. Forshey, B. M.. Garcia. P., Morrison. A. C., Wu, S-J, L. 2014 Evaluation of Dengue NS1 Antigen Rapid Tests and ELISA Kits Using Clinical Samples. PLoS ONE 2014; 9(11): e 1 13411.
15. C Suk-Yin, I Kautner, L Sal-Kit. Detection and serotyping of dengue viruses by PCR: a simple, rapid method for the isolation of viral RNA from infected mosquito larvae. South Asian J Trop Med Publication, 1994.
16. Ahmed NH, Broor S. Comparison of NS1 antigen detection ELISA, real time RT-PCR and virus isolation for rapid diagnosis of dengue infection in acute phase. J Vector Borne Dis. 2014; 51(3):194-9.
17. Anand AM, Sistla S, Dhodapkar R, Hamide A, Biswal N, Srinivasan B.Evaluation of NS1 Antigen Detection for Early Diagnosis of Dengue in a Tertiary Hospital in Southern India. JCDR 2016;10(4): DC01-DC04.