RESEARCH

A STUDY OF NON-SPECIFIC 1 ANTIGEN AND IGM/IGG ANTIBODY IMMUNOCHROMATOGRAPHY TEST WITH ELISA FOR THE EARLY DETECTION OF DENGUE IN THE SUBURBS OF INDORE, MADHYA PRADESH

P. Veerendra Kumar Reddy[1], Ramanath K[2], Surendra Prasad Chauhan[3], Bijay Kumar Mahaseth[4]

[1]Ph.D. Scholar, Department of Microbiology, Malwanchal University, Madhya Pradesh, India
[2]Professor, Department of Microbiology, Index Medical College, Indore, Madhya Pradesh, India.
[3]Assistant Professor, Department of Microbiology, Shri Shankaracharya institute of medical sciences Bhilai, Chhattisgarh, India.
[4]Assistant Professor, Department of Biochemistry, NC Medical College & Hospital, Israna, Panipat, Haryana, India

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Corresponding Author:
Dr. Ramanath K,
Email. ramanath.karicheri@gmail.com
ORCID: 0000-0001-7608-2127

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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 present study was aimed at the assessment of the role of NS1 antigen determination in the diagnosis of dengue. **Materials and Methods:** ICT (Immunochromatography Test) Ex DXTM 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 an 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 of 73.33 % on day four of illness. On comparison of NS1 antigen by ICT has a sensitivity of 95.8% as compared to ELISA but a low specificity of 75.6% as compared to ELISA. **Conclusion:** NS1 antigen ELISA can be implemented in diagnostic laboratories for the diagnosis of dengue in the acute phase of illness and the diagnosis can be made as early as three days after the 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 ICT may give false positive and false negative results as compared to ELISA and thus Microbiology laboratories should confirm all NSW antigen positive results by ICT with ELISA.

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, South-East Asia, Eastern Mediterranean, and Western Pacific regions are currently infected with the disease; the Americas, South-East, Western Pacific, and Asia regions are the most severely afflicted (WHO). Dengue fever has surpassed diarrheal disease and ARDS (acute respiratory distress syndrome) 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 Flaviviridae family and has four antigenically different serotypes: 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 ORF, which are translated into a polyprotein by a single-peptidase in the host

cell, which is then processed into 3 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 infections, including dengue fever (DF), dengue hemorrhagic fever, 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 regions9. The most of one severe outbreak of DF/DHF in our country happened in 1996, with a total of 16,517 cases out of 545 deaths reported, with 10,252 cases out of 423 deaths reported from Delhi. DENV-2 serotype was found to be the source of the outbreak.[10] It was the 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 4 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 7580813. 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. 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 RT-PCR or immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (ELISA), immunoglobulin G (lgG) ELISA, and NSI (non-structural antigen 1) antigen detection.

MATERIALS AND METHODS

Study Design

All the suspected cases of dengue in the study period were included in this study. 130 blood samples were randomly collected from Medicine Department were transported to the Microbiology department of Index hospital, Indore, Madhya Pradesh, India. The clinical information regarding the patients was collected from the Medicine department. The present study was approved by the Institutional Ethics Committee of Index Medical College and associated with Malwanchal University.

ICT (Immunochromatography Test)

ExDXTM Dengue Combo (Ag+Ab) is a rapid qualitative immune chromatographic test for detection of Dengue NS1antigen and differential detection of IgM and IgG antibodies against dengue virus in humans serum.

Procedure:

a. ExDXTM Dengue NS1 Antigen device:
   1. 60µL of serum was added using an antigen test sample dropper into the sample well marked ‘S’
   2. The results were interpreted in 20 minutes. (The results shouldn’t be interpreted after 20 minutes which can give incorrect results)

b. ExDXTM Dengue IgM/IgG Antibody device
   1. 5µL of serum was added using an antibody test sample dropper into the sample well marked ‘S’
   2. Three drops of buffer solution were added to the ports’
   3. The results were interpreted in 20 minutes. (The results shouldn’t be interpreted after 20 minutes which can give incorrect results)

Interpretation:

a. ExDXTM Dengue NS1 Antigen device:

Negative: The appearance of one distinct line in the control region “C” only, indicates that the sample is “Negative” for Dengue NS1 antigen.

Positive: Appearance of colored line, one each in test region “T” and control region “C” indicates that the sample is positive for Dengue NS1 antigen.

Invalid: If the colored control line does not appear, the test is invalid. It is recommended that the specimen has to be retested.

b. ExDXTM Dengue IgM/IgG Antibody device.

Negative: The appearance of one distinct line in the control region “C” only, indicates that no dengue antibodies are present in the sample.

Positive:

IgG positive: Along with the control band, if IgG band alone 7 appears it indicates the test is positive for IgG. This is indicative of past infection.

IgM positive: Along with the control band, if the IgM band alone appears it indicates the test is positive for IgM. This is indicative of primary dengue infection.

IgM and IgG positive: Along with the control band, if IgM and IgG both band appears it indicates the test is positive for both IgM and IgG antibodies. This is indicative of secondary dengue infection.

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Invalid: If the colored control line does not appear, the test is invalid. It is recommended that the specimen has to be retested.

RESULTS

The NS1 antigen was detected from day 1 to day 7 of illness with maximum positivity of 73.33 % on day 4 of illness. [Table 1]

Regarding [Table 2] only the results are displayed here, but no mention of the ELISA (NS1 –Ag) test procedure in the material and method section.

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

On comparison of NS1 antigen by ICT has a sensitivity of 95.8% as compared to ELISA but a low specificity of 75.6% as compared to ELISA. [Table 3]

Table 1: NS1 antigen positivity in the duration of illness by ICT

Duration of illness (Days)	Total Tested	NS1 antigen Positive	
		Number	Percentage
1	2	1	50
2	45	21	46.66
3	40	24	60
4	15	11	73.33
5	18	11	61.11
6	7	3	42.85
7	3	1	33.33

Table 2: Detection of Nonstructural protein-1 antigen by ELISA

	Positive		Negative		Total
NS1 by ELISA	Number	percentage	Number	Percentage	
	63	48.46	67	51.53	130

Table 3: Comparison of NS1 antigen immune-chromatographic test and NS1 antigen ELISA.

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

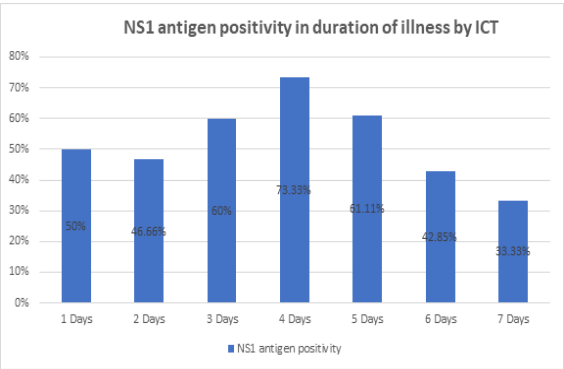

Figure 1: NS1 antigen positivity in the duration of illness by ICT

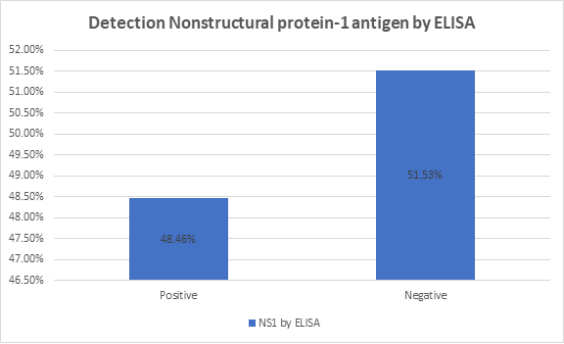

Figure 2: Detection of Nonstructural protein-1 antigen by ELISA

DISCUSSION

All 130 serum samples were tested for NS1 antigen using an 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 first to day seven of illness with maximum positivity of 73.33 % on day 4 of illness. On comparison of NS1 antigen by ICT 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),[14] Anand et al (2016),[15] and Gaikwad et al (2017),[16] suggested that the efficiency, specificity, sensitivity, negative and positive predictive values of NS1 Ag detection ELISA were 83.6, 73.5, 100, 100, and 70%, respectively.

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 beginning 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.[17] RT-PCR detection of viral RNA enables early diagnosis during the febrile period. The RT-PCR can detect dengue viruses quickly, sensitively, and specifically.[18] The advantage of RT – PCR is that it can serotype the viruses. NS1 antigen detection, on the other hand, gives an advantage for the early

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diagnosis of dengue fever in peripheral laboratories and primary health care institutions when PCR equipment is not available. The current study was aimed at the assessment of the role of NS1 antigen determination in the diagnosis of dengue

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 NSW antigen giving positive results by ICT.

CONCLUSION

NS1 antigen ELISA can be applied in diagnostic laboratories for the diagnosis of dengue in the acute phase of illness and the diagnosis can be made as early as days after the start of fever. This test has great possible value for use in epidemic situations, as it facilitates the early screening of patients. However, NS1 antigen detection by ICT may give false positive and false negative results as compared to ELISA and thus Microbiology laboratories should confirm all NSW antigen positive results by ICT with ELISA.

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