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Original Article

Microbiology Section

Comparison of NS1 Antigen Detection by RDT and ELISA and its Concordance with RT-PCR for the Early Diagnosis of Dengue in Ananthapuramu District, Andhra Pradesh, India

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ABSTRACT

Introduction: Early detection of dengue fever is important for effective clinical care and vector control. For the detection of dengue viral antigen and antibodies, several serological techniques based on the concept of immunochromatography and Enzyme-Linked Immunosorbent Assays (ELISA) are routinely utilised. The performance of these tests depends on the sensitivity and specificity.

Aim: To compare Non Structural protein-1 (NS1) antigen detection by Rapid Diagnostic Tests (RDTs) and ELISA and its association with Real-Time Polymerase Chain Reaction (RT-PCR).

Materials and Methods: This diagnostic cross-sectional study was done on 100 clinically suspected cases of Dengue between July-November 2021 at a tertiary care hospital in Ananthapuramu, Andhra Pradesh, India. All the sera samples were collected and subjected to NS1 antigen detection test by rapid test, NS1 ELISA, and RT-PCR, and also serotypes DENV-1, 2, 3, 4 were detected from serum sample by RT-PCR test. The results of rapid and ELISA tests were compared with RT-PCR. Data was analysed by Statistical Package for the Social Sciences (SPSS) version 16.0.

Results: Out of total 100 samples, 29 samples were tested positive by NS1 rapid test, 30 samples were tested positive by NS1 ELISA, and 32 samples were tested positive by RT-PCR. The sensitivity, specificity of dengue NS1 antigen rapid test were 87.50% and 98.52%, respectively when compared to RT-PCR whereas that of NS1 ELISA were 93.75% and 100% when compared to RT-PCR. Out of 32 samples tested positive by RT-PCR, two samples were positive for DENV-1, 26 samples were positive for DENV-2, and four samples were positive were DENV-3.

Conclusion: The NS1 ELISA test requires extra procedures and time. RDTs need a one-step operation that takes roughly 15-30 minutes. Despite the fact that RT-PCR and ELISA have better performance than RDTs, RDTs are more effective for early diagnosis and therapy of dengue fever in countries with limited infrastructure and in remote places.

Keywords: *Aedes aegepti*, Enzyme-linked immunosorbent assay, Non structural protein-1 antigen, Rapid diagnostic test, Reverse transcription polymerase chain reaction

INTRODUCTION

Classical dengue fever has only been observed in India from 1988 [1]. Dengue affects upto 100 million people every year, comprising 500,000 occurrences of Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) and about 30,000 deaths, the majority of which occur in children [2,3]. It is carried by the mosquitoes *Aedes aegypti* and *Aedes albopictus* and is present all over the world, but is most frequent in tropical and subtropical locations [4]. Asymptomatic febrile illness to more severe infection types such as DHF and DSS are among the clinical signs [5]. Acute dengue infection identification is essential for evidence-based diagnosis, treatment, implementation of control measures, surveillance, and research. An ELISA, that identifies anti-Dengue Virus (DENV) IgM or IgG antibodies in patient serum, is the current diagnostic approach for diagnosing dengue. Antibodies are discovered 4-5 days following the onset of symptoms [6]. There are a number of tests available for detecting dengue fever during the acute stage of the illness. The first ELISA capable of detecting DENV NS1 was developed in the year 2000. The amino acid sequence of NS1 is extremely conserved, and it may be found in both membrane and soluble forms [7]. In both primary and secondary DENV infections, NS1 is detected early in the acute phase. The quantity and timing of NS1 levels in human clinical specimens make it a better target for the production of a variety of diagnostic tests [6,8]. Dengue infection is currently diagnosed in affected persons using both quick NS1 and ELISA testing. As a result, such tests can be employed for vector surveillance as well as for therapeutic treatment.

As the dengue cases are more prevalent and patients come to the hospital at a severe stage of Dengue in Ananthapuramu, the present study was carried out to compare quick dengue NS1 antigen detection and NS1 ELISA testing with PCR for early DENV infection detection and to detect serotypes by RT-PCR Serotyping (1-4) kit in a tertiary care hospital, Ananthapuramu.

MATERIALS AND METHODS

A diagnostic cross-sectional study was conducted in the Department of Microbiology at Government Medical College, Ananthapuramu, Andhra Pradesh, India from July-November 2021. Prior to the start of the study, the Institutional Ethics Committee (IEC) approval for the project number 44/2020 was taken. Written informed consent was taken from all the subjects. In the case of paediatric patients, parental consent was acquired.

Inclusion criteria: Clinically probable dengue patients with fever lasting 1-4 days and whom serological diagnosis was requested for dengue infection and patients of all age groups were included in the study.

Exclusion criteria: Patients who have had a fever for more than four days non conclusive reports, already diagnosed cases of dengue (referred or admitted with dengue positive report) were excluded from the study.

Study Procedure

Type of sample and collection: A 3-5 mL of blood was taken aseptically from patients with clinically probable dengue fever who presented to the General Medicine and Paediatric Out Patient Department (OPD) within four days of the beginning of the illness and submitted to the laboratory following written informed permission. In addition, the patient's record file was examined for relevant demographic, clinical, and investigational data. In case record form, the patient's detailed clinical history was recorded.

Sample processing: The serum and plasma were obtained by centrifuging the collected samples at 2500 revolution per minute (rpm) for 15 minutes. The serum was tested for rapid dengue NS1 antigen and NS1 ELISA, and plasma samples were used for real-time RT-PCR with a serotyping kit.

Rapid dengue non structural protein-1 antigen detection test: All 100 samples were examined using a rapid dengue NS1 antigen detection method based on the immunochromatographic approach. The kit used was J.Mitra Co. Pvt Ltd. The process was carried out according to the manufacturer's instructions, and the results were evaluated as positive if a pink coloured line appeared on the test region and control region and negative if a pink line appeared on control region and does not appear on the test region.

Dengue non structural protein-1 antigen detection Enzyme-Linked Immunosorbent Assay (ELISA): All sera samples were tested by NS1 antigen ELISA. The kit used was NS1 antigen J Mitra Co., ELISA. The manufacturer's instructions for performing the test and interpreting the findings were properly followed. Using an ELISA reader, the optical density was determined at 450 nm.

Hi-Media Dengue Serotyping (1-4) Kit Real-Time Probe-Based PCR: All the samples were serotyped using commercially available Hi-Media Dengue Serotyping (1-4) Kit Real-Time Probe-Based PCR. The assay included a super mix for DENV 1-4 RNA specific amplification. DENV-1 with a FAM, DENV-2HEX, DENV-3 with Texas Red probe, and DENV-4 with Cy5 probe were all included in the primer mix. A HEX-labelled probe was added in the primer mix to detect the RNA Internal Control (IC), which was utilised to monitor the extraction process and prevent RT-PCR inhibition. The kit included a positive control. The study was performed as instructed by the manufacturer.

STATISTICAL ANALYSIS

The data was processed and arranged into distribution tables and cross tables using SPSS version 16.0.

RESULTS

Out of 100 samples received in laboratory, 51 samples were of females and 49 samples were of males. The total number of patients tested positive by RT-PCR were 32, out of which 17 (53%) were males and 15 (47%) were females. The largest age group tested positive belonged to 0-10 years followed by age group of 11-20 years [Table/Fig-1].

Age (years)	Male	Positive	Female	Positive	Total positive
0-10	24	13	24	07	20
11-20	10	03	08	06	09
21-30	04	0	06	01	01
31-40	06	01	02	01	02
>40	05	0	11	0	0
Total	49	17	51	15	32

[Table/Fig-1]: Age and gender wise analysis of dengue positive cases by RT-PCR.

Out of total 100 samples, 32 (32%) samples were tested positive by RT-PCR test. Out of 32 positive samples, two samples were positive for DENV-1, 26 samples were positive for DENV-2, and four samples were positive were DENV-3 [Table/Fig-2].

Total no. of samples tested	No. of positives	No. of negatives
100	DENV-1=02	68
	DENV-2=26	
	DENV-3=04	
	Total positives=32	

[Table/Fig-2]: Dengue serotypes by RT-PCR test.

Out of total 100 samples, total positive samples found were 91. Samples tested positive by RT-PCR test were 32, samples tested positive by NS1 antigen test were 29, and samples tested positive by NS1 ELISA were 30.

Sensitivity, specificity, when NS1 was detected by ELISA when compared with RT-PCR were 93.75%, 100%, and sensitivity, specificity, when NS1 was detected by RDT kits when compared with RT-PCR were 87.50%, 98.52% as shown in [Table/Fig-3]. Positive Predictive Value (PPV) for ELISA vs RT-PCR was 100% and for Rapid test vs RT-PCR 96.55% and Negative Predictive Value (NPV) for ELISA vs RT-PCR was 97.14% and for Rapid Vs RT-PCR was 94.44%.

Parameter		RT-PCR		Sensitivity	Specificity
		Positive	Negative		
NS1 by ELISA	Positive	30	0	93.75%	100%
	Negative	2	68		
NS1 by rapid test	Positive	28	1	87.50%	98.52%
	Negative	04	67		

[Table/Fig-3]: Comparison of NS1 antigen by ELISA and rapid test against RT-PCR.

DISCUSSION

Because there is no vaccine to prevent dengue fever, early identification and treatment are suggested for reducing complications and disease control in endemic areas. In addition to the challenges associated with dengue prevention, reliable identification of the virus has proven challenging due to the non specific nature of its symptoms, particularly in the early, acute stages of infection. Dengue infection can be diagnosed precisely using virus isolation and viral RNA detection through RT-PCR, however, this procedure is time-consuming, expensive, and out of reach for even most tertiary care facilities, instead it is diagnosed using dengue-specific antibodies and/or NS1 antigen or ELISA. NS1 is substantially conserved in both membrane and soluble forms. The NS1 antigen is highly specific and may be detected in serum from days 1-9 following the beginning of fever [9].

In the present study, out of 32 positive dengue samples, 17 (53%) were males and 15 (47%) were females. Pardeshi A et al., did a similar study in KEM hospital, Mumbai, and found that out of 420 patients, 236 (56%) were men and 184 (44%) were females [10].

In current study, 32 patients were tested positive for Dengue with the RT-PCR test. There were 26 cases of dengue serotype-2, four cases of DENV 3, and two cases of DENV 1 among the 32 patients. This was consistent with findings from earlier research conducted in Delhi, Uttar Pradesh, and Mumbai [11-13]. This study compared the quick dengue NS1 antigen rapid detection test and NS1 ELISA test to real-time RT-PCR for early dengue diagnosis. In this study, sensitivity and specificity were 93.75% and 100%, respectively, when NS1 was considered on ELISA when compared to RT-PCR, and 87.50% and 98.52%, respectively when NS1 was considered on RDT kits when compared to RT-PCR. According to Hunsperger EA et al., showed NS1 fast diagnostic test had a sensitivity of 38-71% and a specificity of 76-80%, whereas the NS1 antigen ELISA had a

sensitivity of 60-75% and a specificity of 71-80% [14]. Gaikwad S et al., found that the quick dengue NS1 antigen test has a sensitivity and specificity of 81.5% and 66.7%, respectively, whereas the NS1 ELISA had a sensitivity and specificity of 89.9% and 100% [15]. Anand AM et al., found that NS1 rapid detection outperformed RT-PCR in terms of cost, technical performance, and speed [16]. The data from previous literature also showed rapid dengue tests as an effective tool in the diagnosis and patient care of acute dengue infection [Table/Fig-4] [15,17-19].

Author name (Ref no.)	Place of study and year of publication	Positivity of NS1 by rapid test	Positivity of NS1 by ELISA	Positivity of NS1 by RT-PCR
Rajeshwari KG [17]	Bengaluru, 2021	42.9%	55.4%	-
Gaikwad S et al., [15]	Mumbai, 2017	62%	53.5%	59.5%
Gill MK et al., [18]	Amritsar, 2016	22%	27.6%	-
Kalpana S and Varma G [19]	Karnataka, 2021	57.6%	8.46%	-
Present study	Andhra Pradesh, 2022	29%	30%	32%

[Table/Fig-4]: Previous studies showing NS1 antigen positivity by different methods [15,17-19].

Limitation(s)

The current study had the limitation that the performance of test devices can be impacted by various study-specific variables, including the reference technique used (RT-PCR), the kind of samples taken, the Post Symptom Onset (PSO) day, and the circulating serotypes and strains included in a particular assessment panel.

CONCLUSION(S)

In this study, it was found that DENV-2 was the most prevalent in this region. Dengue fever is a mosquito-borne disease that affects people and can be fatal if not treated promptly. The quick RDT kit for NS1 antigen detection that was employed for testing worked on par with an ELISA-based test, according to the findings. Rapid dengue tests can thus play a crucial role in the diagnosis and patient care of acute dengue infection in developing nations like India, where there is a shortage of diagnostic laboratory infrastructure, particularly in rural and isolated locations. These fast tests are very basic, do not require technological skill, are straight forward to administer, and the findings are delivered in minutes, allowing for timely case management.

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