Comparison of NS - 1 Antigen Detection by RDT and ELISA and its Correlation with RT PCR for Early Diagnosis of Dengue and Detection of Concurrent Serotypes DENV - 1, 2, 3 in Ananthapuramu District

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ABSTRACT

INTRODUCTION
Early diagnosis of dengue is important for appropriate clinical management and vector control. Different serological tests based on the principle of immunochromatography and Enzyme - Linked Immune Sorbent Assays (ELISA) is commonly used for detection of antigen and antibodies of dengue virus. The performance of these tests depends on the sensitivity and specificity. Hence, the study was undertaken to compare nonstructural protein - 1 (NS1) antigen detection by rapid and ELISA correlation with Real Time - Polymerase Chain Reaction (RT - PCR) for diagnosis of dengue and to detect serotypes by RTPCR Serotyping (1 - 4) kit at Tertiary care hospital, Ananthapuramu.

MATERIALS AND METHODS
In this study 100 clinical suspected cases of Dengue were enrolled. All the collected sera a sample were subjected for NS1 antigen detection test by rapid test, NS1 ELISA, and RT PCR and also serotypes DENV - 1, 2, 3, 4 are detected from serum sample by RTPCR test. The results of rapid and ELISA tests were compared with real Time

RESULTS
Out of total 100 samples, 29 samples tested positive by NS1 Rapid test, 30 samples tested positive by NS1 ELISA, and 32 samples tested positive by RTPCR. The sensitivity, specificity of rapid dengue NS1 antigen test were 87.50 % & 98.52 % respectively when compared to RTPCR whereas that of NS1 ELISA were 93.75 % and 100 % when compared to RTPCR. Out of 32 samples tested positive by RTPCR, 2 samples were positive for DENV - 1, 26 samples were positive for DENV - 2, and 4 samples were positive were DENV - 3

CONCLUSION
NS_1 ELISA test takes several steps and more time. RDT's require very less time about 15 - 30 minutes single step procedure. Even though RTPCR, ELISA have superior performance than RDT, in countries with fewer infrastructures and in remote areas, RDT's are more useful for early diagnosis and management of dengue with less expertise within a short time.

KEYWORDS
Dengue, NS1 antigen, Rapid, Enzyme linked immunosorbent assay, RTPCR Serotyping

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INTRODUCTION

Dengue fever is a viral infection spread by arthropods that is caused by one of four serotypes of the Dengue virus (DEN1 - 4). It is found all throughout the world, but is most common in tropical and subtropical areas, and is spread by the mosquitoes Aedes aegypti and Aedes albopiticus.[1] Clinical symptoms vary from asymptomatic febrile sickness to more severe infection forms such as Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).[2] Acute dengue infection detection is critical for evidence - based diagnosis, care, control measures implementation, surveillance, and research. An Enzyme - Linked Immune Sorbent Test (ELISA), which assesses anti - dengue virus (DENV) IgM or IgG antibodies in patient serum, is the current diagnostic approach for detecting dengue. These antibodies are detected 4 – 5 days after the beginning of symptoms (PSO).[3] A variety of tests for identifying dengue fever during the acute stage of illness are available on the market. The first ELISA capable of detecting DENV nonstructural protein - 1 (NS1) was developed in the year 2000. NS1 is highly conserved and may be found in both membrane and soluble forms.[4] NS1 is evident early in the acute phase of both primary and secondary DENV infections (day 1 – 8 PSO). The quantity and timing of NS1 levels in human clinical specimens make it an appealing target for the development of numerous diagnostic assays.[5] Both fast NS1 (Based On Immunochromatography Principle) and ELISA tests are now utilised to diagnose dengue infection in afflicted people. As a result, these tests can be used for clinical therapy as well as vector surveillance.

A study was conducted to compare rapid dengue NS1 antigen detection and NS1 ELISA tests with Polymerase Chain Reaction (PCR) for early diagnosis of DENV infection and to detect serotypes by RTPCR Serotyping (1 - 4) kit because rapid dengue NS1 antigen detection is used in private laboratories and emergency care situations.

MATERIAL AND METHODS

A prospective comparative study was conducted in the department of Microbiology at Government Medical College, Ananthapuramu from July 2021 to November 2021 in the Department of Microbiology. Approval of Institutional Ethics Committee was taken before beginning of the study. Before proceeding with any kind of test, informed consent was obtained from the subjects. In case of pediatric patients, assent from the parents was obtained.

Inclusion Criteria

- Clinically suspected cases of dengue presenting with fever from 1 to 4 days along with symptoms and signs of acute dengue like illness and whom serological diagnosis requested for dengue infection.
- Patients of all age groups.

Exclusion Criteria

- Patients presenting with fever > 4 days.
- Non conclusive reports, already diagnosed cases of dengue (referred or admitted with dengue positive report).

Type of Sample and Collection

Approximately, 3 - 5 ml of blood sample was collected aseptically from clinically suspected dengue fever patients who have come to General Medicine and Pediatric OPD within 4 days of fever onset were collected and sent to laboratory after written informed consent. In addition, relevant demographic, clinical, and investigational information was collected from patient's record file. Detailed clinical history of the patient with relevant records was recorded in case record form.

Sample Processing

Collected samples were centrifuged at 2500 rpm for 15 min to obtain serum and plasma. The sera were subjected to rapid dengue NS1 antigen test, and NS1 ELISA, and plasma samples were used for real - time RT - PCR with serotyping kit.

Rapid Dengue Non - Structural Protein - 1 Antigen Detection Test

In all, 100 samples were tested by rapid dengue NS1 antigen detection test based on the principle of immunochromatography. The manufacturer's instructions were followed in the procedure, and the results were interpreted as positive or negative. Dengue nonstructural protein-1 antigen detection enzyme-linked immunosorbent assay: NS1 antigen ELISA was performed on all sera samples (n = 100). The kit used was NS1 antigen J Mitra Co, ELISA. The manufacturer's instructions were strictly followed for performing the test and interpreting the results. The O. D. was measured at 450 nm using ELISA reader.

Hi Media Dengue Serotyping (1 - 4) Kit Real - Time Probe Based PCR: The serotyping of all the samples was carried zout using commercially available.

Hi Media Dengue Serotyping (1 - 4) Kit Real - Time Probe Based PCR: The assay contained a super mix for the specific amplification of DENV 1 – 4 RNA. The primer mix contained primers for DENV - 1 carrying a FAM, DENV -2HEX, DENV - 3 with Texas Red probe, and DENV - 4 carrying Cy5 probe. CY 5.5 The primer mix included a HEX - labeled probe to detect the RNA Internal Control (IC) used to monitor the extraction process and RT - PCR inhibition. A positive control was provided in the kit. The test was carried out according to the manufacturer's instruction.

RESULTS

Out of 100 samples received in laboratory, 51 samples belong to Females & 49 samples belong to males (Table 1).

Gender	Frequency (n)	Percentage (%)
Male	49	49
Female	51	51

Table 1. Gender Distribution.

Out of total 100 samples, 32 (32 %) samples were tested positive by RTPCR test. out of 32 positive samples, 2 samples were positive for DENV - 1, 26 samples were positive for DENV - 2, and 4 samples were positive were DENV – 3 (Table 2).

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

Table 2. RTPCR Tests.

Out of total 100 samples, samples tested positive by RTPCR test were 32 %, samples tested positive by NS1 antigen test were 28 (28 %), and samples tested positive by NS1 ELISA were 30 (30 %) (Table 3).

Diagnostic test	No. of dengue positive samples	Percentage (%)
RTPCR	32	32
NS_1 ELISA	30	30
NS_1 antigen immuno chromatographic test (Rapid test)	29	29

Table 3. Comparison of Result by Different Diagnostic Assays.

Sensitivity, specificity, when only NS1 was considered on ELISA when compared with RTPCR were 93.75 %, 100 %, and Sensitivity, specificity, when only NS1 was considered on RDT kits when compared with RTPCR were 87.50 %, 98.52 % (Table 4).

	RTPCR			Sensitivity	Specificity
		Positive	Negative		
NS_1 ELISA	Positive	30	0	93.75%	100%
	negative	2	68		
NS_1 Rapid test	Positive	28	1	87.50%	98.52%
	negative	4	67		

Table 4. Comparison of NS1 ELISA, NS1 RAPID against RTPCR.

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 (DENV Non - structural Protein 1) is substantially conserved in both membrane and soluble forms. The NS1 antigen is highly specific and may be detected in serum from days 1 to 9 following the beginning of a fever.[6] we compared the quick dengue NS1 antigen detection test and NS1 ELISA test to real - time RTPCR for early dengue diagnosis in this study. Sensitivity and specificity were 93.75 percent and 100 percent, respectively, when only NS1 was considered on ELISA when compared to RTPCR, and 87.50 percent and 98.52 percent, respectively, when just NS1 was considered on RDT kits when compared to RTPCR. According the NS1 fast diagnostic test has a sensitivity of 38 – 71 percent and a specificity of 76 % – 80 %, whereas the NS1 antigen ELISA has a sensitivity of 60 %– 75 % and a specificity of 71 % – 80 %.[7] found that the quick dengue NS1 antigen test has a sensitivity and specificity of 81.5 percent and 66.7 percent, respectively, whereas the NS1 ELISA had a sensitivity and specificity of 89.9 % and 100 %.[8] In their study.[9] found that while both NS1 and RT - PCR are beneficial for early Dengue diagnosis, NS1 quick detection outperformed RT- PCR in terms of cost, technical performance, and speed.

CONCLUSION

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 we employed for testing worked on par with an ELISA - based test, according to our 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 lab infrastructure, particularly in rural and isolated locations. These fast tests are very basic, do not require technological skill, are straightforward to administer, and the findings are delivered in minutes, allowing for timely case management.

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