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Original Research Article

A Comparative Study on NS1 Antigen Detection in Acute Dengue Infection by Rapid Diagnostic Test and Elisa in a Tertiary Care Hospital

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Abstract

Introduction: Early detection of dengue virus (DENV) infection can enhance clinical outcomes by maintaining close monitoring, implementing appropriate supportive medicines, and raising awareness of the risk of haemorrhage or shock. Non-structural glycoprotein-1 (NS1) has been shown to be an effective biomarker for the early detection of dengue. Rapid diagnostic tests (RDTs) and enzyme-linked immunosorbent assays (ELISAs) targeting NS1 antigen (Ag) are now commercially available. The purpose of the study was to improve the early identification of dengue by comparing the immunochromatographic test and ELISA for NS1 antigen.

Materials and Methods: This cross-sectional study was done on 100 clinically suspected cases of Dengue at a tertiary care hospital in Nellore, Andhra Pradesh, India. All the sera samples were collected and subjected to NS1 antigen detection test by rapid test and NS1 ELISA.

Results: Out of total 100 samples, 76 samples were tested positive by NS1 rapid test & 91 samples were tested positive by NS1 ELISA. The sensitivity, specificity of dengue NS1 antigen rapid test were 82.2% and 87.6% when compared to ELISA.

Conclusion: The NS1 ELISA test needs additional procedures and time. RDTs require a one-step procedure that takes 15-30 minutes. Despite the fact that ELISA perform better than RDTs, RDTs are more useful for early diagnosis and treatment of dengue fever in nations with poor infrastructure and in remote locations.

Keywords: Dengue, NS1 Antigen, Enzyme-linked immunosorbent assay, Rapid diagnostic test

Introduction

Dengue fever is a mosquito-borne arboviral disease caused by the Flavivirus genus and transmitted to humans by the bites of infected Aedes mosquitos, most notably Aedes aegypti.[1]

Dengue fever is caused by any of four serologically similar viruses known as DENV-1, DENV-2, DENV-3, and DENV-4 [2]. Infection with either of these serotypes usually results in a mild, self-limiting febrile disease (Classical Dengue fever), but in a rare case, severe life-threatening Dengue Haemorrhagic Fever 1 (DHF) and Dengue Shock Syndrome (DSS) emerge [2]. There are various laboratory approaches for diagnosing dengue infection, including virus isolation, RNA detection, antigen and antibody testing. However, both viral

isolation and viral RNA identification using RT-PCR take time and require a specialised laboratory with expensive methods and well-trained personnel, which may not be readily available in hospital settings [3,4].

In most cases, serologic assays such as ELISA are performed to detect IgM and IgG antibodies. The presence of IgM antibodies during the acute phase implies primary infection, and it appears after viremia or fever has subsided [5]. Detection of nonstructural protein 1 (NS1) antigen in patients with primary and secondary infections has recently been researched in several laboratories across the world [4,6-8]. NS1 is a highly conserved glycoprotein that is generated in both cell membrane-associated and secreted forms across all serotypes [8]. It is required for virus viability or replication, although it has no biological action, and its particular function has yet to be determined [9]. It elicits a strong humour response [10]. NS1 antigen is detected in blood from the first day after fever onset until day 9, and it is also detectable in the presence of IgM antibodies and when viral RNA by RT-PCR is negative [7]. The primary goal of this study was to compare the immunochromatographic test and the ELISA for NS1 antigen in order to enhance the early detection of dengue.

Materials and Methods

A cross-sectional study was conducted in the Department of Microbiology at Narayana Medical College & Research Institute, Nellore, Andhra Pradesh. Prior to the start of the study, the Institutional Ethics Committee (IEC) approval 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: 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. 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 based on the immunochromatographic approach and NS1 ELISA. The kit used was J.Mitra Co. Pvt Ltd. The test procedures were performed according to manufacturers' instructions.

Statistical Analysis

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

Result

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

Table 1: Gender distribution

Gender	Frequency	Percentage
Male	49	49
Female	51	51

Out of total 100 samples, samples tested positive by ELISA test were 91% as shown in Table 2.

Table 2: NS1 Antigen detection by ELISA Method

Findings	Positive	Percentage
NS1 Positive	91	91
NS1 Negative	9	9

Out of total 100 samples, samples tested positive by NS1 Rapid test were 76% as shown in Table 3.

Table 3: NS1 Antigen by RDT (Immuno chromatography Card Method)

Findings	Positive	Percentage
NS1 Positive	76	76
NS1 Negative	24	24

When the rapid ICT test for NS1 Ag was compared with the NS1 Antigen capture ELISA, it showed a sensitivity of 82.2% and specificity of 87.6% which was found to be less sensitive and less specific as shown in Table 4.

Table 4: Comparison of NS1 Rapid test and NS1 ELISA test

Parameter		NS1 ELISA		sensitivity	specificity
		Positive	Percentage		
NS1 RAPID TEST	NS1 Positive	75	1	82.2%	87.6%
	NS1 Negative	16	8		

Discussion

There is no preventive vaccine for dengue. In endemic areas, early diagnosis and treatment are critical for reducing sequelae and disease control. Along with the problems in prevention, correct diagnosis of dengue infection is also challenging due to the lack of distinct symptoms, particularly in the early stages. Dengue infection can be diagnosed by virus isolation and viral RNA detection using RT-PCR, but these technologies are time consuming and are not available in most tertiary care hospitals, hence diagnosis is reliant on the identification of dengue specific NS1 antigen using fast kits or ELISA. When compared to ELISA, the sensitivity of the rapid ICT tests for NS1 Ag in our investigation was more than 80% and the specificity was more than 85%. As a result, the likelihood of a patient contracting acute dengue infection if the tests are positive is nearly identical to that of ELISA-based tests. This study's findings are consistent with prior research that have revealed the effects of quick ICTs [11-13].

Rapid ICTs having the advantage of being simple to conduct, requiring little skill, and being accomplished in minutes [14]. A high number of samples must be processed at the same time to make ELISA cost viable. For an ELISA test, a lab must be equipped with instruments such as an ELISA washer and reader. In comparison to ELISA, quick ICT requires very little technical competence and produces results in minutes. The key benefit of quick ICT is that a single sample can be

conducted without having to wait for the samples. The sensitivity and specificity of various kits on the market in a developing nation like India vary greatly, and this should be borne in mind when doing dengue diagnostic testing.

Conclusion

According to the findings, the rapid RDT kit used for NS1 antigen detection performed similarly to an ELISA-based test. Rapid dengue ICT tests can be employed in early detection and management of acute dengue infection in countries without infrastructure for diagnostic labs in rural regions.

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