e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(7); 473-476

Original Research Article

Comparison of Rapid Immuno- Chromatographic Card Test with Elisa in Diagnosis of Dengue Fever at Tertiary Care Centre

N Raghu Prakash Reddy[1], Satish Kumar Reddy.G[2], B V.V.V. Tejaswani[3]

[1]Associate Professor of Microbiology, Kamineni Institute of Medical Sciences, Hyderabad, Telangana,
[2]Associate Professor of Microbiology, Kamineni Institute of Medical Sciences, Hyderabad, Telangana
[3]Assistant Professor of Microbiology, Kamineni Institute of Medical Sciences, Hyderabad, Telangana

Received: 20-03-2023 / Revised: 21-04-2023 / Accepted: 25-05-2023
Corresponding author: Dr. Satish Kumar Reddy. G
Conflict of interest: Nil

Abstract

Background: As a result of its association with mortality and morbidity, dengue has emerged as a significant public health concern throughout India. It is the most prevalent mosquito-borne viral illness in humans. As a result, early and rapid laboratory diagnosis of dengue is critical. For the quick detection of dengue, commercially available rapid tests that detect the presence of NS1 antigen and anti-dengue antibodies have been developed. Early dengue diagnosis is the only effective means of controlling disease progression. This study was conducted to compare rapid immune-chromatographic test with ELISA for detection of NS1antigen and IgM in suspected dengue patients.

Materials and Methods: From April 2022 to March 2023, a cross-sectional study was conducted in the Department of Microbiology at Kamineni Medical College & Hospital. A total of 175 serum samples from patients suspected of having dengue infection were included in the study. All samples were tested using the rapid ICD test which detects NS1 antigen, IgM and IgG antibodies, as well as the Panbio Dengue Early ELISA, which detects NS1 antigens and the Panbio Dengue IgM Capture ELISA, which identifies IgM antibodies.

Results: The Rapid Dengue Day 1 card Test had a sensitivity and specificity of 93% and 98% for NS1 antigen detection, respectively, and 91% and 100% for IgM Antibody detection, respectively.

Conclusion: Rapid diagnostic assays for the early identification of dengue have a high sensitivity and specificity. Particularly in situations with limited resources, like the periphery health care centre, immunochromatographic testing (ICT) can show to be a very helpful instrument.

Keywords: Dengue fever, ICT, IgM-ELISA, NS1 antigen.

Introduction

Dengue virus is a member of the Flaviviridae family and the Flavivirus genus. Positive sense RNA on a single strand is found in these viruses.[1] It has four serotypes: DEN-1, DEN-2, DEN-3, and DEN-4, and a fifth serotype, DEN-5, was discovered in Bangkok in 2013.[2] The Aedesaegypti mosquito is the primary vector of the viruses that cause dengue. The viruses are transmitted to humans via the bites of an infective female Aedes mosquito that picks up the virus while feeding on the blood of an affected person. These mosquitos also spread diseases such as Yellow fever, Zika fever, and Chikungunya.[3]

Dengue fever is a severe flu-like sickness that primarily affects newborns, young children, and adults, but rarely results in death. Dengue fever is characterised by a high fever of 104°F, severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands, and rash. Symptoms typically persist 2-7 days after an incubation period of 4-10 days following an infected mosquito bite.[4,5] Severe dengue, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), is a potentially fatal consequence that can result in plasma leakage, fluid accumulation, respiratory difficulties, severe bleeding, or organ dysfunction. It mirrors the signs and symptoms of many different diseases due to its vast spectrum of clinical features. As a result, it is difficult for doctors to detect and recognise it in a timely manner. There is no specific treatment for dengue/severe dengue, but early detection and access to proper medical care reduces fatality rates to less than 1% and aids in early patient management and the immediate application of appropriate vector control methods, which can aid in the prevention and control of the infection.[6,7]

The detection of viral nucleic acid, virus, antigens, or antibodies, or a combination of these techniques, may be used in the laboratory to confirm dengue

virus infection. NS1 develops positive on the first day of fever.[8] Anti-DENV IgM and IgG antibodies typically take 4-5 and 1-14 days, respectively, to become detectable, depending on whether the patient has primary or secondary infection.[9] For rapid diagnosis, ELISA assays based on the detection of NS1 antigen and IgM antibodies are often utilised.[10]

These assays are prohibitively expensive, time-consuming, and frequently unavailable in small laboratories. For the quick detection of dengue, commercially available rapid kits detecting the presence of NS1 antigen and/or anti-dengue antibodies have recently been developed. These examinations can be performed even in remote and far-falling places with minimal infrastructure and experience. The only effective strategy to prevent illness progression is to diagnose dengue at an early stage.

The present study was conducted to determine and compare the sensitivity and specificity of rapid diagnostic tests for NS1 Ag and IgM antibody for acute dengue diagnosis to ELISA testing.

Materials and Methods

This is a cross-sectional study done at Kamineni Medical College and Hospital, a tertiary care hospital from April 2022 to March 2023. Institutional ethical committee approval was taken prior to the start of the study.

Inclusion criteria

Patients of all age groups, with clinical symptoms and signs of acute dengue illness and whom serological diagnosis requested for dengue infection

Exclusion criteria

Non-conclusive reports, Already diagnosed cases of dengue (referred or admittedwith dengue positive report)

In the proforma, primary details (age, gender, com-plaints, and medical history) were recorded. Under aseptic conditions, blood samples were obtained, and serum was separated and preserved for subse-quent examination.

Dengue Rapid Immuno-chromatographic Test

The Dengue Check Combo Test is a qualitative Immuno-chromatographic test technique for detecting dengue NS1, IgM, and IgG antibodies to dengue virus in human serum or plasma. It consists of two devices housed in a tray, one for detecting dengue NS1 and the other for detecting IgG and IgM antibodies differentially. The detection system employs the principle of antibody/antiserum agglutination with the relevant antigen in immune-chromatography format, as well as the usage of nano gold particles as an agglutination revealing agent. When the test is completed correctly, a built-in control band in the control are a marked 'C' appears in each NS 1 and IgG/IgM device, regardless of the presence or absence of the dengue NS 1 antigen and or 'anti-dengue virus' antibodies in the specimen. Its purpose is to validate each device's test performance.

Dengue NS1 ELISA

To detect low levels of NS1 in serum, an ELISA kit employs a single enzymatically amplified, two-step sandwich-type immunoassay. Controls and unidentified serum samples are diluted in sample dilution buffer containing secondary antibody and incubated in microtitration wells in this Dengue NS1 ELISA kit. These Dengue NS1 ELISA kit wells were coated with a very efficient NS1 antibody before being blocked. The NS1 antigens found in the samples of this Dengue NS1 ELISA kit are sandwiched between the capture and secondary antibodies. The colorimetric result obtained using an enzyme-conjugated-HRP and liquid TMB substrate confirms the presence of NS1 antigen on this Dengue NS1 ELISA kit.

Dengue IGM Capture ELISA Procedure

Serum Pre-dilution

The strip holder is inserted with the microwells. Positive control (PC), negative control (NC), and cut-off calibrator (CO) in triplicate require 5 microwells. The PC, NC, and CO samples, as well as the patient samples, are diluted in appropriate test tubes or microtiter plates. 1000 l or 1 ml of serum diluent is mixed well with 10 l of serum.

ELISA Procedure: (Instructions as Per Panbio Kit Insert)

Using the antigen diluent, the antigen is diluted 1/250. That is, 10 l of antigen plus 2.5 ml of antigen diluent. Per strip, 0.5 mL of diluted antigen is required. In a test tube, a required volume of diluted antigen is mixed with an equal volume of MAb tracer (Horseradish peroxidase conjugated Monoclonal antibody tracer) and stored at room temperature (20- 25 C) until needed. A total of 100l of diluted patient sample and controls (one positive control, one negative control, and three cut-off calibrators) are pipetted into the assay plate's microwells. 4. Cover the dish and incubate it at 37°C for 1 hour. The plate is rinsed six times with diluted wash buffer after incubation. The antigen-MAb tracer solution is thoroughly mixed before 100l is put to microtiter wells. The plate is covered and incubated at 37 C for 1 hour. After incubation, the plates are washed six times with diluted wash buffer. A 100 l pipette of TMB (Tetramethylbenzidine) is pipetted into each well, causing a blue hue to appear. The plate is incubated at room temperature for 10 minutes.

After 10 minutes, 100 l of stop solution is pipetted into all wells. The blue colour will eventually turn yellow. Using a dual wavelength spectrophotometer, the absorbance of each well is measured at 450 nm with a reference filter of 600-650 nm.

Descriptive statistics were used in the statistical analysis. When NS1 was compared to ELISA, the sensitivity, specificity, and predictive values were calculated.

Results

A total of 175 clinically suspected dengue patients were enrolled in our study, and their serum samples were examined. Out of total sample tested, predominance of males patients, 63% (110) were observed, making it a ratio of 1.7: 1. Out of all the cases, preponderance were seen in age group of 21-30 years (28%) followed by group 11-20 years (22%). (Table 1)

Table 1: Age and Gender wise distribution

Age (years)	Males	%	females	%	total	%
0-10	9	5	4	2	13	7
11-20	23	13	16	9	13	22
21-30	30	17	19	11	49	28
31-40	10	6	5	3	15	9
41-50	17	10	10	6	27	16
51-60	14	8	7	4	21	12
>60	7	4	4	2	11	6
total	110	63	65	37	175	100

Out of total 175 samples. Total number of patients detected positive by NS1 ELISA were 58 & by IgM ELISA were 22. Out of total 175 samples. total number of patients detected positive by NS1 ICT were 54 & by IgM ICT were 23. After analyzing the results a total Sensitivity of 93 percent was seen with NS1 antigen, and Specificity of 98 percent, with Positive predictive valve of 96 percent, while data accuracy was calculated to be around 96 percent as shown in Table 2

Table 2: Comparison of NS1 ELISA and ICT for NS1 antigen

	ELISA +ve	ELISA –ve	Total
RAPID +ive	51	3	54
RAPID –ive	7	114	121
	58	117	175

Sensitivity	93%
Specificity	98%
PPV	96%
NPV	96%
DA	96%

Table 2 depicts a total sensitivity of 91 percent with IgM and a total Specificity of 100 percent. Positive Predictive Value was found to be 100 percent, while Data Accuracy of 99 percent was observed

Table 3: Comparison of capture ELISA and ICT for IgM

	ELISA +ve	ELISA -ve	Total
RAPID +ve	20	3	23
RAPID –ve	2	150	152
	22	153	175

Sensitivity	91%
Specificity	100%
PPV	100%
NPV	98%
DA	99%

Discussion

Because there is no vaccine for dengue, early diagnosis and treatment are essential for reducing complications and disease control in endemic areas. In addition to issues with dengue prevention, reliable diagnosis of the virus has proven challenging due to non-specific symptoms, particularly in the early, acute stage of the infection.

In our investigation, serum samples from 175 probable dengue patients were tested for NS1 antigen and IgM antibodies using the Dengue fast test and ELISA. The majority of the patients (49%) were between the ages of 21 and 30. The current study's findings are consistent with the findings of T. Begum et al[11], who discovered that approximately 43 percent of the patients included in their study were between the ages of 15 and 30. Gore MM et al,[12] Raju BJ et al,[13] NeerjaM et al[14], and Dash PK et al[15] obtained almost identical results.The majority of patients in the current study were males (59.78%) rather than females (40.21%). Similar findings were made by Raju BJ et al. [13] (males 61% versus females 39%) and Dash PK. et al.[15]

Many studies in various hospitals have found a wide range of sensitivity (48.5 to 98.7%) and specificity (71.42 to 100%) of ICT-based RDTs when compared to ELISA; similarly, in our study, we found a sensitivity and specificity of 93% and 98% for NS1 antigen detection and 91% and 100% for IgM Antibody detection. Kaylan D et al. [16] discovered a high positivity rate of 22.5% (9 out of 40 cases) compared to traditional ELISA, which showed 5% positivity (2 out of 40 cases) in acute dengue cases.

Our findings are consistent with those of other studies, and the majority of them suggest that ELISA has a better detection power. Combining the results of the NS1 and IgM Assays can improve diagnostic sensitivity and specificity. The findings of our investigation were indistinguishably comparable to those of other authors.

Conclusion

Rapid dengue ICT tests, particularly in centres without ELISA resources, can play an important role in the diagnosis and management of acute dengue infection. Rapid ICTs are simple to execute and can be utilised as point-of-care testing. We propose that in patients with probable dengue infection, the quick ICT Combo test for NS1Ag, IgM, and IgG detection be employed.

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