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Original Research Article

Diagnostic accuracy of rapid immunochromatographic test compared to ELISA in dengue fever

Pooja P. S.*, Sandeep B. Kokate, Sunanda Shrikhande

Department of Microbiology, Government Medical College, Nagpur, Maharashtra, India

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***Correspondence:**
Dr. Pooja P. S.,
E-mail: poojarohini283@gmail.com

ABSTRACT

Background: Dengue fever is a significant mosquito-borne viral disease affecting tropical and subtropical regions. Early and accurate diagnosis is vital for patient care and outbreak control. While enzyme-linked immunosorbent assay (ELISA) is considered the reference standard for dengue diagnosis, rapid immunochromatographic tests (ICTs) offer quicker, simpler alternatives suitable for point-of-care settings.
Methods: This cross-sectional study evaluated 429 clinically suspected dengue cases from July 2022 to June 2024. Serum samples were tested for NS1 antigen, IgM, and IgG antibodies using both rapid ICTs and ELISA. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using ELISA as the gold standard.
Results: The sensitivity of ICTs for NS1 antigen, IgM, and IgG antibodies was 70.97%, 67.14%, and 68.18%, respectively. The corresponding specificities were 99.50%, 99.44%, and 99.51%. PPVs exceeded 97% for all markers, while NPVs ranged from 89.88% to 90.96%.
Conclusions: Rapid ICTs demonstrate high specificity but moderate sensitivity, supporting their use as initial screening tools. ELISA remains essential for confirmatory diagnosis, especially in cases of strong clinical suspicion.

Keywords: Dengue fever, NS1 antigen, IgM, IgG, ELISA, Rapid diagnostic test, Immunochromatographic test, Sensitivity, Specificity

INTRODUCTION

Dengue fever is a mosquito-borne viral infection caused by the dengue virus, which belongs to the *Flaviviridae* family and is transmitted primarily by *Aedes aegypti* mosquitoes. It poses a significant public health challenge in tropical and subtropical regions worldwide, with an estimated 390 million infections annually, of which about 96 million manifests clinically.[1] Early and accurate diagnosis of dengue fever is critical for timely patient management, appropriate vector control, and prevention of outbreaks.

Laboratory diagnosis of dengue typically relies on the detection of viral components or the host's immune response. ELISA is widely regarded as a gold standard for dengue diagnosis due to its high sensitivity and specificity, particularly for detecting dengue-specific IgM and IgG antibodies, as well as the NS1 antigen in the early phase of infection.[2] However, ELISA requires well-equipped laboratories, skilled personnel, and a longer turnaround time, which limits its utility in resource-limited or point-of-care settings.

Rapid ICTs have emerged as a valuable alternative for dengue diagnosis, offering simplicity, rapid results (usually within 15-20 minutes), and the potential for use at the bedside or in field conditions. These tests typically detect dengue NS1 antigen or IgM/IgG antibodies and can facilitate early case detection, especially in primary healthcare and endemic regions.[3] Despite their advantages, concerns remain regarding the diagnostic accuracy of

rapid ICTs, with varying sensitivity and specificity reported across different studies and brands.[4]

This study aims to compare the diagnostic accuracy of rapid ICTs with ELISA for the diagnosis of dengue fever, assessing their performance in terms of sensitivity, specificity, PPV, and NPV. Understanding these parameters is essential to inform clinical decision-making and optimise dengue surveillance strategies, especially in resource-constrained settings.

METHODS

A cross-sectional study was conducted in the department of microbiology at government medical college, Nagpur, Maharashtra, India, after receiving permission from the institutional ethical committee, from July 2022 to June 2024. Suspected cases of dengue fever were taken up for this study. Case selection was based on the WHO dengue guideline 2011. Cases of febrile illness of 2-7 days duration with features like headache, myalgia, arthralgia, rash, hemorrhagic manifestations, and leucopenia were included.[5] Patients with clinical evidence of urinary tract infection, pneumonia, abscess or any other apparent cause of fever were excluded; patients attending out-patient department (OPD) and those who were not willing to take part were excluded from the study. The sample size was calculated by

$$n=\frac{Z^2_{1-\frac{\propto}{2}}\,p(1-p)}{d^2}$$

Where,

$1-\frac{\propto}{2}$ =95% (Desired confidence level)

p=38.3% (Expected prevalence)

d=5% (Absolute precision)

n=363(Estimated sample size)

Sample size is estimated based on the percentage of seropositivity of suspected dengue cases in a study conducted in Kolkata.[20]

Based on the criteria, around 5 ml of blood sample was collected aseptically from 429 patients by venepuncture. The serum was separated by centrifugation and collected in a serum vial. The specimen was properly labelled with the name of the patient, date of collection and laboratory number.

Samples were immediately tested for dengue NS1 antigen, IgM and IgG antibodies by rapid immunochromatographic method (Figure 1 and 2) and then stored for ELISA at 2-8°C for one week, or frozen at -20°C or lower. (Samples were kept from repeatedly freezing and thawing.) All the samples were tested for the detection of dengue NS1 antigen by DENGUE NS1 Ag MICROLISA and dengue-specific IgM and IgG by capture ELISA. According to the manufacturer's instructions, tests were performed, and calculations were done.

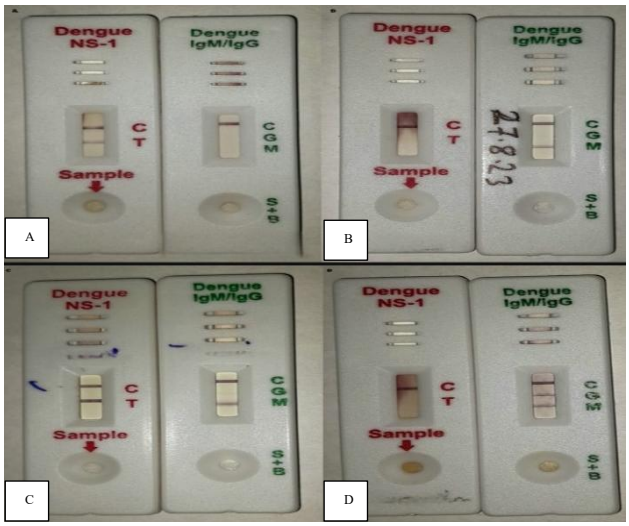

Figure 1 (A-D): ICT cards (A)-positive for NS1 antigen only; (B)-positive for IgM only; (C)-positive for NS1 and IgM; (D)-positive for IgM and IgG.

RESULTS

All 429 samples were tested by rapid ICT and ELISA for all 3 dengue-specific serological markers (NS1 antigen, IgM, and IgG antibodies). The distribution of positive cases by each method is shown in Figure 2.

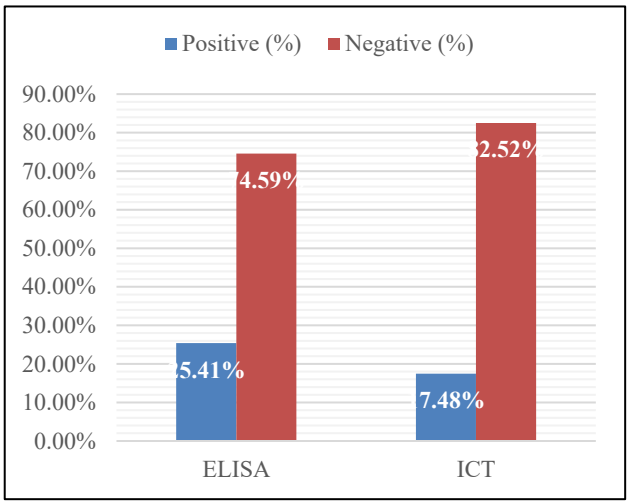

Figure 2: Detection of dengue cases by rapid ICT and ELISA (by any one of the dengue-specific serological markers).

The majority of dengue-positive cases are in the age group of 21-30 years. Out of 109 positive cases detected by ELISA, 54.13% were females and 45.87% were males (Figure 3). 74.31% positive cases belong to urban areas and 25.69% to rural areas.

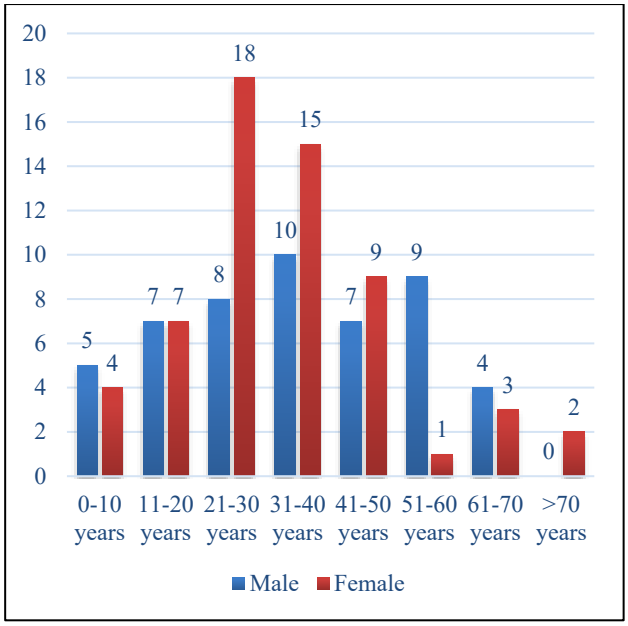

Figure 3: Age-wise and gender-wise distribution of dengue positive cases.

From Table 1, the sensitivity of rapid NS1 ICT was 70.97% when evaluated against NS1 antigen ELISA as a reference test. Specificity was 99.50% compared to NS1 antigen ELISA, and positive and NPVs were 97.96% and 90.96%, respectively.

Table 1: Comparison of dengue NS1 antigen by ICT with ELISA.

Dengue NS1 antigen	ELISA positive	ELISA negative	Total
ICT positive	22	2	24
ICT negative	9	396	405
Total	31	398	429

From Table 2, the sensitivity of rapid IgM ICT was 67.14% when evaluated against dengue IgM antibody capture ELISA as a reference test. Specificity was 99.44% compared to IgM capture ELISA, and positive and NPVs were 97.62% and 89.88%, respectively.

Table 2: Comparison of dengue IgM antibody by ICT with ELISA.

Dengue IgM antibody	ELISA positive	ELISA negative	Total
ICT positive	47	2	49
ICT negative	23	357	380
Total	70	359	429

From Table 3, the sensitivity of rapid IgG ICT was 68.18% when evaluated against dengue IgG antibody capture ELISA as a reference test. Specificity was 99.51% compared to IgG capture ELISA, and positive and NPVs were 97.92% and 90.18%, respectively.

Table 3: Comparison of dengue IgG antibody by ICT with ELISA.

Dengue IgG antibody	ELISA positive	ELISA negative	Total
ICT positive	15	2	17
ICT negative	8	404	412
Total	23	406	429

DISCUSSION

Dengue infection typically manifests similarly to other viral illnesses. The clinical spectrum of dengue infection varies from asymptomatic febrile illness to dengue haemorrhagic fever (DHF), or dengue shock syndrome (DSS). For this reason, it becomes crucial to diagnose and treat the infection promptly. Rapid ICTs are easy assays that do not require complex lab equipment or skilled personnel and can yield results in twenty minutes or less. As a result, these tests may be employed as screening procedures for an early diagnosis.[6]

In our study, ELISA was positive for 25.41% of samples by any one of the dengue-specific serological markers (NS1, IgM or IgG). In comparison, 17.48% of samples were positive for any one of the dengue-specific serological markers by ICT. A total of 14.85% (735/4948) of participants in a study by Kalita et al tested positive for dengue serology using various rapid tests.[7] In a study by Gill et al out of 250 serum samples of the patients suspected of dengue fever, 69 were seropositive by ELISA and 55 were positive by the rapid test.[6] In most studies, dengue seropositivity is higher by ELISA in comparison to rapid ICTs. Hence, in case of high clinical suspicion, it's ideal to test samples by ELISA even if ICT tested negative.

In this study, the sensitivity of NS1 rapid ICT was 70.97% when evaluated against NS1 antigen ELISA as a reference test. Specificity was 99.50% compared to NS1 ELISA, and positive and NPVs were 97.96% and 90.96%, respectively. Dussart et al reported a similarly low sensitivity of 81.5% with a 100% specificity.[8] In contrast to the current investigation, the immunochromatography test for dengue NS1 protein in a study by Zainah et al gave an overall sensitivity of 90.4% and a specificity of 99.5% for rapid detection of dengue NS1 antigen in serum, with reference to the commercial dengue NS1 ELISA. This immunochromatography test had a PPV of 99.6% (284/285) and a NPV of 87.9% (218/248) for the rapid detection of dengue NS1 antigen in serum.[9] Comparably, using the DENV NS1 ELISA as the standard, a study by Shukla et al found that the DENV NS1 RDT has 99.2% sensitivity and 96.0% specificity.[10] According to a study by Hang et al when the target antigen is not available for the monoclonal antibody from ELISA, false-negative results may result from the formation of immunological complexes of NS1 antigen with IgG, particularly during secondary infections.[11] According to Dussart et al rapid assays are being used to identify the dengue NS1 antigen, and they offer a promising substitute for diagnostics that

rely on antibody detection.[8] Zainah et al stated that it would be ideal to have an easy, rapid dengue test that does not require any equipment for detecting acute dengue, whether in an outpatient clinic setting or fieldwork. This intended purpose is fulfilled by the rapid dengue NS1 antigen immunochromatography test.[9]

In the present study, the sensitivity of rapid ICT was 67.14% when evaluated against IgM antibody capture ELISA as a reference test. Specificity was 99.44% compared to ELISA and positive and NPVs were 97.62% and 89.88%, respectively. In research evaluating anti-dengue virus immunoglobulin M kits, Hunsperger et al showed similar low sensitivity and high specificity. In comparison to reference ELISAs, test sensitivities ranged from 21% to 99% and specificities from 77% to 98%. Patients with prior dengue infections or with malaria were shown to have false-positive results.[12] Hasan et al calculated sensitivity, specificity, negative and PPVs of dengue IgM rapid ICT and compared them with those of dengue IgM ELISA and the results obtained were 16.67%, 88.89%, 24.24% and 83.33% respectively.[13] Eight commercially available rapid ICTs for the detection of acute dengue virus infection were chosen for performance evaluation in a study by Blacksell et al. The results obtained were compared with the dengue IgM/IgG ELISA results. Low assay sensitivities, 6.4% to 65.3%, were observed. The specificities observed ranged from 69.1% to 100%. RDTs showed false positive reactivity (4.4% to 34.8%) in samples from individuals infected with the dengue-related flavivirus.[14] According to Peeling et al detecting dengue-specific IgM is a useful surveillance and diagnostic method. The quality of the antigen employed in IgM-based assays has a significant impact on its sensitivity and specificity, which might differ significantly between commercially available kits.[3] Anti-DENV IgM tests have certain limitations, such as their inability to detect the DENV serotype that is causing infection and the likelihood of antibody cross-reactivity with other flaviviruses. IgM assays should not be utilised in dengue endemic countries as confirmatory tests for current illness, since IgM can persist for up to three months or longer. IgM implies that a dengue infection occurred within the last two to three months. The demonstration of a seroconversion (fourfold or higher changes in antibody titres) in paired sera is necessary to diagnose an acute dengue infection.[3,12]

The sensitivity of rapid dengue IgG ICT was 68.18% in the current study when evaluated against IgG antibody capture ELISA as a reference test. Specificity was 99.51%, and positive and NPVs were 97.92% and 90.18%, respectively. The study conducted by Jang et al used the dengue IgG ELISA as a reference, and the results showed that the IgG antibody ICT sensitivities ranged from 72.48% to 82.57%. The range of specificities for dengue-specific IgG ICT was 95.24% to 100%.[15] Hasan et al also showed low sensitivity and high specificity of dengue IgG ICT. In this study, sensitivity, specificity, NPV and PPV of IgG ICT were calculated and compared to dengue IgM ELISA results. The obtained values were 33.33%, 100%, 31.03%, and 10%, respectively.[13] A study by Liberal et al which evaluated the effectiveness of a dengue IgG rapid diagnostic test intended to determine dengue serostatus, reported a high sensitivity and specificity, in contrast to the current study. The OnSite dengue IgG RDT showed 95.3% sensitivity and 98.0% specificity.[16] According to Chakraverti et al the IgG is a less reliable marker for dengue infection. IgG produced by both clinical and subclinical illnesses can last for years and alter the interpretation of test results. Bites from infected mosquitoes in endemic areas may induce elevated IgG levels.[17] Paired sera must be collected within the appropriate time range to allow for the demonstration of seroconversion between acute and convalescent serum samples for dengue-specific IgG-based assays to be employed for detecting both past and present infections. The broad cross-reactivity with other flaviviruses is a limitation of IgG-based ELISA. Its inability to determine the infecting dengue virus serotype is another drawback.[3]

CONCLUSION

Dengue is an important vector-borne disease. In recent decades, dengue has emerged as a notifiable public health problem in terms of its mortality, morbidity, and economic burden, especially in the tropics and subtropics. It is a major public health problem in India as well. Dengue infection has been endemic in many parts of India for over two centuries as a benign and self-limited disease. Dengue epidemics are increasing in frequency, and it is becoming hyperendemic in India. The dengue rapid immunochromatography test has very less sensitivity, but its specificity is satisfactory. The PPV of dengue rapid ICT is satisfactory, but the NPV is less satisfactory. Ideally, dengue should be diagnosed at the primary level of care, and the best tool available is rapid ICT, which can be carried out fast and easily without a sophisticated laboratory. Low sensitivity and potential for cross-reactivity with other flaviviruses are hurdles for us. Hence, the commercially available rapid ICTs device can be used as a screening device during dengue outbreaks. It should not be used as a stand-alone device for the diagnosis of dengue. Cases with higher degrees of clinical suspicion should be subjected to diagnostic tests with higher sensitivity and specificity, like ELISA and reverse transcription polymerase chain reaction (RT-PCR).

This study comparing rapid tests and ELISA for dengue diagnosis has some limitations, including variations in sensitivity, timing of sample collection, and subjective interpretation of rapid tests. Relying on ELISA alone as a reference standard might have missed cases without molecular confirmation. Moreover, the potential for cross-reactivity with other flaviviruses cannot be entirely excluded.

Recommendations

Further large-scale, multicenter studies incorporating RT-PCR as the reference standard are recommended to validate the diagnostic performance of both ELISA and rapid ICTs. Inclusion of molecular diagnostic techniques will provide more definitive insights into the true

sensitivity and specificity of serological assays. Additionally, emphasis should be placed on standardising rapid test kits and evaluating their performance across various stages of dengue infection. Strengthening laboratory infrastructure and enhancing training for healthcare professionals in the interpretation of diagnostic results will also contribute to improved case detection, timely intervention, and more effective management of dengue outbreaks.

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