MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.36478/makrjms.2024.12.151.155

Early Detection of Dengue Fever on Day One Using Clinical Criteria and NS1 Antigen Microelisa: A Comparative Study with Immunochromatography

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Key Words
Dengue, NS1 antigen, microelisa, immunochromatography

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Received: 20 August 2024
Accepted: 14 November 2024
Published: 18 November 2024

Citation: P. Sreelatha, P. Shyam Sunder Rao and B. Nageshwar Rao, 2024. Early Detection of Dengue Fever on Day One Using Clinical Criteria and NS1 Antigen Microelisa: A Comparative Study with Immunochromatography. Res. J. Med. Sci., 18: 151-155, doi: 10.36478/makrjms.2024.12.151.155

ABSTRACT

Dengue is prevalent in tropical and subtropical regions, caused by an RNA virus of the Flaviviridae family and transmitted primarily by Aedes aegypti mosquitoes. While most cases are self-limiting, a small percentage progress to severe forms, including dengue hemorrhagic fever and dengue shock syndrome. This study aims to facilitate early dengue diagnosis, specifically on the first day of fever, using clinical criteria and NS1 antigen detection via MICROELISA, comparing results with immunochromatography for NS1 antigen, IgM and IgG antibodies. With no available vaccine or specific antiviral therapy, early diagnosis on day one is critical for effective patient management. The study included 104 patients presenting with clinical signs of dengue, such as fever, headache, retro-orbital pain, muscle and joint pain, flushed skin, rash, petechiae and bleeding manifestations. Serum samples were collected from patients on the first day of fever and tested for NS1 antigen using MICROELISA and immunochromatography, along with IgM and IgG antibodies by immunochromatography. Among the 104 serum samples, 46 (44%) tested positive for NS1 antigen via MICROELISA and 37 (35%) were positive by NS1 antigen immunochromatography. Three samples (2%) were positive for IgM antibodies and one was positive for IgG antibodies by immunochromatography. Correlation of MICROELISA with immunochromatography highlighted the value of NS1 antigen MICROELISA in early dengue detection. This study emphasizes the effectiveness of NS1 antigen MICROELISA as a reliable method for the day-one diagnosis of dengue fever, offering a timely and accurate diagnostic tool essential for early patient management in the absence of a vaccine or specific antiviral treatment. The findings support NS1 antigen MICROELISA as a critical step in improving outcomes for dengue patients through prompt diagnosis and intervention.

INTRODUCTION

Dengue fever, caused by the dengue virus and transmitted through the Aedes mosquito, is a rapidly spreading infectious disease endemic in over 110 countries[1]. Given its high morbidity and potential for severe complications, early diagnosis is critical for patient management and for controlling outbreaks[2]. Traditional diagnostic methods like virus isolation and RT-PCR, though accurate, are time-intensive and require specialized equipment, which may not be accessible in resource-limited settings[3]. Consequently, rapid, sensitive and accessible diagnostic methods are essential, particularly in regions with a high dengue burden[4]. A promising early diagnostic marker for dengue is the NS1 antigen, a non-structural glycoprotein that circulates in the blood during the acute phase of infection. NS1 can be detected as early as the first day of fever onset, making it an important tool for prompt diagnosis[5]. The Dengue NS1 Ag detection via MICROELISA is one of the methods used to identify this antigen. This technique is advantageous as it is quicker and more accessible compared to molecular methods, allowing for rapid diagnosis during the critical early phase of infection when intervention is most beneficial[6]. Furthermore, the NS1 antigen detection can distinguish dengue infection from other febrile illnesses, aiding in timely and accurate patient management. While studies have demonstrated the effectiveness of NS1 antigen detection, there is limited research on the diagnostic accuracy of NS1 antigen tests specifically on the first day of fever onset[7]. Previous studies have assessed the performance of NS1-based diagnostics across multiple days of illness, often combining results from days 1-5 or later in the disease course, where sensitivity may vary[8]. However, the effectiveness of NS1 MICROELISA specifically on the first day remains under explored. A few studies have indicated high sensitivity and specificity for NS1 antigen detection during early infection phases, but there is insufficient data on its day-one accuracy, which is critical for early intervention. This gap underscores the need to evaluate the reliability of NS1 MICROELISA for day-one diagnosis, particularly in high-prevalence areas where prompt detection can significantly impact clinical outcomes. Early diagnosis based on clinical criteria plays a significant role in managing potential epidemics and implementing vector control measures. While virus isolation and RT-PCR are time-consuming and require specialized laboratory equipment, serological diagnosis is more practical for dengue fever[9]. This can be achieved using NS1 antigen detection via MICROELISA and immunochromatography to detect NS1 antigen, IgM and IgG antibodies of the dengue virus. This study aims to assess the diagnostic accuracy of Dengue NS1 Ag MICROELISA in detecting dengue infection on the first day of fever onset. By focusing on day-one diagnosis, we seek to provide insights into the utility of NS1 antigen detection for early intervention, contributing to the development of more effective diagnostic protocols for managing dengue outbreaks and reducing disease burden.

MATERIALS AND METHODS

This study was conducted on 104 clinically diagnosed cases of dengue fever at Mamata General Hospital. Cases were selected based on WHO clinical criteria, including fever, headache, retro-orbital pain, nausea, vomiting, muscle and joint pain, flushed skin, maculopapular rash, petechiae, bleeding manifestations and abnormal blood counts. Blood samples were collected on the first day of fever along with associated symptoms and were sent to the microbiology department for dengue diagnosis. The serum samples were first subjected to the Dengue Day 1 test by J. Mitra, a rapid, solid-phase immunochromatographic test designed for the qualitative detection of dengue NS1 antigen and differential detection of IgM and IgG antibodies to dengue virus in human serum and plasma. This test serves as an aid for day-one diagnosis of dengue infection. The test kit consists of two devices one for NS1 antigen detection and the other for IgM and IgG antibody detection in serum/plasma samples.

- **NS1 Antigen Device:** This device has two lines: “C” (control line) and “T” (test line for dengue NS1 antigen). When serum containing dengue NS1 antigen is added, it binds to the anti-dengue NS1 gold colloid conjugate, forming an antigen-antibody complex. This complex migrates along the nitrocellulose membrane to the test region, creating a visible pink line at “T,” indicating the presence of NS1 antigen.
- **LgM and LgG Test Device:** This device contains three lines “C” (control line), “M” (IgM test line), and “G” (IgG test line). When serum is added, IgM and IgG antibodies in the sample react with particles coated with dengue proteins. As this complex migrates, it is captured by the corresponding test bands in the device window, causing a pale pink band to form at the IgM or IgG region. The intensity of these bands varies with the amount of antigen or antibody in the sample. A red procedural control line always appears to confirm that the test was performed correctly.

After initial testing, all serum samples were also tested using the NS1 Ag MICROELISA. The NS1 Ag MICROELISA

(from J. Mitra) is a sandwich ELISA designed to detect the NS1 antigen of all four serotypes of dengue virus, with a test procedure lasting two hours.

Dengue NS1 Ag Microelisa Procedure:

- The MICROELISA plates are pre-coated with anti-dengue NS1 antibodies. Fifty microliters of sample diluent is added to each well, followed by 50 microliters of positive and negative controls, calibrators and serum samples.
- A diluted enzyme conjugate (1:20) is prepared, and 100 microliters of the working enzyme conjugate is added to each well.
- The ELISA plate is covered and incubated at 30°C for 90 minutes.
- The plates are washed six times, blotted and incubated at room temperature for 30 minutes in the dark after adding 150 microliters of chromogenic substrate (tetramethylbenzidine, TMB).
- After 30 minutes, the reaction is stopped with a stop solution and the plates are read using an ELISA reader.

Interpretation of Results:

- The cutoff value was calculated based on the optical density (OD) of controls and calibrators, with an index value derived by dividing the sample OD by the cutoff value.
- An index value of <0.9 was considered negative, 0.9-1.0 was equivocal and >1.1 was positive.

In this procedure, the NS1 antigen present in the sample binds to the coated anti-NS1 antibody, forming a complex with the enzyme conjugate and TMB substrate. The enzyme conjugate then reacts with the TMB substrate to produce a color change, indicating a positive result. Samples without NS1 antigen show no color change.

RESULTS AND DISCUSSIONS

A total of 104 serum samples were tested for dengue NS1 antigen using both the NS1 Ag MICROELISA (direct sandwich ELISA, J. Mitra) and immunochromatography (ICT) for NS1 antigen, IgM and IgG antibodies. The results of the dengue NS1 Ag MICROELISA were compared with the immunochromatography results for NS1 antigen and IgM, IgG antibodies.

Table 1: Summary of Dengue-Positive Samples by Diagnostic Test

Diagnostic Test	Number of Positive Serum Samples
Dengue NS1 ELISA	46
Dengue NS1 ICT	37
Dengue IgM ICT	3
Dengue IgG ICT	1

All 37 samples that were positive for dengue NS1 antigen by ICT were also positive by NS1 Ag MICROELISA. Additionally, the three samples positive by dengue IgM ICT also tested positive with NS1 Ag MICROELISA. Only one sample was positive for both NS1 Ag MICROELISA and dengue NS1 ICT and another single sample was positive for dengue IgG antibody and IgM ICT (Table 1).

Table 2: Comparative Evaluation of Dengue Day 1 NS1 Ag MICROELISA vs. Dengue Day 1 NS1 Ag and Ab Combi Card

Parameter	Calculation	Result
Sensitivity (True Positives)	True Positives (37) / False Negatives (46) x 100	80.4%
Specificity (True Negatives)	True Negatives (58) / False Positives (58) x 100	100%
Positive Predictive Value (PPV)	True Positives = (True Positives (37) / False Positives (37)) x 100	100%
Negative Predictive Value (NPV)	True Negatives = (True Negatives (58) / False Negatives (67)) x 100	86.5%

(Table 2) discusses the comparative evaluation of dengue NS1 Ag MICROELISA versus the NS1 Ag and Ab Combi Card, indicating higher false negatives with the Combi Card compared to the NS1 Ag MICROELISA. Based on WHO clinical criteria, 104 patients presenting with fever and other dengue symptoms on the first day were included in the study. NS1 Ag MICROELISA detected 46 cases as positive for dengue fever. All 46 patients presented with high, unremitting fever, flushed skin, headache and muscle pains. Additional symptoms were observed as follows:

Table 3: Clinical Criteria and Patient Symptoms

Clinical Criteria	Number of Cases	Percentage
Fever	46	100%
Headache	46	100%
Flushed Skin	46	100%
Muscle Pains	46	100%
Retro-orbital Pain	40	86%
Nausea and Vomiting	35	76%
Joint Pains	21	45%
Maculopapular Rash	17	56%
Petechiae and Bleeding Manifestations	1	2.1%

Out of all patients, only one case showed abnormal blood counts. This female patient presented with high fever, severe muscle and joint pains, headache, petechiae, epistaxis, conjunctival ecchymosis and bleeding per vagina. She tested positive for NS1 Ag in both MICROELISA and ICT, as well as for IgM and IgG antibodies. Unfortunately, this patient succumbed to complications due to vaginal bleeding two weeks after presentation. The remaining patients recovered within 1-3 weeks (Table 3). These findings suggest that NS1 Ag MICROELISA is a reliable method for early diagnosis, with a higher sensitivity compared to the Combi Card device, particularly in the initial stages of dengue infection.

The diagnosis of dengue is typically based on clinical symptoms and physical examination, especially in endemic regions. However, early-stage dengue can be challenging to distinguish from other viral infections. A probable dengue diagnosis is generally made when a

patient presents with fever plus two of the following symptoms: headache, retro-orbital pain, nausea and vomiting, muscle and joint pains, generalized pains, maculopapular rash, a positive tourniquet test, petechiae, or other bleeding manifestations. Low blood counts or warning signs in a patient living in an endemic area further support the diagnosis[10]. Common warning signs include abdominal pain, persistent vomiting, liver enlargement, mucosal bleeding, high hematocrit with low platelets and lethargy, which often appear before the onset of severe dengue. It can also be difficult to differentiate between dengue fever and chikungunya, as both diseases present with similar symptoms and are prevalent in similar tropical and subtropical regions[11]. Laboratory investigations are often conducted to exclude other conditions that mimic dengue symptoms, such as malaria, leptospirosis, typhoid fever and meningococcal disease. The earliest laboratory indicator of dengue is a low white blood cell count, which may progress to low platelet levels and metabolic acidosis. In severe cases, plasma leakage can cause hemoconcentration (indicated by a rising hematocrit) and hypoalbuminemia. Ultrasound can be helpful in some cases but is often limited by lack of availability in many settings[12]. Dengue fever can be confirmed by microbiological testing, including virus isolation in cell cultures, nucleic acid detection via PCR, viral antigen detection, or specific antibody serology. While virus isolation and nucleic acid detection are more accurate, they are less widely available due to higher costs. Previous studies have found NS1 antigen presence in 82-83% of dengue patients from day 1 up to days 9-18 after the onset of fever[13]. The study highlights the utility of Dengue NS1 Ag MICROELISA for diagnosing dengue on the first day of fever onset. The higher sensitivity (80.4%) and specificity (100%) of the MICROELISA in detecting NS1 antigen compared to the immunochromatographic test (ICT) suggest that it is a superior choice for early dengue detection. The results showed that MICROELISA detected more positive cases (46) than the ICT method (37), underscoring its potential in early and accurate diagnosis. In comparing our findings with prior studies, similar sensitivity trends for NS1 antigen detection have been observed. For example, studies by Guzmán *et al.* and Dussart *et al.* support NS1 antigen's effectiveness as a reliable marker in the early phase of dengue infection, showing high specificity in acute infections[14,15]. These studies also noted that NS1 antigen presence declines as the illness progresses, which may limit its use in later stages[15]. However, our study specifically assessed day-one sensitivity, which is critical in regions with high dengue prevalence, where early intervention can drastically impact patient outcomes. The primary advantage of MICROELISA over ICT methods lies in its reduced false-negative rate. Previous studies indicated that ICT methods often miss low-antigen-level cases in the initial stages, leading to potential misdiagnosis or delays in treatment. In our study, MICROELISA detected all 37 cases initially identified by ICT, along with additional cases that ICT missed, thus reinforcing its role as a more sensitive tool for early dengue detection. Given the clinical symptoms observed among patients high fever, headache, muscle pain and flushed skin MICROELISA's ability to confirm dengue on day one aids in differentiating it from other febrile illnesses, which present with overlapping symptoms. Additionally, early detection allows for timely treatment and implementation of control measures, especially important during dengue outbreaks. Furthermore, the MICROELISA's performance aligns with the WHO's guidelines emphasizing accessible and accurate diagnostic methods, particularly in resource-limited areas where rapid and affordable diagnostics are essential[16]. The reliable diagnosis provided by MICROELISA can assist healthcare providers in prompt decision-making, minimizing severe dengue complications and potentially reducing mortality rates.

CONCLUSION

The Dengue NS1 Ag MICROELISA demonstrates high diagnostic accuracy for early dengue detection, outperforming ICT methods, especially on the first day of fever onset. Given its higher sensitivity, it is a practical and effective tool for early diagnosis in endemic areas, thereby improving patient outcomes. Future studies should focus on large-scale evaluations of MICROELISA's efficacy across diverse patient demographics and varied disease stages to further solidify its clinical utility in managing dengue fever.

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