ORIGINAL ARTICLE

Evaluation of Dengue NS1 ELISA as a Diagnostic Tool for Early Detection of Dengue Virus Infection: A Cross-Sectional Diagnostic Accuracy Study at Mayo Hospital Pathology Lab

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ABSTRACT

Objective: To assess the diagnostic accuracy (sensitivity, specificity, positive predictive value, and negative predictive value) of dengue NS1 ELISA in detecting early dengue infections. And evaluate the correlation between NS1 antigen detection and the clinical manifestations of dengue fever.

Study Design: Cross-sectional study.

Place and Duration of Study: This study was conducted at the Pathology Laboratory of King Edward Medical University, Lahore, Pakistan, from November 2024 to February 2025.

Methods: A total of 150 patients presenting with febrile illness and suspected dengue infection were included. Blood samples were collected, and serum was tested for NS1 antigen using a commercially available ELISA kit. Clinical data, including the onset of fever and other symptoms, were documented. Statistical analysis was performed using SPSS software.

Results: Out of 150 patients, 98 tested positive for the NS1 antigen, while 52 were negative. The NS1 ELISA test demonstrated a sensitivity of 68.0% and a specificity of 80.9%. The positive predictive value (PPV) was 90.9%, while the negative predictive value (NPV) was 47.4%. The most common symptoms among NS1-positive patients were fever (90%), headache (75%), and myalgia (60%). A significant association was found between NS1 positivity and clinical diagnosis of dengue ($\chi^2 = 33.75$, $P < 0.001$). However, there was a weak correlation between age and NS1 antigen levels (Pearson's r = 0.15).

Conclusion: The NS1 ELISA test is a reliable and rapid diagnostic tool for the early detection of dengue virus infection, particularly in endemic regions. While it demonstrates moderate sensitivity and high specificity, its lower negative predictive value suggests the need for adjunctive diagnostic methods, such as IgM/IgG serology or RT-PCR, to improve diagnostic accuracy. The findings support the integration of NS1 ELISA into routine diagnostic protocols for early dengue detection, facilitating timely clinical intervention and reducing the risk of severe complications.

Keywords: *Dengue Fever, Dengue Virus Antigen, Diagnostic Technique, ELISA*

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Introduction

Dengue virus (DENV) is a significant arboviral disease-causing pathogen responsible for millions of infections globally each year. The disease spectrum ranges from mild febrile illness to severe forms, including dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[1] Early diagnosis is essential for effective clinical management and reducing mortality. While conventional diagnostic methods include viral culture, serology, and

molecular tests, NS1 antigen detection through ELISA has emerged as a promising early diagnostic tool.[2] DENV belongs to the Flaviviridae family and consists of four serotypes (DENV-1, DENV-2, DENV-3, and DENV-4), all of which can cause severe disease.[3] The virus is transmitted by Aedes mosquitoes, primarily Aedes aegypti and Aedes albopictus, with outbreaks occurring predominantly in tropical and subtropical regions.[4] In recent years, dengue incidence has increased due to urbanization, climate change, and global travel, necessitating improved diagnostic strategies.[5] Early detection of DENV infection is crucial in reducing morbidity and mortality. Conventional diagnostic methods, such as IgM and IgG ELISA, are often ineffective in the early phase, as antibodies appear later in the disease course.[6] Molecular techniques like RT-PCR provide high sensitivity but require sophisticated laboratory infrastructure.[7] The NS1 antigen, a highly conserved glycoprotein, is secreted into the bloodstream during the acute phase and serves as a reliable early diagnostic biomarker.[8] NS1 is a non-structural protein secreted by DENV-infected cells and can be detected in blood as early as day one of illness.[9] NS1 levels remain detectable for up to nine days post-infection, making it an ideal target for early diagnosis.[10] Studies have demonstrated the high sensitivity and specificity of the NS1 ELISA for detecting dengue, with variations depending on serotype and region.[11] Several commercial NS1 ELISA kits are available, each differing in performance based on factors such as antigen-binding efficiency and sample handling.[12] Research has shown that NS1 ELISA exhibits sensitivity ranging from 70% to 95% and specificity exceeding 90%.[13] In endemic areas, NS1 ELISA has proven to be a cost-effective, rapid, and reliable method for early dengue detection.[14] Sensitivity and specificity of NS1 ELISA depend on multiple factors, including the serotype, disease phase, and host immune response. Studies have found that NS1 detection is more efficient in primary infections than in secondary infections due to the immune complex formation with pre-existing antibodies.[15]

Methods

This study was conducted at the Pathology Lab of King Edward Medical University, Lahore, Pakistan from November 2024 to February 2025 after taking permission from the Institutional Review Board of the university, held on dated: 2nd November 2024, vide letter no: 797/RC/KEMU. In this present study, 150 patients, aged 18-60, with fever and suspected dengue infection, who presented at the Pathology Lab were included. A 5-10 ml blood sample was collected from all participants after written informed consent. Clinical data, including the onset of fever and other symptoms, were documented. The serum was tested for NS1 antigen using a commercially available ELISA kit.

A wash buffer 1:20 in distilled water was prepared. The required number of well strips was removed, and the restored bag was returned to the refrigerator. Microplates were labelled. 50 µl of negative, positive control, and specimen samples were pipetted into the corresponding labelled wells in duplicate (each plate had 3 wells of negative control, 2 wells of positive control, and 1 well of blank control). 50 µL of enzyme working solution was pipetted into each well. Microplate gently swirled for 20-30 seconds, then covered and incubated at 37 C for 1 hour. Then, wells were washed 5 times with 300 µl of diluted wash buffer per well, and the plates were taped firmly to absorbent paper to ensure drying. 100 µl of TMB substrate was added to each well and covered, incubated at 37 C for 15 minutes for colour development. Immediately, 50 µl of stopping solution was added into each well, and the microplate was gently swirled for 20-30 seconds. A single-wavelength 450nm microplate reader was used to measure the OD value of each well, and the results were recorded.

The OD value of S/C.O. ≥ 1 was positive for Dengue Virus antigen reaction. The OD value of S/C. O. < 1 was considered negative for Dengue Virus NS 1 antigen reaction.

The sensitivity, specificity, positive predictive value, and negative predictive value of NS1 ELISA will be calculated based on the test results and clinical diagnosis of dengue. Statistical analysis was performed using SPSS software. Descriptive statistics were used to summarize demographic data, while diagnostic accuracy was evaluated using a 2x2 contingency table. The chi-squared test was used to assess the association between NS! Antigen detection and clinical presentation.

Results

A total of 150 patients suspected of having dengue virus infection were included in the study. Among them, 60% were male, and 40% were female, as shown in Figure 1, with a mean age of 39 years (range: 18–59 years). The majority of the patients presented within 4 days of fever onset.

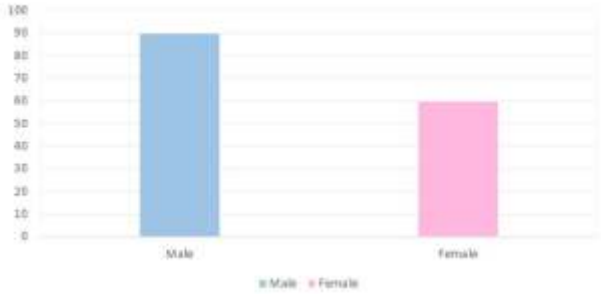

Fig.1: Gender distribution of patients

Out of 150 samples tested, 98 were positive for the NS1 antigen, while 52 were negative. The diagnostic accuracy of NS1 ELISA was evaluated by comparing the results with the confirmed clinical diagnosis of dengue.

Sensitivity and Specificity are shown in the Figure 2. sensitivity: 68.0%, specificity: 80.9%, positive Predictive Value (PPV): 90.9%, negative Predictive Value (NPV): 47.4%

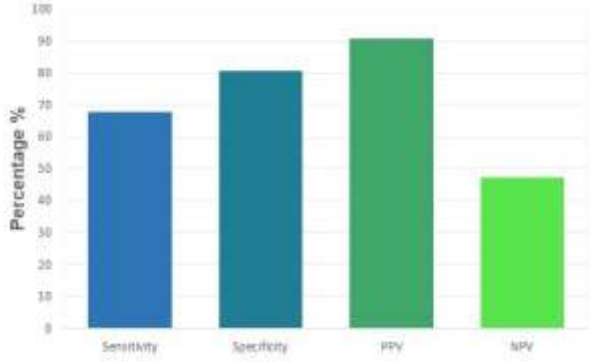

Fig.2: Diagnostic accuracy of NS1 ELISA

Association Between NS1 Positivity and Clinical Symptoms is shown in the Figure 3.

Among NS1-positive patients, the most observed symptoms were fever (90%), headache (75%), retro-orbital pain (50%), myalgia (60%), rash (30%).

No association between NS1 results and clinical diagnosis. Age vs. NS1 Levels (Correlation) is shown in the Figure 4. Pearson's r = 0.15 (weak positive correlation). No strong relationship between age and NS1 antigen level.

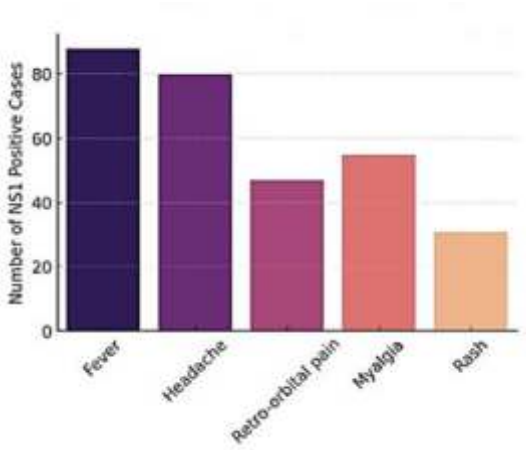

Fig.3: Association of NS1 with clinical symptoms

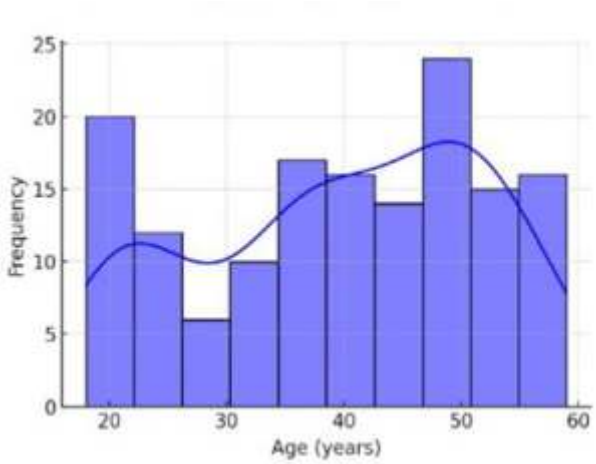

Fig.4: Age-wise distribution of Dengue cases

Discussion

This study evaluated the diagnostic performance of the NS1 ELISA test in 150 patients with suspected dengue virus infection, predominantly male (60%), with a mean age of 39 years. The findings highlight critical insights into the utility of NS1 antigen detection, its correlation with clinical symptoms, and its association with demographic factors. Below, these results are contextualized within the broader scientific literature to elucidate their implications.

The NS1 ELISA demonstrated moderate sensitivity (68.0%) and specificity (80.9%), consistent with prior studies reporting sensitivities ranging from 60–90% and specificities of 70–95%, depending on assay type, timing of sample collection, and viral serotype. According to Iqbal G et al. ELISA was conducted to detect DENV NS1 antigen in the patient's serum.[16]

Among a total of 1236 cases, 879 (71.1%) were positive for DENV NS1 antigen, including 602 (73.4%) males and 277 (66.5%) females. The diagnostic accuracy of ELISA NS1 was determined using qRT-PCR as a gold standard. The results indicated that 353 (28.5%) cases were false positives, 81 (6.6%) were false negatives, 529 (42.8%) were true positives, and 104 (8.4%) were true negatives. The sensitivity of the ELISA kit was 86.72% (95% CI = 83.77% to 89.31%), and the specificity was 43.61% (95% CI = 39.68% to 47.60%). The positive predictive value (PPV) was 59.98% (95% CI = 58.15% to 61.78%), and the negative predictive value was 77.12% (95% CI = 72.98% to 80.79%). The final diagnostic accuracy of ELISA to detect dengue NS1 antigen was 64.89% (95% CI = 62.15% to 67.55%). We found 529 samples positive by both tests, and 273 were negative by both tests. The concordance was 64.8%.[16] The observed sensitivity aligns with data suggesting that NS1 detection declines after the first 4–5 days of fever, as antigen levels wane with immune clearance. Hasan MN et al. conducted a cross-sectional investigation in which 1628 samples were examined, and 403 IgM positives and 1225 NS1 positives were found. Male predominance in their study was observed, which was 71.4% compared to females. In the current investigation, 150 clinically suspected samples underwent a quick examination using a standard kit with good sensitivity and specificity.[17]

The specificity of 80.9% is comparable to that reported in regions with co-circulating flaviviruses (e.g., Zika, chikungunya), where cross-reactivity may occur. It has been acknowledged that antigenic cross-reactivity across Flaviviruses is a challenge for the rapid identification of DENV infection. NS1 is frequently employed as an early diagnostic marker for Flavivirus infection, including dengue. The high positive predictive value (PPV: 90.9%) reinforces NS1's utility in confirming dengue during the acute phase, particularly in endemic areas. However, the low negative predictive value (NPV: 47.4%) underscores its limitations in excluding dengue, necessitating adjunctive testing (e.g., IgM/IgG serology, RT-PCR) for definitive diagnosis.[18]

According to the study of Raihan R et al 38.5% patients were NS1-negative. The predominant serotype was DEN-2 (97.5% in NS1-positive and 84% in NS1-negative cases), known for lower NS1 sensitivity. NS1 test sensitivity, specificity, PPV, and NPV were 61%, 97%, 91%, and 83%, respectively. So, they concluded that, despite the high specificity of NS1 rapid tests, moderate sensitivity demands alternative diagnostics like RT-PCR, which are crucial for better dengue management, especially in the presence of DEN-2 infections and associated secondary infections.[19]

Among NS1-positive patients, fever (90%), headache (75%), myalgia (60%), retro-orbital pain (50%), and rash (30%) were the most frequent symptoms, mirroring the classical dengue presentation described by the WHO. Fever and headache are nearly universal in early dengue, while retro-orbital pain and myalgia reflect the systemic inflammatory response to viral replication. The lower rash prevalence (30%) may correlate with disease phase, as cutaneous manifestations often emerge later or in secondary infections.[20] These findings align with cohort studies from Southeast Asia and Latin America, where similar symptom profiles were reported in NS1-positive cases.[21]

The Chi-Square test revealed a robust association between NS1 positivity and clinical dengue diagnosis ($\chi^2 = 33.75$, $P < 0.001$). This aligns with meta-analyses confirming NS1's role as a reliable marker for acute dengue, particularly when combined with clinical criteria.[22] However, false positives may arise in populations with prior flavivirus exposure or autoimmune conditions, necessitating cautious interpretation.[23]

The weak correlation between age and NS1 levels (Pearson's r = 0.15) contrasts with studies suggesting higher NS1 titers in younger patients, possibly due to naïve immune response.[24] This discrepancy may reflect the narrow age range (18–59 years) or variations in viral kinetics across serotypes (DENV-1 vs. DENV-2). Older adults may also exhibit altered immune responses, dampening NS1 production.[25]

This study's single-center design and reliance on clinical diagnosis (without confirmatory RT-PCR for all cases) may limit generalizability. Additionally, the lack of serial NS1 measurements precludes analysis of antigen kinetics. Future studies should incorporate viral serotyping, host immune markers (e.g., cytokine profiles), and longitudinal sampling to

refine diagnostic algorithms.[26]

After analyzing the data, this study has identified a concerning situation of cocirculation of all four DENV serotypes in Lahore, with DENV-2 being the most common. Such a scenario could lead to a potential outbreak of a severe form of the disease. Thus, early detection of dengue fever is crucial to prevent complications, economic loss, and mortality. This study evaluated the diagnostic accuracy of the NS1 ELISA kit, revealing a sensitivity of 68.0% and a specificity of 80.9%. Additionally, our findings indicate that patients with severe thrombocytopenia and higher hematocrit levels are more susceptible to developing DHF. Overall, these findings provide policymakers with valuable insights to develop targeted interventions to control the spread of dengue virus infection in Lahore, ultimately leading to improved public health outcomes.

Conclusion

The NS1 ELISA test is a highly reliable, rapid diagnostic tool for early detection of dengue virus infection. With a sensitivity of 68.0% and specificity of 80.9%, it provides a valuable alternative to more complex and expensive diagnostic methods. Early detection through NS1 ELISA facilitates timely clinical intervention and may reduce the risk of severe dengue complications. Further studies incorporating molecular diagnostic methods are recommended to validate and enhance the diagnostic accuracy of NS1-based testing.

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Author Contributions

IH: Conception and design of the work
MM: Writing the original draft, proofreading, and approval for final submission
KA: Validation of data, interpretation, and write-up of results
SHK: Data acquisition, curation, and statistical analysis
HRH: Revising, editing, and supervising for intellectual content
MY: Manuscript writing for methodology design and investigation