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COMPARATIVE EVALUATION OF ELISA, PCR, AND NS1 ANTIGEN DETECTION FOR DENGUE VIRUS DIAGNOSIS

Original Research

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ABSTRACT

Background: Dengue fever is a rapidly spreading mosquito-borne viral disease, posing a significant public health challenge in endemic regions like Pakistan. The disease presents with a range of clinical manifestations, from mild febrile illness to severe complications such as dengue hemorrhagic fever and dengue shock syndrome. Early and accurate diagnosis is essential for effective patient management and outbreak control. Molecular, antigen-based, and serological tests serve as primary diagnostic tools; however, their feasibility and accuracy in resource-limited settings remain areas of concern.

Objective: This study aimed to evaluate the diagnostic performance, feasibility, and cost-effectiveness of enzyme-linked immunosorbent assay (ELISA), non-structural protein 1 (NS1) antigen detection, and reverse transcription polymerase chain reaction (RT-PCR) for dengue virus detection at Chaudhry Muhammad Akram Teaching & Research Hospital, Lahore.

Methods: A cross-sectional study was conducted over six months at Chaudhry Muhammad Akram Teaching & Research Hospital. A total of 440 patients with suspected dengue fever were tested using NS1 antigen detection, IgM and IgG ELISA, and RT-PCR. Blood samples were collected, processed, and stored at -20°C. Statistical analysis was performed using SPSS software to assess the sensitivity, specificity, and diagnostic efficiency of each modality.

Results: The NS1 antigen was detected in 18 cases, primarily within the first week of illness, with platelet counts ranging from 50,000 to 150,000 cells/µL. IgM ELISA identified 44 recent infections, with platelet counts between 70,000 and 130,000 cells/µL. IgG ELISA confirmed 31 past or secondary infections, with platelet counts between 80,000 and 140,000 cells/µL. A total of 250 patients exhibited clinical symptoms suggestive of dengue but tested negative in laboratory evaluations. Additionally, 97 patients had inconclusive results.

Conclusion: This study highlights the importance of combining NS1 antigen detection, ELISA, and RT-PCR in a structured diagnostic approach for effective dengue management. Improving diagnostic capacity in resource-limited settings can enhance patient outcomes and strengthen public health surveillance.

Keywords: Dengue virus, Diagnosis, ELISA, NS1 antigen, PCR, Sensitivity, Specificity.

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INTRODUCTION

Dengue fever is a rapidly expanding global health threat, particularly in tropical and subtropical regions where the *Aedes aegypti* mosquito is prevalent. As a mosquito-borne viral infection, dengue is caused by four distinct serotypes of the Dengue virus, each belonging to the *Flaviviridae* family. The disease presents a broad clinical spectrum, ranging from mild febrile illness to severe forms such as dengue hemorrhagic fever and dengue shock syndrome. Reinfection with a different serotype increases the risk of severe disease due to antibody-dependent enhancement. Globally, an estimated 390 million infections occur annually, with approximately 96 million manifesting clinical symptoms, posing a significant socioeconomic burden, especially in low- and middle-income countries with limited healthcare infrastructure(1, 2). In Pakistan, the incidence of dengue has surged sharply over recent decades, with recurrent outbreaks posing a substantial public health challenge. Urban and peri-urban areas, particularly in major cities like Lahore, are highly susceptible due to dense population, favorable breeding conditions for mosquitoes, and insufficient preventive measures. Chaudhry Muhammad Akram Teaching & Research Hospital, Lahore, frequently encounters dengue outbreaks, with contributing factors such as rapid urbanization, inadequate sanitation, and limited healthcare access exacerbating the problem(3, 4).

Given the high prevalence of dengue and the challenges in clinical diagnosis, robust laboratory confirmation is essential to differentiate dengue from other febrile illnesses such as malaria, chikungunya, typhoid, and leptospirosis. Molecular, antigen-based, and serological diagnostic tests play pivotal roles at various stages of infection, ensuring accurate detection and facilitating appropriate patient management(5, 6). Early-phase diagnostics primarily include molecular techniques like reverse transcription polymerase chain reaction (RT-PCR), which detects viral RNA with high sensitivity and specificity. NS1 antigen detection, another early diagnostic tool, identifies the non-structural protein 1 of the dengue virus, signaling active infection. As the disease progresses, serological assays such as enzyme-linked immunosorbent assay (ELISA) are employed to detect dengue-specific immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies, distinguishing between recent and past infections(7, 8). However, implementing these diagnostic modalities in resource-limited settings remains a challenge due to cost, infrastructure requirements, and the need for skilled personnel. In facilities like Chaudhry Muhammad Akram Teaching & Research Hospital, accessible, cost-effective, and accurate diagnostic solutions are urgently needed to enhance patient care and improve outbreak control(9, 10). This study aims to evaluate the diagnostic performance, feasibility, and cost-effectiveness of RT-PCR, NS1 antigen detection, and ELISA at Chaudhry Muhammad Akram Teaching & Research Hospital, Lahore. By assessing these diagnostic methods, the research seeks to provide evidence-based recommendations to optimize dengue diagnosis and improve healthcare delivery in resource-constrained environments(11, 12).

METHODS

The study employed a cross-sectional design and was conducted at Chaudhry Muhammad Akram Teaching & Research Hospital, Lahore, Pakistan. The study population consisted of patients presenting with clinical symptoms suggestive of dengue fever, including high-grade fever, headache, retro-orbital pain, myalgia, and rash. Ethical approval was obtained from the institutional review board, and written informed consent was secured from all participants prior to enrollment. The study was conducted over a six-month period, adhering to ethical guidelines and principles of human research(13). A total of 440 patients were enrolled in the study based on clinical suspicion of dengue infection. Blood samples were collected using aseptic venipuncture, with approximately 5 mL of blood drawn from each participant. The collected samples were divided into two portions: one portion was centrifuged to obtain serum for enzyme-linked immunosorbent assay (ELISA) and NS1 antigen detection, while the other was preserved in ethylenediaminetetraacetic acid (EDTA) tubes for RNA extraction and reverse transcription polymerase chain reaction (RT-PCR) testing. All serum and EDTA blood samples were stored at -20°C until further processing to maintain sample integrity and ensure diagnostic accuracy(14). The NS1 antigen test was performed using a commercially available rapid immunochromatographic assay kit according to the manufacturer's protocol. This test detects the presence of non-structural protein 1 (NS1), a viral antigen that appears in the early stages of dengue infection. Test results were interpreted based on the visibility of the test lines, with positive and negative outcomes determined according to predefined cut-off values(15). Dengue-specific immunoglobulin M (IgM) and immunoglobulin G (IgG) were measured using ELISA to differentiate between primary and secondary dengue infections. A commercially available ELISA kit was used following the manufacturer's specifications, applying acceptance and rejection criteria to validate results. Calibration factors were applied to enhance result accuracy and distinguish between acute and past infections(15).

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RT-PCR was employed for the detection and identification of dengue virus RNA. RNA extraction was carried out using a commercially available RNA extraction kit, followed by reverse transcription to synthesize complementary DNA (cDNA). PCR amplification was performed using dengue virus-specific primers and deoxynucleotide triphosphates (dNTPs). The amplified products were visualized using gel electrophoresis or quantified using real-time PCR. In addition to detecting the presence of the virus, RT-PCR facilitated serotype identification(16). All diagnostic test results were analyzed statistically to assess their sensitivity, specificity, and diagnostic efficiency. The diagnostic performance of the NS1 antigen test and ELISA was compared against RT-PCR as the reference standard for dengue diagnosis in the acute phase. Descriptive and inferential statistical analyses were conducted using SPSS software. Patient characteristics, clinical presentations, and diagnostic outcomes were analyzed to establish correlations and differences among the various diagnostic approaches, ensuring a comprehensive evaluation of each testing modality(17).

RESULTS

A total of 440 patients were tested for dengue at Chaudhry Muhammad Akram Teaching & Research Hospital, Lahore, in 2024. Patients presented with clinical symptoms suggestive of dengue fever, including fever, headache, myalgia, rash, and other associated manifestations. Various diagnostic modalities, including NS1 antigen testing, IgM and IgG ELISA, and clinical assessment, were employed to determine dengue infection status. Platelet counts varied across different diagnostic groups, ranging from normal to significantly low levels. Among the total tested patients, 18 were found positive for the NS1 antigen test. This test, primarily used for early dengue diagnosis within the first seven days of symptom onset, identified cases with platelet counts ranging from 50,000 to 150,000 cells/µL, indicating thrombocytopenia commonly associated with dengue virus infection.

ELISA testing for IgM antibodies identified 44 positive cases, indicating recent dengue infections occurring four to five days after symptom onset. Platelet counts in this group ranged from 70,000 to 130,000 cells/µL, supporting the presence of active or recent dengue infection. IgG antibody testing was positive in 31 patients, suggesting past dengue exposure or secondary infections. Platelet counts in this group ranged between 80,000 and 140,000 cells/µL, with most patients maintaining relatively stable platelet levels. Clinical symptoms suggestive of dengue were observed in 250 patients; however, these cases did not fulfill the laboratory criteria for dengue confirmation. These patients either tested negative on all laboratory investigations or were diagnosed with other febrile illnesses such as malaria or typhoid fever. Platelet counts in this group varied from normal to low, highlighting the importance of distinguishing between different febrile illnesses in regions where multiple endemic infections overlap.

Additionally, 97 patients remained undiagnosed due to inconclusive laboratory findings. Possible reasons for these unclear results included suboptimal sample quality, late-stage testing beyond diagnostic windows, or non-specific findings. Platelet counts in this group generally remained within normal ranges, suggesting that additional follow-up or advanced diagnostic testing may be necessary for definitive classification. These results underscore the need for a comprehensive diagnostic strategy combining NS1 antigen detection, IgM, and IgG ELISA for effective dengue detection across various disease phases. The variation in thrombocytopenia among different diagnostic groups highlights its significance as a supportive diagnostic marker. The high number of symptomatic yet unconfirmed cases reflects the need for improved diagnostic capacity and enhanced surveillance, particularly in resource-limited settings like Chaudhry Muhammad Akram Teaching & Research Hospital. The findings contribute valuable insights to the epidemiological understanding of dengue and provide a foundation for strengthening diagnostic protocols and public health interventions in the region.

Table 1

Category	Number of Patients	Platelet Count (Range)	Diagnostic Status
Total Patients Tested	440	Normal to Low	Total Tested
NS1 Antigen Positive	18	50,000–150,000	Confirmed Dengue (NS1)
IgM Positive	44	70,000–130,000	Confirmed Dengue (IgM)
IgG Positive	31	80,000–140,000	Past Dengue (IgG)
Only Symptoms (Not Confirmed Dengue)	250	Normal to Low	Symptomatic without Lab Confirmation
No Result	97	Normal	No Diagnostic Outcome

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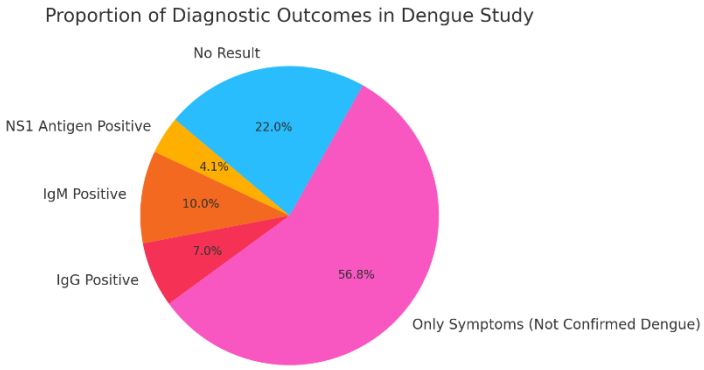

Figure 2 Proportion of Diagnostic Outcomes in Dengue Study

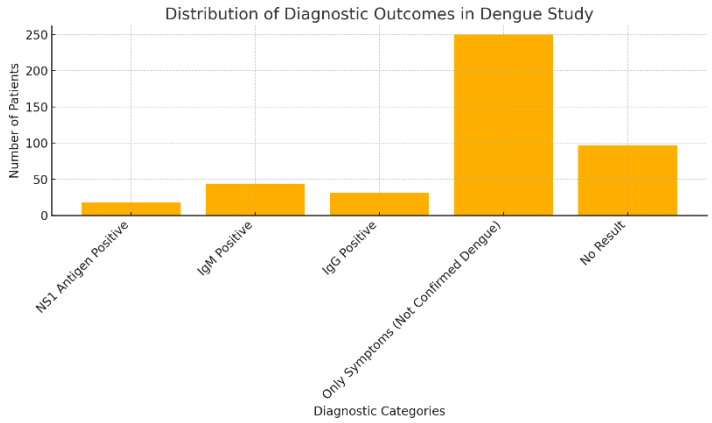

Figure 1 Distribution of Diagnostic Outcomes in Dengue Study

DISCUSSION

The findings of the present study on diagnostic modalities for dengue virus at Chaudhry Muhammad Akram Teaching & Research Hospital, Lahore align with and contrast against various recent studies published in the last four years, highlighting both consistencies and deviations in diagnostic performance across different methodologies. In this study, NS1 antigen detection emerged as a valuable tool for early dengue diagnosis, with a positivity rate of 18 cases among 440 patients, confirming its utility during the acute phase of infection. These findings are consistent with a study conducted in Indore, India, where NS1 antigen ELISA demonstrated a sensitivity of 95.8% and a specificity of 75.6% compared to ELISA-based assays. This underlines the importance of follow-up testing since NS1 testing alone may yield inconclusive results, necessitating confirmation through RT-PCR or ELISA-based serological assays(18, 19). Furthermore, ELISA-based IgM and IgG detection in this study identified 44 IgM-positive cases, indicative of recent infections, and 31 IgG-positive cases, suggestive of past exposure or secondary infections. These results are in line with a study conducted in Ananthapuramu, India, where NS1 antigen rapid tests showed 87.5% sensitivity and 98.5% specificity compared to RT-PCR, while ELISA demonstrated superior diagnostic accuracy over rapid testing. Similarly, a study conducted in Peru evaluating the SD Dengue DUO rapid test showed that IgM detection sensitivity was 68%, increasing to 75% when performed within the first three days of symptom onset. These findings reinforce the importance of utilizing multiple diagnostic methods, as delayed seroconversion of IgM antibodies in dengue patients requires comprehensive testing across different stages of infection(20, 21).

The study also confirmed the importance of RT-PCR as the gold standard for definitive dengue diagnosis; however, its application remains limited in resource-constrained settings such as Chaudhry Muhammad Akram Teaching & Research Hospital due to high costs and the need for specialized equipment. A study from Thailand demonstrated the effectiveness of microfluidic immuno-magnetic agglutination assays for NS1 antigen detection, reporting a sensitivity of 85.5%, making it a promising alternative to RT-PCR for early diagnosis, particularly in areas with limited laboratory infrastructure. Similarly, research evaluating VIDAS® diagnostic assays showed that NS1 antigen detection had a 95.6% agreement with competitor ELISA kits and exhibited high sensitivity relative to RT-PCR. These findings suggest that automated and microfluidic-based rapid tests could be viable alternatives for early dengue detection in resource-limited healthcare settings(22, 23). The strengths of this study lie in its comprehensive diagnostic approach using multiple modalities across a sample size of 440 patients, providing insights into the strengths and limitations of each method. However, certain limitations persist, including reliance on conventional ELISA and NS1 antigen tests without incorporating newer diagnostic techniques like fluorescence immunoassays or fully automated systems, which have shown improved accuracy in recent studies. Additionally, due to infrastructural constraints, RT-PCR testing could not be applied to all patients, possibly resulting in undiagnosed cases(24-26).

Overall, the findings emphasize the importance of integrating NS1 antigen detection, ELISA-based serology, and molecular testing within a structured diagnostic algorithm to improve the accuracy of dengue diagnosis. This study supports the global consensus that while NS1 antigen detection remains a reliable tool for early-phase detection, it should be complemented with ELISA and RT-PCR for definitive confirmation. Moving forward, efforts should focus on improving access to molecular diagnostics and exploring cost-effective, high-sensitivity alternatives—such as microfluidic and fluorescence-based assays—to optimize dengue detection in endemic regions like Lahore.

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CONCLUSION

This study underscores the critical need for a structured diagnostic approach in dengue-endemic regions like Lahore, where timely and accurate detection is essential for effective clinical management and outbreak control. By evaluating the performance of NS1 antigen detection, ELISA-based serology, and RT-PCR, the findings highlight the strengths and limitations of each diagnostic modality and emphasize the necessity of an integrated testing algorithm. While NS1 antigen serves as a reliable marker for early-phase detection, its diagnostic accuracy improves significantly when combined with ELISA and molecular methods. The study reinforces the importance of making diagnostic tools accessible, cost-effective, and highly sensitive, particularly in resource-limited settings like Chaudhry Muhammad Akram Teaching & Research Hospital. This approach can enhance case identification, reduce misdiagnosis, and strengthen public health interventions. The insights from this research contribute to ongoing efforts to optimize dengue surveillance and improve patient outcomes in regions that frequently face recurrent outbreaks of the disease.

AUTHOR CONTRIBUTIONS

Author	Contribution
Zeeshan Hussain*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Sidra Batool	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Asad Ullah Khan	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Rehana Shaheen	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Muhammad Asif	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Ali Ghulam	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published

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