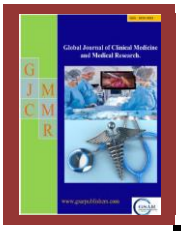

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ELISA, PCR, & NS1 ANTIGEN FOR DENGUE VIRUS DETECTION AT SAIDU TEACHING HOSPITAL, SWAT

By

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Abstract

This paper aims to compare the results of NS1 antigen, ELISA, and PCR in the diagnosis of dengue virus in Saidu Group of Teaching Hospital, SGOTH district Swat. Out of all the patients, 1,100 patients with clinical manifestations of dengue were included in the study. Outcomes shed light on the necessity of using a diagnostic suite. Of the total patients, 45 patients were positive by NS1 antigen test, 111 were positive by ELISA for dengue IgM antibodies, and 77 were positive for IgG, indicating a history of dengue. Clinical suspicion without evidence from laboratory testing was found in 643 patients, and laboratory investigations provided indeterminate results in 224 patients. In confirmed cases with COVID-19, there was a reduction in platelets with a count of 50 000 – 150 000 /micro, which was noted. Early-phase NS1 antigen was most helpful in the acute phase, while IgM and IgG were detected by enzyme-linked immunosorbent assays (ELISA) in the convalescent phase. PCR revealed high specificity and sensitivity within the earlier stages of the disease. These findings call for strengthening the diagnostic tools, incorporating efficient diagnostic approaches, and strengthening public health infrastructure to contain dengue in low-income settings such as Saidu Group of Teaching Hospital and SGOTH district Swat.

Keywords: *Dengue Fever, Diagnosis, NS1 Antigen, ELISA, PCR, Timber gauge.*

INTRODUCTION

Dengue fever is a severe disease recognized worldwide due to the increased incidence of disease rates and lethality, especially in tropical and sub-tropical climates where the Aedes aegypti mosquito is most predominant. It is in the Flaviviridae family and comprises four serotypes (Dengue virus type 1, type 2, type 3, and type 4). Dengue may be asymptomatic to severe manifestations like DHF or DSS. This is a key reason why reinfections with a different serotype generally raise the severity of the disease because it is an ADE (Guzman & Harris, 2015). Dengue fever is known to occur in over a hundred countries; the estimated number of new infections yearly for dengue is around 390 million, of which ninety-six million show clinical signs (Bhatt et al., 2013). The disease has a significant socio-economic impact, especially in the developing world, where the healthcare infrastructure is already stretched (Shepard et al., 2016). The rapid transmission of the vector is threatening Pakistan's integral health. Similarly to other developing countries, dengue has increased in the last decades as a public health problem mainly affecting peri-urban and densely populated areas (Khan et al., 2018). Some areas, such as Saidu Group of Teaching Hospital and SGOTH district Swat Khyber Pakhtunkhwa, continuously have dengue outbreaks because of environmental conduct and minimal access to health facilities (Ahmed et al., 2020). Dengue usually presents clinically as a high-grade fever. The common symptoms of dengue are onset

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with severe headache, mainly behind the eyes, eye pain, muscle and joint pains, skin rashes, vomiting, bleeding gum, and in severe cases, petechiae (WHO, 2009). Proper diagnosis can significantly help manage the ailment, prevent further serious development of the illness, and simultaneously limit the transmission of the virus during an epidemic. Moreover, diagnosis of dengue has always been problematic because its clinical manifestations are like other febrile diseases, including malaria, chikungunya, typhoid fever, and leptospirosis, among others (Centres for Disease Control and Prevention [CDC], 2023). Laboratory confirmation is. Therefore, mandatory molecular tests, antigen detection assays, and serological tests are all used at different infection periods. Methods like RT-PCR and NAATs are very sensitive and specific during the early clinical phase when viral RNA is present in the blood (Gubler, 1998). The third diagnostic technique that may be employed to diagnose dengue during the early phase is the NS1 antigen test utilizing the non-structural protein 1 (NS1) antigen (Hunsperger et al., 2016). Serological tests commonly used during the convalescent stage of the disease involving ELISA identify dengue-specific IgM and IgG (CDC, 2023). Despite being highly effective, these methods are limited in resource-constrained environments by cost, infrastructure, and personnel requiring specialized technical input (Wilder-Smith et al., 2019). Saidu Group of Teaching Hospital, SGOTH district Swat, is an important healthcare centre in Dir Lower, and the health facility receives many patients during dengue outbreak seasons. Molecular test procedures, antigen-based tests, and serology are unavailable in the hospital, ensuring a proper diagnosis of dengue. This work assessed the efficiency of these diagnostic techniques in recognizing dengue cases, especially in acute and convalescent stages. Accordingly, the study intends to determine the diagnostic performance, feasibility, and cost of implementing RT-PCR, NS1 antigen testing, and ELISA to obtain a clearer understanding of how to improve the diagnosis in resource-limited current like Saidu Group of Teaching Hospital, SGOTH district Swat Furthermore, they explained the need for an integrative approach in case of finding improvement. They detailed the need to boost the tangible findings in patients. These help policymakers and HC practitioners improve laboratory diagnosis services and establish efficient, cheap algorithms for diagnosing dengue in endemic areas (Shepard et al., 2016). Laboratory diagnosis for dengue virus includes assessing the patient's medical history, travel history, CDC travel advisories about diseases and viruses, and the patient's immunization history, principally yellow fever and Japanese encephalitis, to estimate the likelihood that the present or recent illness is because of infection with the dengue virus (CDC, 2023). Proper diagnosis can significantly help manage the ailment, prevent further serious development of the illness, and simultaneously limit the transmission of the virus during an epidemic. Moreover, diagnosis of dengue has always been problematic because its clinical manifestations are like other febrile diseases, including malaria, chikungunya, typhoid fever, and leptospirosis, among others (Centres for Disease Control and Prevention [CDC], 2023). Laboratory confirmation is. Therefore, mandatory molecular tests, antigen detection assays, and serological tests are all used at different infection periods. 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Laboratory diagnosis for dengue virus includes assessing the patient's medical history, travel history, CDC travel advisories about diseases and viruses, and immunization history, principally yellow fever and Japanese encephalitis, to estimate the likelihood that the present or recent illness is caused by infection with the dengue virus (CDC, 2023). NAAT or NS1-negative patients during the acute phase should have sequential samples again tested for IgM antibodies to conclude recent dengue virus infection (CDC, 2023). The ambiguity between dengue and other flaviviruses cannot be overemphasized due to the interference of some serologic tests. Serum cross-reactivity of flaviviruses, including Japanese encephalitis, St. Louis encephalitis, West Nile, yellow fever, zika, and dengue, has been observed (94). In many regions where multiple flaviviruses are circulating, sensitive and specific laboratory tests, like PRNT, should be used to distinguish between dengue and other flaviviruses (Fong et al., 2016). Women with symptoms who have ever been to or lived in areas with a Zika virus risk should also be considered for NAAT testing for Zika, together with dengue virus testing (Wilder-Smith et al., 2019). Molecular tests are most favourable in the first week of the disease since they target viral nucleic acids – the NAAT assay (CDC, 2023). The results of these tests are positive if the patient is currently having an active form of dengue. A negative result, however,

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requires testing for IgM antibodies or a follow-up test in the subsequent phase. Most commercially available rapid diagnostic tests, including NS1, detect the non-structural protein of the virus produced during the early stage of dengue infection. Thus, the NS1 tests are non-inferior to molecular tests during the first week and are less sensitive in the subsequent weeks. NS1 and IgM antibody testing offers rapid diagnosis during the acute phase of the disease (Hunsperger et al., 2016). A serological test such as IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) can be rightfully used for diagnostic purposes starting from the 4th – 5th day after onset of symptoms. These tests identify the recent infection and may cross-reactivity; however, in certain instances, PRNT must be used (WHO, 2009). NAAT and IHC analysis tissue-based diagnostic techniques primarily apply in retrospective studies and forensic epidemiology (CDC, 2023). IHC techniques are very selective about the specimen, not causing complications in specimen transport, which makes it suitable for diagnosing dengue in paraffin embedded=formalin fixed tissues (Hunsperger et al., 2016). As a result of the studies discussed in this article, the necessity of developing a phase-sensitive diagnostic strategy for dengue virus infection becomes clear. For diagnosing the disease during the acute phase, molecular and antigen detection methods are most suitable for their high sensitivity, including RT-PCR and NS1 (Hunsperger et al., 2016). Serological tests such as ELISA and confirmatory tests such as PCR are more sensitive in the convalescent stage of the disease. Correct sample collection, processing, and transportation are followed in laboratories to diagnose dengue, with importance laid on the sample cold chain to get accurate results (CDC, 2023).

Materials and Methods

This type of study was a cross-sectional one carried out at the Saidu Group of Teaching Hospital facility, SGOTH district Swat Khyber Pakhtunkhwa, Pakistan. These patients had clinical findings of dengue fever that included high-grade fever, headache, retro-orbital pain, myalgia, and rash. Ethical clearance was sought, and the study was conducted over six months from [insert dates]. Self-administrated and written informed consent was obtained before enrolling patients suspected of dengue fever. Data collection and analysis were carried out in compliance with the principles of ethical conduct approved by the institutional review board.

The ELISA (Enzyme-Linked Immunosorbent Assay) test is a gold standard for detecting specific antibodies or antigens in a sample. The process begins by coating the wells of a microtiter plate with a targeted antigen or antibody, followed by a blocking step to prevent unwanted interactions. After adding and incubating the sample, enzyme-linked secondary antibodies are introduced to bind to the antigen-antibody complex. A substrate is then added, triggering a colour change that indicates the presence of the target. The intensity of the colour, measured using a spectrophotometer, correlates with the concentration of the substance being tested, making ELISA a powerful diagnostic tool.

Polymerase Chain Reaction (PCR) is a revolutionary technique to amplify specific DNA sequences for accurate pathogen identification. The test begins with extracting DNA or RNA from the sample, followed by preparing a reaction mix containing primers, polymerase, and nucleotides. The DNA is denatured through a series of thermal cycles, primers anneal to target regions, and new strands are synthesized in the extension phase. These cycles are repeated 25–40 times to achieve billions of copies of the target sequence, which can be visualized through gel electrophoresis or fluorescence. PCR's high sensitivity and specificity make it indispensable for diagnosing infectious diseases.

The NS1 antigen test offers a quick and reliable method for early dengue diagnosis. A small blood sample is applied to a test cassette, followed by adding a buffer solution to facilitate antigen detection. Within 15–20 minutes, the test reveals results: two lines indicate a positive result, one line confirms a negative result, and the absence of a control line necessitates retesting. This user-friendly and rapid diagnostic tool is particularly valuable in dengue-endemic regions, allowing for timely intervention and management of the disease.

Aseptic venipuncture was performed, and blood samples of about 5 mL each were drawn from each patient. The blood samples were divided into two parts: One part was centrifuged for serum collection for ELISA and NSI antigen tests, and the second part was put in EDTA tubes for RNA extraction and PCR test. The serum and EDTA blood samples were refrigerated at -20°C until they could be tested further.

The NS1 antigen test was done using a commercially available rapid immunochromatographic test kit (if available, mention the company). This test is used to identify the presence of the NS1 protein, which is usually present from the onset of dengue virus infection. The test was performed according to the manufacturer's guidelines. Cut-off was noted based on visible lines on the test strip, with positive or negative results.

Dengue-specific IgM and IgG were measured by enzyme-linked immunosorbent assay (ELISA). A commercially available enzyme-linked immunosorbent cell culture assay kit from (specify the manufacturer) was used following the manufacturer's description. This method gave qualitative semi-quantitative data that helped classify whether it was a primary or secondary dengue infection. Acceptance criteria, rejection criteria, and calibration factors were applied to confirm the results. In this regard, the results provided the basis to differentiate between acute and past infection.

Real-time PCR was employed to identify dengue virus RNA molecules. From the EDTA-collected blood, the RNA was extracted using an RNA extraction kit from a certain manufacturer. The RNA extract was then subjected to reverse transcription and polymerase chain reaction using deoxy nucleotide triphosphate (DNPs) with dengue virus-specific primers. After the PCR reaction, the PCR products were resolved on gel or quantified using real-time PCR. In addition to the virus detection, this method was also used to determine the serotype.

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All diagnostic tests were analysed statistically to assess their built-in sensitivity, specificity, and diagnostic efficiency. The NS1 Antigen Test and ELISA gave similar results when compared with PCR for diagnosing dengue at the acute phase of the disease. Descriptive and inferential analyses were done using statistical software such as SPSS. By assessing patient characteristics, clinical picture, and diagnostic results, the authors tried to establish similarities and differences.

Results

The study conducted at Saidu Group of Teaching Hospital, SGOTH district Swat, in 2024 found that 1,100 patients were tested for dengue. Some patients developed typical signs like fever, headache, muscle pain, rash, and dengue fever. Diagnostic parameters such as NS1 antigen, IgM, and IgG ELISA, and clinical strength were used to determine the presence or absence of dengue infection. The platelet count was normal up to low, which is observed in the clinic depending on the patients.

Among the total number of tested patients, 45 were found to be NS1 antigen positive. This test is a sensitive diagnostic test administered in the early stage of the dengue infection (first 7 days of symptoms). Platelet counts of these patients were significantly low and varied from 50000 to 150000 cells/µL, confirming thrombocytopenia in dengue virus infections. These are cases of PAR in which the diagnosis is confirmed at the early history of the disease.

ELISA revealed 111 patients positive for dengue-specific IgM antibodies. This test is usually done during or in the later part of the acute phase of the disease since IgM antibodies can be detected 4 to 5 days after onset. The platelet counts of this group seemed to distribute between 70,000 to 130,000 cells/µL, which supported active or recent dengue infections. These cases describe an immunological response to the virus and demonstrate the optimal confirmation means in the subacute phase.

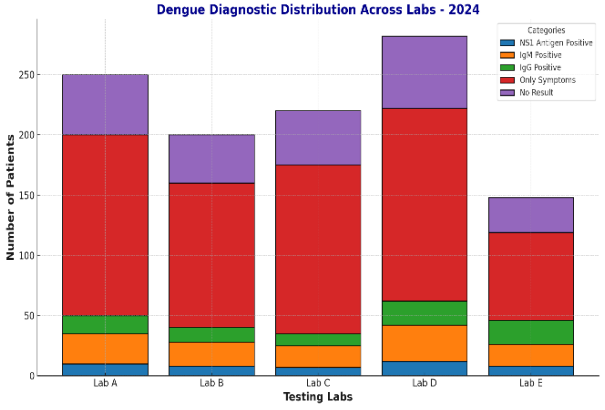
Out of 120 patients, only 77 tested positive for IgG, which is common in those who have had dengue viruses in the past or secondary dengue attacks. The IgG test enables differentiation of primary and secondary infections, which is important since the latter are associated with higher risks of severe manifestations. Considering the platelet values of this group, the counts were between 80000 and 140000 cells/µL, and most of the patients had normal platelets. These findings are important from the epidemiological perspective and regarding the population immunity levels.

The remaining 643 patients tested positive only for clinical features indicative of dengue but did not fulfil the laboratory criteria for dengue disease. These patients either had negative results on all the investigations performed, or their clinical manifestations were blamed on other febrile diseases, like malaria or typhoid. Platelet count in this group was low, normal, or decreased due to the different ethology of the complaint. These cases support the call to distinguish febrile diseases in areas where more than one fever usually exists.

Last of all, 224 patients did not get a clear explanation for the symptoms by the existing diagnostic methods. In these cases, the diagnostic was problematic, for example, due to low sample quality, testing performed after the patient had left, or non-specific results. These patients' platelet counts did not show any deviation from regular, and more follow-up or a higher level of testing may be necessary to develop a definitive diagnosis. The findings from 1,100 patients sampled in 2024 emphasize the continued need for a 3-dimensional diagnostic strategy for dengue. This covers all the phases of dengue; therefore, the three, NS1 antigen, IgM and IgG, are helpful. The fact that the plates exhibit differences in thrombocytopenia within various categories establishes its importance as a supportive diagnostic feature. However, many symptomatic cases were unconfirmed, focusing on heightening diagnostic capacity and surveillance in such a low-resource Saidu Group of Teaching Hospital, SGOTH district Swat. The results help to improve the knowledge of dengue epidemiology and support the development of intervention

Summary

Out of the total 1100 patients tested, 45 were NS1 Antigen positive, with platelet counts ranging from 50,000 to 150,000, indicating confirmed dengue (NS1). 111 patients tested positive for IgM, with platelet counts ranging between 70,000 and 130,000, also confirming dengue (IgM). A further 77 patients tested positive for IgG, with platelet counts ranging from 80,000 to 140,000, indicating past dengue (IgG). Additionally, 643 patients showed symptoms of dengue but did not have lab confirmation, with platelet counts ranging from normal to low. Finally, 224 patients had no diagnostic outcome, with normal platelet counts, resulting in no confirmed diagnosis.



Category	Number of Patients	Platelet Count (Range)	Diagnostic Status		
Total Patients Tested	1100	Normal to Low	Total Tested		
NS1 Antigen Positive	45	50,000-150,000	Confirmed Dengue (NS1)		

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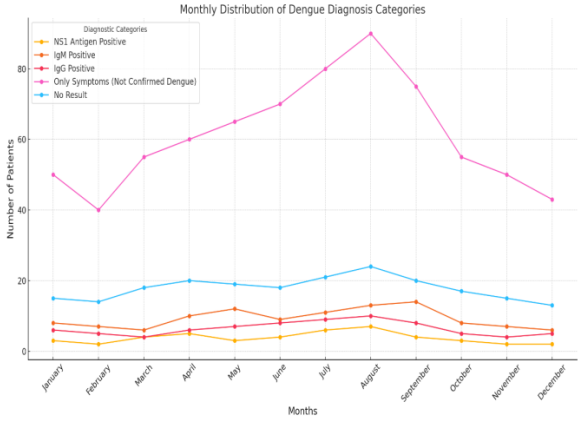
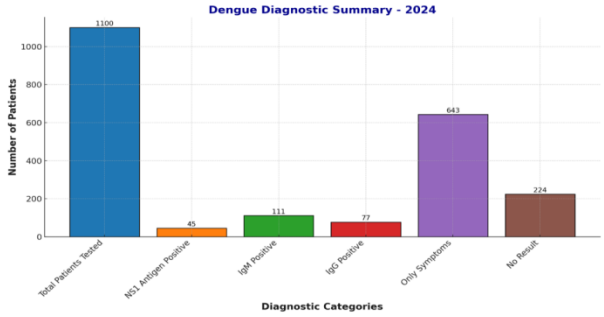
IgM Positive	111	70,000-130,000	Confirmed Dengue (IgM)	
IgG Positive	77	80,000-140,000	Past Dengue (IgG)	
Only Symptoms (Not Confirmed Dengue)	643	Normal to Low	Symptomatic without Lab Confirmation	
No Result	224	Normal	No Diagnostic Outcome	





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