Research Article

The NS1 Antigen Positivity and its Association with Day of Illness among Suspected Dengue Patients: A Hospital-Based Study

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Abstract: Background: The dengue fever has remained a significant health in Pakistan due to its endemic outbreaks. NS1 glycoprotein is an important serological biomarker play an important role in disease progression. Its early identification can optimize the diagnostic testing and result interpretation in suspected dengue patients.

Objective: To associate NS1 antigen positivity with day of illness in suspected dengue patients.

Materials and Methods: This hospital based retrospective cross-sectional study was conducted from 1st June 2025 to 30th November 2025, at Pakistan Institute of Medical Sciences, Islamabad after obtaining ethical approval from the ethical review committee of PIMS under ERC# F-5-2/2024 (ERRC) PIMS, dated: 15-11-2025. A hospital record of total 364 suspected dengue patients was collected and analyzed. The association between NS1 antigen positivity and day of illness were evaluated by using Chi-square. Age was presented as mean ± S.D while frequency was observed for gender distribution.

Result: Among 364 suspected dengue cases, 31.9% tested positive for NS1 antigen, with a higher prevalence in males (67.3%) and a mean age of 36.75 years. NS1 positivity was highest in patients presenting on days 5–7 of illness (33.7%) and reached 100% in those presenting on day 8 or later. Platelet counts showed no significant variation across different days of illness ($p = 0.475$).

Conclusion: NS1 antigen testing is a valuable early diagnostic tool for dengue, and its accuracy may improve when combined with serological and hematological markers. Future longitudinal studies are needed to better understand NS1 dynamics throughout the illness.

Keywords: Dengue, NS1 antigen, Serological markers, Disease progression, Epidemiology.

INTRODUCTION

Dengue is one of the most widely spread mosquito-borne infectious disease, caused by any one of the four dengue serotypes: DENVs 1–4 [1]. This disease is mostly predominant in the tropical and sub-tropical parts of the world and is communicated to the human body by the bite of female Aedes aegypti mosquito [2]. Dengue is usually a self-limiting and can be presented with wide variety of symptoms ranging from mild dengue fever lasting for about 5- 7 days to dengue hemorrhagic fever and dengue shock syndrome associated with myalgia, arthralgia, vomiting and bleeding complications with an increase in its severity [3]. In the year 2024, a record 14.1 million cases of dengue fever were reported globally presenting a massive surge in cases [4], while in Pakistan alone, 2,795 new cases were reported in the same year [5]. Dengue fever is endemic to Pakistan, as the country experiences year-round transmission with seasonal upsurge. Dengue Virus Serotype 2 (DENV-2) is the most prevailing and frequently reported serotype in Pakistan, accountable for major outbreaks across the country [6]. Early detection and diagnosis of the disease is vital to reduce the chances of complications and that can significantly lower the morbidity and mortality and reduce the burden of disease among the overall health care system of Pakistan.

A multifunctional Nonstructural protein 1 (NS1) plays a vital role in the viral replication, immune evasion and pathogenesis of disease [7]. NS1 protein serves as an imperative bio-marker for dengue fever as it is freely present in the circulation released by infected cells in contrast to other non-structural glycoproteins that remains inside the host cells. During the acute phase of infection (from day 1-9), this glycoprotein is secreted in large amount and can be detected in blood even before the formation of antibodies [8]. However, the test results are mainly reliant on multiple factors such as timing of collection of samples, the immune status of patient i-e whether the patient is having primary or secondary infection, the serotype infecting the patient and the viral load. The patients with primary infection are likely to have an increased frequency of positivity of NS1 protein as compared to those having pre-existing dengue- reactive IgG antibodies [9]. The sensitivity of NS1 protein is dependent on the dengue serotypes. Literature has revealed that DENV- 1 and DENV-3 have higher positivity rates in contrast to DENV-2 and DENV-4 [10].

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The health care system of Pakistan suffers extreme stress and pressure during epidemics of dengue [11]. Rapid NS1 antigen testing is used for an initial diagnosis in most of the Pakistani hospitals. Nevertheless, the day of illness from the onset of symptoms considerably changes the sensitivity of NS1 antigen. If NS1 fast antigen tests are repeated outside of the diagnostic time frame, numerous false negatives can occur.

Despite of dengue upsurge and an over load of disease with seasonal patterns, the regional data is scarce to address the association of NS1 antigen with day of illness in adult population. The local studies have focused on pediatric cohort that lacks the temporal dynamics of NS1 antigen in routine clinical investigation. This study presents information regarding the variation in NS1 positive with disease duration that is pertinent to the local community in adult set of population. It is intended to assist public health professionals with epidemiological tracking, enhance the diagnostic precision and timing of NS1 testing, and direct clinicians when to administer or rerun the test.

MATERIAL AND METHODS

This was a hospital-based retrospective cross-sectional study, conducted for a duration of six months, from 1st June 2025 to 30th November'2025, at Pakistan Institute of Medical Sciences, Islamabad. The ethical approval was sought from the ethical review committee of PIMS under ERC# F-5-2/2024 (ERRC) PIMS, dated: 15-11-2025. Keeping an anticipated frequency of 37% of dengue fever in Pakistani population, the sample size was found to be 364 at 95% confidence interval and 5% margin of error [12]. The samples were collected through stratified sampling technique. This stratified sampling approach was applied based on the day of illness of presentation to justify the primary objective of the study. The stratification of patients was made on the following predefined criteria: early phase (day 1-4), intermediate phase (days 5-7) and late phase (≥8 days).

The inclusion criteria were patients of any age who presented to hospital in the latest season suffering from suspected dengue, patients who underwent NS1 antigen testing and files from hospital record with documented date or day of symptom onset and date or day of sample collection. The suspected cases of dengue were classified as per guidelines given by World Health Organization (WHO) in 2025. Accordingly, patients presenting with acute febrile illness lasting for 2 to 7 days with following symptoms: high grade fever (40°C/104°F), severe headache, retro-orbital pain, severe myalgia, arthralgia, nausea, vomiting, rash or any other warning sign were considered as suspected cases of dengue [13].

The exclusion criteria included those patients with missed or improbable record files, flawed test results of NS1 antigens, and/or records not meeting suspected dengue criteria. The hospital and laboratory record of suspected dengue patients, who were tested for NS1 antigens during the recently occurring dengue outbreaks, were kept as necessary data. The day on which the sample was taken during the course of disease was considered as exposure variable and was defined as the difference between the date of appearance of first symptom and the date on which the patient was tested. The positivity of NS1 antigen by lab results was considered as a dependent variable. The detection of NS1 antigen in the collected samples was performed through a commercial Enzyme Linked Immunosorbent assay (ELISA). This test was carried out as a part of routine investigation in the lab facility of hospital. The test was performed in accordance with given instruction of manufacturer and as per the guidelines, the assay shows high diagnostic sensitivity during first 5 days of illness, with 70-90% sensitivity and 95% specificity.

A standardized data extraction sheet was used to record data, and strict confidentiality was maintained with data accessible to the researchers only.

STATISTICAL ANALYSIS

Data was analyzed by using SPSS v.25. The association between NS1 antigen positivity and day of illness were evaluated by using Chi-square. In cases where expected cell counts were less than 5, Fischer's exact test was applied. A p-value < 0.05 was considered statistically significant. Age was presented as mean ± S.D while frequency was observed for gender distribution. The distribution of platelet counts across different days of illness was evaluated by using Kruskal-Wallis test and a p-value of < 0.05 was considered significant for analysis. A Chi-square test was used to determine the association between warning signs with NS1-negative and NS1-positive patients

RESULT

The current investigation included 364 participants that were suspected cases of dengue fever and had undergone NS1 antigen testing. The study participants were predominantly males (67.3%) as compared to females (32.7%). The participants' mean age was found to be 36.75 ± 13.25 years, suggesting disease prevalence affecting the economically active age group as shown in Table **1**.

Table 1. Gender Distribution and Age Characteristics of Study Participants (n = 364).

Gender	Frequency (n)	Percentage (%)
Male	245	67.3
Female	119	32.7
Total	364	100.0
Age (years)		
Mean ± S.D	36.75 ± 13.25	

Based upon the hospital and laboratory record of suspected dengue patients, 116 (31.9% at 95% CI: 27.2–36.9%) were tested positive for NS1 antigen while 68.1% were confirmed as negative during antigen testing. Table **2** is presenting the distribution of illness according to the day of illness. During the early phase of disease (days 1-4), NS-1 positivity was observed

in 79 patients (30.6%). From days 5-7, a slight increase in the positivity rate was observed and 35 patients (33.7%) were tested positively. Patients presenting later during the course of disease, day 8 or later included only 2 and both were tested positive for the antigen (100%).

Table 2. Association of NS1 Antigen Positivity with Day of Illness.

Day of Illness (days)	NS1 Negative (n)	NS1 Positive (n)	Indeterminate	Total (n)	NS1 Positivity %*	P-value
1-4	175	79	4	258	30.6	0.1575
5-7	65	35	4	104	33.7	
≥8	0	2	0	2	100	

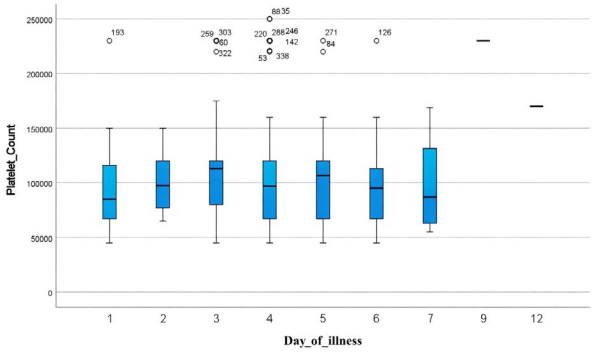

Fig. (1). Boxplot Showing Platelet Count Distribution across Days of Illness among Suspected Dengue Patients.

Fig. (**1**) is demonstrating the non-significant distribution of platelet counts across different days of illness among suspected dengue patients (p-value=0.475).

Table 3. Distribution of Warning Signs According to NS1 Antigen Status.

Warning Sign	NS1 Negative (n = 240)	NS1 Positive (n = 122)	% in NS1 Negative	% in NS1 Positive	p-value
Abdominal pain	1	2	0.42	1.64	0.0059*
persistent vomiting	1	5	0.42	4.10	
Mucosal bleeding	10	0	4.17	0.00	
liver enlargement	0	1	0.00	0.82	
No warning signs	206	107	85.83	87.70	

*Statistically significant.

The distribution of warning signs differed significantly between NS1-negative and NS1-positive patients (p-value = 0.0059). Persistent vomiting and abdominal pain were more frequent among NS1-positive cases, while mucosal bleeding was observed only in NS1-negative patients. The majority of patients in both groups presented without warning signs (Table **3**). These results offer the foundation for discussing the diagnostic performance of NS1 antigen testing in context to duration of illness.

DISCUSSION

This was a hospital-based retrospective study that involved 362 suspected cases of dengue fever and were tested for NS1 antigen to associate it with day of illness, furthermore demographic characteristics and hematological parameters were also observed. The cumulative positivity rate of NS1 antigen was found to be 31.9% while majority were tested negative and a fewer unspecified results. A possible reason for lower positivity rate seen in our set of population could be the predominant presence of DENV-2 serotype in our set of population which usually results has lowered sensitivity for NS-1 antigen [14]. Another hospital-based study conducted in the dengue endemic region found to have comparatively modest positivity rates of NS1 antigen [15]. Some other factors attributed to the insignificant levels can be difference in timing of patient presenting, variation in immune response of patients and secondary dengue infection. During routine clinical investigation, NS1 ELISA is usually performed in patients presenting at variable points in their course of illness that also includes beyond the early febrile phase when the antigen levels begin to fall and therefore, 30-40% of positivity rates are commonly reported, validating the present study results [16].

The present study found an insignificant association between NS1 antigen positivity and day of illness. Although a slightly raised proportion of NS1 positivity was observed in days 5-7 as compared to days 1-4, but still these numerical variations did not conclude statistically meaningful and should be interpreted cautiously. The positivity rates were slightly increased from days 1-4 and later 5-7 but couldn't reached significant levels. This finding is contrasting to existing literature, which has shown a positive correlation between antigen levels and day of illness especially in pediatric population [17]. A study conducted by Duong *et al.* [18], reported peak levels of antigens and positivity in first few days of illness followed by a decline in this trend as disease progresses further. The present investigation also demonstrated small number of patients presenting in the ≥8-day category (n = 2), which further limits the meaningful interpretation of the observed positivity in this subgroup. The small subset of population in this particular group may have decreased the statistical power in order to represent a real association between NS1 positivity and the duration of illness.

The demographic data presented in the existing study is in line with the literature exhibiting a male predominance and a significant involvement of economically active age group. Few regional studies have reported the same findings reflecting an increased exposure risk, health seeking behaviors and other social determinants that differ by age and gender [19, 20].

The box plot in the present study represented the correspondence of plate count in the early, intermediate, and late phase in the course of illness. Although low platelet counts or thrombocytopenia is a notable clinical feature of dengue infection, the results of current study demonstrate that this cell count may not follow a homogenous pattern during variable stages of disease in a clinical setting. A study conducted by Khazali *et al.* has reported that low platelet count is positively correlated with NS1 positivity and other serological markers and not be strictly correlated with the day of illness in each dataset [21]. The variability of platelet count may also be influenced by the patients' immunological status, serotype of dengue virus and any other concurrent comorbidity [22]. It highlights the importance of having longitudinal study design to focus the changes occurring in the hematological parameters [23].

The results of earlier studies are consistent with the present study unveiling a noteworthy linkage between NS1 antigen and the presence of warning signs occurring with dengue fever. The literature has cited that NS1 positivity is often reported more commonly earlier in the course of disease, specifically the gastrointestinal symptoms like nausea, vomiting and abdominal pain, validating the results of the present study [24]. Sirisena *et al.* has reported mucosal bleeding more commonly presented in the advanced stage of the illness irrespective of status of NS1 antigen [25], this could be a contrasting finding but may be explained with the fact that majority of study population were found to have negative NS1 antigens in the present cohort. The potential differences in the timing of presentation of patients, dengue serotypes, and host-immune reaction could be some important factors responsible for variations in clinical presentation across many research studies [26].

The results of the current investigation highlight the importance of diagnostic precision and clinical decision making. The relatively moderately positive NS1 antigens and an insignificant association between the levels of antigen with days of illness signifies that physicians should not only rely on these biomarkers alone but they need to correlate them with antibody status and clinical presentation of the patients. This can greatly impact the sensitivity and specificity of laboratory tests and can provide an infection profile of patients during varied phases of disease.

LIMITATIONS

The current study has few limitations that should be taken into account while interpreting the findings. The retrospective study design is dependent on the existing data available in laboratory settings of hospital, which may limit the access to accurate data. The confirmatory molecular test i-e Polymerase chain reaction (PCR) was not available for each suspected case of dengue fever. An uneven distribution of patients, specifically those presenting after day eight, restricted to reach a statistically meaningful conclusion.

CONCLUSION

The clinical evaluation of NS1 antigen remains a validated diagnostic investigation especially during early phases of disease. Considering the moderate positivity rate and the absence of a statistically significant association between NS1 positivity with day of illness, the testing of this biomarker needs to be coalesce with serological and hematological parameters. In future, more prospective studies with longitudinal study designs are needed to give a better picture of sequential dynamics of NS1 positivity and platelet counts across the course of dengue infection.

AUTHORS' CONTRIBUTION

Shaista Faheem and **Muhammad Usama**: Conceptualization, Study design, Writing draft.

Badeeya Rashid: Conceptualization, Methodology, Data analysis and interpretation, Critical review and revision the manuscript.

Maria Zafar: Conceptualization, Study design, Methodology, Data analysis and interpretation, Writing draft.

Muhammad Talha Tariq: Methodology, Data analysis and interpretation, Critical review and revision the manuscript, Final approval, final proof to be published.

Samina Rashid: Writing draft, Critical review and revision the manuscript.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Mr. Muhammad Arsalan Sharif for his valuable assistance in translating the manuscript.

ETHICAL DECLARATIONS

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

Ethical approval for this study was obtained from the Institutional Review Board of Pakistan Institute of Medical Sciences, Islamabad (Approval No: ERC# F-5-2/2024(ERRC) PIMS, Dated: 15-11-2025.

Consent to Participate

Written informed consent was obtained from all participants prior to enrollment in the study.

Consent for Publication

Consent for publication was obtained from all individual participants included in the study.

Conflict of Interest

Declared none.

Competing Interest/Funding:

This research received no external funding.

Use of AI-Assisted Technologies

AI tools (grammerly/ consensus) were solely used for grammatical refinement and language editing. The author confirm that the scientific content, data interpretation and conclusions are entirely their own.

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Received: February 14, 2026 Revised: March 13, 2026 Accepted: March 24, 2026