



The Dynamics of NS1 Antigen and Platelet Levels in the Acute Phase of Dengue Hemorrhagic Fever

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A B S T R A C T

Introduction: Dengue hemorrhagic fever (DHF) is a significant global health concern, particularly in tropical and subtropical regions. The early diagnosis and effective management of DHF are crucial to reduce morbidity and mortality. The NS1 antigen and platelet count are two key laboratory parameters used in the diagnosis and monitoring of DHF. This study aimed to investigate the dynamics of NS1 antigen and platelet levels during the acute phase of DHF and their correlation with disease severity. **Methods:** A prospective cohort study was conducted at a tertiary care hospital in Central Java, Indonesia. Patients presenting with suspected DHF were enrolled and followed up during their hospitalization. Blood samples were collected at admission and at regular intervals to measure NS1 antigen levels and platelet counts. Clinical and laboratory data were collected to assess disease severity. **Results:** A total of 150 patients were included in the study. The majority of patients were adults (median age, 32 years) with a female predominance (58%). The median duration of fever at presentation was 4 days. NS1 antigen levels were significantly higher in patients with DHF compared to those with dengue fever (DF) ($p < 0.001$). Platelet counts were significantly lower in patients with DHF compared to those with DF ($p < 0.001$). NS1 antigen levels showed a negative correlation with platelet counts ($r = -0.62$, $p < 0.001$). The levels of NS1 antigen and platelet count were also significantly associated with disease severity. **Conclusion:** The dynamics of NS1 antigen and platelet levels during the acute phase of DHF provide valuable insights into disease pathogenesis and can aid in the early diagnosis and prediction of disease severity. The negative correlation between NS1 antigen and platelet levels suggests a potential role of NS1 in the development of thrombocytopenia in DHF. Further studies are needed to elucidate the exact mechanisms underlying these observations.

1. Introduction

Dengue, a mosquito-borne viral illness caused by the dengue virus and transmitted primarily by the *Aedes aegypti* mosquito, poses a significant global health challenge, particularly in tropical and subtropical regions. The World Health Organization estimates an annual incidence of 390 million dengue infections worldwide, with a significant proportion occurring in Southeast Asia and the Western Pacific.¹ The clinical manifestations of dengue infection range from asymptomatic or mild dengue fever (DF) to severe

dengue, encompassing dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).² The progression to severe dengue is associated with increased vascular permeability, thrombocytopenia, and coagulopathy, potentially leading to life-threatening complications such as shock, organ failure, and hemorrhage.³ The dynamic interplay of viral factors, host immune responses, and secondary infections contributes to the complex pathogenesis of severe dengue.⁴ The early and accurate diagnosis of dengue infection, particularly the identification of

individuals at risk of developing severe dengue, is crucial for timely clinical management and the prevention of complications. The diagnosis of dengue relies on a combination of clinical presentation, laboratory findings, and epidemiological context.⁵ The World Health Organization has established criteria for the diagnosis of dengue and its severity classification, which include fever, hemorrhagic manifestations, thrombocytopenia, and evidence of plasma leakage.⁶ Laboratory tests, such as the detection of NS1 antigen, viral RNA, and IgM/IgG antibodies, play a pivotal role in confirming the diagnosis and monitoring the progression of dengue infection.⁷

The NS1 antigen, a non-structural glycoprotein produced by the dengue virus during its replication cycle, is secreted into the bloodstream during the early phase of infection and can be detected in the serum of infected individuals.⁸ The NS1 antigen test offers a rapid and sensitive diagnostic tool for dengue infection, particularly in the first few days of illness when viral RNA levels may be declining and IgM/IgG antibody responses may not yet be detectable.⁹ Several studies have demonstrated the diagnostic utility of NS1 antigen testing in various clinical settings, with sensitivities ranging from 60% to 90% and specificities exceeding 90%.^{10,11} The NS1 antigen test is particularly valuable in resource-limited settings where access to molecular diagnostic techniques may be limited.^{1,2} Thrombocytopenia, defined as a platelet count below 100,000/ μ L, is a hallmark of severe dengue and is associated with increased disease severity, risk of bleeding, and mortality.^{1,3} The mechanisms underlying thrombocytopenia in dengue are multifaceted and involve a complex interplay of viral and host factors. The dengue virus can directly infect megakaryocytes, the platelet precursor cells in the bone marrow, leading to impaired platelet production.^{1,4} Additionally, the virus can induce immune-mediated destruction of platelets through antibody-dependent enhancement and complement activation.^{1,5}

Furthermore, the inflammatory response triggered by dengue infection can lead to increased platelet consumption and sequestration in the spleen and liver.^{1,6} The correlation between NS1 antigen levels

and platelet counts in dengue infection has been investigated in several studies, with varying results. Some studies have reported a negative correlation between NS1 antigen levels and platelet counts, suggesting a potential role of NS1 in the development of thrombocytopenia.^{7,8} Other studies, however, have not found a significant correlation between these two parameters.^{9,10} The discrepancies in these findings may be attributed to differences in study design, patient populations, and laboratory techniques used to measure NS1 antigen and platelet counts. The dynamics of NS1 antigen and platelet levels during the acute phase of dengue infection, particularly in the context of DHF, remain incompletely understood. A comprehensive understanding of these dynamics is crucial for the development of improved diagnostic and prognostic tools for dengue, as well as for the identification of potential therapeutic targets. This study aimed to investigate the temporal changes in NS1 antigen and platelet levels during the acute phase of DHF and their correlation with disease severity.

2. Methods

The present study employed a prospective cohort design, meticulously executed within the confines of a tertiary care hospital situated in the Central Java province of Indonesia. The research spanned the duration from January 2022 to December 2022, and ethical oversight was ensured through the acquisition of requisite approvals from the Institutional Review Board of the designated hospital. The prospective nature of the study design enabled the researchers to follow a cohort of patients from the point of their initial presentation with suspected dengue hemorrhagic fever (DHF) through the course of their illness, facilitating the dynamic tracking of NS1 antigen and platelet levels. The selection of a tertiary care hospital as the study setting was strategic, as such institutions typically cater to a diverse patient population, including those with severe manifestations of dengue, thereby enhancing the generalizability of the findings. The year-long study duration allowed for the inclusion of a substantial number of patients, bolstering the statistical power of the analyses. The study population encompassed individuals who presented at the

hospital with clinical manifestations suggestive of DHF. The eligibility criteria were thoughtfully crafted to ensure the inclusion of a homogenous group of patients while minimizing the potential for confounding factors. The inclusion criteria stipulated that participants must be 18 years of age or older, exhibit clinical features congruent with the World Health Organization's (WHO) definition of DHF, and yield a positive result on either the NS1 antigen test or the dengue IgM antibody test. The age criterion was implemented to focus on the adult population, where DHF tends to be more prevalent and severe. The requirement for clinical compatibility with the WHO definition of DHF ensured that only patients with a confirmed diagnosis of DHF were included in the study. The positive NS1 antigen or dengue IgM antibody test served as an additional confirmatory measure for dengue infection. The final inclusion criterion, which pertained to the willingness to participate and provide informed consent, underscored the ethical considerations of the study. Conversely, the exclusion criteria were designed to eliminate individuals who might introduce extraneous variables that could potentially distort the study findings. Pregnant women were excluded due to the physiological changes associated with pregnancy that could influence platelet counts and NS1 antigen levels. Patients with coexisting medical conditions known to impact these parameters, such as liver disease or hematological disorders, were also excluded to prevent confounding. Similarly, individuals using medications that could affect platelet counts or NS1 antigen levels, such as aspirin or heparin, were excluded for the same reason.

A comprehensive data collection protocol was implemented to capture pertinent demographic, clinical, and laboratory information. Upon admission, demographic data, including age and gender, were recorded. Clinical data, such as the duration of fever and the presence of hemorrhagic manifestations, were also documented. The collection of blood samples was a pivotal aspect of the study, with samples obtained at the time of admission and at predetermined intervals on days 3, 5, and 7 of hospitalization. This serial sampling strategy enabled the researchers to monitor

the temporal changes in NS1 antigen and platelet levels throughout the acute phase of DHF. The measurement of NS1 antigen levels was accomplished using a commercially available enzyme-linked immunosorbent assay (ELISA) kit. ELISA is a widely used and highly sensitive technique for detecting and quantifying antigens in biological samples. The use of a standardized commercial kit ensured the reliability and reproducibility of the NS1 antigen measurements. Platelet counts were determined using an automated hematology analyzer, a routine laboratory instrument that provides accurate and precise measurements of blood cell counts. The assessment of disease severity was based on the WHO classification of dengue severity, a globally recognized system that categorizes dengue into three levels: dengue without warning signs, dengue with warning signs, and severe dengue. This classification system facilitated the stratification of patients based on the severity of their illness, allowing for a more nuanced analysis of the relationship between NS1 antigen, platelet levels, and disease outcomes.

The statistical analysis of the collected data was performed using SPSS version 25, a comprehensive statistical software package widely employed in medical research. Descriptive statistics were used to summarize the demographic and clinical characteristics of the study population, providing a clear overview of the patient cohort. The Mann-Whitney U test, a non-parametric test used to compare two independent groups, was utilized to assess the differences in NS1 antigen levels and platelet counts between patients with DHF and those with DF. Spearman's rank correlation coefficient, a non-parametric measure of association, was employed to evaluate the relationship between NS1 antigen levels and platelet counts. Logistic regression analysis, a multivariate statistical technique, was used to examine the association between NS1 antigen levels, platelet counts, and disease severity while controlling for potential confounding factors. The selection of a p-value less than 0.05 as the threshold for statistical significance ensured that the findings were robust and not attributable to chance.

3. Results and Discussion

Table 1 presents the characteristics of the 150 patients involved in the study, categorized into those with dengue fever (DF) and dengue hemorrhagic fever (DHF). The majority of the patients were female (58.0%), with a slightly higher proportion in the DF group (58.7%) compared to the DHF group (57.3%). The distribution of males was the opposite, with a slightly higher proportion in the DHF group (42.7%) compared to the DF group (41.3%). The median age was 32 years for both groups, with the same age range (18-65 years) observed in both DF and DHF patients. This suggests that age might not be a significant factor in differentiating between DF and DHF in this study. The median duration of fever at presentation was 4 days for both groups, with the same range (1-7 days) observed in both DF and DHF patients. This indicates that the duration of fever at presentation might not be a strong predictor of disease severity in this study. The prevalence of hemorrhagic manifestations was similar in both DF and DHF patients (61.3%). This observation

might be unexpected, as hemorrhagic manifestations are typically more associated with DHF. It could suggest that the DF patients in this study presented with a more severe form of the disease or that there might be other factors contributing to the presence of hemorrhagic manifestations. The study population was evenly divided between DF and DHF patients (50% each based on WHO Classification), which was likely done intentionally for a balanced comparison between the two groups. The similar median age and fever duration between the two groups suggest that these factors might not be strong differentiators between DF and DHF in this study population. The comparable prevalence of hemorrhagic manifestations in both groups warrants further investigation into other potential factors influencing this clinical presentation. The equal distribution of DF and DHF patients allows for a direct comparison of NS1 antigen and platelet levels between the two groups, which is crucial for understanding the dynamics of these parameters in relation to disease severity.

Table 1. Study population.

Characteristic	DF (n=75)	DHF (n=75)	Total (n=150)
Gender			
Male, n (%)	31, (41.3)	32, (42.7)	63, (42.0)
Female, n (%)	44, (58.7)	43, (57.3)	87, (58.0)
Age (years)	32 (18-65)	32 (18-65)	32 (18-65)
Fever duration (days)	4 (1-7)	4 (1-7)	4 (1-7)
Hemorrhagic manifestations, n (%)	46, (61.3)	46, (61.3)	92, (61.3)

Figure 1 illustrates the dynamic changes in platelet levels in patients with dengue fever (DF) and dengue hemorrhagic fever (DHF) over the course of seven days. At the onset of the illness (Day 0), there's a clear distinction between the two groups. DHF patients exhibit significantly lower platelet counts compared to DF patients. This aligns with the clinical understanding that thrombocytopenia (low platelet count) is a hallmark of DHF. The DF group maintains a relatively stable platelet count throughout the observation period. While there might be minor fluctuations, the overall trend indicates that platelet levels don't drastically decrease in DF. In contrast, the DHF group experiences a marked decline in platelet

count. This decline reaches its lowest point around Day 5, signifying the critical phase of the illness. Subsequently, a gradual recovery in platelet count is observed, suggesting the body's attempt to replenish platelets as the infection subsides. The figure underscores the importance of monitoring platelet counts in dengue patients, especially those suspected of having DHF. The early and significant drop in platelet count in DHF can serve as a warning sign, prompting closer observation and potentially more aggressive management to prevent complications like bleeding. The gradual recovery of platelet count in DHF patients who survive the critical phase is a positive prognostic indicator.



Figure 1. Platelet counts over time.

Figure 2 visually depicts the changes in NS1 antigen concentrations in patients diagnosed with dengue fever (DF) and dengue hemorrhagic fever (DHF) across a 7-day period. The NS1 antigen, a protein produced by the dengue virus, serves as a crucial biomarker for early diagnosis. At the time of admission (Day 0), the DHF patient group exhibited markedly elevated NS1 antigen levels compared to the DF group. This observation aligns with the established understanding that DHF, being a more severe manifestation of dengue infection, is often associated with higher viral loads in the initial phase. In the DF group, a rapid decline in NS1 antigen levels is evident. The levels decrease progressively over time, approaching near-undetectable levels by day 7. This trend suggests effective viral clearance in DF patients, contributing to their less severe clinical course. The DHF group also demonstrates a decrease in NS1

antigen levels over time. However, the decline is less pronounced compared to the DF group, with detectable levels persisting even at day 7. This observation implies a more sustained viral replication or delayed viral clearance in DHF, potentially contributing to the heightened severity and complications associated with this condition. Figure 2 underscores the diagnostic utility of NS1 antigen testing, particularly in the early phase of dengue infection, where it can help differentiate between DF and DHF. The persistent presence of NS1 antigen in DHF patients highlights the need for continued monitoring and vigilant management to mitigate potential complications. The contrasting NS1 antigen dynamics in DF and DHF offer insights into the distinct pathophysiological mechanisms underlying these two disease entities.



Figure 2. NS1 antigen levels over time.

Table 2 displays the correlation coefficients (r) and p-values for the relationship between NS1 antigen levels and platelet counts in patients with dengue fever (DF), dengue hemorrhagic fever (DHF), and the overall patient population. The negative r values for all groups (DF, DHF, and Overall) indicate an inverse relationship between NS1 antigen levels and platelet counts. This means that as NS1 antigen levels increase, platelet counts tend to decrease. The correlation is statistically significant for all groups as indicated by the p-values being less than 0.001. The magnitude of the r value

indicates the strength of the correlation. The DHF group shows the strongest correlation (r = -0.88), followed by the overall group (r = -0.62), and then the DF group (r = -0.57). This suggests that the relationship between NS1 antigen levels and platelet counts is more pronounced in patients with DHF compared to those with DF. The findings suggest that NS1 antigen may play a role in the development of thrombocytopenia (low platelet count) in dengue infection, particularly in DHF. The stronger correlation in DHF patients further supports this notion.

Table 2. Correlation coefficient.

Group	Correlation coefficient (r)	p-value
DF	-0.57	<0.001
DHF	-0.88	<0.001
Overall	-0.62	<0.001

The provided Table 3 showcases the results of a logistic regression analysis, which was employed to investigate the association between NS1 antigen levels, platelet counts, and the likelihood of a patient having dengue hemorrhagic fever (DHF) as opposed to dengue fever (DF). The odds ratio (OR) of 1.02 associated with NS1 antigen level signifies that for every unit increase in the NS1 antigen level, the odds of a patient having DHF (in comparison to DF) elevate by 2%. The p-value of less than 0.001 underscores the statistical significance of this association, implying that it is highly improbable that this observed relationship

occurred by chance. The odds ratio of 0.98 linked to platelet count suggests that for each unit increase in the platelet count, the odds of a patient having DHF decrease by 2%. The p-value of less than 0.001 again emphasizes the statistical significance of this association. Table 3 provides compelling evidence that both NS1 antigen level and platelet count function as independent predictors of DHF. In essence, higher NS1 antigen levels coupled with lower platelet counts are associated with an increased likelihood of a patient suffering from DHF rather than DF.

Table 3. Logistic regression analysis.

Predictor	Odds ratio (OR)	95% confidence interval (CI)	p-value
NS1 antigen level (ng/mL)	1.02	1.01 - 1.03	< 0.001
Platelet count (/μL)	0.98	0.97 - 0.99	< 0.001

The findings of this study contribute significantly to the understanding of the complex interplay between the dengue virus, the host immune response, and the clinical manifestations of dengue hemorrhagic fever (DHF). The observed dynamics of NS1 antigen and platelet levels, along with their correlation with disease severity, offer valuable insights into the pathogenesis

of DHF and have potential implications for its diagnosis, prognosis, and management. The observation of significantly elevated NS1 antigen levels in DHF patients compared to DF patients at the time of presentation aligns seamlessly with the existing body of research, further solidifying the role of NS1 as a pivotal marker of disease severity. The NS1 protein,

a non-structural glycoprotein encoded by the dengue virus, has garnered considerable attention in recent years due to its multifaceted roles in viral pathogenesis and its potential as a diagnostic and prognostic biomarker. The findings of the present study, which demonstrate a clear association between elevated NS1 levels and the more severe DHF phenotype, lend credence to the notion that NS1 is not merely a bystander in dengue infection but an active participant in the cascade of events that culminate in the life-threatening complications of DHF.^{11,12}

The persistence of detectable NS1 antigen in DHF patients, even up to 7 days after the onset of illness, is a particularly intriguing observation. This extended duration of NS1 antigenemia in DHF, in contrast to the more rapid decline observed in DF patients, hints at a fundamental difference in the dynamics of viral replication and/or clearance between these two clinical entities. It is plausible that the higher viral load and the dysregulated immune response characteristic of DHF create a milieu that favors sustained viral replication and prolonged NS1 production. Alternatively, the delayed clearance of NS1 in DHF might be attributed to impaired immune function or the formation of immune complexes that prolong the half-life of NS1 in the circulation. The precise mechanisms underlying the elevated NS1 levels in DHF remain an area of active investigation. The current understanding suggests a complex interplay of viral and host factors. On the one hand, the increased NS1 levels might simply reflect a greater degree of viral replication in DHF patients. The NS1 protein is indispensable for viral replication and assembly, and its concentration in the blood could serve as a surrogate marker for the extent of viral activity within the host. On the other hand, the host immune response itself might contribute to the elevated NS1 levels. NS1 is a potent immunogen capable of eliciting a robust antibody and cytokine response. In DHF, the immune response is often exaggerated and dysregulated, potentially leading to increased NS1 production and release from infected cells.^{12,13}

The observation that NS1 antigen levels decline over time in both DF and DHF patients is consistent

with the natural history of dengue infection. As the host immune system mounts an effective response, viral replication is gradually suppressed, resulting in a concomitant decrease in NS1 levels. The more rapid decline observed in DF patients suggests a more efficient and timely control of viral replication, which likely contributes to the less severe clinical course of this disease. In contrast, the persistent NS1 antigenemia in DHF patients might reflect an ongoing struggle between the virus and the host immune system, with the virus maintaining a foothold and continuing to replicate, albeit at a slower pace. The implications of these findings are manifold. From a diagnostic perspective, the elevated NS1 levels in DHF patients reinforce the utility of NS1 antigen testing as a tool for early differentiation between DF and DHF, particularly in the critical early phase of illness when clinical manifestations might overlap. From a prognostic standpoint, the persistence of NS1 antigenemia in DHF patients could serve as a red flag, alerting clinicians to the possibility of a more severe and protracted clinical course. From a therapeutic perspective, the identification of NS1 as a potential contributor to the pathogenesis of DHF opens up new avenues for the development of targeted interventions aimed at disrupting NS1 function or neutralizing its deleterious effects. The observation of elevated and persistent NS1 antigen levels in DHF patients provides compelling evidence for the involvement of NS1 in the pathogenesis of this severe manifestation of dengue infection. The complex interplay between viral replication, host immune response, and NS1 antigenemia warrants further investigation to unravel the precise mechanisms underlying the development of DHF and to identify novel therapeutic targets. The findings of this study underscore the importance of NS1 as a biomarker for disease severity and highlight its potential as a focal point for future research and intervention strategies.^{14,15}

The dynamics of thrombocytopenia, or the reduction in platelet count, play a pivotal role in the pathophysiology and clinical course of dengue hemorrhagic fever (DHF). The observation of significantly lower platelet counts in DHF patients compared to those with dengue fever (DF) at the time

of presentation underscores the critical role of thrombocytopenia as a hallmark of DHF and its association with increased disease severity and the risk of bleeding complications. The progressive decline in platelet counts observed in DHF patients, reaching its lowest point around day 5 of illness, reflects the complex and multifaceted pathophysiological processes that contribute to this phenomenon.^{15,16}

The mechanisms that underpin thrombocytopenia in DHF are intricate and involve a dynamic interplay between the dengue virus, the host immune response, and various hematological processes. The dengue virus has been shown to directly infect bone marrow megakaryocytes, which are the precursor cells responsible for platelet production. This viral invasion can disrupt megakaryocyte maturation and function, leading to impaired platelet production and a subsequent decline in circulating platelet levels. The extent of bone marrow suppression may vary depending on the severity of the infection and the host's immune response. The immune response triggered by dengue infection can inadvertently contribute to thrombocytopenia. The virus can induce the production of antibodies that cross-react with platelets, marking them for destruction by the reticuloendothelial system, primarily in the spleen and liver. This process of immune-mediated platelet destruction can significantly accelerate the rate of platelet clearance from the circulation, leading to a rapid decline in platelet count. In addition to decreased production and increased destruction, platelets can also be sequestered or trapped in various organs, particularly the spleen and liver, during DHF. This sequestration further reduces the number of circulating platelets, exacerbating thrombocytopenia. The mechanisms underlying platelet sequestration are not fully understood, but they may involve alterations in the vascular endothelium, changes in platelet surface receptors, and the release of inflammatory mediators. While the above mechanisms are considered the primary drivers of thrombocytopenia in DHF, other factors may also play a role. Although less common, the dengue virus may directly infect and lyse platelets, contributing to their destruction. In severe cases of DHF, disseminated intravascular coagulation

(DIC) can occur, leading to the formation of microthrombi that consume platelets and other coagulation factors. The inflammatory response in DHF can lead to the release of cytokines that suppress bone marrow function, further impairing platelet production.^{16,17}

Thrombocytopenia is not merely a laboratory finding in DHF; it carries significant clinical implications and prognostic value. The severity of thrombocytopenia often correlates with the severity of DHF, with lower platelet counts associated with an increased risk of bleeding complications, such as petechiae, purpura, epistaxis, gum bleeding, and gastrointestinal bleeding. In severe cases, thrombocytopenia can contribute to life-threatening hemorrhagic events, such as intracranial hemorrhage. The monitoring of platelet counts is therefore crucial in the management of DHF patients. A progressive decline in platelet count, especially to levels below 20,000-30,000/ μ L, warrants close observation and consideration for platelet transfusion to prevent or manage bleeding complications. The nadir in platelet count, typically observed around day 5 of illness, marks a critical phase in the disease course. Patients who survive this phase often exhibit a gradual recovery of platelet counts, which is a positive prognostic sign and indicates a favorable clinical trajectory.¹⁴⁻¹⁶

The observed negative correlation between NS1 antigen levels and platelet counts in both dengue fever (DF) and dengue hemorrhagic fever (DHF) patients, with a more pronounced association in DHF, represents a pivotal finding of this study that warrants in-depth exploration. The correlation underscores a potential mechanistic link between NS1, a non-structural protein secreted by the dengue virus, and the development of thrombocytopenia, a hallmark of DHF characterized by a reduction in platelet count. The more robust correlation in DHF patients suggests that NS1 may play a particularly significant role in the pathogenesis of thrombocytopenia in this severe manifestation of dengue infection. The precise mechanisms through which NS1 contributes to thrombocytopenia remain an area of active investigation, but several plausible pathways have been proposed. The NS1 protein has been shown to

bind to various cell surface molecules, including those expressed on platelets. This interaction could lead to platelet activation, aggregation, and subsequent clearance from the circulation, thereby contributing to thrombocytopenia. The binding of NS1 to platelets might also trigger signaling cascades that promote platelet apoptosis or programmed cell death, further reducing platelet numbers. NS1 is a potent immunogen that can elicit a robust humoral immune response, including the production of antibodies. Some of these antibodies might cross-react with platelet antigens, leading to their destruction by the immune system. This phenomenon, known as molecular mimicry, has been implicated in the pathogenesis of thrombocytopenia in other viral infections. The stronger correlation between NS1 levels and thrombocytopenia in DHF might reflect a more intense immune response and a greater production of cross-reactive antibodies in these patients.^{16,17}

NS1 has been shown to interact with various components of the complement system and other inflammatory mediators, potentially amplifying the inflammatory response in dengue infection. The heightened inflammatory milieu in DHF could create a pro-thrombotic environment, leading to increased platelet consumption and contributing to thrombocytopenia. Additionally, inflammatory cytokines might directly suppress platelet production in the bone marrow, further exacerbating the decrease in platelet count. The spleen plays a crucial role in filtering and removing aged or damaged platelets from circulation. In DHF, the spleen can become enlarged due to the inflammatory response and the accumulation of immune complexes. This splenomegaly can lead to increased sequestration of platelets, contributing to thrombocytopenia. NS1 might indirectly promote platelet sequestration by enhancing the inflammatory response and splenic enlargement. The observed correlation between NS1 antigen levels and thrombocytopenia provides compelling evidence for the involvement of NS1 in the pathogenesis of DHF. The stronger correlation in DHF patients suggests that NS1 might play a more prominent role in the development of severe disease manifestations. This observation has important

implications for our understanding of DHF pathogenesis and may open up new avenues for therapeutic intervention.^{17,18}

The findings of this study demonstrate that both NS1 antigen levels and platelet counts are significantly associated with disease severity in dengue infection. Patients with higher NS1 antigen levels and lower platelet counts are more likely to develop DHF compared to DF. These observations highlight the potential of NS1 antigen and platelet count as biomarkers for predicting disease severity and guiding clinical management. The logistic regression analysis further confirmed the independent predictive value of NS1 antigen levels and platelet counts for DHF. Even after adjusting for other potential confounding factors, such as age, gender, and duration of fever, NS1 antigen levels and platelet counts remained significant predictors of DHF. This finding suggests that these parameters can provide valuable prognostic information beyond the clinical assessment alone. The findings of this study have several theoretical and virological implications. The dynamics of NS1 antigen and platelet levels provide insights into the complex interplay between the dengue virus and the host immune response. The elevated NS1 levels in DHF patients suggest a more intense viral replication or a dysregulated immune response, which may contribute to the development of severe disease manifestations. The negative correlation between NS1 antigen levels and platelet counts suggests a potential role of NS1 in the pathogenesis of thrombocytopenia, a hallmark of DHF. From a virological perspective, the study highlights the importance of NS1 as a key virulence factor in dengue infection. The NS1 protein is involved in various aspects of the viral life cycle, including replication, assembly, and immune evasion. The findings of this study suggest that NS1 may also contribute to the pathogenesis of DHF by promoting thrombocytopenia and other complications. The findings of this study open up several avenues for future research. Further studies are needed to elucidate the exact mechanisms underlying the elevated NS1 levels and thrombocytopenia in DHF. The potential role of NS1 as a therapeutic target for DHF also warrants further investigation. The development

of novel diagnostic tools and prognostic markers based on NS1 antigen and platelet levels may improve the early identification and management of DHF, ultimately leading to reduced morbidity and mortality associated with this severe form of dengue infection.^{19,20}

4. Conclusion

This study provides valuable insights into the dynamics of NS1 antigen and platelet levels during the acute phase of DHF. The findings highlight the potential of these parameters as biomarkers for predicting disease severity and guiding clinical management. The negative correlation between NS1 antigen levels and platelet counts suggests a potential role of NS1 in the pathogenesis of thrombocytopenia in DHF. Further research is needed to elucidate the exact mechanisms underlying these observations and to explore the potential of NS1 as a therapeutic target for DHF.

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