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The Role of NS1 Gene Mutations in Dengue Virus Serotype 2 and Their Association with Clinical Severity of Dengue Hemorrhagic Fever: A Systematic Review

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Abstract

NS1 gene of Dengue Virus Serotype 2 (DENV-2) plays a significant role in the pathogenesis and clinical severity of dengue infections, including Dengue Hemorrhagic Fever. Mutations in the NS1 gene of DENV-2 are closely associated with the clinical severity of dengue. Methods: This review article employs a systematic review methodology, utilizing Scopus, PubMed and Web of Science from 2015 to 2025. Results: The T164S mutation was consistently associated with increased severity through elevated secreted NS1 (sNS1) production, complement activation, and inflammation. Conversely, the S103T mutation appeared in milder cases. NS1 mutations also correlated with immune hyperactivation, vascular leakage, and, in some cases, neurological symptoms such as encephalitis. Despite these findings, direct causality between specific mutations and clinical outcomes requires further investigation. Conclusion: Mutations in the NS1 gene of DENV-2 affect the stability, secretion, and immunogenic properties of the NS1 protein, leading to increased vascular leakage and severe disease outcomes

Keywords: *Dengue Virus Serotype 2, Disease Severity, NS1 Mutations, Vascular Leakage.*

INTRODUCTION

The global incidence of dengue fever has reached unprecedented levels, with the 2024-2025 period experiencing a significant escalation in both the frequency and severity of outbreaks. In 2024, a new, alarmingly high baseline for transmission was established, with over 13 million cases reported worldwide, surpassing all previous records. This alarming trend has persisted into 2025, resulting in millions of additional infections and thousands of deaths across major tropical regions. An analysis published in July 2024 indicated that the primary regions for dengue transmission were Southeast Asia (50.4%), South Central Asia (14.9%), the Caribbean (10.9%), and South America (9.2%). The median age of affected individuals was 33 years, with travel particularly tourism being the most common reason for exposure, accounting for 67.3% of cases (Carlson, 2023).

The dengue virus (DENV) is mainly spread through mosquito bites and can lead to various health problems, ranging from mild dengue fever to more serious conditions such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Of the four dengue virus serotypes (DENV-1 to DENV-4), DENV-2 is most frequently associated with severe disease and large outbreaks globally (Bhatt et al., 2013; Cañari-Casaño et al.,

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2024). The increasing incidence of dengue hemorrhagic fever (DHF) poses a significant public health challenge, particularly in tropical and subtropical regions where the disease is most prevalent.

The non-structural protein 1 (NS1) is a key factor in the pathogenesis of severe dengue. It facilitates viral replication, helps the virus evade the immune response, and damages the lining of blood vessels. Unlike other non-structural proteins, NS1 is secreted outside the infected cells (sNS1) and can directly interact with host cells and immune components, contributing to disease severity (Muller & Young, 2013). Elevated sNS1 levels are linked to increased vascular permeability, a hallmark of Dengue Hemorrhagic Fever (DHF). These heightened levels also indicate activation of the complement system and an increase in pro-inflammatory cytokines, all of which exacerbate disease severity (Puerta-Guardo et al., 2016).

Recent research indicates that mutations in the NS1 gene can substantially affect the virus's characteristics, including its replication rate, interactions with the immune system, and potential to cause severe illness (Fang et al., 2023). For example, the T164S mutation in the NS1 protein has been associated with elevated sNS1 levels and a heightened inflammatory response, which may lead to increased disease severity and mortality in animal studies (Chan et al., 2019). Some mutations, like S103T, are more commonly found in milder dengue cases, indicating that variations in the NS1 protein may have diverse effects on disease severity (Hapuarachchi et al., 2015).

Despite increasing evidence highlighting the significance of NS1 mutations in dengue virus pathogenesis, the exact relationship between specific NS1 gene variations and disease severity in DENV-2 infections remains unclear. A thorough synthesis of current research is essential to clarify these associations. We conducted this review because no previous studies have examined the effects of DENV-2 NS1 protein mutations on its effects. Consequently, this systematic review aims to investigate the impact of NS1 gene mutations in Dengue Virus Serotype 2 from current studies and its correlation with the clinical severity of Dengue Hemorrhagic Fever that will occur.

METHODS

In this research, a thorough review of scientific literature was performed covering publications from January 2015 to May 2025, including all available results up to that date. To ensure a comprehensive search, three major databases Scopus, PubMed, and Web of Science were queried. The search utilized specific Boolean operators to combine relevant keywords: ("NS1" OR "non-structural protein 1" OR "DENV NS1" OR "Dengue virus protein") AND ("gene mutation" OR "mutation" OR "genetic alteration" OR "genetic variation") AND ("Dengue virus" OR "DENV" OR "Dengue" OR "Dengue serotype 2") AND ("Dengue Hemorrhagic Fever" OR "DHF" OR "hemorrhagic fever" OR "severe dengue"). No direct contact was made with researchers, and unpublished data were excluded from the review. The methodology adhered to the PRISMA guidelines, focusing solely on database searches and registry reviews. Manuscripts were selected through screening of titles and abstracts, followed by full-text evaluations.

A comprehensive search was conducted using PubMed (n = 450), Scopus (n = 520), Web of Science (n = 380), and trial registers (n = 1,350), yielding a total of 2,700 records. Following the consolidation of the search results, a total of 895 duplicate records were eliminated prior to the screening process. The initial screening stage was conducted on 895 records, with the selection process based on a thorough evaluation of titles and abstracts. A total of 780 records were excluded on the basis of irrelevance to the research topic, leaving 115 records for full-text retrieval. However, eight full-text articles could not be retrieved, resulting in a total of 107 articles for the subsequent eligibility

assessment.During the eligibility stage, the full-text articles were meticulously evaluated against the predefined inclusion criteria. A total of 75 articles were excluded for the following reasons: no correlation with clinical severity (n = 25), no specific NS1 gene analysis (n = 22), not related to DENV serotype/genotype (n = 15), review articles (n = 11), and text not available in English (n = 4). Following a thorough evaluation, 30 articles were deemed to meet all the necessary inclusion criteria and were thus incorporated into the systematic review. The sequence of events in the article selection process is illustrated in Figure 1.

RESULT

In the initial phase, 2,700 records were identified across four sources: PubMed 450, Scopus 520, Web of Science 380, and clinical trial registries 1,350. After removing 895 duplicates, 1,805 unique records remained for screening based on titles and abstracts. Of these, 780 did not meet the inclusion criteria and were excluded, leaving 1,025 articles for full-text review. Eight articles could not be retrieved, resulting in 1,017 articles assessed for eligibility according to predefined criteria. Subsequently, 75 articles were excluded due to reasons such as lack of correlation between NS1 mutation and clinical severity 25 , absence of specific NS1 gene analysis 22, studies not related to DENV serotype 2 15, review articles 11, and publications in non-English languages 4. Finally, 30 studies were included in the final systematic review. Figure 1. PRISMA Flow Diagram of the systematic review examining the association between NS1 gene mutations in DENV-2 and the clinical outcomes or severity of dengue hemorrhagic fever.

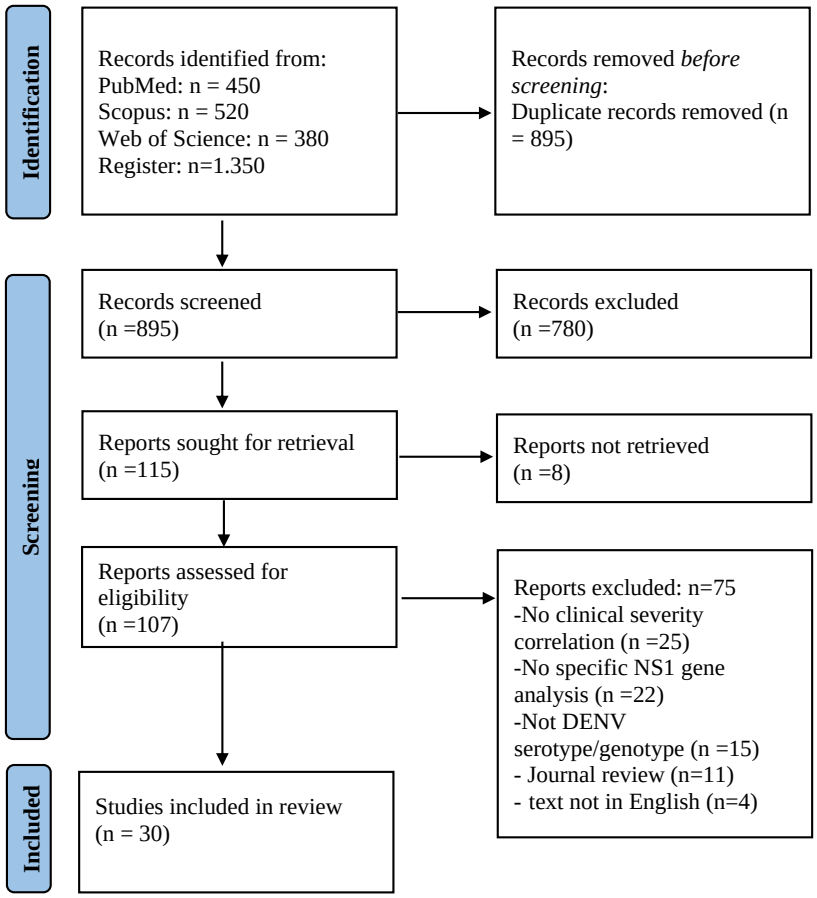

Figure 1. PRISMA flow diagram showing the study selection process for the systematic review of NS1 gene mutations in DENV-2 and their association with clinical severity of DHF

Table 1. NS1 gene mutations in Dengue Virus Serotype 2 (DENV-2), associated clinical outcomes, molecular mechanisms, and supporting primary studies

NS1 Gene Mutations in DENV-2	Population	Clinical Manifestation/Clinical Symptoms	Mechanism	Study
T164S	Americas, AG129 mice, Aedes aegypti	Severe disease, increased complement activation, tissue inflammation, rapid mortality	Increased sNS1 production, up-regulation of genes linked to vascular leakage, higher proinflammatory cytokines	(Chan et al., 2019)
a) Nonsense mutations at site 585 (T to C) in most isolates and at site 936 (C to T) in isolate HNNS201806 b) 68 base mutations	Sera patients with dengue fever, Hunan Province, China in 2018	Mild symptoms to severe and potentially fatal complications	Immune system interactions, genetic factors, and the presence of concurrent infections	(Feng et al., 2020)
Lys272, Lys324	La Reunion, Tanzania	Poor NS1 stability and secretion	Low NS1 secretion in hepatocytes	(Ogire et al., 2021)
S103T	Singapore, DF patients	Mild disease outcome	Associated with less virulent DENV-2 strains	(Hapuarachchi et al., 2015)
Val236➔Ala	Various (ELISA study)	Decreased NS1 detection	Reduced NS1 production and secretion	(Ghosh et al., 2022)
F103S, T146I	Laboratory strains, HMEC-1 cells	Increased vascular leakage	Higher sNS1 secretion, changes in amino acid properties	(Singh et al., 2017)
Not specified	Brazil, 68 patient cases	Severe disease, warning signs	Constrained diversity in NS2B gene, potential epistatic interactions	(Torres et al., 2021)

Not specified	Sri Lanka, 91 patients	Severe dengue, higher viremia levels	Higher NS1 antigen levels, correlated with TNF-a, IL-4, and MCP-1	(Nwe et al., 2023)
K272R	Taiwan 2015 Outbreak (Cosmopolitan)	Increased likelihood of severe dengue (DHF/DSS) in infected individuals and Potential for more pronounced vascular leakage (due to heightened pro-inflammatory cytokines)	Enhanced viral replication; increased sNS1 secretion; suppressed host interferon response (STAT1 phosphorylation).	(Hee et al., 2025)
G53D	DENV-2 16681 strain	-	Affected viral infection and dissemination in both mosquito and mammalian cells.	(Choy et al., 2020)

Clinical Manifestations Associated with NS1 Gene Mutations

1. NS1 mutations in Dengue Virus Serotype 2

Recent whole genome sequencing studies have uncovered considerable genetic diversity within the NS1 gene of Dengue Virus Serotype 2 (DENV-2), which has important consequences for the virus's ability to cause disease and the severity of clinical outcomes. A genomic analysis in China identified both sense and nonsense mutations in the NS1 gene of DENV-2 isolates. Phylogenetic analysis demonstrated a close relationship to strains circulating in Southeast Asia, suggesting regional spread and evolutionary connections (Feng et al., 2020). A dedicated research study on Pakistan's Punjab Province revealed a significant number of genetic mutations not only in the NS1 region but also in neighboring areas such as NS2A and NS2B. The study identified 25 mutations in NS2A and 20 in NS2B, including several novel variants, indicating potential mutation hotspots that could be crucial for understanding viral evolution and developing targeted interventions (Mushtaq et al., 2024).

Analysis of DENV-2 genomes from various isolates reveals a total of 2667 mutations, with a significant number being non-synonymous. Notably, these mutations occur more frequently in isolates from severe dengue cases, supporting the hypothesis that specific NS1 mutations could be linked to increased disease severity (Ravi et al., 2025). In Taiwan, a compensatory mutation in the NS5 gene (V357E) has been identified to markedly increase viral replication and pathogenicity, highlighting the significant role of non-structural gene mutations in viral behavior. (Ko et al., 2024). Recent research on a DENV-2 outbreak in Mexico has identified the emergence of new viral lineages. These strains exhibit concurrent mutations in the NS2A and NS5 regions, which may indicate adaptive evolution and the development of more virulent, locally adapted strains (Rodriguez et al., 2022).

Research indicates that mutations are not limited to the NS1 protein but are also prevalent across the DENV-2 genome, including NS2A, NS2B, and NS5 regions. These mutations often cluster in more severe or evolving strains, implying a pattern of co-mutation that may work synergistically to enhance disease severity, facilitate immune evasion, and complicate diagnostic efforts. Understanding these mutation patterns is crucial for developing more effective diagnostic tools and treatments (Samune et al., 2024).

2. Clinical Severity

The correlation between mutations in the NS1 gene of Dengue Virus Serotype 2 (DENV-2) and the severity of Dengue Hemorrhagic Fever (DHF) has been extensively studied through genomic and epidemiological approaches. Analysis of 1,023 DENV-2 genomes revealed a higher frequency of mutations in severe dengue cases compared to milder forms. Specifically, 56 mutations, many situated within the NS1 gene region, were significantly linked to increased disease severity, suggesting their potential role in enhancing viral virulence and immune system evasion (Ravi et al., 2025). This genome-wide association aligns with previous findings that identified the T164S mutation in the NS1 protein, which has been associated with heightened vascular leakage and more severe dengue outbreaks across the Americas. These results highlight the significance of NS1 mutations as molecular markers of disease severity and their role in dengue fever pathogenesis (Chan et al., 2019).

Although some research indicates a straightforward relationship, other studies reveal a more intricate interaction between viral characteristics and host responses. A comparative genomic analysis of DENV-2 strains isolated from patients with dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) identified distinct viral lineages associated with milder disease outcomes. This suggests that the progression to severe conditions like DHF and DSS is influenced not only by viral mutations but also significantly by the host's immune response and genetic factors (Katzelnick et al., 2015).

Recent research into the evolution of dengue virus (DENV-2) outbreaks has revealed a compensatory mutation in the NS5 gene that markedly increases viral replication and disease severity. Although this mutation is not located within the NS1 gene itself, its influence on overall pathogenicity underscores the potential for synergistic interactions among various non-structural proteins, including NS1, to exacerbate disease outcomes (Ko et al., 2024). Intrahost viral diversity indicates that variations in viral populations among patients with dengue fever, dengue with warning signs, and severe dengue are primarily affected by the timing of infection and selective pressures rather than the severity of the disease itself. These insights imply that although certain NS1 gene mutations might serve as indicators of disease severity, the overall clinical outcomes of dengue infection are likely shaped by a complex interplay of viral genetics, host immune responses, and evolutionary factors (Torres et al., 2021).

3. Immune Response

Previous studies indicate that cases of Severe Dengue tend to have lower viral loads, suggesting that the host's immune response its role and diversity are crucial factors influencing disease severity (Wang et al., 2018). There has been a rise in severe dengue cases among individuals experiencing secondary infections with different heterologous DENV serotypes. In these cases, the Antibody dependent enhancement (ADE) immune response does not effectively neutralize the virus particles, leading to increased disease severity (Katzelnick et al., 2017).

The NS1 protein of Dengue Virus Serotype 2 (DENV-2) is crucial not only for viral replication but also for its ability to modulate the host immune response. During DENV-2 infection, the immune system produces anti-NS1 antibodies, which are linked to the development of protective immunity. This highlights NS1 as a significant target for immune intervention. Additionally, vaccine approaches that utilize recombinant NS1 protein, especially when combined with adjuvants like non-toxic LT derivatives, have demonstrated the ability to induce robust humoral immune responses and provide protection in experimental models (Glasner et al., 2018; Sanchez et al., 2024).

Within the scope of immunopathogenesis, NS1-specific T cells have been identified as contributors to vascular leakage observed in severe dengue cases. This indicates that the immune response targeting NS1 might not always be protective and, in some instances, could worsen disease severity. Additionally, the presence of anti-NS1 antibodies has been linked to clinical severity and may serve as a predictive biomarker for adverse outcomes. The NS1 protein features epitope regions that are not only recognized by the humoral immune response but may also interact with host factors or influence viral replication, highlighting its complex role in host–virus interactions (Glasner et al., 2017).

Although the immunological significance of the NS1 protein in dengue virus (DENV) infection is well recognized, the specific effects of mutations within the NS1 gene on the host immune response are not yet fully understood. Genetic variations in DENV, including alterations in the NS1 region, can influence the virus's virulence and its interactions with the host immune system. However, current research has not definitively linked particular NS1 mutations to changes in immune recognition or response strength. Consequently, while it is hypothesized that NS1 mutations may impact immunopathology and vaccine effectiveness, more targeted empirical studies are necessary to clarify these relationships and their implications for disease management and vaccine development (Kraivong et al., 2022; Scaturro et al., 2015).

NS1 gene mutations in DENV-2 impact the virus's ability to cause Dengue Hemorrhagic Fever

Recent research indicates that specific mutations in the NS1 gene of Dengue Virus Serotype 2 (DENV-2) play a crucial role in increasing the virus's ability to induce Dengue Hemorrhagic Fever (DHF). Notably, the T164S mutation has been identified as a key factor, as it enhances the secretion of the NS1 protein (sNS1). Elevated levels of sNS1 are directly linked to increased vascular permeability and endothelial cell dysfunction, which are characteristic features of severe dengue cases such as DHF (Chan et al., 2019). In vitro experiments utilizing mammalian cell lines and human peripheral blood mononuclear cells (PBMCs) revealed that the T164S mutation leads to an increased expression of inflammatory genes without enhancing viral RNA replication. This indicates a pathogenic effect of the mutated NS1 protein that is independent of viral replication. In animal models, this mutation was associated with more severe disease manifestations, including tissue inflammation, activation of the complement system, and increased mortality rates, compared to wild-type strains (Chan et al., 2019).

Conversely, the NS1-S103T mutation has been primarily identified in patients experiencing mild dengue fever, suggesting that specific genetic variations in the NS1 protein could influence disease severity. These mutations may lead to decreased inflammatory responses or lower levels of soluble NS1, thereby contributing to less severe clinical outcomes. The differing mutation patterns underscore the role of NS1 genetic diversity in modulating virulence, either by intensifying vascular damage in severe cases or by supporting subclinical, mild infections (Hapuarachchi et al., 2015).

Mutations in the NS1 gene, such as T164S, have been linked to enhanced viral fitness and transmission capabilities. In Aedes mosquitoes infected with these strains, higher viral titers and increased sNS1 levels have been observed, potentially improving the efficiency of virus acquisition and spread. This may contribute to more extensive and severe dengue outbreaks, emphasizing the importance of monitoring genetic variations in dengue virus strains (Chan et al., 2019).

Clinical Symptoms Associated with NS1 Gene Mutations in Dengue Virus Serotype 2 Infection

Recent research indicates that mutations in the NS1 gene of Dengue Virus Serotype 2 (DENV-2) are increasingly linked to more severe clinical outcomes. These genetic variations may contribute to complications such as neurological issues, vascular leakage, heightened inflammatory responses, and overall increased disease severity, highlighting their potential impact on disease progression beyond typical dengue fever symptoms (Beatty et al., 2015; Chan et al., 2019; Puerta et al., 2016).

Neurological symptoms, especially dengue encephalitis, represent a significant clinical concern. Studies have shown that DENV-2 strains isolated from cerebrospinal fluid and serum of affected patients contain high viral loads, indicating the virus's capacity to breach the blood-brain barrier. These viral isolates are linked to severe neurological manifestations, and in some cases, have resulted in rapid patient deterioration and death. This suggests that mutations in the NS1 protein may enhance the virus's neurotropism and ability to invade the central nervous system (Tun et al., 2020).

The T164S mutation in the NS1 gene has emerged as a critical marker of disease severity in dengue infections. This mutation markedly enhances the secretion of the NS1 protein (sNS1), which plays a key role in vascular leakage a hallmark of Dengue Hemorrhagic Fever (DHF). In vivo studies reveal that mice infected with DENV-2 harboring the T164S mutation exhibit heightened complement activation, tissue inflammation, and rapid mortality. In vitro experiments with human peripheral blood mononuclear cells (PBMCs) further confirm that this mutation promotes the release of pro-inflammatory cytokines, thereby intensifying immune-mediated tissue damage. Notably, severe dengue cases show increased levels of cytokines such as TNF-α, VEGF-A, IFN-γ, IL-6, and IL-10, which contribute to increased vascular permeability and cell death (Chan et al., 2019).

Furthermore, the T164S mutation was identified during a significant dengue outbreak in Cuba, where it quickly became predominant within circulating viral strains. This rapid fixation indicates a potential selective advantage related to enhanced replication and transmission capabilities. These epidemiological and functional insights reinforce the hypothesis that specific NS1 mutations not only exacerbate clinical symptoms but also increase the epidemic potential of the virus (Chan et al., 2019).

Beyond T164S, other NS1 gene changes such as amino acid substitutions involving lysine at position 324 have also been linked to oxidative stress and elevated cytokine production in human hepatoma cells, further implicating NS1 mutations in the immunopathogenesis of severe dengue (Ogire et al., 2021, 2023)

DISCUSSION

Mutations in the non-structural protein 1 (NS1) gene of Dengue Virus Serotype 2 (DENV-2) are crucial factors influencing the virus's ability to cause disease and the severity of clinical symptoms, especially in cases of Dengue Hemorrhagic Fever (DHF). This review emphasizes the high mutation rate observed in DENV-2 strains, with key mutations such as T164S and S103T identified in multiple studies. Whole-genome

sequencing of clinical samples shows a greater occurrence of non-synonymous mutations in severe dengue cases, indicating that genetic variability particularly within NS1 and nearby non-structural regions may affect viral virulence. The evolutionary patterns of DENV-2 suggest that compensatory mutations across the genome, including those in NS1, enhance viral replication, transmission, and ability to evade immune responses.

The link between NS1 mutations and clinical severity is particularly pronounced with the T164S variant, which increases the secretion of NS1 protein (sNS1) and results in vascular leakage, a hallmark of DHF (Chan et al., 2019). Experimental models have demonstrated that this mutation intensifies inflammatory responses, activates complement, and disrupts the endothelium, leading to more severe disease manifestations and increased mortality (Chan et al., 2019). In contrast, the S103T mutation appears to be more common in mild dengue cases, suggesting a potential inverse relationship between specific NS1 mutations and disease severity (Hapuarachchi et al., 2015). These findings indicate that NS1 mutations do not uniformly increase virulence, but instead exhibit differential impacts depending on their position and molecular effects.

NS1 mutations not only affect disease severity but also influence host immune reactions. Although anti-NS1 antibodies are generally linked to protective immunity, higher levels have been detected in DHF patients, suggesting possible involvement in immune-related pathology. NS1-specific T cell responses might induce vascular leakage through cytokine-driven endothelial damage. However, the exact impact of individual NS1 mutations on immune cell activation or specific antibody binding remains unclear, highlighting the necessity for more in-depth immunological and structural studies.

From a clinical perspective, NS1 mutations have also been linked to distinct symptom profiles, including neurological complications such as dengue encephalitis, which may reflect the virus's enhanced capacity to cross the blood-brain barrier (OhAinle et al., 2011). The ability of certain NS1 mutations to increase viral fitness in both human and mosquito hosts raises additional concerns regarding transmission efficiency and epidemic potential (Chan et al., 2019). Therefore, genomic surveillance focusing on NS1 variants, coupled with immunological monitoring, is essential for early detection of high-risk strains, guiding vaccine development, and informing public health responses. Continued research is required to fully elucidate the mechanistic pathways through which NS1 mutations alter the course of dengue infection and to determine their value as predictive markers of disease progression.

CONCLUSION

Mutations in the NS1 gene in Dengue Virus Serotype 2 are associated with the clinical severity of dengue fever infection, particularly through mechanisms involving suboptimal immune activation in eliminating the virus. Specific mutations in T164S cause increased production of secreted NS1, which contributes to the manifestation of severe symptoms of DHF through a vascular leakage. These findings highlight the importance of NS1 as a pathogenic determinant and a potential target for diagnostics, surveillance, and strategies in the discovery of therapies or challenges in the discovery of vaccines as one of the future prevention methods.

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