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Genomic Insights into SARS-CoV-2 Evolution: Nucleocapsid Gene Mutations in West Sumatra, Indonesia

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ABSTRACT

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic has necessitated a comprehensive understanding of viral mutations, particularly in the nucleocapsid protein, which plays a crucial role in the virus's life cycle and interaction with the host immune system. This study aims to analyze the nucleocapsid (N) gene mutations in SARS-CoV-2 variants isolated from West Sumatra during the pandemic. A dataset of 352 SARS-CoV-2 sequences from local COVID-19 cases was examined to identify N gene mutations and assess their prevalence across different variants. The primary mutations identified were R203K and G204R in the ancestral and Omicron variants. However, the D63G and D377Y mutation is present in the Delta variant. These mutations are implicated in enhancing viral infectivity and potential immune evasion. Notably, the Omicron variant showed additional deletions and mutations (P13L, E31-, R32-, S33-, and S413R) that harm protein expansion for structural stability and functionality of the nucleocapsid protein. The conservation of specific mutations across variants suggests their fundamental role in viral replication and immune interaction. Variant-specific mutations may represent adaptive responses to immune pressure or other environmental factors, with significant implications for viral transmissibility and pathogenicity. Understanding the impact of nucleocapsid protein mutations provides critical insights into the evolutionary dynamics of SARS-CoV-2 and aids in developing effective vaccines and therapeutics. Continued surveillance and detailed functional studies are essential to manage the pandemic and prepare for future viral outbreaks.

***Keywords*:** SARS-CoV-2, nucleocapsid protein, N gene, mutations, viral evolution.

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the causative agent of the global coronavirus disease 2019 (COVID-19) pandemic. This virus belongs to the Coronaviridae family and is characterized by its single-stranded RNA genome and a distinctive corona-like halo formed by spike proteins on its envelope. The nucleocapsid protein is crucial to the lifecycle of the virus. It performs essential regulatory operations during the same viral infection process, including

replication and virulence[1]. The nucleocapsid (N) gene is a highly conservative element in the circle of life of the coronavirus. In the beginning, it binds to the viral genome and forms a ribonucleoprotein complex, which is a significant component of the replication process and the packaging of the new virion particles[2]. This protein is fundamental for structural purposes and critical in modulating the host's immune response. The nucleocapsid protein interferes with the host cell's signaling pathways, which may suppress the innate immune response and facilitate viral replication[3].

Mutations in the nucleocapsid protein are of particular interest due to their capacity to affect critical aspects of the SARS-CoV-2 lifecycle and their potential impact on the course of the COVID-19 pandemic. These mutations may lead to changes in viral infectivity, influence transmission patterns, and facilitate immune escape, all of which are central to the ongoing management and understanding of this global health crisis[4]. Global surveys of SARS-CoV-2 mutations face significant challenges in regions with limited genomic data representation. West Sumatra, Indonesia, is one of the areas where a comprehensive analysis of the virus's genomic evolution is much needed but scarcely available. Research analyzing the dynamics of SARS-CoV-2 N gene mutations in West Sumatra during different pandemic waves is non-existent. This knowledge gap hinders a detailed understanding of the virus's evolutionary path, especially in regions containing different variants. Integrating data from underrepresented areas like West Sumatra is crucial to compiling a comprehensive picture of the pandemic's evolution and providing information for targeted interventions locally and globally. In addition, the emergence of West Sumatra-specific variants could impact global vaccine development strategies and public health policies[5-7].

This study aims to address the lack of comprehensive genomic analysis in West Sumatra by analyzing the development of N gene mutations during three different pandemic waves. This can provide insights into the dynamics of SARS-CoV-2 evolution, mainly focusing on the possibility of unique N gene variants that may emerge in West Sumatra. Understanding the virus's genetic diversity in this region is important for local efforts in combating the pandemic and contributing valuable data to global surveillance initiatives.

MATERIALS AND METHODS

The study employed an observational analytical design with a cross-sectional methodology.

Data Collection:

The study analyzed 352 SARS-CoV-2 sequences from next-generation sequencing on an Illumina MiSeq platform, including samples from West Sumatra collected randomly between March 2020 and November 2022[8]. These samples were collected through a partnership with local healthcare agencies and The Center for Infectious Disease Diagnostic and Research (PDRPI) at the Faculty of Medicine, Universitas Andalas. To maintain patient confidentiality, all samples were anonymized by ethical research standards. The sequences consist of clades 19A (n=3), 20A (n=123), and 20B (n=28), which are classified under the early lineages of the ancestral SARS-CoV-2 virus. Subsequently, the Delta variant comprising clades 21I (n=7) and 21J (n=30), and the Omicron variant included clades 21K (n=49), 21L (n=36), 22A (n=1), 22B (n=60), 22D (n=2), 22E (n=5), and 22F (n=8).

Sequence Analysis:

The final genome consensus sequences were aligned with the reference SARS-CoV-2 genome (GenBank accession MN908947, Wuhan-Hu-1 isolate) using CLC Genomics Workbench® version 21.0.3 focusing on identifying mutations in the N gene and their impacts on the N protein's structure and functionality[9].

RESULTS

Nucleotide Mutations in the Nucleocapsid Gene of SARS-CoV-2 Variants

The average nucleotide mutations in the N gene show a tendency of increasing nucleotide mutations with the emergence of new variants of SARS-CoV-2 during the pandemic in West Sumatra, compared to the original MN908947 Severe Acute Respiratory Syndrome Coronavirus 2 isolate Wuhan-Hu-1 variant. The highest nucleotide mutations occurred in the Omicron variant (22E) at a rate of 1.19% (Figure 1).

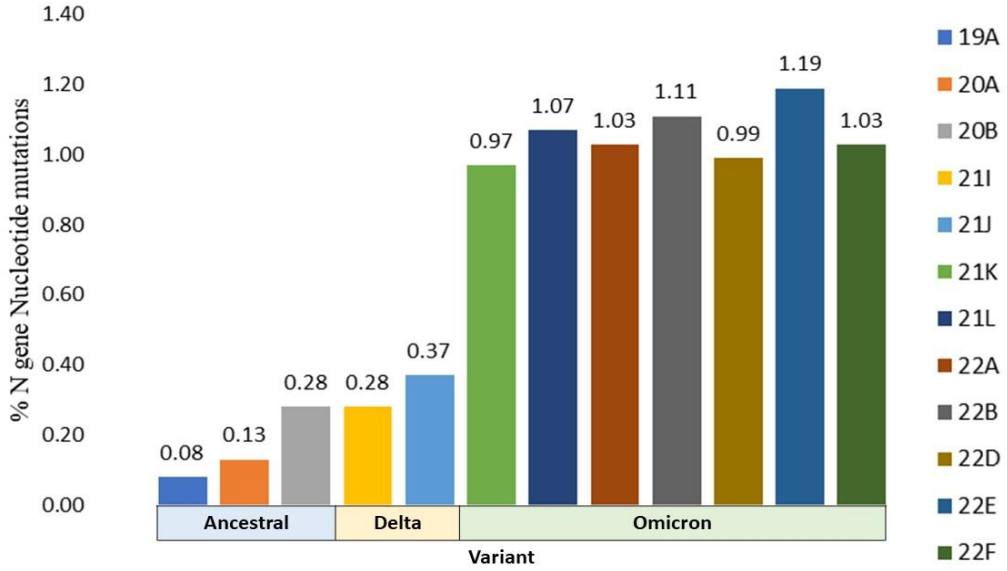

Figure 1 The percentage of Nucleotide Mutations in the N Gene of SARS-CoV-2 Isolates from West Sumatera

Amino Acid Mutations in the Nucleocapsid Gene of SARS-CoV-2 Variants

Our study identified several key amino acid mutations in the nucleocapsid (N) gene across different SARS-CoV-2 variants, with specific mutations characterizing ancestral and emergent strains such as Delta and Omicron (figure 2).

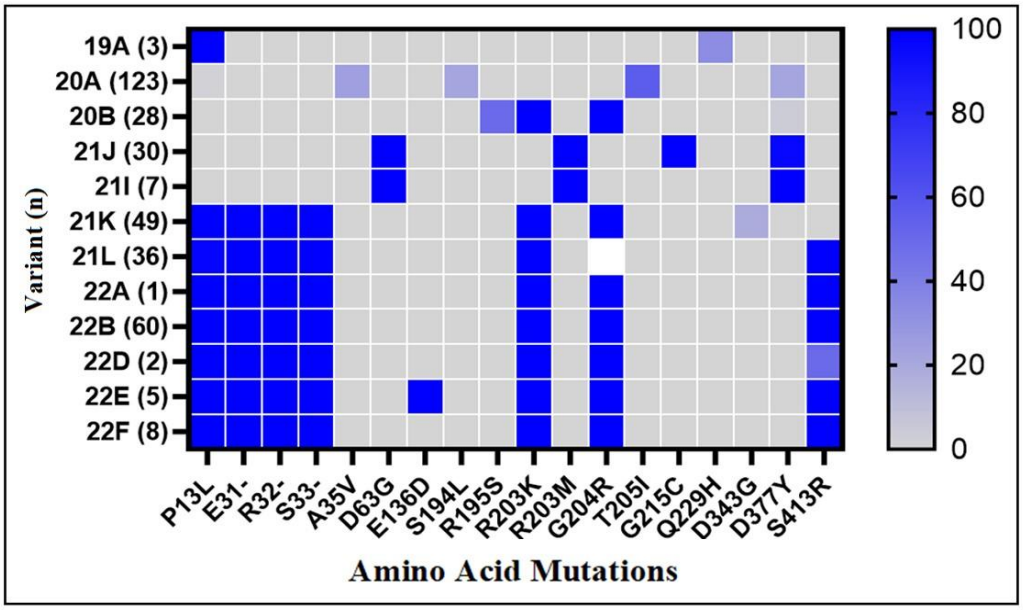

Figure 2. The percentage of Amino Acid Mutations in the N Gene of SARS-CoV-2 Isolates from West Sumatera.

Ancestral Variant Mutations: Clade 20B as the ancestral variant of SARS-CoV-2 displayed mutations R203K and G204R in the N gene. These mutations were also observed persistently across subsequent variants, including the Omicron variant. This suggests that these mutations may confer a fitness advantage or have a functional impact preserved as the virus evolved.

Delta Variant Mutations: The Delta variant (clade 21J and 21I) of the virus showed some distinct changes in its genetic makeup, such as D63G, R203M, D377Y, and G215C. Interestingly, the mutation D377Y was only found in a specific subgroup called sublineage 21J of the Delta variant, which suggests it might have explicitly adapted within this lineage.

Omicron Variant Mutations: The Omicron variant had some unique changes in its N gene, like P13L, and deletions at positions E31, R32, and S33, in addition to the already-known mutations R203K and G204R, and a new one called S413R. The deletions at E31, R32, and S33 are significant because they might suggest alterations in the structure of the nucleocapsid protein. These changes could affect how the virus multiplies or our immune system recognizes it. Another noteworthy mutation, R203M, differed from R203K found in both the ancestral and Omicron variants. This could mean it has a different effect on how the virus behaves or interacts with the body's immune system.

DISCUSSION

The mutations found in the N gene of SARS-CoV-2 in different variants, like the ancestral, Delta, and Omicron, show essential paths in the virus's evolution. These paths could affect how the virus spreads, evades the immune system, and even how we detect it through testing. The persistence of mutations R203K and G204R across multiple variants, including the ancestral and Omicron strains, suggests that these alterations may confer advantageous properties to the virus. Earlier research linked these mutations to the virus spreading more efficiently and maybe avoiding the immune system. Keeping these mutations might mean they're important for the virus to do its essential tasks, like keeping its RNA stable and packing it up properly, which are crucial for it to make more copies of itself efficiently[3, 10].

The R203K and G204R mutations occur in specific regions crucial to RNA binding. They are suggested to increase the nucleocapsid protein's linkage to the RNA structure, allowing the virus to be effectively replicated. The mutations may also influence the nucleocapsid protein's connection to the immune system of the host and thus modify its immunogens and the capacity for immune evasion[4, 11]. The structured pattern of the mutations shall aid the nucleocapsid's increased ability in its lifecycle and viral survival, increasing the amenability, adaptability, and virulence of the SARS-CoV-2[12, 13]. The mutation's commonality throughout the variants presents that mutations are crucial to its lifecycle, offering a probable agent for therapeutic intervention. Such information shall be helpful in developing antiviral agents that target these structured relationships and, therefore, provide broad-spectrum efficacy agents that are also adaptable across multiple variants and subtypes and could be targeted by vaccines to elicit a robust immune response. Since these mutations enhance the virus's stability and potential immunogenicity, vaccines that can induce strong and broad immune responses against these conserved regions might offer cross-protection against multiple variants[14].

The particular mutations found in the Delta variant, like D63G, R203M, D377Y, and G215C, are significant. Among these, D377Y, which is only found in the 21J subgroup, is notable. It suggests that the virus might be reacting locally to the immune system or adjusting to the conditions within the host cells. These mutations could impact how the nucleocapsid protein works, affecting its ability to package and release new viral particles. Specifically, D377Y in the Delta variant indicates changes that could make the virus spread more quickly or

become more harmful, which might explain the severe outbreaks linked to these strains[3]. Knowing about these changes is essential for public health actions and preparing for pandemics. It helps us monitor the virus closely and develop better plans to control it. The mutation R203M, which is different from R203K found in other versions of the virus, could be a result of unique pressures specific to the Delta variant's evolution. This change might influence how the nucleocapsid protein interacts with the body's cells. These mutations, particular to this lineage, might help explain why the Delta variant spreads quickly and causes more severe illness. It suggests a direct link between specific changes in the nucleocapsid and the virus being better at spreading and causing harm[15, 16]. The change from R203K seen in other versions of the virus to R203M in the Delta variant could also show a subtle difference in how the protein interacts with the body's cells. This could impact how the virus lives and spreads in the body and how severe the illness it causes is[17].

The mutations in the N gene of the Omicron variant, like the deletions at E31, R32, and S33, along with P13L and S413R, could change how the nucleocapsid protein is built. These deletions might mess with the protein's ability to attach to RNA or interact with other proteins in the virus or the body's cells, which could mess with how the virus lives and grows. These changes could also affect how the immune system recognizes the protein, possibly making vaccines less effective[18]. More studies are needed to see how these mutations affect how well vaccines work and how our immune system watches out for the virus. The S413R mutation might give the nucleocapsid protein new abilities, like changing how well it attaches to things or creating new ways to interact with other proteins in the virus or our bodies[19]. The S413R mutation makes a change that might influence how the protein interacts with the body's defense systems, making it easier for the virus to avoid detection by the immune system or change how the body responds to inflammation. This mutation swaps a Serine, a neutral molecule, with an Arginine, which is charged. This also removes phosphorylation from Serine. The absence of phosphorylation at specific sites could potentially hinder the RNA packaging process into the nucleocapsid envelope. Residue S413 serves as one of these phosphorylation sites. On the one hand, it remains uncertain whether the S413R mutation positively influences viral particle assembly by altering the major residue or exerts a negative impact by eliminating phosphorylation[20]. Mutation P13L could result in surges of viral impact seen globally, followed by a decline as more deaths occur. Additionally, mutations like P13L, enhancing transmission, might decrease fatality rates, potentially leading to the virus persisting longer and becoming more established within the human-microbial environment[13, 21].

A trend observed from the analysis is that mutations in the nucleocapsid protein, particularly those conserved across multiple variants, tend to enhance viral stability and efficiency in host interaction. Conversely, variant-specific mutations often confer unique advantages that may be contextual to the epidemiological and immunological landscape faced by that variant. These findings suggest that while some mutations are fundamental to viral function, others are adaptations to environmental and selective pressures. The results also significantly affect vaccine design and efficacy. The ongoing evolution of the nucleocapsid protein and its impact on immune recognition underscores the need for vaccines that can elicit broad and robust immune responses, possibly targeting multiple viral proteins. Also, examining mutations in the nucleocapsid protein can help us predict if vaccines might have trouble working against new strains. This allows us to make vaccines that can adjust to new versions of the virus[22].

Looking at it from a treatment angle, the way mutations in the nucleocapsid protein affect how the virus multiplies and interacts with the immune system shows that focusing on

these processes could be an excellent way to treat the virus. Small molecules that can stop the nucleocapsid protein from working with viral RNA or the body's cells might help lower the amount of virus in the body and make the illness less severe. The specific mutations found in variants like Delta and Omicron give us new ideas for drugs that could interfere with these interactions[23].

When testing for the virus, understanding how mutations in the nucleocapsid protein affect the virus's structure can help make tests more accurate. Mutations that change how the protein looks to our immune system might cause tests that spot this protein to miss the virus. Developing tests that target different parts of the nucleocapsid protein or other viral proteins could make tests better at catching the virus, especially as it keeps changing[19].

In managing a pandemic, keeping an eye on genetic changes in the virus is super important. By tracking how the virus's genetic makeup changes, like in its nucleocapsid protein, health experts can predict how it might spread more easily or dodge our immune systems. This helps them tweak their plans to keep us safe. It's like being proactive rather than reactive, which is crucial for dealing with the current pandemic and any new ones that might pop up.

CONCLUSION

Our study on how SARS-CoV-2's nucleocapsid protein mutates gives us a better understanding of how the virus evolves. Keeping an ongoing watch on these mutations will be critical in how we respond globally to COVID-19, ensuring our vaccines and treatments stay effective as the virus changes. By staying on top of these changes, we can better protect public health and lessen the impact of this global health crisis.

Declaration by Authors

Ethical Approval: Approved by the Ethical Committee of the Faculty of Medicine, Universitas Andalas (approval number: 664/UN.16.2/KEP-FK/2022)

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Conflict of Interest: The authors declare no conflict of interest.

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