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Investigation of SARS-CoV-2 Genomic Variants in Two Patients: Insights into Initial Isolates from West Kalimantan, Indonesia

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ABSTRACT

Backgrounds: The COVID-19 (coronavirus disease-2019) pandemic caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) has led to significant global health impacts. Understanding the genomic evolution and mutation dynamics of SARS-CoV-2 is crucial for devising effective control measures. This study aimed to perform genomic analysis of early 2020 SARS-CoV-2 isolates from West Kalimantan, Indonesia, focusing on the presence of the D614G mutation.

Materials & Methods: Samples were collected from individuals whose COVID-19 was confirmed using RT-PCR at the Microbiology Laboratory of Tanjungpura University Hospital during October to November 2020. Samples were sequenced following standard protocols using a GridION sequencer at the Genetica Science Sequencing Services Company in Jakarta. Additionally, 53,109 SARS-CoV-2 genome sequences from Indonesia (January 2020- June 2023) were retrieved from the GISAID database. A phylogenetic tree and 3D (dimensional) protein structure were constructed using MEGA X software and Iterative Threading Assembly Refinement (I-TASSER).

Findings: Genomic sequencing and phylogenetic analysis showed that both West Kalimantan isolates were closely related to the Wuhan Hu-1 strain and belonged to the B.1.459 lineage. Both samples exhibited the D614G mutation in the spike protein, a mutation later found in dominant variants including Delta and Omicron in Indonesia. The identification of B.1.459 in this region contributes to understanding its spread across the archipelago.

Conclusion: This study provides genomic evidence of the first SARS-CoV-2 isolates in West Kalimantan, supporting the role of the D614G mutation in increased transmission. It also enhances understanding of the emergence and geographic distribution of the B.1.459 lineage in Indonesia, highlighting the importance of continued genomic surveillance.

Keywords: COVID-19, Mutation, Public health surveillance, SARS-CoV-2

CITATION LINKS

[1] World Health Organization... [2] Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, et al. Genome... [3] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical... [4] Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical... [5] Gao Z, Xu Y, Sun C, Wang X, Guo Y, Qiu S, et al. A systematic... [6] Durland J, Ahmadian-Moghadam H. Genetics... [7] Coronaviridae Study Group of the... [8] Tai W, He L, Zhang X, Pu J, Voronin D, Jiang S, et al. Characterization... [9] Magazine N, Zhang T, Wu Y, McGee MC, Veggiani G, Huang W. Mutations... [10] Harvey WT, et al. SARS-CoV-2... [11] Dong J, Zost S, Greaney A, et al. Genetic and... [12] Banerjee A, Doxey AC, Mossman K, Irving AT. Unraveling... [13] Meredith LW, et al. Rapid implementation... [14] Wibmer CK, et al. SARS-CoV-2... [15] Zhang L, et al. SARS-CoV-2... [16] Schoeman D, Fielding BC. Coronavirus... [17] Oktavianthi S, et al. Whole-genome... [18] Wijayanti N, et al. Evolutionary... [19] Shi AC, Xie X. Making sense of... [20] Plante JA, et al. Spike mutation... [21] Novella IS, Duarte EA, Elena SF, Moya A, Domingo E, Holland JJ. Exponential... [22] Muazir S, Lestari L, Alhamdani M, Nurhamsyah M. Regional... [23] Kusumawati RL, et al. Clinical epidemiology... [24] Sam IC, et al. Changing predominant... [25] De Virgiliis F, Di Giovanni S. Lung innervation... [26] Mukherjee S, Pahan K. Is COVID-19... [27] Rao SN, Manissero D, Steele VR, Pareja J. A systematic... [28] Dadras O, et al. The relationship... [29] Rabaan AA, et al. Viral dynamics... [30] Yurkovetskiy L, et al. Structural and... [31] Zhang J, et al. Structural impact... [32] Korber B, et al. Tracking changes... [33] Ahmadi AS, et al. Comparison of... [34] Dorji T, Dorji K, Wangchuk T, Pelki T, Gyeltshen S. Genetic... [35] Negi SS, Sharma K, Bhargava A, Singh P. A comprehensive... [36] Liana DF, Novianry V, Andriani A, Mahyarudin M, Astuti P. Disappearance... [37] Aleem A, Akbar Samad AB, Vaqar S. Emerging... [38] Ozono S, Zhang Y, Ode H, Sano K, Tan TS, Imai K, et al. SARS-CoV-2... [39] Yang TJ, Yu PY, Chang YC, Hsu ST. D614G... [40] Chakraborty C, Sharma AR, Bhattacharya M, Lee SS. A detailed...

Introduction

Over the past years, around 7 million people have died, and 777 million have been affected by the COVID-19 (coronavirus disease-2019) pandemic (as of March 2025) [1]. The outbreak, which began in December 2019, was caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a large RNA virus belonging to the coronavirus family. SARS-CoV-2 shares 79 and 50% genomic similarity to SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV), respectively [2]. Since the beginning of the pandemic, SARS-CoV-2 has rapidly spread worldwide, causing mild to severe symptoms such as fever, fatigue, dry cough, headache, diarrhea, hypoxia, dyspnea, respiratory failure, and pneumonia, which could lead to death [3, 4]China, was c aused by a novel betacoronavirus, the 2019 novel coronavirus (2019-nCo V. However, most COVID-19 patients have been asymptomatic but could still transmit the virus, contributing to the rapid spread of SARS-CoV-2 [5].

The capacity of SARS-CoV-2 to transmit and infect host cells has evolved through genomic mutation, which is a form of adaptation that could alter protein function and lead to characteristic changes [6]. SARS-CoV-2 possesses a genome of 29.8 – 29.9 kb, composed of positive-sense single-stranded RNA protected by an envelope [7]. Around 70% of the viral genome is in the form of ORF1a and ORF1b (open reading frames), encoding 15 non-structural proteins (NSPs) [8]. The remaining genome encodes structural proteins, i.e., membrane (M), envelope (E), nucleocapsid (N), and spike (S). Research on SARS-CoV-2 has primarily focused on the spike protein, playing a key role in viral entry [9]. The spike (S) protein is essential for binding to host target cells and mediating immune activation and serves as a target for COVID-19 therapy and vaccination [10, 11] RNA viruses such as SARS-CoV-2 naturally undergo adaptation through mutations that arise as part of their replication cycle, resulting in various viral lineages. Mutations in SARS-CoV-2 may potentially alter its characteristics, leading to enhanced pathogenicity and decreased susceptibility to immune responses or therapeutic agents. To monitor and understand these changes, genomic surveillance of SARS-CoV-2 serves multiple purposes, including tracking the origin of the virus [12], exploring transmission routes [13], conducting epidemiological studies, and most importantly, investigating viral evolution and its escape from neutralizing antibodies [14].

An example of such characteristic changes is the D614G mutation in the carboxy(C)-terminal region of the S1 domain, which is believed to confer a fitness advantage to the lineage and has become prevalent since April 2020 [9, 15]. The D614G mutation, which substitutes aspartic acid (D) with glycine (G), was initially identified in Argentina in January 2020 and subsequently reported in Indonesia in March 2020 [16]. In Indonesia, this mutation has become highly prevalent, with 97% of isolates exhibiting this mutation, contributing to the rapid spread of the virus in the second wave of the pandemic [17]. While the spread and dominance of the D614G mutation have been documented in several regions of Indonesia, such as Yogyakarta and Central Java [18], limited data are available regarding its emergence and circulation in the western part of the archipelago, particularly West Kalimantan. This study aimed to close that gap by providing genomic surveillance data from an underrepresented region, resulting in a more comprehensive understanding of SARS-CoV-2 evolution and transmission dynamics across Indonesia.

Investigating the D614G mutation in the SARS-CoV-2 spike protein is critical to understanding and monitoring the virus be-

cause of its significant impact on transmissibility, viral fitness, and vaccine efficacy. This mutation has become globally dominant within circulating SARS-CoV-2 variants, suggesting a selective advantage that enhances the virus's ability to spread among human hosts. Although the D614G mutation presents challenges for monitoring and controlling the pandemic, it offers insights into the virus's evolution and potential targets for therapeutic interventions. Understanding these dynamics is crucial for an effective public health response [19, 20].

In this context, genomic research has played a pivotal role in understanding and combating COVID-19. However, there is a notable scarcity of genomic surveillance of SARS-CoV-2 in Indonesia, particularly concerning the D614G mutation. Most existing studies have primarily focused on sequences from Java Island. Therefore, this study aimed to perform genomic surveillance of early SARS-CoV-2 isolates from West Kalimantan (outside Java Island), with particular attention to the D614G mutation.

Objectives: This study marked the first isolation and genomic characterization of SARS-CoV-2 sequences from West Kalimantan during the early stages of the 2020 pandemic, specifically examining the emergence and presence of the D614G mutation in Indonesia over time.

Materials and Methods

Sample collection: Samples were collected from individuals exhibiting COVID-19 symptoms as well as randomly-selected healthy individuals who were swabbed in densely-populated areas as part of epidemiological surveillance during October and November 2020. Samples were confirmed positive for COVID-19 at the Microbiology Laboratory of Tanjungpura University Hospital. To be included in the study, samples had to meet specific criteria: having a positive result for COVID-19 based on real-time polymerase chain reaction (RT-PCR) analysis, having a cycle threshold (Ct) value < 30, and originating from West Kalimantan. Nasopharyngeal and oropharyngeal samples were collected using sterile cotton swabs, submerged in viral transport medium (VTM), and then stored in a refrigerator at -80 oC until analysis. Sample collection procedures were approved by the Health Research Ethics Committee of the Faculty of Medicine, Tanjungpura University (registration number: 8179/UN22.9/PG/2022).

Nucleic acid extraction and SARS-CoV-2 detection by real-time polymerase chain reaction (RT-PCR): Nucleic acid was extracted from two nasopharyngeal and oropharyngeal samples using an extraction kit following the manufacturer's protocol (Bioneer viral RNA Extraction Kit, Korea). The process involved utilizing 200 µL of samples to yield 100 µL of total RNA. Total RNA was then used for RT-PCR analysis and whole-genome analysis. For the detection of SARS-CoV-2 nucleic acid, RT-PCR was performed using the Biosewoom Real-Q 2019-nCoV Detection Kit (2020.03.25) with slight modifications. Oligonucleotide primers and probes used to detect SARS-CoV-2 were selected from regions of the viral RNA-dependent RNA polymerase (RdRP) gene and envelope (E) gene. An additional primer/probe set was also included in the kit to detect the human RNase P gene (HRP) in clinical specimens. The fluorescent dye of the probes utilized was FAM for the RdRp gene, HEX for the E gene, and Cy5 for the human RNase P gene. About 20 µL of the master mix was dispensed into strip tubes or plates, then 10 µL of sample RNA and positive control were added into each well. RNA-free water served as a negative control. The RT-PCR process was conducted using the QuantStudioTM 5 real-time PCR system (Thermo Fisher Scientific) with the following

cycling parameters: one cycle of 50 oC for 30 min and 95 oC for 15 min, followed by 40 cycles of 95 oC for 15 s and 62 oC for 45 s.
Quantification, integrity, and quality of RNA: The quantification, integrity, and quality of RNA were verified by fluorometric method using a Qubit 4 fluorometer. RNA analysis followed the protocol outlined in the Qubit RNA HS Assay Kit for RNA quantification and the Qubit RNA IQ Assay Kit for RNA integrity and quality measurement (Thermo Fisher Scientific, Willow Creek Rd, CA, USA).
Library preparation and sequencing: PCR products obtained from RT-PCR were sequenced according to standard protocols using a GridION DNA sequencer (Oxford Nanopore Technologies) at the Genetica Science Sequencing Services Company in Jakarta. Sequencing library preparation was conducted according to the manufacturer's instructions for the Rapid Barcoding Kit (SQK-RBK110.96, Oxford Nanopore Technologies) and Midnight RT PCR Expansion Kit (EXP-MRT001, Oxford Nanopore Technologies). A total of 8 µL of samples were used for sequencing analysis. Barcoding reactions were performed on individual samples before pooling and cleanup using SPRI beads. The sequencing mixture with a total volume of 75 µL comprised 12 µL of DNA library [1 µL of RAP (rapid adapter) and 10 µL of pooled samples (800 ng/µL)], 37,5 µL of sequencing buffer, and 25,5 µL of loading solution. Sequencing was carried out using a GridION sequencer (Oxford Nanopore Technologies), and base-calling was performed using MinKNOW operating software. Demultiplexing and identification of SARS-CoV-2 were performed using the EPI2ME cloud-based bioinformatics platform. Data were processed using Fastq QC + ARTIC + NextClade 2022.07.19-15399 with a default minimum q-score of 8. Sequences were identified based on the Pangolin and GISAID (global initiative on sharing all influenza data) databases.
Phylogenetic analysis and molecular docking: Phylogenetic analyses were conducted using the ClustalW method [17]. The sequences of our samples were aligned with the reference genome (hCoV-19/Wuhan/Hu-1/2019) and other variants, including Beta B.1.351, Delta B.1, Gamma P.1, Alpha B.1.1.7, Lambda C.37, Omicron B.1.1.529, and XBB. A phylogenetic tree was constructed using the maximum likelihood (ML) method with a bootstrap consensus tree of 1000 replicates and the Tamura-Nei model in MEGA X software to visualize the evolutionary relationships between our samples and other SARS-CoV-2 isolates deposited in GISAID. Additionally, the 3D structure of S-protein in both samples was predicted using I-TASSER (iterative threading assembly refinement) for further analysis.
Prevalence analysis of the D614G mutation in Indonesia: The temporal prevalence and epidemiological development of the D614G mutation in SARS-CoV-2 isolates in Indonesia were analyzed through retrospective genome analysis using publicly accessible sequence data. A total of 53,109 SARS-CoV-2 genome sequences, originating from cases in Indonesia, were obtained from the GISAID database. This dataset covers the period from March 2020 to June 2023, spanning from the beginning of the COVID-19 outbreak to the official declaration of the end of the pandemic phase in Indonesia. The monthly prevalence of the D614G mutation was determined by calculating the proportion of sequences with the D614G amino acid substitution relative to the total number of SARS-CoV-2 sequences submitted to the database from Indonesia during each month of the study period.

Findings

Patient characteristics and molecular analysis: During the first year of the

pandemic, samples were collected from two COVID-19 patients residing in West Kalimantan. Sample X was collected from a young female patient on November 28, 2020, while sample Y was obtained from an older male patient from Mempawah Regency, who tested positive for COVID-19 on October 15, 2020. The patient corresponding to sample Y experienced mild symptoms, such as fatigue and headache, and had no underlying comorbidities. In contrast, sample X was taken from an asymptomatic patient with no comorbidities. To ensure high-quality sequencing, a low cycle threshold (Ct) value is necessary. Therefore, RT-PCR was performed to detect SARS-CoV-2 (Table 1), and both samples exhibited low Ct values for the ORF1ab and RdRp genes.

Phylogenetic analysis of SARS-CoV-2 isolates from West Kalimantan in 2020: The evolutionary history was inferred using the maximum likelihood method with a bootstrap consensus tree of 1000 replicates (Figure 1). The phylogenetic tree analysis results showed that both samples studied shared a close ancestral lineage with the original Wuhan Hu 1 reference strain, representing the earliest identified SARS-CoV-2 genome. Furthermore, these samples also exhibited a close genetic relationship with the two primary variants of concern, namely Beta (B.1351) and Delta (B.1), suggesting that they might have evolved from the same ancestral node in the SARS-CoV-2 phylogenetic tree. In contrast, the phylogenetic analysis revealed that both samples shared a more distant lineage with several other major variants of concern and interest, including Gamma (P.1), Alpha (B.1.1.7), Lambda (C.37), Omicron (B.1.1.529), and the recombinant XBB lineage. These variants appear to have acquired additional mutations over time, as evidenced by their divergence in several genomic sequences, particularly in the spike protein gene and other regions associated with viral infectivity and immune escape.

Genomic mutation analysis of SARS-CoV-2 isolates from West Kalimantan in 2020: To explore mutations, viral genomes were sequenced using next-generation sequencing (NGS). The resulting data were pairwise aligned with the reference strain hCoV-19/Wuha n/WIV04/2019, which was sequenced in China on December 30, 2019. Alignment was conducted using Molecular Evolutionary Genetics Analysis (MEGA) software (Ver. 10.2.2), and the sequences were subsequently submitted to GISAID.

Table 1) Sample characteristics and RT-PCR test result for SARS-CoV-2

Variable	Sample X	Sample Y
Sample characteristics		
Gender	Female	Male
Age	32	55
City of Origin	Pontianak	Mempawah
Clinical symptom	Asymptomatic	Fatigue, headache
Comorbidities	No comorbidities	No comorbidities
Suspected source of infection	Direct contact with COVID-19 patient	-
Patient status	Alive	Alive
RT-PCR Results		
Cycle Threshold (Ct) of ORF1ab gene	18.47	25.26
Cycle Threshold (Ct) of RdRp gene	17.38	23.89

Sample X was assigned the accession number EPI_ISL_911708, while sample Y was assigned the accession number EPI_ISL_902921. Both samples exhibited high sequencing coverage, with 97% of nucleotides sequenced, as stretches of NNNs were less than 3%. After submission to GISAID, both samples were identified as belonging to the B.1.459 lineage. Then mutations in three proteins, including the spike (S) protein, nucleocapsid (N) protein, and non-structural proteins (NSPs), were identified in both samples (Table 2). Sample Y had mutations at D614G and L5F of the S protein, whereas sample X had additional mutations at A260V, S689R, and M731I. In the N protein, both samples had one mutation each: A119S in sample X and R203M in sample Y. Furthermore, several mutations were found in non-structural proteins (NSPs) in both samples, including P822L (nsp3), P323L (nsp12), and Q57H (ns3). Additionally, sample X displayed mutations at H82R and G49V, while sample Y had an additional mutation at G172C.

Spike protein mutations: Among the mutations identified in the samples, those in the spike protein were subjected to further investigation in this study. The spike protein of SARS-CoV-2 plays a pivotal role in receptor recognition, viral attachment, and entry into the host cell. Thus, mutations in this protein sequence may impact viral pathogenicity. Samples X and Y were aligned with the reference strain hCoV-19/Wuhan/WIV04/2019 using Molecular Evolutionary Genetics Analysis (MEGA). As shown in Figure 2a, a change was observed in the amino acid at position 614 from aspartate (D) in the reference sequence to glycine (G) in both samples. Subsequently, the 3D structure of the spike protein was predicted for further analysis using Iterative Threading Assembly Refinement (I-TASSER). The 3D structure of sample X was aligned with the reference protein 7CWL (SARS-CoV-2 spike protein and P17 fab complex with one RBD in close state) [20], revealing a distance of 1.1 Å between the aspartate residue in 7CWL and the glycine residue in sample X. Comparatively, when compared to the reference protein 6VSB [21], sample Y exhibited a longer distance of 2.1 Å.

Spread of the D614G mutation in SARS-CoV-2 variants during the pandemic in Indonesia: The D614G mutation, a point mutation in the spike protein, has garnered attention due to its impact on viral infectivity. Since this mutation was identified in the studied samples, 53.109 sequence samples from Indonesia, submitted to GISAID up to June 2023, were collected to monitor the presence of D614G. The prevalence of the D614G mutation was presented as the percentage of its occurrence in seven variants: Alpha, Beta, Delta, Eta, Kappa, Omicron, and "n/a" (lineages not

Table 2) Mutation of sample X and Y based on whole genome sequencing (WGS) analysis

Location of Mutation					
Spike protein		Nucleocapsid protein		Non-structural protein	
Sample X	Sample Y	Sample X	Sample Y	Sample X	Sample Y
A260V	-	A119S	-	P822L	P822L
D614G	D614G	-	R203M	P323L	P323L
S689R	-			H82R	-
M731I	-			G49V	-
	L5F			Q57H	Q57H
				-	G172C

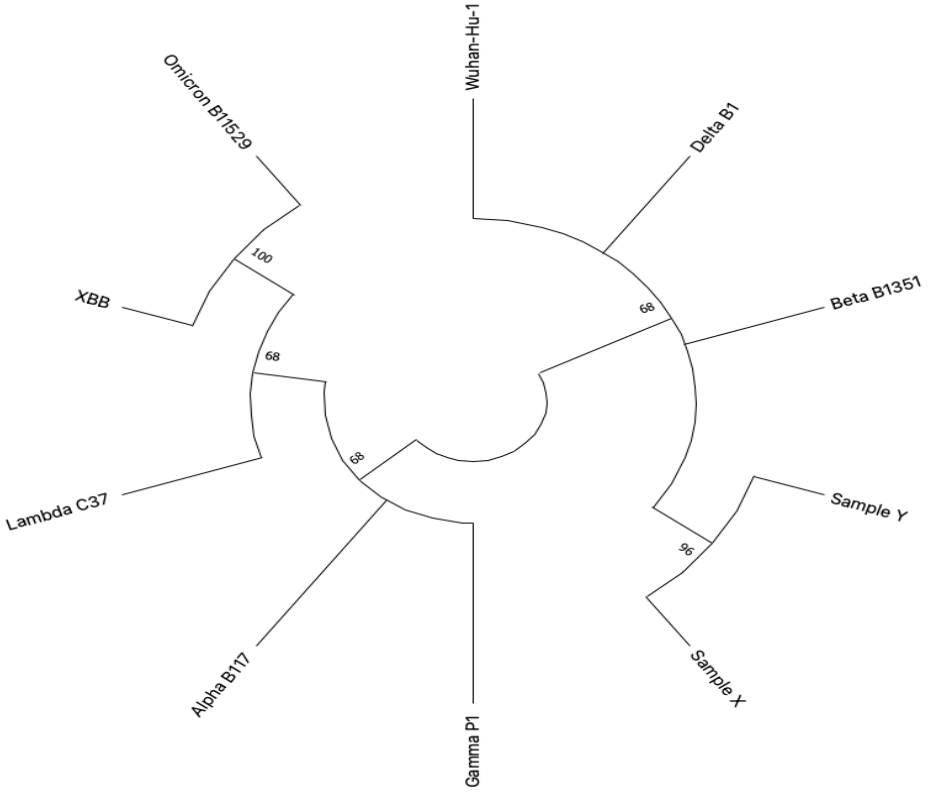

Figure 1) The evolutionary analysis indicates that the two samples share a common ancestral origin with the Wuhan-Hu1- strain, as well as with the former variants of concern Beta (B.1.351) and Delta (B.1). In contrast, they exhibit distinct evolutionary lineages from the Gamma (P.1), Alpha (B.1.1.7), Lambda (C.37), Omicron (B.1.1.529), and XBB variants.

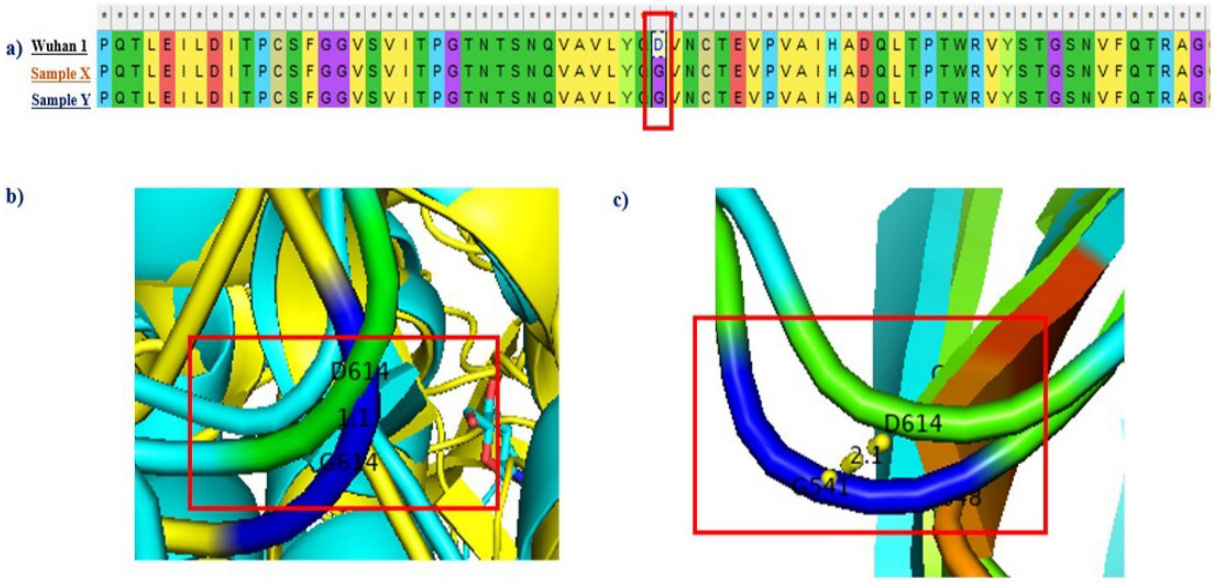

Figure 2) a)The alignment panel of Spike protein mutation in sequence 614, mutated from Aspartate(D) into Glysine (G) in both samples. b) The S-protein from samples X (dark blue) aligned with protein reference 7CWL (in green) using PyMol software. c) 3D model of S-protein from sample Y (dark blue) aligned with protein reference 6VSB (in green).

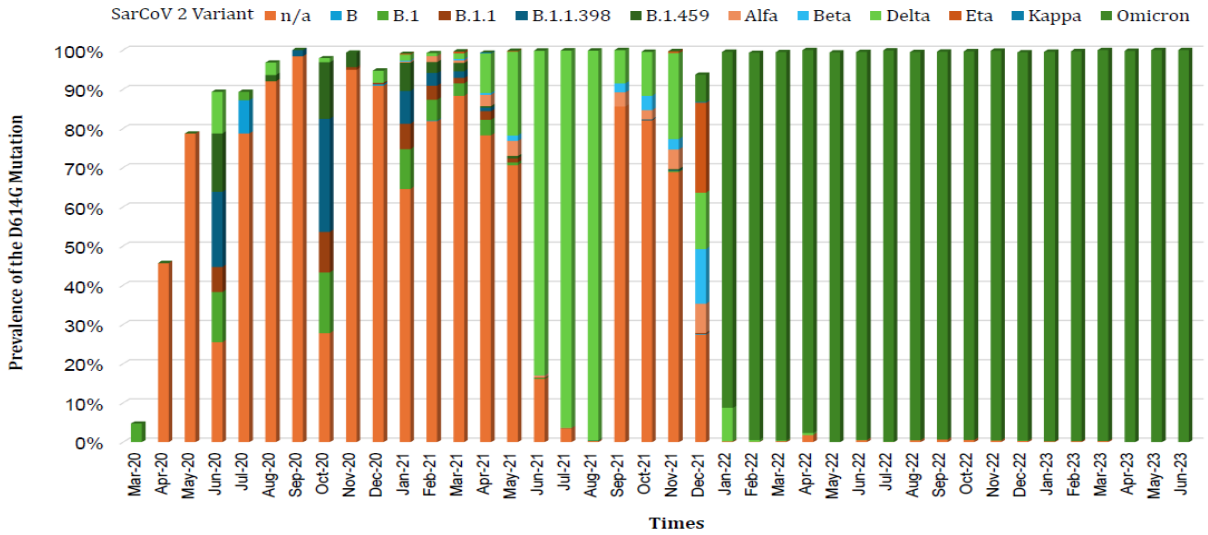

Figure 3) Prevalence of the D614G mutation among seven SARS-CoV2- variants identified in Indonesia between 2020 and 2023

belonging to any known variants) (Figure 3). As illustrated in Figure 3, the prevalence of the D614G mutation gradually increased since March 2020, reaching a steady presence in nearly all sequences throughout the pandemic. Initially, the D614G mutation appeared in sequences classified as "n/a". When the Delta variant dominated Indonesia in 2021, the mutation was detected in the majority of Delta variant sequences (> 90% of cases). In the same year, the D614G mutation was also found in minority variants such as Alpha, Eta, and Kappa. By 2022 and 2023 (the end of the pandemic), the D614G mutation was identified in all Omicron variant sequences.

Discussion

The pandemic, which began in 2019, shows no signs of ending soon. As a global phenomenon, SARS-CoV-2 mutations have primarily emerged independently in various countries. The rapid genomic changes of SARS-CoV-2 may act as a double-edged sword for an RNA virus; large population passages often result in fitness gains through advantageous mutations [21]; however, these same mutations may also hinder host immune defenses and pose challenges to vaccination strategies.

In April 2020, West Kalimantan reported its first confirmed SARS-CoV-2 cases through RT-PCR testing. Between August and December 2020, confirmed cases increased by 4.17 times [22], raising concerns about possible viral mutations in the region. Although the first case appeared in April, a significant increase in cases towards the end of the year prompted us to collect samples in October and November to investigate potential genomic changes. However, limited resources and facilities at the time restricted us to analyzing only two samples by the end of the year, which is a limitation of the study. The detection of D614G in the studied samples, representing the first genomic data from West Kalimantan, aligns with its rapid spread across Indonesia during the early stages of the pandemic up to June 2023. After submitting sequencing data to GISAID, both samples were identified as belonging to the B.1.459 lineage, which was later detected in North Sumatera (Indonesia) in June 2021 [23]. The B.1.459 lineage was also reported in January 2021 in the Sarawak region of Malaysia, directly bordering West Kalimantan [24]. The fact that these samples were from COVID-19 cases that significantly

increased during October and November suggests that the D614G mutation might have played a role in increasing virus transmission in the region. This highlights how even a small number of genomic sequences, when contextualized with epidemiological data, could provide important insights into the dynamics of variant spread [15].

Samples were collected from individuals of different genders without any comorbidities. The symptoms such as headache and fatigue experienced by patient Y might have been associated with his age and gender [25, 26]. However, none of the patients experienced severe COVID-19. Although studies on the relationship between low cycle threshold (Ct) values and COVID-19 severity have remained inconclusive, they have been reported to be strongly associated with viral transmission [27, 28]. This explains why patient X did not experience any symptoms despite having a lower Ct value compared to patient Y. The low Ct values observed in the studied samples may be due to various factors, including specimen type, extraction and amplification methods, as well as pre- and post-analysis variables [29].

SARS-CoV-2 virus enters host cells via its spike (S) glycoprotein through transmembrane fusion; therefore, alterations in the amino acid sequence of the spike protein might influence infectivity and viral interactions with host target cells [10]. In this study, five mutations were identified in the spike protein of the studied samples: L5F, A260V, D614G, S689R, and M731I. Among these, the D614G mutation is particularly noteworthy for its role in augmenting SARS-CoV-2 infectivity in humans [15]. This mutation was the first significant mutation that attracted global attention due to its persistence in subsequent variants, potentially contributing to the increase in the prevalence of COVID-19 cases [15]. A mutation at position 614th affects the structure of the spike protein and enhances its binding to angiotensin converting enzyme 2 (ACE2) [30]. Substitution of aspartic acid (D) with glycine (G) enhances the stability of S trimer formation, leading to increased viral entry through the spike protein [31]. Korber et al. (2020) reported that the D614G mutation elevated viral load in upper respiratory tract cells, although it was not associated with worsened disease severity [32]. In this study, approximately 89% of SARS-CoV-2 viruses in Indonesia carried the D614G mutation, making it the most prominent mutation in 2020 (Figure 3). The domination of the D614G mutation was also observed in SARS-CoV-2 genomes from India, Bhutan, and Iran [33–36]. For the next two years, the D614G mutation remained globally dominant and, at the time of writing this manuscript, was present in 99.65% of sequences submitted to GISAID.

Another mutation in the spike protein, the A260V mutation, is located in the S1 subunit, specifically in the N-terminal domain. The S1 subunit interacts with the ACE2 receptor in the aminopeptidase N region. Mutations in key residues could affect interaction with ACE2 [30]. However, there is limited information on how this particular mutation influences the function of the S1 subunit. The S689R and M731L mutations are found in the S2 subunit, positioned before the furin cleavage (FP) site. Limited information is available regarding the L5F mutation, but this mutation has also been reported in other variants, such as the Eta and Iota variants [37].

Since August 2020, the number of confirmed COVID-19 cases has been steadily increasing. However, due to limited resources, only a whole-genome sequencing analysis was performed in December 2020, with the earliest sample collected in October 2020. The findings of the D614G mutation in samples collected in October and November aligned with published sequencing data analyzing the evolution of variants during

that period. Moreover, it might have contributed to the increase in COVID-19 cases in West Kalimantan.

The continued presence of the D614G mutation from 2020 to the present highlights the evolving nature and adaptations of the SARS-CoV-2 virus over time. This mutation is one of the factors contributing to increased binding affinity to ACE2, which is associated with higher infectivity in humans [38]. It also enhances viral fitness, reduces sensitivity to temperature-dependent denaturation and folding stability, increases environmental resistance [39], and improves immune escape capability [40].

Conclusion

In conclusion, analyzing only two samples is insufficient to fully capture the evolutionary trajectory and mutation dynamics of SARS-CoV-2. However, despite this limited sample size, the findings contribute to understanding variant dynamics in West Kalimantan during the early stages of the pandemic. Notably, both samples exhibited the D614G mutation in the spike protein, which later became the dominant mutation until the end of the pandemic in Indonesia. Expanding genomic surveillance through broader sample collection across multiple islands in Indonesia will provide a more comprehensive understanding of SARS-CoV-2 evolution and mutation patterns. These efforts are essential to strengthen public health responses and inform preparedness strategies for future pandemics.

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Ethical permissions: Ethical approval for this study was obtained from the Ethical Clearance Committee Faculty of Medicine Tanjungpura University, approval number No. 8179/UN22.9/PG/2022.

Authors' contributions: The research paper benefited from a collaborative effort among its authors. PA and MM provided valuable insights into the conception and design of the study, while PAM, MF, and MAR played key roles in data acquisition. SSS, DFL, VN contributed significantly to the analysis and interpretation of the data. DFL, MM, and PA collectively drafted the article, synthesizing the findings into a cohesive narrative. Overall, each author's unique expertise and contribution were instrumental in shaping the research and final manuscript, demonstrating a comprehensive and collaborative approach to scientific inquiry.

Conflicts of interests: The authors declare that they have no conflicts of interest to disclose regarding this manuscript.

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Verbal consent for sample collection was obtained by authorized public health officers in accordance with national public health regulations.

All samples and accompanying data were

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References

1. World Health Organization. WHO COVID-19 dashboard. Geneva: World Health Organization; 2025.
2. Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, et al. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host Microbe. 2020;27(3):325–8.
3. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet Lond Engl. 2020;395(10223):497–506.
4. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020;323(11):1061–9.
5. Gao Z, Xu Y, Sun C, Wang X, Guo Y, Qiu S, et al. A systematic review of asymptomatic infections with COVID-19. J Microbiol Immunol Infect. 2021;54(1):12–6.
6. Durland J, Ahmadian-Moghadam H. Genetics, mutagenesis. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022.
7. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species severe acute respiratory syndrome-related coronavirus: Classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020;5(4):536–44.
8. Tai W, He L, Zhang X, Pu J, Voronin D, Jiang S, et al. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: Implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell Mol Immunol. 2020;17(6):613–20.
9. Magazine N, Zhang T, Wu Y, McGee MC, Veggiani G, Huang W. Mutations and evolution of the SARS-CoV-2 spike protein. Viruses. 2022;14(3):640.
10. Harvey WT, Carabelli AM, Jackson B, Gupta RK, Thomson EC, Harrison EM, et al. SARS-CoV-2 variants, spike mutations, and immune escape. Nat Rev Microbiol. 2021;19(7):409–24.
11. Dong J, Zost S, Greaney A, Starr TN, Dingens AS, Chen EC, et al. Genetic and structural basis for recognition of SARS-CoV-2 spike prote by a two-antibody cocktail. bioRxiv. 2021.
12. Banerjee A, Doxey AC, Mossman K, Irving AT. Unraveling the zoonotic origin and transmission of SARS-CoV-2. Trends Ecol Evol. 2021;36(3):180–4.
13. Meredith LW, Hamilton WL, Warne B, Houldcroft CJ, Hosmillo M, Jahun AS, et al. Rapid implementation of SARS-CoV-2 sequencing to investigate cases of health-care associated COVID-19: A prospective genomic surveillance study. Lancet Infect Dis. 2020;20(11):1263–71.
14. Wibmer CK, Ayres F, Hermanus T, Madzivhandila M, Kgagudi P, Oosthuysen B, et al. SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. Nat Med. 2021;27(4):622–5.
15. Zhang L, Jackson CB, Mou H, Ojha A, Peng H, Quinlan BD, et al. SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. Nat Commun. 2020;11(1):6013.
16. Schoeman D, Fielding BC. Coronavirus envelope protein: Current knowledge. Virol J. 2019;16(1):69.
17. Oktavianthi S, Lages AC, Kusuma R, Kurniasih TS, Trimarsanto H, Andriani F, et al. Whole-genome sequencing and mutation analyses of SARS-CoV-2 isolates from Indonesia. Pathogens. 2024;13(4):279.
18. Wijayanti N, Gazali FM, Supriyati E, Hakim MS, Arguni E, Daniwijaya ME, et al. Evolutionary dynamics of SARS-CoV-2 circulating in Yogyakarta and Central Java, Indonesia: Sequence analysis covering furin cleavage site (FCS) region of the spike protein. Int Microbiol. 2022;25(3):531–40.
19. Shi AC, Xie X. Making sense of spike D614G in SARS-CoV-2 transmission. Sci China Life Sci. 2021;64(7):1062–7.
20. Plante JA, Liu Y, Liu J, Xia H, Johnson BA, Lokugamage KG, et al. Spike mutation D614G alters SARS-CoV-2 fitness. Nature. 2021;592(7852):116–21.
21. Novella IS, Duarte EA, Elena SF, Moya A, Domingo E, Holland JJ. Exponential increases of RNA virus fitness during large population transmissions. Proc Natl Acad Sci. 1995;92(13):5841–4.
22. Muazir S, Lestari L, Alhamdani M, Nurhamsyah M. Regional network (centrality) and Covid-19 spread in West Kalimantan. Appl Eng Technol. 2022;1(1):47–55.
23. Kusumawati RL, Lubis IN, Kumaheri MA, Pradipta A, Faksri K, Mutiara M, et al. Clinical epidemiology of pediatric COVID-19 Delta variant cases from North Sumatra, Indonesia. Front Pediatr. 2022;10:810404.
24. Sam IC, Chong YM, Abdullah A, Fu JY, Hasan MS, Jamaluddin FH, et al. Changing predominant SARS-CoV-2 lineages drives successive COVID-19 waves in Malaysia, February 2020 to March 2021. J Med Virol. 2022;94(3):1146–53.
25. De Virgiliis F, Di Giovanni S. Lung innervation in the eye of a cytokine storm: Neuroimmune interactions and COVID-19. Nat Rev Neurol. 2020;16(11):645–52.
26. Mukherjee S, Pahan K. Is COVID-19 gender-sensitive? J Neuroimmune Pharmacol.

2021;16(1):38–47.
27. Rao SN, Manissero D, Steele VR, Pareja J. A systematic review of the clinical utility of cycle threshold values in the context of COVID-19. Infect Dis Ther. 2020;9(3):573–86.
28. Dadras O, Afsahi AM, Pashaei Z, Mojdeganlou H, Karimi A, Habibi P, et al. The relationship between COVID-19 viral load and disease severity: A systematic review. Immun Inflamm Dis. 2022;10(3):e580.
29. Rabaan AA, Tirupathi R, Sule AA, Aldali J, Mutair AA, Alhumaid S, et al. Viral dynamics and real-time RT-PCR Ct values correlation with disease severity in COVID-19. Diagnostics. 2021;11(6):1091.
30. Yurkovetskiy L, Wang X, Pascal KE, Tomkins-Tinch C, Nyalile TP, Wang Y, et al. Structural and functional analysis of the D614G SARS-CoV-2 spike protein variant. Cell. 2020;183(3):739-51.
31. Zhang J, Cai Y, Xiao T, Lu J, Peng H, Sterling SM, et al. Structural impact on SARS-CoV-2 spike protein by D614G substitution. Science. 2021;372(6541):525–30.
32. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, et al. Tracking changes in SARS-CoV-2 spike: Evidence that D614G increases infectivity of the COVID-19 virus. Cell. 2020;182(4):812-27.
33. Ahmadi AS, Shafiei-Jandaghi NZ, Sadeghi K, Nejati A, Zadheidar S, Mokhtari-Azad T, et al. Comparison of circulating variants during the beginning, middle, and the end of the 4th wave of COVID-19 in Tehran province, Iran in 2021. Iran J Public Health. 2023;52(12):2621–9.
34. Dorji T, Dorji K, Wangchuk T, Pelki T, Gyeltshen S. Genetic diversity and evolutionary patterns of SARS-CoV-2 among the Bhutanese population during the pandemic. Osong Public Health Res Perspect. 2023;14(6):494–507.
35. Negi SS, Sharma K, Bhargava A, Singh P. A comprehensive profile of SARS-CoV-2 variants spreading during the COVID-19 pandemic: A genomic characterization study from Chhattisgarh State, India. Arch Microbiol. 2024;206(2):68.
36. Liana DF, Novianry V, Andriani A, Mahyarudin M, Astuti P. Disappearance of imported cases of Omicron lineage BA.2.40 in West Kalimantan, Indonesia. Iran J Med Sci. 2024;49(3):176–85.
37. Aleem A, Akbar Samad AB, Vaqar S. Emerging variants of SARS-CoV-2 and novel therapeutics against coronavirus (COVID-19). In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2025.
38. Ozono S, Zhang Y, Ode H, Sano K, Tan TS, Imai K, et al. SARS-CoV-2 D614G spike mutation increases entry efficiency with enhanced ACE2-binding affinity. Nat Commun. 2021;12(1):848.
39. Yang TJ, Yu PY, Chang YC, Hsu ST. D614G mutation in the SARS-CoV-2 spike protein enhances viral fitness by desensitizing it to temperature-dependent denaturation. J Biol Chem. 2021;297(4):101238.
40. Chakraborty C, Sharma AR, Bhattacharya M, Lee SS. A detailed overview of immune escape, antibody escape, partial vaccine escape of SARS-CoV-2 and their emerging variants with escape mutations. Front Immunol. 2022;13:801522.