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Original Article

Disappearance of Imported Cases of Omicron Lineage BA.2.40 in West Kalimantan, Indonesia

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Abstract

Background: The World Health Organization has declared Omicron as the fifth variant of concern with more than 50 mutations, particularly in the spike protein. Given increased viral infectivity due to mutations, worldwide genomic surveillance and detection of severe acute respiratory syndrome 2 (SARS-CoV-2) is essential. The present study aimed to track Omicron lineage BA.2.40 in West Kalimantan, Indonesia.

Methods: In May-August 2022, nasopharyngeal swab samples (n=3,642) were collected from international travelers to West Kalimantan (active surveillance), and patients hospitalized due to SARS-CoV-2 infection (baseline surveillance). The samples were tested for Omicron lineages based on ORF1ab, N, and HV69-70del genes, followed by whole-genome sequencing. The sequences were then identified using two genomic databases, aligned against the reference genome (Wuhan/Hu-1/2019), and then compared with BA.2.40 lineage detected across the world. Phylogenetic analysis between the samples and other SARS-CoV-2 isolates was performed using molecular evolutionary genetics analysis software.

Results: Based on the genomic databases, 10 isolates were identified as BA.2.40. All samples tested positive for the ORF1ab and N genes, but negative for the HV69-70del gene, which is a marker to detect the Omicron variant. Phylogenetic analysis showed the isolates were closely related to an isolate from Malaysia, an area dominated by BA.2.40.

Conclusion: Omicron lineage BA.2.40 has no HV69-70 deletion in the spike protein, a marker used to screen for the Omicron variant. BA.2.40 showed a high similarity to an isolate from Malaysia and was detected only during certain periods, indicating the effect of internationally imported cases.

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Keywords ● SARS-CoV-2 ● Indonesia ● Mutation ● COVID-19 ● Spike glycoprotein, coronavirus

What's Known

- Omicron is declared a variant of concern due to its increased viral infectivity, higher transmission rate, and worldwide spread.
- Genomic surveillance of coronavirus disease-19 as a prevention and control strategy is essential, since genetic mutations are associated with disease severity and the spread of viral infections.

What's New

- BA.2.40 has no HV69-70 deletion in the spike protein, a marker used to screen for the Omicron variant. Therefore, the use of this marker as a screening tool should be re-evaluated.
- BA.2.40 in West Kalimantan (Indonesia) had the same origin as the variant in Malaysia, indicating the effect of internationally imported cases.

Introduction

Among all variants of severe acute respiratory syndrome 2 (SARS-CoV-2), on 24 November 2021, the World Health Organization (WHO) declared Omicron as the fifth variant of concern (VOC). Previously declared variants were Alfa, Beta, Delta, and Gamma. VOC is defined when a virus (e.g., SARS-CoV-2) mutates into variants that spread more easily, become more virulent, change clinical presentations, or reduce the effectiveness of available diagnostics, vaccines, and treatments.[1] So far, Omicron has 50

genetic mutations, of which 30 are spike protein mutations; making it more infectious but less severe than previous variants.[2]

Omicron is currently the dominant variant worldwide. It poses a global threat due to its high contagiousness compared to other variants, with 7.9 million genomic sequences recorded in the Global Initiative on Sharing All Influenza Data (GISAID) EpiCoV™ database.[3] Omicron variant (B.1.1.529) was first identified in South Africa, followed by lineages BA.1, BA.2, BA.3, BA.4, BA.5, and descendent sublineages.[1] BA.1, BA.1.1, BA.2, and their sublineages are considered the most important variants and are closely monitored. BA.2 has shown a higher transmission rate than BA.1, but with similar disease severity.[4]

Rapid mutation of SARS-CoV-2 variants has necessitated the use of genomic surveillance as the main approach to closely monitor the growth of circulating lineages. A comprehensive understanding of the SARS-CoV-2 genomic evolution serves as an early sign for epidemiological monitoring and provides information to policymakers who can then adjust their strategies to manage the pandemic. The socio-economic implications of the disease have been grave. Social distancing, travel restrictions, and lockdowns have severely affected public and private businesses, the transportation industry, and the employment rate. In Indonesia, thousands of people have lost their jobs due to the pandemic. Consequently, extensive travel back and forth by Indonesian workers between West Kalimantan and Malaysia. This in turn has affected the epidemiological pattern of the disease in Indonesia, especially in West Kalimantan, enforcing targeted genomic surveillance of international travelers. The present study aimed to track Omicron variant BA.2.40 in West Kalimantan from May to August 2022. The target population was suspected cross-border workers with symptoms of coronavirus disease-19 (COVID-19), and patients hospitalized due to the disease.

Materials and Methods

Both baseline and active surveillance of the disease were carried out. Active surveillance was conducted of travelers arriving at the international entry point of West Kalimantan with COVID-19 symptoms. Baseline surveillance was carried out on all patients hospitalized for SARS-CoV-2 infection. From May to August 2022, a total of 3,642 nasopharyngeal swab samples were collected and tested for SARS-CoV-2. Of these, 159 samples were screened for Omicron variants in the Laboratory of Microbiology, Tanjungpura University Hospital (Pontianak, Indonesia). All samples with cycle threshold (Ct) values below 33 were included in the study. Whole-genome sequencing was performed in the Department of Biochemistry and Molecular Biology, School of Medicine, Tanjungpura University Hospital. The study design and sample collection were approved by the Ethics Committee of Tanjungpura University Hospital (number: 8179/UN22.9/PG/2022).

Detection of Omicron Variant

DNA/RNA extraction kit was used to extract viral DNA/RNA from nasopharyngeal swab samples using GeneRotex 96 rotary nucleic acid extractor (Tianlong Science and Technology, Xi'an, China). RNA extraction was performed using 200 uL of samples to extract 60 uL of total RNA. RNA amplification was performed using QuantStudio™ 5 real-time polymerase chain reaction (RT-PCR) system (Applied Biosystems, Thermo Fisher Scientific, Massachusetts, US). The PCR test was performed using a total volume of 25 uL to detect three target genes, namely ORF1ab, N, and HV69-70del. The P732H multiplex detection kit (Tianlong Science and Technology, Xi'an, China) contained 12.5 uL reaction solution, 1 uL enzyme mix, 6.5 uL primer and probe mix, and 5 uL RNA negative and positive controls. ORF1ab is the common gene in the Beta variant and is widely used in SARS-CoV-2 detection due to its high sensitivity, specificity, and positive predictive value.[5] The nucleocapsid (N) protein protects the viral genome and is commonly used in COVID-19 detection.[6] HV69-70del gene is one of the key features of Omicron lineage B.1.1.7, and is widely used to differentiate B.1.1.7 from other lineages.[7, 8]

The results of Omicron screening were categorized into two groups, namely probable samples (positive result on ORF1ab, N, and HV69-70del genes) and nonprobable samples (negative result on HV69-70del gene). Both probable and nonprobable samples were further analyzed using whole-genome sequencing.

Library Preparation and Sequencing

RNA library preparation for sequencing was performed using Rapid Barcoding kit SQK-RBK110.96 and Midnight RT-PCR expansion kit EXP-MRT001 (Oxford Nanopore Technologies; Oxford, UK). The input volume for each sample was 8 µL. After barcoding the reaction in each well plate, all samples were pooled and purified with solid-phase reversible immobilization (SPRI) beads using a microcentrifuge tube

magnetic separation rack. One microliter of rapid adapter (RAP) was added to 10 µL of the pooled eluate (800 ng), and the library was kept on ice until loaded onto the flow cell. A total of 75 µL solution contained 12 µL DNA library, 37.5 µL sequencing buffer, and 25.5 µL loading solution.

The sequencing was performed using a MinION sequencer (Oxford Nanopore Technologies, Oxford, UK), and local base-calling was performed using the MinKNOW software. Demultiplexing and identification of SARS-CoV-2 were performed using the EPI2ME cloud-based bioinformatics platform (https://labs.epi2me.io/; Oxford Nanopore Technologies, Oxford, UK). The analysis was performed using Fastq QC+ARTIC+NextClade 2022.07.19-15399 with a default minimum Q-score threshold of 8. The MinION ran for up to six hours (28,000 sequence reads). The sequences were identified using Pangolin (https://pangolin.cog-uk.io/) and GISAID (https://gisaid.org/) databases.

Phylogenetic Sequence Analysis

The sequences were aligned against the reference genome (Wuhan/Hu-1/2019) and six other genomes (Beta B.1.351, Delta B.1, Gamma P.1, Alpha B.1.1.7, Lambda C.37, and Omicron B.1.1.529) using the MAFFT method.[9] The phylogenetic tree was constructed using the neighbor-joining (NJ) method with Molecular Evolutionary Genetics Analysis (MEGA X) software (Philadelphia, US) to visualize the evolutionary relationship between our original samples and other SARS-CoV-2 isolates. The distribution of the data from the GISAID database and isolates from the swabs was visualized on a world map. In addition, the mutation point of BA.2 was compared with Omicron, Omicron BA.5, Omicron BQ.1, Omicron BQ.1, Omicron XBB.1, Omicron BA.2.75, Alpha, Beta, Delta, and Gamma variants using a web-based application (https://outbreak.info/compare-lineages).

Results

Sample Characteristic and Surveillance Strategy

Of the 3,642 nasopharyngeal swab samples, 159 isolates were screened for Omicron followed by whole-genome sequencing analysis. Of these, based on pangolin and GISAID databases, 10 isolates were identified as Omicron lineage BA.2.40 (table 1). Except for isolate 01, all other isolates belonged to Indonesian workers traveling from Tebedu (Sarawak, Malaysia) to Entikong (West Kalimantan, Indonesia) between June and July 2022 (active surveillance). Isolate 01 was also Omicron lineage BA.2.40, but from a child hospitalized following RT-PCR testing. This patient was included in the baseline surveillance category due to age and low Ct value (table 2).

Table 1: Characteristics of isolates with Omicron lineage BA.2.40 in West Kalimantan

Isolates	Sex	Age (years)	Sampling date	Origin	Surveillance
01	Female	4	09-05-2022	Pontianak	Baseline
02	Male	21	23-06-2022	Sanggau	Active
03	Male	56	23-06-2022	Sanggau	Active
04	Female	51	23-06-2022	Sanggau	Active
05	Male	27	23-06-2022	Sanggau	Active
06	Male	25	29-06-2022	Sanggau	Active
07	Male	43	01-07-2022	Sanggau	Active
08	Male	39	01-07-2022	Sanggau	Active
09	Male	18	01-07-2022	Sanggau	Active
10	Male	24	01-07-2022	Sanggau	Active

Table 2: The results of real-time polymerase chain reaction and whole-genome sequencing for detecting targeted genes

Isolates	Ct values of targeted genes			Sample status	WGS
	ORF1ab	N	HV69-70del		
01	22.66	21.88	0.00	Nonprobable	BA.2.40
02	17.63	19.28	0.00	Nonprobable	BA.2.40
03	17.64	19.64	0.00	Nonprobable	BA.2.40
04	22.36	23.34	0.00	Nonprobable	BA.2.40
05	28.44	30.29	0.00	Nonprobable	BA.2.40
06	29.65	31.10	0.00	Nonprobable	BA.2.40
07	29.65	31.10	0.00	Nonprobable	BA.2.40
08	25.98	26.88	0.00	Nonprobable	BA.2.40
09	29.74	31.46	0.00	Nonprobable	BA.2.40
10	26.78	28.64	0.00	Nonprobable	BA.2.40

Ct: Cycle threshold; ORF: Open reading frame; N: Nucleocapsid; HV69-70del: Deletion at sites 69 (histidine) and 70 (valine); WGS: Whole-genome sequencing

Detection of Omicron Variant using RT-PCR and Whole-genome Sequencing

All samples tested positive for ORF1ab and N genes with Ct values ranging from 17.63 to 29.74 and 19.28 to 31.46, respectively. However, in contrast with Omicron lineage B.1.1.7, none of the samples showed HV69-70del mutation (Ct=0). Therefore, isolates without HV69-70del mutation were classified as nonprobable samples (table 2).

The search in the GISAID database did not reveal the emergence of BA.2.40 in other Indonesian provinces. Therefore, the search was extended to other countries. In line with a previous study, the results showed that of the variants deposited in GISAID, 124 isolates were from Malaysia, 11 from Australia, three each from Germany and USA, and one each from Austria, Nigeria, Colombia, Israel, Denmark, and Thailand.[10] Seven reference genomes were used, namely Wuhan-Hu-1, Beta (B.1.351), Delta (B.1), Gamma (P.1), Alpha (B.1.1.7), Lambda (C.37), and Omicron (B.1.1.529) variants. The final dataset included 30,326 positions. As shown in figure 1, all samples with BA.2.40 had originated from Omicron B.1.1.529. We also found that the origin of the identified isolate was very similar to the isolate from Malaysia. Based on the GISAID database, the first case of BA.2.40 was identified in Sarawak (Malaysia) in February 2022. Although BA.2.40 is widespread across the world, 81% of the cases were detected in Malaysia (figure 2). Whole-genome sequencing is still performed in Indonesia, however, the last detection of BA.2.40 dates back to July 1, 2022. The corresponding samples (n=4) belonged to those traveling from Malaysia (table 1).

Spike Protein Mutation in BA.2.40 from West Kalimantan

Genomic sequencing analysis of all isolates from West Kalimantan was performed and submitted to GISAID for data sharing and epidemiological data analysis. The sequencing coverage of all samples was in the range of 3.81-6.13%, with the exception of isolate 06 with 17% coverage. The results confirmed that the 10 isolates from West Kalimantan had 97.4% similarity with the lineage BA.2.40 compared to the reference strain hCoV-19/Wuhan/WIV04/2019, the first SARS-CoV-2 genome strain detected in Wuhan, China.

The results showed 31 mutations in the spike protein, of which 21 mutations in non-structural protein, 7 in N protein, 2 in membrane (M) protein, and a single mutation in NS and E proteins.[11] However, in this study, we only focused on mutation in the spike protein. Among the 31 mutations, three deletions occurred at sites 24, 25, and 26 for leucine and proline, respectively. As shown in table 3, other mutations caused a change in the amino acid of the spike protein. Most mutations were found in subunit 1 (26 site mutations), 16 of which were specifically found in the receptor-binding domain (RBD) of the spike protein, and

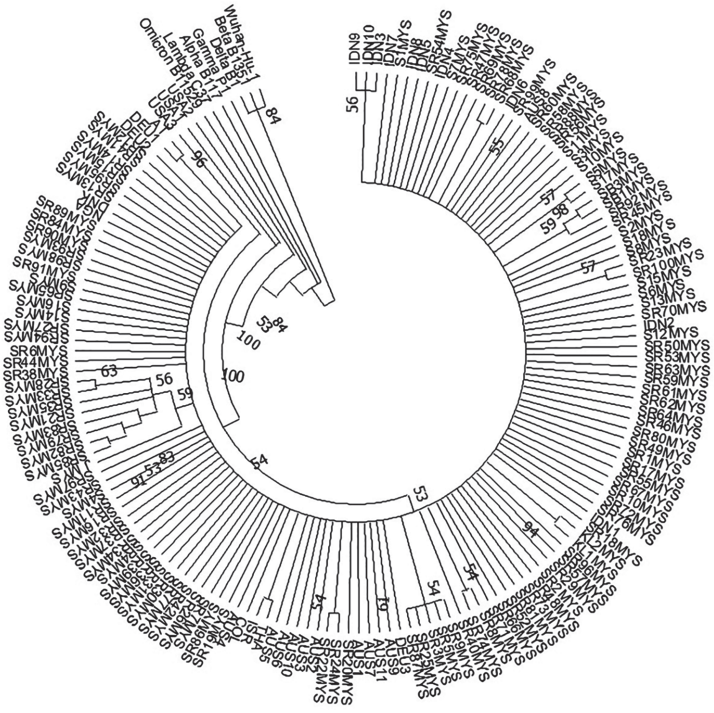

Figure 1: The Phylogenetic Tree of Indonesian BA.2.40 variants compared with all BA.24.40 variants from Malaysia (MYS), Australia (AUS), Germany (DEU), United States of America (USA), Austria (AUT), Nigeria (NGA), Colombia (COL), Israel (ISR), Denmark (DNK), and Thailand (THA)

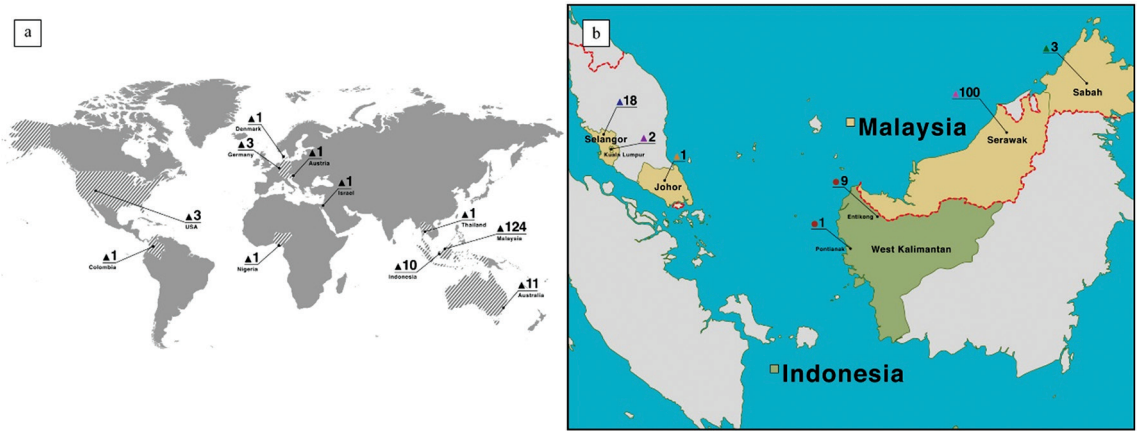

Figure 2: a) The worldwide distribution of BA.2.40 variants (data was retrieved from GISAID database) **b)** most of BA.2.40 varaints was found in Malaysia (as 6 December 2022)

Table 3: Spike protein mutations of Omicron lineage BA.2.40 in isolates from West Kalimantan

Number	Mutation	Mutation type	AA replacement		Occurrence based on GISAID*	
			Original AA	Mutated AA	Rate (%)	Number of affected countries
1	T19I	AA replacement	Threonine	Isoleucine	28.27	178
2	L24del	Deletion	-	-	27.93	176
3	P25del	Deletion	-	-	27.94	176
4	P26del	Deletion	-	-	27.95	176
5	A27S	AA replacement	Alanine	Serine	28.15	186
6	G142D	AA replacement	Glycine	Aspartic acid	67.12	211
7	V213G	AA replacement	Valine	Glycine	28.99	177
8	G339D	AA replacement	Glycine	Aspartic acid	45.04	207
9	S371F	AA replacement	Serine	Phenylalanine	28.14	179
10	S373P	AA replacement	Serine	Proline	44.25	205
11	S375F	AA replacement	Serine	Phenylalanine	44.13	205
12	T376A	AA replacement	Threonine	Alanine	28.18	178
13	D405N	AA replacement	Aspartic acid	Asparagine	28.56	179
14	R408S	AA replacement	Arginine	Serine	27.22	178
15	K417N	AA replacement	Lysine	Asparagine	39.88	207
16	N440K	AA replacement	Asparagine	Lysine	38.70	203
17	S477N	AA replacement	Serine	Asparagine	44.63	207
18	T478K	AA replacement	Threonine	Lysine	75.97	213
19	E484A	AA replacement	Glutamic acid	Alanine	44.12	206
20	Q493R	AA replacement	Glutamine	Arginine	31.16	200
21	Q498R	AA replacement	Glutamine	Arginine	43.12	204
22	N501Y	AA replacement	Asparagine	Tyrosine	53.23	213
23	Y505H	AA replacement	Tyrosine	Histidine	43.25	204
24	D614G	AA replacement	Aspartic acid	Glycine	99.23	216
25	H655Y	AA replacement	Histidine	Tyrosine	48.54	210
26	N679K(674)	AA replacement	Asparagine	Lysine	47.46	208
27	P681H(674)	AA replacement	Proline	Histidine	56.64	214
28	N764K	AA replacement	Asparagine	Lysine	44.64	207
29	D796Y	AA replacement	Aspartic acid	Tyrosine	46.80	206
30	Q954H	AA replacement	Glutamine	Histidine	45.53	205
31	N969K	AA replacement	Asparagine	Lysine	46.62	205

GISAID: Global initiative on sharing all influenza data; AA: Amino acid; *GISAID database per 6 December 2022.

5 mutations in subunit 2 (S2). Based on records retrieved from GISAID (as of December 6, 2022), the mutation rate in our samples ranged from 27.22% to 99.23%. Five mutations with a rate >50% were D614G (99.23%), T478K (75.97%), G142D (67.12%), P681H (56.64%), and N501Y (53.23%). The classification of SARS-CoV-2 variants was made based on the occurrence of a mutation. Omicron lineage BA.2 has similar mutations to other Omicron lineages, with the exception of the Q493R mutation and no HV69-70 deletion (figure 3).

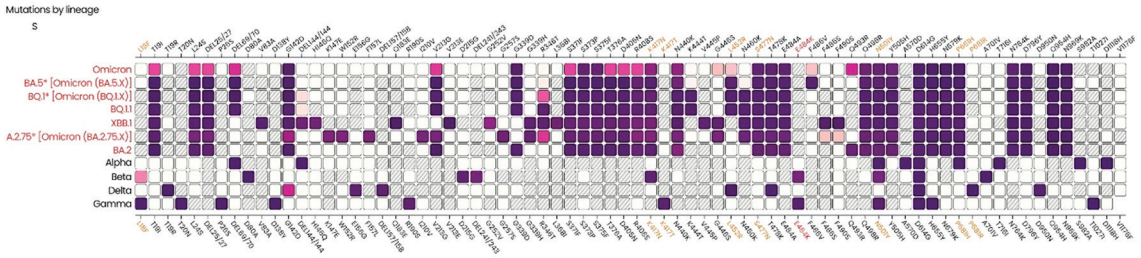

Figure 3: Comparison of Mutation Point of BA.2 with Omicron, Omicron BA.5, Omicron BQ.1, Omicron BQ.1, Omicron XBB.1, Omicron BA.2.75, Alpha, Beta, Delta, and Gamma Variants

Discussion

For the first time, genomic surveillance of SARS-CoV-2 was conducted in mid-2022 in West Kalimantan. By August 2022, we sequenced 159 isolates, of which 10 isolates were classified as Omicron lineage BA.2.40. This is the first time that cases of BA.2.40, a descendent of BA.2 lineage, have been reported from West Kalimantan. Although BA.2.40 is widespread in the world, only a total of 157 isolates across 11 countries were reported in the GISAID database.[11] Our genomic analysis showed that all samples with BA.2.40 were of the same parental lineage B.1.1.529, a VOC with 62 site mutations. Currently, Omicron is the dominant variant in the world, posing a serious threat due to its high rate of contagiousness compared to other VOC variants.[12] BA.1 and BA.2 lineages have similar disease severity. However, BA.2 is closely monitored by the WHO because of its high transmission rate.[4]

In 2022, we performed genomic surveillance of SARS-CoV-2, and 10 isolates were identified as BA.2.40, of which nine isolates were from Indonesian workers traveling from Sarawak (Malaysia) to West Kalimantan (Indonesia). In Malaysia, 124 isolates with BA.2.40 were detected from February to March 2022. The samples were collected mainly in Sarawak province, followed by Selangor, Sabah, and Johor provinces. The first cases of BA.2.40 in Indonesia were detected in West Kalimantan, but it had almost disappeared from the region by May 2022, with the last case reported on August 31, 2022. During the same period, based on our laboratory data deposited in GISAID, variant BA.5 followed by XBB became the dominant strain in the region.[10] These variants are more transmissible than the previous variants[13, 14] and also designated as VOC. This may explain why these two variants became dominant over other variants, including BA.2.40. A similar trend was also observed in several other countries, e.g., a rapid disappearance of the Kappa variant and regional dominance by the Delta variant due to higher infection and transmissibility rates.[15]

Based on travel history reports and phylogenetic analysis, we found that the presence of BA.2.40 in West Kalimantan was primarily due to travelers from Sarawak (Malaysia). Many countries are at risk of COVID-19 due to the effect of internationally imported cases, e.g., Hong Kong and Taiwan.[16] Shandong province (China) was also at high risk of COVID-19 from imported cases due to the peak of the pandemic in South Korea and Japan.[17] However, in contrast, another study reported that relaxation of border control measures for inbound travelers from low-risk countries did not pose a higher risk of COVID-19 outbreak than tighter controls for high-risk countries.[18]

Nucleotide mutation is the survival mechanism of coronavirus to evade the host's immune system.[19] The first reported mutation (D614G) that rapidly spread across the world was identified in February 2020.[20] D614G mutation enhances viral load in the upper respiratory tract, replicates lung epithelial cells, lowers RT-PCR cycle thresholds, and increases interhuman transmission. This discovery was alarming, given that possible future mutations could lead to a more difficult situation.[21-24] Genomic surveillance of SARS-CoV-2 allows optimum contact tracing, as well as tracing the transcontinental origin and history of the virus. During the past two years, the WHO has defined at least 12 variants, namely Alpha, Beta, Gamma, Delta, Epsilon, Zeta, Eta, Theta, Iota, Kappa, Lambda, and Omicron.[25] Omicron is the latest VOC reported in late November 2021 and classified as B.1.1.529, BA.1, BA.2, BA.3, BA.4, BA.5, and descendent sublineages.[1]

The main impact of SARS-CoV-2 mutations is related to mutation of the spike protein, responsible for viral binding to the host cell.[26] Spike protein has a crown-like appearance on the surface of the SARS-CoV-2 virus, allowing it to interact with host cells by binding to receptor angiotensin-converting enzyme 2 (ACE2).[26] Spike protein consists of two subunits, namely subunit 1 (S1) and subunit 2 (S2). S1 binds the virus to the host cell receptors to initiate virus infection. RBD is located in S1 and binds with

cell receptor ACE2.[25] Mutation in this area is crucial for the interaction with receptors and subsequently immune recognition. On the other hand, S2 facilitates viral fusion to the target cell membrane and viral entry.[27] BA.2.40 has 31 sites of mutation on the spike protein, 26 sites of mutation in S1, and five sites in S2. Several mutations of the spike protein have been reported to be associated with host antibodies. Modification of nucleotide barcode in G339D and N440K allows the virus to escape the host-neutralizing antibodies.[28] N440K is reported to be involved in cases of reinfection associated with immune escape and lower vaccine efficiency.[29, 30] These two, together with S373P and S375F mutations, are reported to significantly enhance the interaction between spike protein and ACE2 receptor in the host.[31] Mutations in S371F, D405N, and R408S are reported to reduce the effectiveness of sarbecovirus neutralizing antibodies.[32] Mutation of T19I is associated with mortality,[33] which is specifically found in BA.2 but not in Omicron B.1.617.2, BA.1.1, and BA.1.[27] Together with T19I, K417N and G142D have lower affinity for antibodies.[31] Another interesting mutation has been reported in T376A that reduced the ability of the virus to infect host cells.[34] This means that some variants do not affect the severity of COVID-19.

Several studies reported the impact of spike protein mutations. A previous study showed N501Y and S477N mutations significantly decreased the neutralization activity of some monoclonal antibodies, resulting in immune escape. Furthermore, N501Y and T478K mutations in RBD increased the binding affinity of spike protein for human ACE2.[35] Amino acids, specifically at E484 and N501 sites, increased binding affinity between RBD and ACE2.[36] An *in-silico* study used a method to predict the effect of mutations on spike protein stability. They reported that all amino acid changes caused a decrease in structural stability. Based on functional effect analysis, they showed that E484A, Y505H, N764K, and N969K mutations decrease spike protein's function. Mutation of E484A, located in the RBD, affects viral transmission and Y505H mutation was predicted to negatively affect spike functionality and susceptibility to the disease. On the other hand, mutation of N764K and N969K affected spike functionality, but at the same time exacerbated disease severity.[37] E484A mutation has less affinity for ACE2 than N501Y and was predicted to impact the immunogenicity of RBD protein and decrease the pathogenicity and probability of diseases induced by the protein.[36] A point mutation on Q493R, Q498R, and H655Y decreases spike protein stability but does not disrupt spike functionality and disease severity.[37] Another study reported that the D614G mutation increases the infectivity of the COVID-19 virus.[21]

In the present study, as shown in table 2 and figure 3, we did not find HV69-70 deletion in our samples (nonprobable). This finding is in contrast with other omicron variants that had HV69-70 deletion. BA.2 and its descendants, as well as XBB (another Omicron sublineage), do not have HV69-70 deletion within the spike protein, which is used as a marker to detect an Omicron variant (figure 3). HV69-70 deletion in spike protein allows Omicron variants to be easily identified using the proxy marker of S-gene target failure (SGTF).[38] SGTF has high accuracy in screening for Alpha (98%) and Omicron (100%) variants. It is therefore strongly recommended for early screening of Omicron worldwide. Since January 2022, the Indonesian Ministry of Health has also recommended the SGTF method for early detection of Omicron variants. However, despite its widespread use, this marker should be re-evaluated for screening purposes since our results showed no HV69-70 deletion in the spike protein of BA.2.40.

The main limitation of the study was incomplete clinical data related to travel history reports, limiting our observational analysis. In addition, we could not fully verify that travelers arriving at the international border crossing point had COVID-19.

Conclusion

Omicron lineage BA.2.40 was only detected in West Kalimantan, Indonesia. Phylogenetic analysis showed that BA.2.40 had the same origin as the variant in Malaysia, indicating the effect of internationally imported cases. Genomic analysis of BA.2.40 showed 31 spike mutations in various proteins that differed from other Omicron variants, e.g., Q493R mutation. The use of the SGTF method for early detection of Omicron variants should be re-evaluated since no HV69-70 deletion in the spike protein of BA.2.40 was detected.

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Authors' Contribution

DF.L, A.P: Study concept, data interpretation,

drafting, and revising of the manuscript. M.M: Data analysis and interpretation, drafting and revising of the manuscript. V.N, A.A: Data collection, molecular testing, screening, and sequencing data. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

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