IJID Regions 8 (2023) S1–S7

Contents lists available at ScienceDirect

IJID Regions

journal homepage: www.elsevier.com/locate/ijregi

SARS-CoV-2 lineages circulating during the first wave of the pandemic in North Sumatra, Indonesia

Ramadhan Bestari[1], Irbah Rea Alvieda Nainggolan[2], Mirzan Hasibuan[2], Rima Ratnanggana[3], Krisnoadi Rahardjo[3], Aldise Mareta Nastri[3], Jezzy Renova Dewantari[3], Soetjipto Soetjipto[3], Maria Inge Lusida[3], Yasuko Mori[4], Kazufumi Shimizu[4], R Lia Kusumawati[2], Muhammad Ichwan[2], Inke Nadia Diniyanti Lubis[2,*]

[1] *Faculty of Medicine, Universitas Islam Sumatera Utara, Medan, Indonesia*
[2] *Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia*
[3] *Institute of Tropical Disease, Airlangga University, Surabaya, Indonesia*
[4] *Center for Infectious Diseases, Kobe University Graduate School of Medicine, Chuo-ku, Japan*

ARTICLE INFO

Keywords:
SARS-CoV-2
Whole-genome sequence
Lineage B.1.468
D614G
North Sumatera

ABSTRACT

Objectives: To determine the lineage distribution of the virus during the first wave of the pandemic in North Sumatra, Indonesia.
Methods: A total of 20 samples with positive results based on reverse transcription-polymerase chain reaction were selected for virus culture and then performed whole-genome sequence analysis using next-generation sequencing which was applied by the Illumina MiSeq instrument.
Results: Whole-genome sequence analysis revealed that the majority of our samples belong to lineages B.1.468 (n = 10), B.1 (n = 5), B.1.1 (n = 2), B.1.1.398 (n = 2), and B.6 (n = 1). Other unique amino acid mutations found in our samples were present in A58T on non-structural protein (NSP3) (70%), P323L on NSP12 (95%), Q57H on NS3 protein (75%), and D614G on S (100%).
Conclusion: The SARS-CoV-2 lineage B.1.468 may be the main virus variant circulating in North Sumatra at the beginning of the emergence of COVID-19 cases in this province.

Introduction

SARS-CoV-2 was discovered in Wuhan, China, in late 2019, and COVID-19, the disease caused by SARS-CoV-2 [1], rapidly developed into a global pandemic and also affected the Indonesian population. The first two cases in Indonesia were discovered on March 02, 2020 [2], then spread rapidly to 34 provinces. By June 2023, Indonesia had been the country with the most confirmed cases of COVID-19 in Southeast Asia [3].

The first wave of the SARS-CoV-2 pandemic in Indonesia occurred on March 02, 2020, to April 30, 2021. The second wave, dominated by the Delta variant, peaked in July 2021, followed by the third wave, which peaked in February 2022 with the dominance of the Omicron variant. Most of the cases and deaths in Indonesia were reported on the island of Java [4]. North Sumatra province contributed the highest confirmed cases of COVID-19 outside Java [5].

The Indonesian government implemented social/physical distancing measures by closing schools in a limited manner and encouraged people to work from home. However, without strict precautions, the number of deaths due to the coronavirus in Indonesia had soared to the highest in Southeast Asia [6]. The government finally declared a national health emergency by imposing a partial lockdown Pembatasan Sosial Berskala Besar, regional with harsh measures such as shutting down public venues, isolating public transportation, imposing limitations on travel to other zones, limiting commercial travel, and closing the international borders [7]. However, the number of cases is continued to increase.

SARS-CoV-2's first genome was published in January 2020, and since then, several studies have emerged to track its evolution worldwide [8]. Viruses evolved into a high-level phylogenetic grouping called the PANGO Lineage (CoV-Lineage) nomenclature [9] that complements the other two SARS-CoV-2 nomenclature systems NextStrain [10] and Global Initiative on Sharing All Influenza Data (GISAID) [11], which focus on broader phylogenetic clades incorporating specific criteria based on whole-genome sequence (WGS) analysis. The PANGO Lineage is hierarchical and small-scale and designed to look

* Corresponding author:
E-mail address: inke.nadia@usu.ac.id (I.N.D. Lubis).

https://doi.org/10.1016/j.ijregi.2023.07.006
Received 2 January 2023; Received in revised form 12 July 2023; Accepted 16 July 2023

at patterns of pandemic transmission which can recognize different geographic areas through the ongoing evolution of the virus that occurs [12].

Analyzing WGSs was essential to provide insight into this new virus to contain its outbreaks. Genomic epidemiology based on WGS analysis helps explore transmission patterns that play a crucial role in understanding the rapid emergence and global spread of SARS-CoV-2. Epidemiological analyses of the genome have concluded the evolutionary relationship of the virus using whole-genome sequences and phylogenetic approaches that have yielded important insights into the beginning of the COVID-19 pandemic in many countries [13]. However, the limited supply of reagents, trained laboratory personnel, and the availability of well-equipped laboratories are the main challenges for detecting and whole-genome sequence analysis of SARS-CoV-2 in developing countries, especially Indonesia [14].

Some of the SARS-CoV-2 whole-genome sequences from Indonesia were submitted to the GISAID database, classified into several CoV lineages or clades and amino acid mutations that occur. Many sequences have been entered into GISAID, but this is the first report of the SARS-CoV-2 North Sumatra, Indonesia local isolates. So this study aims to determine the type of lineages SARS-CoV-2 in North Sumatra province during the first pandemic through genomic surveillance.

Methods

Samples were collected from nasopharyngeal-oropharyngeal swabs in viral transport media (VTM) of COVID-19 suspected patients and of contact tracing purposes from those attending any health facility in North Sumatra province. Viral RNA was extracted from the VTM of COVID-19 suspected patients using the Maccura - Mag-Bind RNA Extraction Kit (Maccura Biotechnology, Chengdu, Republic of China). The isolation process was done with 300 µl VTM added to lysis buffer, followed by nucleic acid binding, washing buffer, and finally elution of RNA for reverse transcription-polymerase chain reaction using Maccura - SARS-CoV-2 Fluorescent polymerase chain reaction kit (Maccura Biotechnology, Chengdu, Republic of China) using the LightCycler® 96 Instrument (Roche Diagnostic Corporation, Indianapolis, USA) according to the manufacturer's instructions at the Microbiology Laboratory, Faculty of Medicine, Universitas Sumatera Utara in Medan. The Maccura kit targeted three genes, E, N, and ORF1ab, with cycle threshold (Ct) value <37 for the E gene and <38 for both N and ORF1ab genes was defined as the cut-off to confirm a positive result for SARS-CoV-2.

A total of 20 positive specimens were selected if the Ct value >20 and without degradation of the VTM quality indicated by a yellowish color or VTM volume <600 µl. Samples were then sent to the Institute of Tropical Diseases, Universitas Airlangga, Surabaya, East Java, Indonesia to perform a WGS analysis. The study flowchart can be seen in Figure 1.

WGS has comprehensive utility in virus discovery; this technique provides high sensitivity which makes it prone to contamination. The combination of viral culture and WGS can increase the sensitivity and accuracy of WGS analysis, which can enable amplification and enrichment of viral genetic material, isolation of certain viral strains, reduction of host contamination, and preparation of high-quality viral nucleic acids, which will result in understanding of the genomic data of SARS-CoV- 2 [15–17].

The viral culture was performed by inoculating the sample onto confluent monolayers of African green monkey kidney Vero cells (ATCC, Virginia, USA) grown in growth medium (Gibco, Thermo Fisher Scientific, Massachusetts, USA) supplemented with fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific, Massachusetts, USA) in a cell culture dish (Corning, New York, USA). Cell culture disk moved from the 37°C incubator to a Class III Biosafety cabinet within a Biosafety Level-3 laboratory environment. Samples were prepared by diluting a reduced serum growth medium without FBS and filtering over a filter. Collect 150-200 µl of filtered patient material inoculated onto a detached confluent cell monolayer and transfer it to the new tube along with the negative control. Samples were absorbed onto cells by incubating tubes for 1 hour at 37°C before cultures were re-fed with 2 ml of pre-warmed (37°C) reduced serum growth medium. Cultures were incubated for 2-7 days until observed signs of cytopathic effect (CPE). Cell culture is suspected of hosting virus replication based on the presence of CPE, including damage to the monolayer, cell clearing, and morphological changes.

Total viral RNA was extracted from samples using a QIAamp Viral Mini kit (Qiagen, Tokyo, Japan). As a transfer RNA wave, we used a linear polyacrylamide as the carrier. The RNA library was prepared using TruSeq RNA Sample Preparation Kit v2 (Illumina, California, USA). WGS was performed using next-generation sequencing applied in the Illumina MiSeq instrument (Illumina, California, USA) and loaded in a 300-cycle cell stream with Illumina MiSeq Reagent Kit v2 (Illumina, California, USA). The multiplexed library sequencing (2 × 150 bp) barcode was performed on the Illumina MiSeq platform (Illumina, California, USA). MiSeq generates a FASTQ file in which the primary sequence and adapter are trimmed to remove the adapter sequences automatically itself when demultiplexing process. All files were then analyzed in the CLC genomics workbench v8.1 (Qiagen, Hilden, Germany). Total reads are filtered to remove reads of poor quality (<Q30, length <26 bp, or contain more than two consecutive ambiguous bases). The filtered readings were then mapped to the genome sequence of the SARS-CoV-2 Wuhan-Hu-1 virus (GISAID ID EPI_ISL_402124) using the CLC Genomic Workbench to obtain a consensus sequence for the SARS-CoV-2 virus in North Sumatra. Full-genome sequences of the SARS-CoV-2 virus in North Sumatra were submitted to the GISAID.

Phylogenetic analysis using a dataset of available SARS-CoV-2 virus genomes that have complete genome, high coverage, and no stretches of repetitive unknown nucleotide (NNNN) for the phylogenetic dataset from genome sequences of 20 lineages identified in this study, and 125 available SARS-CoV-2 genomes database (76 sequences from Indonesia, and 50 sequences from other countries) was retrieved from GISAID up to February 16, 2023, via **doi.org/10.55876/gis8.230622zt**. Sequence alignment for multiple nucleotide sequence alignment and the phylogenetic tree was constructed using 29,409 nucleotide length starting from the ORF1ab gene of SARS-CoV-2 using a neighbor-joining method with 1,000 bootstrap replications were performed in Genetyx version 10 software (Genetyx, Tokyo, Japan). Because the purpose of this phylogenetic analysis was to determine the evolutionary relationships between our virus samples and the other SARS-CoV-2 viruses, the phylogenetic tree was rooted in the oldest virus, hCoV-19/Wuhan/WIV04/2019 (GISAID ID EPI_ISL_402124).

This study was conducted after receiving ethical clearance from the Faculty of Medicine, University of North Sumatra Ethical Committee, by 713/KEP/USU/2020. We affirm that our research adhered to the applicable guidelines and regulations specified in the approval. The oropharyngeal and nasopharyngeal swab samples utilized in this study were obtained from patients who provided written informed consent, expressing their agreement to contribute their samples for research objectives.

Results

During this study period, 20 samples from North Sumatra province, Indonesia, were performed viral cultured. Seventeen samples were successfully performed based on the presence of CPE, while the other three failed. The SARS-CoV-2 genome sequence was submitted via the GISAID platform (https://www.gisaid.org/). Five samples had complete SARS-CoV-2 genome sequences, while the other samples, although not complete genome sequences, still consisted of 11 SARS-CoV-2 genes (ORF1ab, S, ORF3a, E, M, ORF6, ORF7a, ORF7b, ORF8, N, and ORF10). Sequences of this sample were compared with the reference hCoV-19/Wuhan/WIV04/2019 (GISAID ID EPI_ISL_402124). The amino acid mutation of our samples is A58T on non-structural protein-3 (NSP3) (n = 14), P323L on NSP12 (n = 19), Q57H on NS3 protein (n = 15), and all samples had amino acid substitution (D614G) on spike protein (S-protein) (n = 20) (Figure 2). The WGS results revealed that 10 sam-

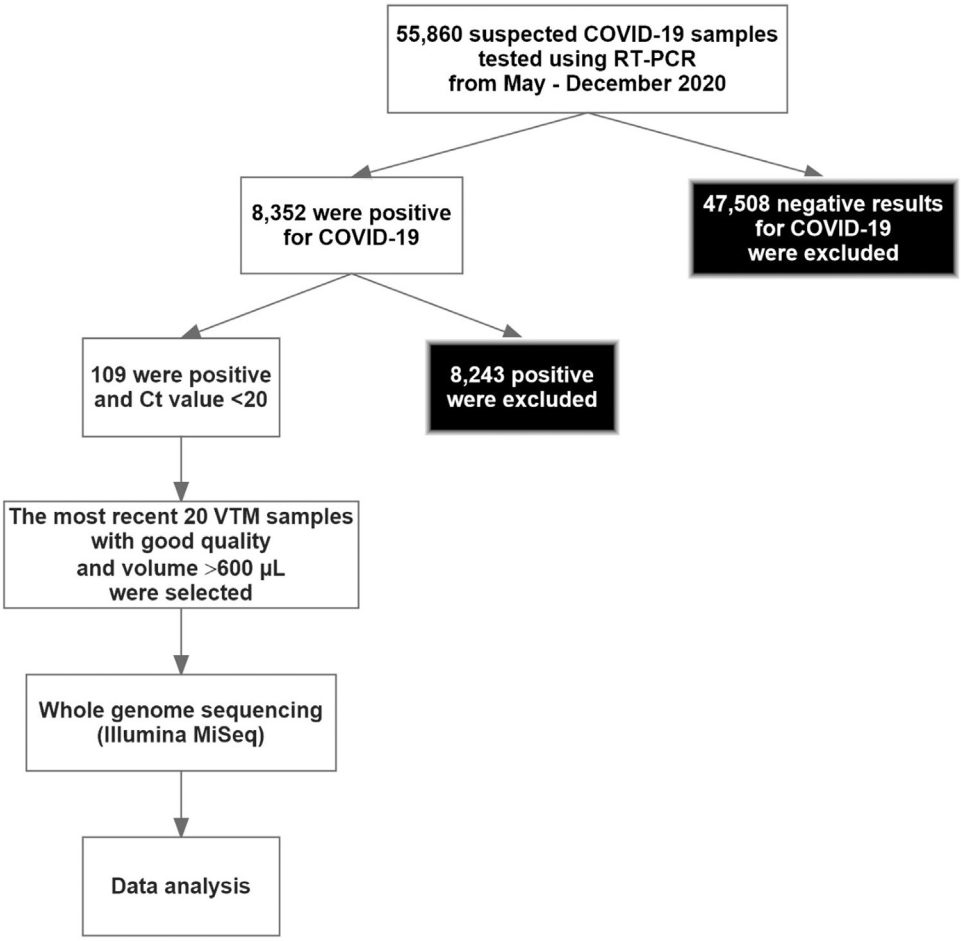

Figure 1. Study flowchart. Ct, cycle threshold; RT-PCR, reverse transcription-polymerase chain reaction; VTM, viral transport media.

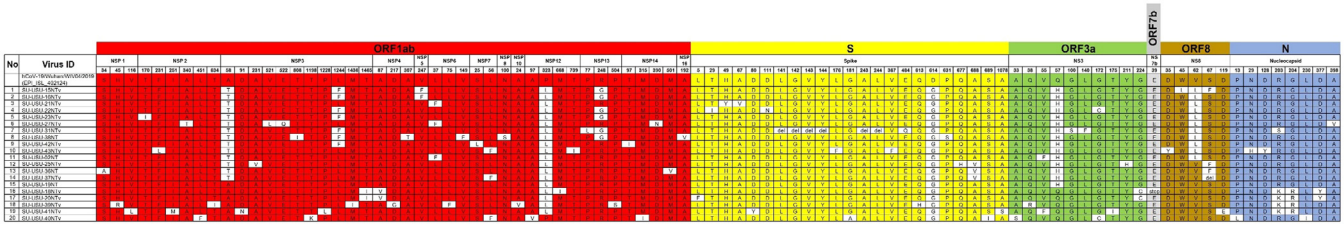

Figure 2. Amino acid variations of SARS-CoV-2 virus samples from North Sumatra aligned with reference genome. (White spot remarked the amino acid mutation which shown only from the translated region of each protein)

ples were B.1.468 lineage, five were B.1 lineage, two were B.1.1 lineage, two were B.1.1.398 lineage, and one was B.6 lineage. All SARS-CoV-2 genomes analyzed belong to the B lineages, with most samples clustered into lineage B.1.468. The phylogenetic tree of all viruses is presented in Figure 3. The other characteristics of the samples can be seen in Table 1.

Discussion

The initial pandemic showed that only two CoV lineages, A and B, are the most dominant lineages circulating worldwide based on WGS analysis of COVID-19 confirmed cases. The ancestor of the two lineages originally came from China, but A lineage spread from Asia throughout the world, while B lineage mainly spread in Europe [18]. After being discovered for the first time, the B lineage developed further into sub-lineages and spread dominantly during the first wave of the pandemic in Indonesia [19].

During the first wave of the pandemic in Indonesia, the lineages that were commonly found in Indonesia were B.1.466.2 and B.1.470 [20], but the lineage that is most commonly found for each province in Indonesia are different (Table 2), such as B. 1.468 which is commonly found in North Sumatra, and B.1.470 which is commonly found in East Java, apart from that during this first wave period, B.1466.2 was also quite commonly found in Bali, before finally spreading throughout the world and being identified as Indonesian lineages. Only B.1.466.2 is still being found after the first wave of the pandemic has passed [21].

During the second wave period, there was a marked shift in the dominant strain of Indonesian SARS-CoV-2 infection, namely the Delta variant (B.1.617.2 and AY.xx sub-lineages) [22], then shift to the Omicron variant (B.1.1.529 and all sub-lineages) in the third wave period which spreads rapidly and is a common variant evenly in all provinces in Indonesia [23]. This phenomenon reflects fierce competition between populations of different variants of the same virus [24]. The transition of SARS-CoV-2 variants within a specific geographical area is a natural progression of the virus as it adjusts to its surroundings. Consequently, new variants may arise as the virus undergoes genetic mutations, potentially enhancing its adaptability and ability to spread [25].

Figure 3. Phylogenetic analysis of SARS-CoV-2 genomes. The clustering of certain lineages is indicative of the extent of genomic variations. (Samples from North Sumatra are indicated in pink color)

Table 1
Characteristics of 20 patients with COVID-19 and SARS-CoV-2 virus samples from North Sumatra.

No	Virus ID (GISAID accession ID)	Sex	Age (year)	Sample origin	Collection date (2020)	Consensus length	Depth coverage	Total Amino acid replacement	CoV lineages
1	hCoV-19/Indonesia/SU-USU-15NTv/2020 (**EPI_ISL_1335883**)	Male	43	Virus Culture	20 September	29903	2235x	10	B.1.468
2	hCoV-19/Indonesia/SU-USU-16NTv/2020 (**EPI_ISL_1069153**)	Female	58	Virus Culture	25 September	29903	2158x	8	B.1.468
3	hCoV-19/Indonesia/SU-USU-21NTv/2020 (**EPI_ISL_1072963**)	Female	33	Virus Culture	15 October	29902	2839x	8	B.1.468
4	hCoV-19/Indonesia/SU-USU-22NTv/2020 (**EPI_ISL_1335885**)	Male	41	Virus Culture	3 October	29892	416x	10	B.1.468
5	hCoV-19/Indonesia/SU-USU-23NTv/2020 (**EPI_ISL_1335886**)	Male	48	Virus Culture	18 October	29899	1066x	8	B.1.468
6	hCoV-19/Indonesia/SU-USU-27NTv/2020 (**EPI_ISL_1335887**)	Female	55	Virus Culture	17 October	29903	986x	11	B.1.468
7	hCoV-19/Indonesia/SU-USU-31NTv/2020 (**EPI_ISL_1335888**)	Male	55	Virus Culture	04 November	29882	555x	18	B.1.468
8	hCoV-19/Indonesia/SU-USU-38NT/2020 (**EPI_ISL_1335898**)	Male	36	Original Swab	17 December	29900	1012x	13	B.1.468
9	hCoV-19/Indonesia/SU-USU-42NTv/2020 (**EPI_ISL_1335892**)	Female	14	Virus Culture	21 December	29903	1504x	8	B.1.468
10	hCoV-19/Indonesia/SU-USU-43NTv/2020 (**EPI_ISL_1335893**)	Female	60	Virus Culture	3 December	29899	929x	13	B.1.468
11	hCoV-19/Indonesia/SU-USU-02NT/2020 (**EPI_ISL_1335896**)	Male	36	Original Swab	18 July	29898	211x	7	B.1
12	hCoV-19/Indonesia/SU-USU-25NTv/2020 (**EPI_ISL_1072964**)	Male	22	Virus Culture	9 October	29902	1836x	9	B.1
13	hCoV-19/Indonesia/SU-USU-36NT/2020 (**EPI_ISL_1335895**)	Female	57	Original Swab	04 November	29888	44x	8	B.1
14	hCoV-19/Indonesia/SU-USU-37NTv/2020 (**EPI_ISL_1335889**)	Male	53	Virus Culture	7 December	29878	1425x	7	B.1
15	hCoV-19/Indonesia/SU-USU-19NT/2020 (**EPI_ISL_1335894**)	Male	23	Original Swab	14 November	29902	148x	3	B.1
16	hCoV-19/Indonesia/SU-USU-18NTv/2020 (**EPI_ISL_1072962**)	Female	40	Virus Culture	23 September	29889	2733x	10	B.1.1
17	hCoV-19/Indonesia/SU-USU-20NTv/2020 (**EPI_ISL_1335884**)	Female	44	Virus Culture	24 November	29903	4664x	9	B.1.1
18	hCoV-19/Indonesia/SU-USU-39NTv/2020 (**EPI_ISL_1072986**)	Female	50	Virus Culture	19 December	29901	1983x	13	B.1.1.398
19	hCoV-19/Indonesia/SU-USU-41NTv/2020 (**EPI_ISL_1335891**)	Male	57	Virus Culture	7 December	29902	663x	12	B.1.1.398
20	hCoV-19/Indonesia/SU-USU-40NTv/2020 (**EPI_ISL_1335890**)	Female	43	Virus Culture	18 December	29894	1188x	12	B.6

The B.1.468 lineage observed in this study was also widespread in Indonesia during the first wave of the pandemic between March 2020 and December 2020. It was also found in several other countries [26–28]. This B.1.468 lineage was first reported in Saudi Arabia on February 13, 2020, before spreading to other parts of the world, including Indonesia. Until June 2023, Indonesia was the country that reported the most findings of the B.1.468 lineage, of which North Sumatra province has reported the most findings of this lineage [29].

The SARS-CoV-2 genome sample from North Sumatra province, which represents the B.1.468 lineage, has several amino acid mutations, including A58T in NSP3, P323L in NSP12, Q57H in NS3, and D614G in S-protein. Through evolutionary and spatial examinations, it was observed that amino acid substitution plays an essential role in viral replication (A58T in NSP3), altering interactions with other components of the replication/transcription machinery or with the RNA template (P323L in NSP12), inducing conformational changes from the binding site (Q57H on NS3), and increased transmission of the virus (D614G on S) which has the potential to affect the process of viral replication and transcription so that it is known to spread more efficiently [30]. This mutation changes the structural stability of the protein. It significantly

Table 2
Lineage distribution in Indonesia during 2019 to 2020, number of entries in GISAID EpiCoV database with high coverage, complete of collection date.

Province	Lineage distribution in Indonesia during 2019 to 2020, number of entries in GISAID EpiCoV database (% of total from Indonesia for each lineage)																											
	B		B.6		B.50		B.56		B.1		B.1.1		B.1.1.398		B.1.36.19		B.1.456		B.1.459		B.1.466.2		B.1.468		B.1.470		Others	Total
West Java	3	(5)	2	(13)	1	(11)	2	(6)	15	(16)	**43**	**(48)**	**64**	**(51)**	3	(11)	**7**	**(30)**	**20**	**(25)**	8	(23)	4	(9)	24	(18)	1	198
East Java	**29**	**(48)**	**3**	**(20)**	3	(33)	**14**	**(39)**	**42**	**(45)**	1	(1)	2	(2)	3	(11)	**7**	**(30)**	2	(3)	2	(6)	1	(2)	**61**	**(45)**	2	172
Jakarta	14	(23)	2	(13)	**4**	**(44)**	**14**	**(39)**	5	(5)	13	(15)	26	(21)	0	(0)	1	(4)	14	(18)	2	(6)	2	(4)	9	(7)	1	106
Bali	0	(0)	0	(0)	0	(0)	0	(0)	2	(2)	20	(22)	3	(2)	1	(4)	6	(26)	0	(0)	**10**	**(29)**	0	(0)	17	(13)	1	60
West Sumatra	0	(0)	2	(13)	0	(0)	1	(3)	6	(6)	2	(2)	15	(12)	**15**	**(56)**	1	(4)	4	(5)	1	(3)	11	(24)	1	(1)	1	60
North Sumatra	0	(0)	1	(7)	0	(0)	0	(0)	2	(2)	3	(3)	2	(2)	0	(0)	0	(0)	1	(1)	0	(0)	**20**	**(44)**	0	(0)	0	29
Yogyakarta	3	(5)	0	(0)	0	(0)	0	(0)	4	(4)	1	(1)	3	(2)	1	(4)	1	(4)	15	(19)	0	(0)	3	(7)	10	(7)	0	41
Banten	2	(3)	0	(0)	0	(0)	2	(6)	2	(2)	2	(2)	5	(4)	0	(0)	0	(0)	10	(13)	5	(14)	0	(0)	8	(6)	1	37
Central Java	1	(2)	1	(7)	0	(0)	0	(0)	0	(0)	1	(1)	0	(0)	0	(0)	0	(0)	10	(13)	0	(0)	0	(0)	0	(0)	0	13
Others	9	(15)	4	(27)	1	(11)	3	(8)	16	(17)	3	(3)	5	(4)	4	(15)	0	(0)	4	(5)	7	(20)	4	(9)	6	(4)	2	68
Total	61	(100)	15	(100)	9	(100)	36	(100)	94	(100)	89	(100)	125	(100)	27	(100)	23	(100)	80	(100)	35	(100)	45	(100)	136	(100)	9	784
(% of World)	(1.5)		(2.3)		(82)		***(78)***		(0.16)		(0.3)		***(66)***		***(48)***		***(70)***		***(66)***		***(78)***		***(53)***		***(84)***		(0.0023)	(0.16)
World	4103		659		11		46		57,485		29,658		188		56		33		122		45		85		162		396,914	499,567

impacts the biological function of the protein by increasing the interaction between the receptor binding domain on the S-protein and the angiotensin-converting enzyme 2 receptor so that it has a higher transmission ability [31,32].

At the time of the study, the availability of WGS analysis in Indonesia was only in the institutes based on Java Island, Indonesia. While stemming further transmission of COVID-19, especially those caused by new variants, appropriate steps are needed to find a variant of the SARS-CoV-2 virus based on genome analysis, which is the most appropriate SARS-CoV-2 detection system during this pandemic. Therefore, this study evaluated the evolution of the SARS-CoV-2 circulating in the province and whether a new mutation emerged within the population. A genomic epidemiological approach should be part of the COVID-19 evaluation to determine further control measures to prevent the increasing wave of cases.

Conclusion

These findings indicate that the SARS-CoV-2 lineage B.1.468 may be the main virus variant circulating in North Sumatra, especially at the beginning of the emergence of COVID-19 cases in this province.

Limitation

The limitation of this study is that the number of samples analyzed was only a tiny proportion of all the SARS-CoV-2 confirmations in North Sumatra and did not correlate the SARS-CoV-2 variant with disease severity or another clinical outcome.

Declarations of competing interest

The authors have no competing interests to declare.

Funding

This study was supported by the Ministry of Education, Culture, Research and Technology Republic of Indonesia, Universitas Sumatera Utara (World Class Research 2020 scheme: 1879/UN5.1.R/SK/PPM/2020) and received financial support from Universitas Sumatera Utara (ICTROMI Funding).

Transparency declaration

This article is part of a supplement entitled ‘Proceedings from the 3rd International Conference on Tropical Medicine & Infectious Diseases’ published with support from the Universitas Sumatera Utara, Medan, Indonesia.

References

[1] Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. *Nature* 2020;**579**:265–9. doi:10.1038/s41586-020-2008-3.

[2] Setiawaty V, Kosasih H, Mardian Y, Ajis E, Prasetyowati EB, Siswanto, et al. The identification of first COVID-19 cluster in Indonesia. *Am J Trop Med Hyg* 2020;**103**:2339–42. doi:10.4269/ajtmh.20-0554.

[3] World Health Organization, World Health Organization. Coronavirus (COVID-19) dashboard, https://covid19.who.int/table; 2023 [accessed 25 June 2023].

[4] Surendra H, Paramita D, Arista NN, Putri AI, Siregar AA, Puspaningrum E, et al. Geographical variations and district-level factors associated with COVID-19 mortality in Indonesia: a nationwide ecological study. *BMC Public Health* 2023;**23**:103. doi:10.1186/s12889-023-15015-0.

[5] CNN Indonesia. Pasien positif Covid-19 pertama di sumut sembuh, https://www.cnnindonesia.com/nasional/20200404222831-20-490375/pasien-positif-covid-19-pertama-di-sumut-sembuh; 2020 [accessed 25 June 2023].

[6] Roziqin A, Mas’udi SYF, Sihidi IT. An analysis of Indonesian government policies against COVID-19. *Public Adm. Policy* 2021;**24**:92–107. doi:10.1108/PAP-08-2020-0039.

[7] Alam MM, Fawzi AM, Islam MM, Said J. Impacts of COVID-19 pandemic on national security issues: Indonesia as a case study. *Secur J* 2022;**35**:1067–86. doi:10.1057/s41284-021-00314-1.

[8] Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020;**579**:270–3. doi:10.1038/s41586-020-2012-7.
[9] Rambaut A, Holmes EC, Á O'Toole, Hill V, McCrone JT, Ruis C, et al. Addendum: a dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nat Microbiol* 2021;**6**:415. doi:10.1038/s41564-021-00872-5.
[10] Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, et al. Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics* 2018;**34**:4121–3. doi:10.1093/bioinformatics/bty407.
[11] Shu Y, McCauley J. GISAID: global initiative on sharing all influenza data – from vision to reality. *Eurosurveillance* 2017;**22**:30494. doi:10.2807/1560-7917.ES.2017.22.13.30494.
[12] O'Toole Á, Scher E, Underwood A, Jackson B, Hill V, McCrone JT, et al. Assignment of epidemiological lineages in an emerging pandemic using the pangolin tool. *Virus Evol* 2021;**7** veab064. doi:10.1093/ve/veab064.
[13] Ladner JT, Grubaugh ND, Pybus OG, Andersen KG. Precision epidemiology for infectious disease control. *Nat Med* 2019;**25**:206–11. doi:10.1038/s41591-019-0345-2.
[14] Bauer DC, Tay AP, Wilson LOW, Reti D, Hosking C, McAuley AJ, et al. Supporting pandemic response using genomics and bioinformatics: a case study on the emergent SARS-CoV-2 outbreak. *Transbound Emerg Dis* 2020;**67**:1453–62. doi:10.1111/tbed.13588.
[15] Cassedy A, Parle-McDermott A, O'Kennedy R. Virus detection: a review of the current and emerging molecular and immunological methods. *Front Mol Biosci* 2021;**76**:637559. doi:10.3389/fmolb.2021.637559.
[16] Dolskiy AA, Grishchenko IV, Yudkin DV. Cell cultures for virology: usability, advantages, and prospects. *Int J Mol Sci* 2020;**21**:7978. doi:10.3390/ijms21217978.
[17] Chen H, Yin Y, Gao H, Guo Y, Dong Z, Wang X, et al. Clinical utility of in-house metagenomic next-generation sequencing for the diagnosis of lower respiratory tract infections and analysis of the host immune response. *Clin Infect Dis* 2020;**71**:S416–26. doi:10.1093/cid/ciaa1516.
[18] Rambaut A, Holmes EC, Á O'Toole, Hill V, McCrone JT, Ruis C, et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nat Microbiol* 2020;**5**:1403–7. doi:10.1038/s41564-020-0770-5.
[19] Fibriani A, Stephanie R, Alfiantie AA, Siregar ALF, Pradani GAP, Yamahoki N, et al. Analysis of SARS-CoV-2 genomes from west Java, Indonesia. *Viruses* 2021;**13**:2097. doi:10.3390/v13102097.
[20] Cahyani I, Putro EW, Ridwanuloh AM, Wibowo S, Hariyatun H, Syahputra G, et al. Genome profiling of SARS-CoV-2 in Indonesia, ASEAN and the neighbouring East Asian Countries: features, challenges and achievements. *Viruses* 2022;**14**:778. doi:10.3390/v14040778.
[21] Zhu M, Zeng Q, Saputro BIL, Chew SP, Chew I, Frendy H, et al. Tracking the molecular evolution and transmission patterns of SARS-CoV-2 lineage B.1.466.2 in Indonesia based on genomic surveillance data. *Virol J* 2022;**19**:103. doi:10.1186/s12985-022-01830-1.
[22] Prasetyoputri A, Dharmayanthi AB, Iryanto SB, Andriani A, Nuryana I, Wardiana A, et al. The dynamics of circulating SARS-CoV-2 lineages in Bogor and surrounding areas reflect variant shifting during the first and second waves of COVID-19 in Indonesia. *PeerJ* 2022;**10**:e13132. doi:10.7717/peerj.13132.
[23] Kadir A, Deby S, Sunarno AM. A systematic review of omicron outbreak in Indonesia: a case record and Howthe country is weathering the new variant of COVID-19. *Eur J Mol Clin Med* 2022;**9**:364–73.
[24] Chen J, Gu C, Ruan Z, Tang M. Competition of SARS-CoV-2 variants on the pandemic transmission dynamics. *Chaos Solitons Fractals* 2023;**169**:113193. doi:10.1016/j.chaos.2023.113193.
[25] 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**:409–24. doi:10.1038/s41579-021-00573-0.
[26] Gu H, Chu DKW, Chang LDJ, Cheuk SSY, Gurung S, Krishnan P, et al. Genetic diversity of SARS-CoV-2 among travelers arriving in Hong Kong. *Emerg Infect Dis* 2021;**27**:2666–8. doi:10.3201/eid2710.211028.
[27] Pattabiraman C, Prasad P, George AK, Sreenivas D, Rasheed R, Reddy NVK, et al. Importation, circulation, and emergence of variants of SARS-CoV-2 in the south Indian State of Karnataka. *Wellcome Open Res* 2021;**6**:110. doi:10.12688/wellcomeopenres.16768.2.
[28] Zainulabid UA, Kamarudin N, Zulkifly AH, Gan HM, Tay DD, Siew SW, et al. Near-complete genome sequences of nine SARS-CoV-2 strains harboring the D614G mutation in Malaysia. *Microbiol Resour Announc* 2021;**10**:e0065721. doi:10.1128/MRA.00657-21.
[29] GISAID. GISAID Global Initiative, https://www.epicov.org/epi3/frontend#638fa; 2023 [accessed 25 June 2023].
[30] Mohammadi E, Shafiee F, Shahzamani K, Ranjbar MM, Alibakhshi A, Ahangarzadeh S, et al. Novel and emerging mutations of SARS-CoV-2: biomedical implications. *Biomed Pharmacother* 2021;**139**:111599. doi:10.1016/j.biopha.2021.111599.
[31] Arora P, Pöhlmann S, Hoffmann M. Mutation D614G increases SARS-CoV-2 transmission. *Signal Transduct Target Ther* 2021;**6**:101. doi:10.1038/s41392-021-00502-w.
[32] Kannan SR, Spratt AN, Quinn TP, Heng X, Lorson CL, Sönnerborg A, et al. Infectivity of SARS-CoV-2: there is something more than D614G? *J Neuroimmune Pharmacol* 2020;**15**:574–7. doi:10.1007/s11481-020-09954-3.