Sys Rev Pharm 2020;11(8): 203-213
A multifaceted review journal in the field of pharmacy

Investigation of the D614G Mutation and Antibody-Dependent Enhancement Sequences in Indonesian SARS-CoV-2 Isolates and Comparison to Southeast Asian Isolates

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ABSTRACT

Introduction: SARS-CoV-2 is a rapidly spreading virus that poses a major burden on global human health and the economy. Therefore, it is essential to develop COVID-19 vaccines. Vaccine construction might not be easy, as a consequence of mutations and antibody-dependent enhancement (ADE). **Objective**: We first reported the D614G mutation and ADE sequences in Indonesian SARS-CoV-2 isolates and compared these isolates to those from other Southeast Asian countries. **Methods**: In this study, we extracted the SARS-CoV-2 genome of 40 Indonesian isolates from the GISAID EpiCoV database and the Wuhan-Hu-1 isolate (reference sequence) from GenBank, NCBI. We used BioEdit v7.2.5 to identify the D614G mutation and ADE sequences in the spike protein. Then, we rendered the spike protein using the SWISS-MODEL web server and PyMOL v2.4. **Results**: We identified the D614G missense mutation in 23 Indonesian SARS-CoV-2 isolates and isolates from six other Southeast Asian countries. In addition, we identified the ADE sequence ^{611}LYQDVNC^{617} in the Wuhan-Hu-1 isolate, which had changed into ^{611}LYQGVNC^{617} in recent mutated isolates. **Conclusion**: We conclude that the D614G mutation might affect ADE activities. A rapid but cautious approach to the vaccine development and other therapies developed for COVID-19 seems needed until we have more data on the risks of the D614G mutation and ADE. However, further studies including *in vitro* and *in vivo* assessments are relevant for validation of these results.

Keywords: antibody-dependent enhancement, COVID-19, genetic mutation, SARS-CoV-2

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INTRODUCTION

SARS-CoV-2 was firstly identified in China and emerged sporadically all over the world[1]. In March 2020, the WHO announced that the infection was a pandemic. The sudden outbreak and rapid dispersion of COVID-19 have endangered global health and the economy. This crisis has called for extensive scientific mobilization of studies on SARS-CoV-2 concerning its clinical aspects, characteristics, and mechanism of transmission, with the ultimate aim of counteracting the devastating outcomes[2,3]. To date, the seventh coronavirus has infected approximately 28 million people globally with more than 900,000 deaths worldwide. In addition, there are more than 200,000 cases and around 8,500 deaths in Indonesia. These data are based on the Johns Hopkins University online website that tracks COVID-19 cases in real-time[4].

Animals and humans can be infected by coronaviruses. The coronavirus family is composed of four different genera: *Deltacoronavirus*, *Gammacoronavirus*, *Betacoronavirus*, and *Alphacoronavirus*. SARS-CoV-2 belongs to *Coronaviridae*[5]. The SARS-CoV-2 genome is a single-stranded positive-sense RNA of roughly 30,000 nucleotides. This genome encodes four structural proteins: membrane (M), envelope (E), nucleocapsid (N), and spike (S)[6]. The spike protein has recently emerged as the primary target antigen in the formulation of a SARS-CoV-2 vaccine[7]. Previously, we identified the candidate for a peptide-based vaccine against COVID-19 based on the spike protein[8]. In addition, the interaction between the host and the virus that causes infection involves a complex response of the immune system. In the normal state, the immune system of the host will respond to the presence of viruses/antigens by activating the complement pathway that will destroy infected cells. Antibodies, as one of the main components of the host's defense system, can facilitate the virus entry into the host cells, causing a lot of damage and resulting in quite severe clinical impacts. This paradoxical phenomenon is known as antibody-dependent enhancement (ADE). Recently, ADE has become a tipping point in the cultivation of antibody-based therapies and vaccines. Furthermore,

ADE sequences have developed in MERS-CoV, SARS-CoV-1, HIV, Dengue, Ebola, and Zika virus infection and vaccination[9,10].

The molecular epidemiological data of the Indonesian SARS-CoV-2 outbreak remains unclear. Furthermore, a vital tool for discovering new emerging viruses is research on molecular epidemiology[11]. There is an exigency to set up advanced studies in molecular epidemiology to comprehend the anticipated impacts of COVID-19[12]. In addition, D614G is a common amino acid mutation in the SARS-CoV-2 spike protein globally[13]. Zhang *et al*. described that this spike protein mutation transmits more efficiently[14] and is associated with enhanced viral loads in COVID-19 patients[13]. Therefore, we investigated the D614G mutation and ADE sequences in Indonesian SARS-CoV-2 isolates compared to other Southeast Asian countries.

MATERIALS AND METHODS

SARS-CoV-2 genome retrieval

We retrieved Indonesian SARS-CoV-2 isolate genomes from the GISAID EpiCoV database and used the reference virus Wuhan-Hu-1, extracted from GenBank, NCBI (Table 1). In addition, we used other isolates and other coronaviruses from GISAID EpiCoV and GenBank, NCBI (Table 3 and Table 4). In this study, we focused on the spike protein gene (3,822 bp) of SARS-CoV-2.

D614G mutation analysis

We performed the translation process of the genome using BioEdit v7.2.5 and then identified the D614G missense mutation status of all isolates.

ADE sequences analysis

The multiple sequence alignment was created by BioEdit v7.2.5 using the ClustalW algorithm. ADE sequences were analyzed according to Wang and Zand[9].

Spike protein visualization analysis

We performed 3D structure visualization by employing the SWISS-MODEL web server and PyMOL v2.4. We then edited the schematic diagram with BioRender.

RESULTS

We generated a representational 3D structure visualization of the spike protein from the Special Region of the Yogyakarta, Indonesia (YO-202449) isolate and, as a reference, the Wuhan-Hu-1 isolate from Wuhan, China. The protein model was rendered using the SWISS-MODEL web server and PyMOL v2.4, then edited using BioRender (Figure 1). We evaluated the sequences for the presence of the D614G missense mutation in the Indonesian isolates (Table 2) and other isolates (Table 3 and Table 4). In addition, we analyzed the ADE sequences of Indonesian isolates (Figure 2 and Table 2) and other isolates (Figure 2, Table 3, and Table 4).

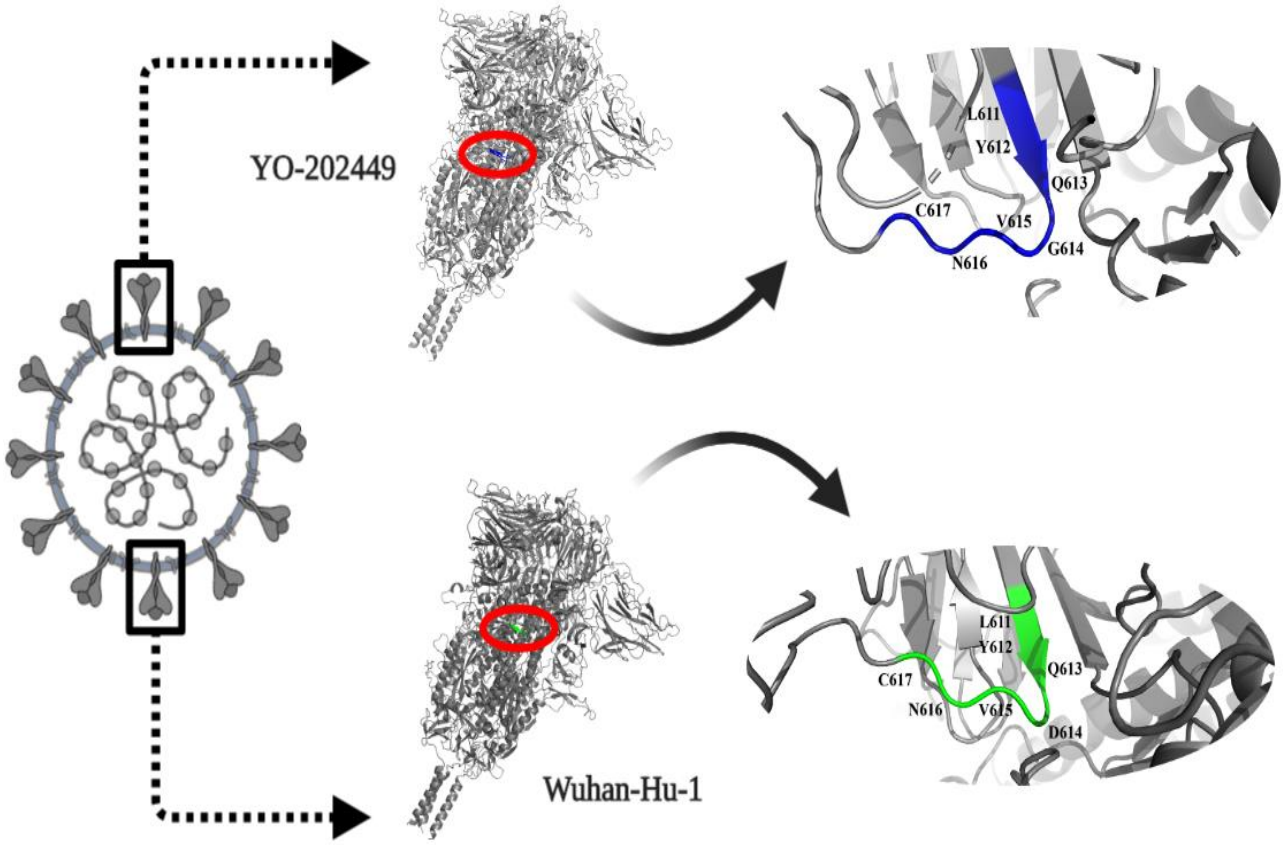

Figure 1. Representational 3D structure visualization of the YO-202449 isolate spike protein from the Special Region of Yogyakarta, Indonesia and Wuhan-Hu-1 isolate from Wuhan, China. "^{611}LYQGVNC^{617}" was the ADE sequence with D614G mutation shown in blue. We generated the structure using the SWISS-MODEL web server and PyMOL v2.4. This schematic diagram was edited with BioRender.

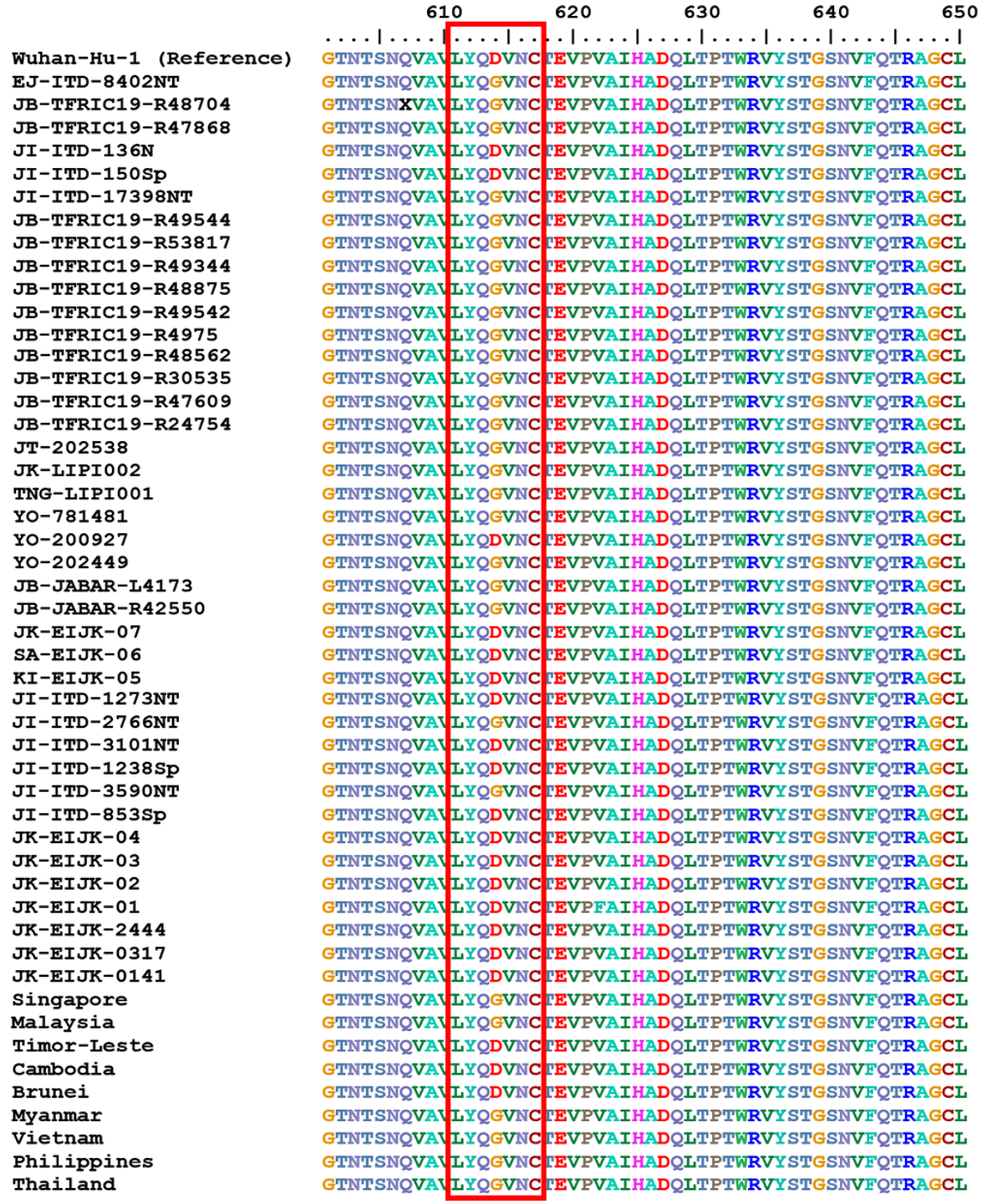

Figure 2. D614G mutation and ADE sequences in isolates from Indonesia and other Southeast Asian countries.

DISCUSSION

SARS-CoV-2, a novel coronavirus, is now widespread globally[15]. Since the virus emerged in Wuhan, China, the number of cases has been increasing rapidly[16]. In addition, human-to-human transmission of the novel virus has been reported[7] and the chance of aerosol infection was recognized by the WHO[17]. The novel virus has been collected from saliva, throat, bronchoalveolar-lavage, oropharyngeal swab, nasopharyngeal swab, and sputum[7]. We extracted 40 Indonesian isolate sequences from the database (Table 1). Based on the data, nasopharyngeal swab, oropharyngeal swab, and sputum methods were used to collect the virus and were submitted by many collaborators between research centers and universities in Indonesia. Currently, the GISAID EpiCoV database has recognized seven subtypes of SARS-CoV-2, specifically V, S, O, L, GR, GH, and G clades. Interestingly, Indonesian isolates were grouped in the G, GH, GR, L, and O clades. On the other hand, our molecular phylogenetic study revealed the relationship of SARS-CoV-2 and other *Coronaviridae* based on the four structural protein genes[8,11]. In line with this, SARS-CoV-2 is closest to *Rhinolophus affinis* coronavirus RaTG13, followed by pangolin coronavirus[18]. Thus, Malayan pangolin is assumed to be the intermediate host before the virus began infecting humans[19].

This study is the first to report the analysis of spike protein in Indonesian SARS-CoV-2 isolates in regards to the D614G mutation and the ADE sequence. These data might support the advancement of studies of biological aspects of SARS-CoV-2, vaccine design and antibody-based therapy, as well as epidemiological studies of markers of disease severity in secondary viral infections. Interestingly, Zhou *et al*. mentioned that the SARS-CoV-2 genome shares approximately 80% of its genome with SARS-CoV[20]. In addition, without any experimental data available, our analysis of the limited data of Indonesian SARS-CoV-2 isolates will potentially offer endless advantages. Duffy suggests that the mutation rates in RNA viruses are much higher than in most other microorganisms. An increased mutation rate might result in enhanced virulence and a higher prospect for adaptive evolution. This ability boosts the potency of zoonotic viral pathogens to establish human-to-human transmission and allows them to enhance their virulence[21]. Our study provides basic data for studies into the medication and prevention of COVID-19. Rapid discovery of mutations of SARS-CoV-2 is compulsory for the understanding of the COVID-19 pandemic in Indonesia.

Our previous study demonstrated the extensive *in silico* study of the structural protein genes of Indonesian SARS-CoV-2 isolates. We discovered peptide-based vaccines contrary to SARS-CoV-2 and many mutations were detected in Indonesian SARS-CoV-2 isolates[8,11]. Researchers around the world have been racing to develop COVID-19 vaccines, with at least 166 vaccine candidates in preclinical and clinical development. Currently, there were 27 vaccine candidates for COVID-19 in clinical evaluation and 139 vaccines in preclinical development. Of the 27 vaccines undergoing clinical evaluation the three lead candidates are viral-vectored and mRNA-based vaccines. A new pandemic vaccine development paradigm has been proposed that compresses the development timeline from 10-15 years to 1-2 years. In addition, vaccine design concerns the selection of antigens, vaccination routes, and vaccine platforms. Generally, vaccine platforms are divided into six groups, such as virus-like particles (VLPs), live attenuated virus, nucleic acid-based (DNA or mRNA) vaccines, recombinant viral-vectored vaccines, inactivated or killed virus, and protein subunit vaccines (Table 5)[22].

Researchers all over the world have reported mutations in the viral genome[7]. The rapid transmission and infectivity of the virus is associated with specific mutations in the genome[23]. Furthermore, one of the most notable amino acid mutations is the D614G. According to a recent study, the D614G mutation is related to the virulence of the virus and increased viral loads in COVID-19 patients[13,14]. Based on currently available information, there are several ways the D614G mutation can impact infectivity as well as increase receptor binding, fusion activation, or ADE enhancement. Another mechanism for mutation to the next form of D614G may only be through antibody escape facilitated by antigenic drift. If the D614G mutation in SARS-CoV-2 impacts neutralizing antibody sensitivity, or, alternatively, the ADE activity observed in the SARS-CoV study, D614G could also be an intermediate antibody escape mechanism that makes individuals more susceptible to second infections[24]. On the other hand, we also reported that the type of mutation emerged in the virus isolates originated from *Mus musculus*, canine, the environment, *Felis catus*, *Mustela lutreola*, and *Panthera tigris jacksoni* (Table 4). In this study, we reported the D614G mutation in Indonesian isolates, including JI-ITD-3590NT, JI-ITD-2766NT, JB-JABAR-R42550, JB-JABAR-L4173, YO-202449, YO-781481, TNG-LIPI001, JK-LIPI002, JT-202538, JB-TFRIC19-R24754, JB-TFRIC19-R47609, JB-TFRIC19-R30535, JB-TFRIC19-R48562, JB-TFRIC19-R4975, JB-TFRIC19-R49542, JB-TFRIC19-R49344, JB-TFRIC19-R48875, JB-TFRIC19-R53817, JB-TFRIC19-

Table 5. Immunological characteristics of COVID-19 candidate vaccine platforms according to Jeyanathan *et al*.[22].

Vaccine Platform	Antigens	Immunogenicity	Neutralizing Antibody Response
Virus-like particle	Multiple viral antigens	Weak, but greater than for protein subunits; requires repeated vaccination	Strong induction
Protein subunit vaccine	Spike protein or RBD	Weak; requires repeated vaccination	Strong induction
Inactivated virus	Multiple viral antigens	Weak; requires repeated vaccination	Strong induction
Live attenuated virus	Multiple viral antigens	Requires only a single delivery	Strong induction
DNA-based vaccine	Spike protein	Weaker than mRNA- based vaccine; requires repeated delivery	Response not as strong as for some of the viral vectors
mRNA-based vaccine	S protein or RBD encapsulated with nanoparticle	Requires repeated delivery	Depends on choice of adjuvant and formulation
Chimpanzee adenovirus (non-replicating; viral-vectored vaccines)	Spike protein	Unimpeded owing to lack of pre-existing antivector immunity	Strong with single delivery
Human serotype 26 adenovirus (non-replicating; viral-vectored vaccines)	Spike protein	Durability and quality affected by pre-existing antivector immunity	Weak; requires repeated or heterologous boost vaccination
Human serotype 5 adenovirus (non-replicating; viral-vectored vaccines)	Spike protein	Durability and quality affected by pre-existing antivector immunity	Strong with single delivery but hindered by pre-existing antivector immunity

R49544, JI-ITD-17398NT, JB-TFRIC19-R47868, JB-TFRIC19-R48704, and EJ-ITD-8402NT. All of the isolates originated from Java island (Central Java, Special Capital Region of Jakarta, Special Region of Yogyakarta, West Java, East Java, and Banten Provinces; Table 2). We also investigated the virus isolates from Southeast Asian countries and we found that the mutation occurred in six other Southeast Asian countries, including Singapore, Malaysia, Myanmar, Vietnam, Philippines, and Thailand. There were no available data of SARS-CoV-2 in Laos (Table 3). In addition, we evaluated the 3D structure to investigate the changing of amino acid residue 614 (Figure 1).

COVID-19 might quickly turn into acute respiratory distress syndrome (ARDS) in elderly patients, over sixty years old, with high mortality, and notably in individuals with comorbidities, such as diabetes, cancer, pulmonary diseases, and hypertension[9,25]. Moreover, coronaviruses that reportedly use ADE as another strategy to infect host cells are associated with facilitating viral entry and replication in the host cells[24]. Neutralization of viruses by antibodies occurs when antibody molecules bind to the surface epitope of the virus and block the process of viral attachment to receptor cells so that the virus is unable to enter the host cell. In the ADE phenomenon, antibodies that bind to virus particles fail to neutralize[26]. ADE of infection is a phenomenon that is a result of the interaction of virus–antibody immune complexes with Fcγ and/or complement receptors on host cells[27]. This can lead to viral fusion and entry in monocytes, B cells, and macrophages, enhancing viral generation, and reducing viral clearance[9] (Figure 3). In addition, the internalized immune complexes regulate the host immune system in order to increase viral replication and provoke illness severity[28]. The intrinsic and extrinsic mechanisms of ADE simultaneously assist in the escalation of viral replication and correspondingly higher viremia levels[9,10]. In the case of SARS-CoV, infected macrophages showed little or no interferon-β induction, which then led to the hypothesis that viral suppression of the immune response results in uncontrolled viral replication in respiratory epithelial cells. This can result in a high viral load leading to more tissue damage[29]. ADE is associated with triggering the cytokine storm, which implicates the robust generation of inflammatory cytokines and other chemical mediators[9,10].

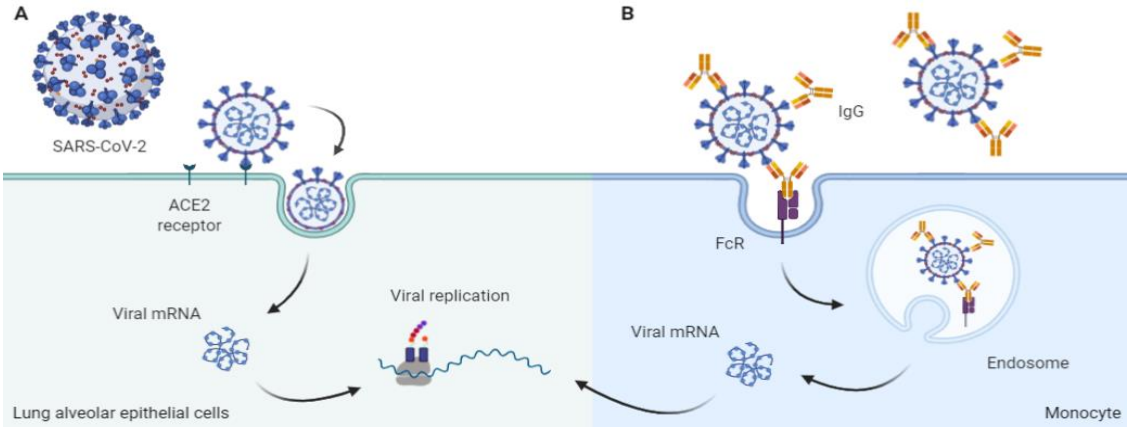

Figure 3. SARS-CoV-2 and ADE. (A) Mechanism of normal viral fusion is shown with binding of the SARS-CoV-2 spike protein to ACE2 in host cells. (B) In ADE, antibody binding to the spike protein both facilitates cell binding via the FcRγ and induces a conformational change in the spike protein, exposing the fusion domain. This figure was edited using BioRender according to Wang and Zand[9].

The implications of ADE for vaccine design and development have been described in several previous studies. Some examples of the vaccination-induced effects of ADE are respiratory syncytial virus (RSV) and measles, where severe disease was more common after vaccination with inactive virions[30]. Interestingly, the protein sequences responsible for ADE have been revealed on the SARS-CoV-2 spike protein[9]. In the present study, we identified that the ADE sequence is "^{611}LYQDVNC^{617}" in the Wuhan-Hu-1 isolate and changed into "^{611}LYQGVNC^{617}" in the recent mutated isolates, including Indonesian isolates and isolates from six other Southeast Asian countries (Table 2 and Table 3). Presently, the same pattern of ADE sequence is also identified in SARS-CoV-2 that infected animals and has been found in the environment globally (Table 4). We suggest that the development of therapeutic products, especially vaccination and antibody-based drug therapy should consider the ADE phenomenon as fundamentally associated with the efficacy and safety of therapeutic products. However, further studies related to this phenomenon should be studied more completely.

The impact of viral mutations on ADE risk remains unclear. In previous studies, mutations were known to block antibody binding to the Fcγ receptor, including LALA (L234A L235A), LALA-PG (L234A L235A P329G), and the eradication of glycosylation sites at N29731. Modification of the Fc portion can be made not only to eradicate binding to the Fcγ receptor, but also to enable for an increased immune response to the virus. The F241A mutation in the Fc region produces antibodies with a stronger endogenous immune reaction due to more systematic uptake of CD23 and greater immunogen generation[32]. However, how the mutation that causes the immune enhancement alters the risk of ADE is currently unknown. Likewise, the D614G mutation in SARS-CoV-2, which has an ADE sequence, has an impact on viral pathogenesis and response to the immune system still needs to be studied in more dept.

CONCLUSION

We conclude that the D614G mutation might affect ADE. We suggest that a high-speed, but cautious approach to the development of vaccines and other antibody-based

therapies for COVID-19 is needed until we have more data on the risks of the D614G mutation and ADE. However, advanced studies such as *in vitro* and *in vivo* assessment are relevant for validation.

FINANCIAL SUPPORT

This study was fully supported by the Professor Nidom Foundation, Indonesia (Grant Number: 003/PNF-RF/07/2020).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENT

We would like to take this opportunity to express our deepest condolences to the victims of COVID-19. We would also like to pay tribute to our frontline heroes who have fought continuously to this day. We additionally thank International Science Editing, Ireland for help in editing the manuscript.

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Table 1. Indonesian SARS-CoV-2 isolates extracted from the database.

No	Accession ID	Virus Name	GISAID Clade	Origin	Specimen Source	Sequencing Technology
1	NC_045512.2 (GenBank/Reference)	Wuhan-Hu-1	-	Wuhan (China)	Unknown	Illumina
2	EPI_ISL_529138	EJ-ITD-8402NT	GH	East Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina MiSeq
3	EPI_ISL_529718	JB-TFRIC19-R48704	GH	West Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina MiSeq
4	EPI_ISL_529719	JB-TFRIC19-R47868	G	West Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina MiSeq
5	EPI_ISL_529961	JI-ITD-136N	L	East Java (Indonesia)	Nasopharyngeal swab	Illumina MiSeq
6	EPI_ISL_529962	JI-ITD-150Sp	L	East Java (Indonesia)	Sputum	Illumina MiSeq
7	EPI_ISL_529963	JI-ITD-17398NT	GH	East Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina MiSeq
8	EPI_ISL_528759	JB-TFRIC19-R49544	GH	West Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina MiSeq
9	EPI_ISL_528753	JB-TFRIC19-R53817	GH	West Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina MiSeq
10	EPI_ISL_528752	JB-TFRIC19-R48875	GH	West Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina MiSeq
11	EPI_ISL_528751	JB-TFRIC19-R49344	GH	West Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina MiSeq
12	EPI_ISL_528750	JB-TFRIC19-R49542	GH	West Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina MiSeq
13	EPI_ISL_528749	JB-TFRIC19-R4975	GH	West Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina MiSeq
14	EPI_ISL_528748	JB-TFRIC19-R48562	G	West Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina MiSeq
15	EPI_ISL_528747	JB-TFRIC19-R30535	GR	West Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina MiSeq
16	EPI_ISL_528746	JB-TFRIC19-R47609	GH	West Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina MiSeq
17	EPI_ISL_528745	JB-TFRIC19-R24754	GH	West Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina MiSeq
18	EPI_ISL_525492	JT-202538	GH	Central Java (Indonesia)	Nasopharyngeal swab	Illumina MiSeq
19	EPI_ISL_518820	JK-LIPI002	GR	Special Capital Region of Jakarta (Indonesia)	Oropharyngeal and nasopharyngeal swab	Oxford Nanopore
20	EPI_ISL_518819	TNG-LIPI001	GH	Banten (Indonesia)	Oropharyngeal and nasopharyngeal swab	Oxford Nanopore
21	EPI_ISL_516829	YO-781481	GH	Special Region of Yogyakarta (Indonesia)	Nasopharyngeal swab	Illumina Miseq
22	EPI_ISL_516806	YO-200927	L	Special Region of Yogyakarta (Indonesia)	Nasopharyngeal swab	Illumina Miseq

23	EPI_ISL_516800	YO-202449	GH	Special Region of Yogyakarta (Indonesia)	Nasopharyngeal swab	Illumina Miseq
24	EPI_ISL_511879	JB-JABAR-L4173	GH	West Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina Miseq
25	EPI_ISL_511878	JB-JABAR-R42550	GH	West Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina Miseq
26	EPI_ISL_467376	JK-EIJK-07	L	Jakarta (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina Miseq
27	EPI_ISL_467375	SA-EIJK-06	L	North Sulawesi (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina Miseq
28	EPI_ISL_467374	KI-EIJK-05	L	East Kalimantan (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina Miseq
29	EPI_ISL_458081	JI-ITD-1273NT	L	East Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina Miseq
30	EPI_ISL_458082	JI-ITD-2766NT	GH	East Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina Miseq
31	EPI_ISL_458083	JI-ITD-3101NT	L	East Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina Miseq
32	EPI_ISL_458079	JI-ITD-1238Sp	L	East Java (Indonesia)	Sputum	Illumina Miseq
33	EPI_ISL_437188	JI-ITD-3590NT	GH	East Java (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina Miseq
34	EPI_ISL_437187	JI-ITD-853Sp	L	East Java (Indonesia)	Sputum	Illumina Miseq
35	EPI_ISL_437192	JK-EIJK-04	L	Special Capital Region of Jakarta (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina NextSeq 550
36	EPI_ISL_437191	JK-EIJK-03	L	Special Capital Region of Jakarta (Indonesia)	Nasopharyngeal swab	Illumina NextSeq 550
37	EPI_ISL_437190	JK-EIJK-02	L	Special Capital Region of Jakarta (Indonesia)	Nasopharyngeal swab	Illumina NextSeq 550
38	EPI_ISL_437189	JK-EIJK-01	L	Special Capital Region of Jakarta (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina NextSeq 550
39	EPI_ISL_435283	JK-EIJK-2444	O	Special Capital Region of Jakarta (Indonesia)	Nasopharyngeal swab	Illumina NextSeq 550
40	EPI_ISL_435282	JK-EIJK-0317	L	Special Capital Region of Jakarta (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina NextSeq 550
41	EPI_ISL_435281	JK-EIJK-0141	L	Special Capital Region of Jakarta (Indonesia)	Oropharyngeal and nasopharyngeal swab	Illumina NextSeq 550

Table 2. D614G mutation status and ADE sequences in Indonesian isolates.

No	Virus Name	D614G Mutation	ADE Sequence
1	Wuhan-Hu-1 (Reference)	D	LYQDVNC
2	EJ-ITD-8402NT	G	LYQGVNC
3	JB-TFRIC19-R48704	G	LYQGVNC
4	JB-TFRIC19-R47868	G	LYQGVNC
5	JI-ITD-136N	D	LYQDVNC
6	JI-ITD-150Sp	D	LYQDVNC
7	JI-ITD-17398NT	G	LYQGVNC
8	JB-TFRIC19-R49544	G	LYQGVNC
9	JB-TFRIC19-R53817	G	LYQGVNC
10	JB-TFRIC19-R48875	G	LYQGVNC
11	JB-TFRIC19-R49344	G	LYQGVNC
12	JB-TFRIC19-R49542	G	LYQGVNC
13	JB-TFRIC19-R4975	G	LYQGVNC
14	JB-TFRIC19-R48562	G	LYQGVNC
15	JB-TFRIC19-R30535	G	LYQGVNC
16	JB-TFRIC19-R47609	G	LYQGVNC
17	JB-TFRIC19-R24754	G	LYQGVNC
18	JT-202538	G	LYQGVNC
19	JK-LIPI002	G	LYQGVNC
20	JI-ITD-3590NT	G	LYQGVNC
21	TNG-LIPI001	G	LYQGVNC
22	YO-781481	G	LYQGVNC
23	JI-ITD-2766NT	G	LYQGVNC
24	YO-202449	G	LYQGVNC
25	JB-JABAR-L4173	G	LYQGVNC
26	JB-JABAR-R42550	G	LYQGVNC
27	YO-200927	D	LYQDVNC
28	JK-EIJK-07	D	LYQDVNC
29	SA-EIJK-06	D	LYQDVNC
30	KI-EIJK-05	D	LYQDVNC
31	JI-ITD-1273NT	D	LYQDVNC
32	JI-ITD-3101NT	D	LYQDVNC
33	JI-ITD-1238Sp	D	LYQDVNC
34	JI-ITD-853Sp	D	LYQDVNC
35	JK-EIJK-04	D	LYQDVNC
36	JK-EIJK-03	D	LYQDVNC
37	JK-EIJK-02	D	LYQDVNC
38	JK-EIJK-01	D	LYQDVNC
39	JK-EIJK-2444	D	LYQDVNC
40	JK-EIJK-0317	D	LYQDVNC
41	JK-EIJK-0141	D	LYQDVNC

Table 3. D614G mutation and ADE sequences in Southeast Asian countries.

No	Accession ID	Origin	D614G Mutation	ADE Sequence
1	NC_045512.2 (GenBank/Reference)	Wuhan (China)	D	LYQDVNC
2	EPI_ISL_516820	Singapore	G	LYQGVNC
3	EPI_ISL_516823	Singapore	G	LYQGVNC
4	EPI_ISL_516828	Singapore	G	LYQGVNC
5	EPI_ISL_524449	Singapore	D	LYQDVNC
6	EPI_ISL_524448	Singapore	D	LYQDVNC
7	EPI_ISL_501222	Malaysia	G	LYQGVNC
8	EPI_ISL_501207	Malaysia	G	LYQGVNC
9	EPI_ISL_501204	Malaysia	G	LYQGVNC
10	EPI_ISL_507000	Malaysia	D	LYQDVNC
11	EPI_ISL_506999	Malaysia	D	LYQDVNC
12	EPI_ISL_480602	Timor-Leste	D	LYQDVNC
13	EPI_ISL_480601	Timor-Leste	D	LYQDVNC
14	EPI_ISL_456612	Timor-Leste	D	LYQDVNC
15	EPI_ISL_456611	Timor-Leste	D	LYQDVNC
16	EPI_ISL_456610	Timor-Leste	D	LYQDVNC
17	EPI_ISL_411902	Cambodia	D	LYQDVNC
18	EPI_ISL_443187	Brunei	D	LYQDVNC
19	EPI_ISL_435677	Brunei	D	LYQDVNC
20	EPI_ISL_435676	Brunei	D	LYQDVNC
21	EPI_ISL_435675	Brunei	D	LYQDVNC
22	EPI_ISL_435674	Brunei	D	LYQDVNC
23	EPI_ISL_434709	Myanmar	G	LYQGVNC
24	EPI_ISL_512844	Myanmar	D	LYQDVNC
25	EPI_ISL_498191	Vietnam	G	LYQGVNC
26	EPI_ISL_498192	Vietnam	G	LYQGVNC
27	EPI_ISL_511891	Vietnam	G	LYQGVNC
28	EPI_ISL_511893	Vietnam	D	LYQDVNC
29	EPI_ISL_511892	Vietnam	D	LYQDVNC
30	EPI_ISL_491298	Philippines	G	LYQGVNC
31	EPI_ISL_491475	Philippines	D	LYQDVNC
32	EPI_ISL_491474	Philippines	D	LYQDVNC
33	EPI_ISL_491473	Philippines	D	LYQDVNC
34	EPI_ISL_491472	Philippines	D	LYQDVNC
35	EPI_ISL_515464	Thailand	G	LYQGVNC
36	EPI_ISL_512866	Thailand	G	LYQGVNC
37	EPI_ISL_512867	Thailand	G	LYQGVNC
38	EPI_ISL_512869	Thailand	G	LYQGVNC
39	EPI_ISL_512872	Thailand	D	LYQDVNC

Table 4. ADE sequence in coronaviruses.

No	Accession ID	Virus	Origin	Host	ADE Sequence
1	EPI_ISL_459910	SARS-CoV-2	China	*Mus musculus*	LYQDVNC
2	EPI_ISL_414518	SARS-CoV-2	Hong Kong	Canine	LYQDVNC
3	EPI_ISL_515398	SARS-CoV-2	USA	Environment	LYQGVNC
4	EPI_ISL_469256	SARS-CoV-2	China	Environment	LYQGVNC
5	EPI_ISL_429874	SARS-CoV-2	Italy	Environment	LYQGVNC
6	EPI_ISL_487275	SARS-CoV-2	Belgium	*Felis catus*	LYQGVNC
7	EPI_ISL_482820	SARS-CoV-2	Spain	*Felis catus*	LYQGVNC
8	EPI_ISL_483064	SARS-CoV-2	France	*Felis catus*	LYQGVNC
9	EPI_ISL_523111	SARS-CoV-2	Netherlands	*Mustela lutreola*	LYQGVNC
10	EPI_ISL_420293	SARS-CoV-2	USA	*Panthera tigris jacksoni*	LYQGVNC
11	EPI_ISL_410539	Pangolin Coronavirus	China	*Manis javanica*	LYQDVNC
12	EPI_ISL_402131	Bat Coronavirus RaTG13	China	*Rhinolophus affinis*	LYQDVNC
13	AY502924.1	SARS Coronavirus TW11	Taiwan	*Homo sapiens*	LYQDVNC
14	AY278488.2	SARS Coronavirus BJ01	China	*Homo sapiens*	LYQDVNC
15	AY390556.1	SARS Coronavirus GZ02	China	*Homo sapiens*	LYQDVNC
16	NC _019843.3	MERS Coronavirus HCoV-EMC/2012	Saudi Arabia	*Homo sapiens*	LFGSVAC
17	KF686346.1	Human Coronavirus HKU1	USA	*Homo sapiens*	LYRNLKC
18	KX344031.1	Human Coronavirus OC43	Mexico	*Homo sapiens*	LFRNIKC
19	NC_002645.1	Human Coronavirus 229E	Germany	*Homo sapiens*	VVGAMLS