

Detection of Representative Mutant Strains and a Case of Prolonged Infection by SARS-CoV-2 with Spike 69/70 Deletion in Japan

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Abstract: There is a concern that the spread of mutant strains of SARS-CoV-2 will increase the number of severe COVID-19 patients and weaken vaccine effectiveness in the world. The mutants of the spike region of SARS-CoV-2, such as N501Y, E484K, P681H, and deletion H69/V70 (del 69/70), were studied in 25 COVID-19 patients admitted from December 2020 to April 2021; there were no patients with N501Y and P681H, but nine patients had E484K alone. Their symptoms, laboratory data, and course of their disease were similar to those of the other patients with the non-mutant virus. One patient with del69/70 alone showed prolonged inflammation, viral excretion, and late exacerbation 18 days after onset. Del69/70 may be independently associated with evasion of immunity, as previously reported, and it is present in Japan.

Keywords: COVID-19, N501Y, E484K, P681H, PCR, remdesivir, vaccine

There has been a worldwide pandemic of coronavirus disease 2019 (COVID-19), including Japan, since February 2020, and the spread of mutant viral strains, such as N501Y and E484K, of the spike protein of SARS-CoV-2 has become a major issue.^{1,2}

N501Y was first reported from the United Kingdom as a representative mutant in the B.1.1.7 lineage (now called Alpha variant), and it was suggested that it has increased infectivity.^{2,3} In the B.1.1.7 lineage, P681H and deletion of H69/V70 (del 69/70) were also reported to be resistant to the anti-viral agent remdesivir and to have an allosterically changed spike protein conformation, respectively.³⁻⁵ E484K was found in South Africa and Brazil with the N501Y mutant, and they appear to be associated with decreased vaccine efficacy.² N501Y and E484K are representative and well-known mutant, but P681H and del69/70 are also important, because they could directly affect the treatment and pathophysiological features of the patients.

In Japan, it has been reported that the N501Y mutant and severe COVID-19 patients had increased rapidly in the western area, including the Osaka City area, and that the E484K alone mutant appeared in the eastern area, especially around the Tokyo Metropolitan area in April 2021.⁶ However, the prevalence of other mutants, such as P681H and del69/70, is unknown.

Therefore, the prevalence of these mutants was analyzed in 25 COVID-19 patients admitted to our hospital located in Tohoku rural area from December 2020 to April 2021 by polymerase chain reaction (PCR)-based detection

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kits (VirSNiP-SAS B1351 (484K+501Y), SARS-CoV-2 Spike P681Y, and del H69/N70, respectively; Roche, Basel, Switzerland).

Table 1 shows the results. There were no patients with N501Y and P681H, though some patients showed severe disease and delayed improvement with administration of remdesivir. In contrast, nine patients had the E484K mutation without N501Y and other mutants. These nine patients had mild to severe disease and ranged in age from young to old; therefore, there were no common clinical features.

However, one patient was found to have del69/70 alone, and this young patient with no underlying diseases showed prolonged symptoms and late exacerbation over two weeks after onset despite appropriate treatment. His symptoms, including dyspnea and general fatigue, increased on Day 18. The physical examination findings were as follows: temperature 39.2°C, blood pressure 110/72 mmHg, respiratory rate 24 breaths/min, and SpO₂ 94% on room air. His initial white

blood cell (WBC) count was 13,500/uL. The C-reactive protein (CRP) value was 23.67 mg/dL, and D-dimer was 3.57 mg/dL. However, almost no pneumonia and abnormalities, including ground glass opacities, were found on imaging examinations (Figure 1A and B). In addition, no superinfecting bacteria and fungi were detected. He received remdesivir and dexamethasone from Day 1, and, fortunately, recovered rapidly. As previously reported, del69/70 mutant independently might lead to evasion of immunity and resulted in prolonged viral excretion.

It is important to be vigilant regarding the existence of mutant strains other than N501Y and E484K if patients show delayed improvement despite receiving appropriate treatment in Japan and other areas where the mutants are not highly prevalent. This is a commentary from the single facility; therefore, we should plan the further region-wide investigations to detect the critical mutants associated with the pathogenesis of the COVID-19 in each area.

Table 1 Patients' Characteristics and Mutations Detected

Patient No.	Male/ Female	Age	WBC	CRP (mg/dl)	D-Dimer (ug/mL)	Duration of Symptoms (Days)	Mutation			
							del H69/ V70	P681H	E484K	N501Y
1	F	85	7200	1.15	0.88	5	None	None	None	None
2	M	71	12,800	2.42	7.91	4	None	None	None	None
3	M	86	11,700	7.53	1.64	5	None	None	None	None
4	M	74	6900	9.4	8.84	2	None	None	None	None
5	F	27	5900	0.02	0.51	4	None	None	None	None
6	F	50	8900	5.16	0.67	5	None	None	None	None
7	F	85	2400	3.84	0.83	4	None	None	None	None
8	M	64	4400	6.18	0.86	3	None	None	None	None
9	M	74	7300	0.71	1.41	2	None	None	None	None
10	F	81	2700	0.94	0.96	3	None	None	None	None
11	M	86	4400	7.79	2.19	3	None	None	None	None
12	F	25	4900	0.18	1.2	7	None	None	Positive	None
13	M	77	7800	2.12	2.67	4	None	None	Positive	None
14	F	67	3100	1.15	0.57	4	None	None	Positive	None
15	M	72	6000	0.29	8.41	3	None	None	None	None
16	F	75	3500	8.97	1.3	7	None	None	Positive	None
17	M	65	10,500	6.89	1.43	2	None	None	Positive	None
18	M	76	8600	12.01	1.68	3	None	None	Positive	None
19	M	87	5100	5.34	2.53	3	None	None	Positive	None
20	M	46	4700	0.02	0.12	2	None	None	Positive	None
21	M	68	5700	0.32	1.35	2	None	None	Positive	None
22	M	88	4800	0.38	0.96	2	None	None	None	None
23	M	79	3000	4.68	1.57	9	None	None	None	None
24	F	51	5900	3.2	1.34	5	None	None	None	None
25	M	39	13,400	25.94	3.37	18	Positive	None	None	None

Abbreviations: M, male; F, female; WBC, white blood cells; CRP, C-reactive protein.

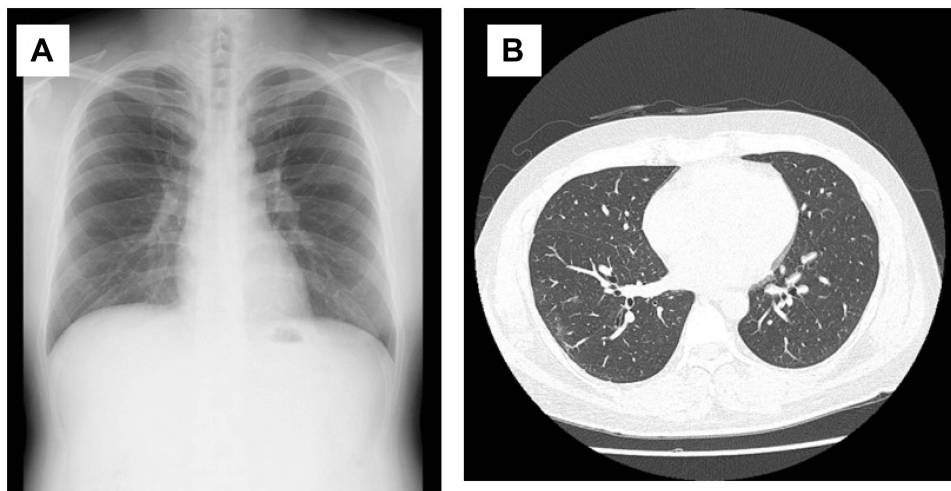


Figure 1 The chest X-ray (A) and computed tomography (CT) (B) findings of a 39-year-old COVID-19 patient. Almost no abnormal findings, including ground glass opacities (GGOs), are seen.

Ethics

The analysis was approved by the Committee for Clinical Scientific Research of Tohoku Medical and Pharmaceutical University Hospital on April 7, 2021 and July 14, 2021 (No. ID 2020-6-006 and 2021-2-032), respectively, and the patients provided written, informed consent for use of their specimens, although the samples were collected as part of routine laboratory analyses. The patient with del 69/70 was also provided written informed consent for the his cases details to be published.

Disclosure

The authors report no conflicts of interest in this work.

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