Viral kinetics of SARS-CoV-2 in patients with COVID-19

Da Young Kim[1], Ju-Hyung Lee[2], Hyeon Jeong Oh[2], Jun-Won Seo[2], Na-Ra Yoon[2], Choon-Mee Kim[2], and Dong-Min Kim[2]

[1]Chosun University College of Medicine
[2]Affiliation not available

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Abstract

As only few studies have analyzed viral kinetics between the incubation and symptomatic periods of COVID-19 patients, we investigated the viral kinetics and compared viral loads between patients with mild and severe COVID-19. We determined the viral kinetics of 10 patients diagnosed with COVID-19 at Chosun University Hospital. Six patients were classified into the "mild" group and 4 into the "severe" group according to supplemental oxygen use during admission. Samples were collected via nasopharyngeal swabs and sputum specimens. SARS-CoV-2 was detected using real-time reverse transcription-polymerase chain reaction (RT-PCR). Chest radiograph scores during hospitalization were obtained. Ct values of the upper respiratory tract specimens were low during the early stages after symptom onset but gradually increased over time in both groups. The severe group had lower Ct values than the mild group. The Ct values of the RdRP and E genes on day 6 after symptom onset were significantly lower in the severe group than in the mild group ($p < 0.05$). Three of 6 patients had positive results on RT-PCR even before symptom onset; 2 of them had the lowest Ct values. The chest radiograph scores were higher in the severe group than in the mild group, and the score in the severe group was the highest at approximately 3 weeks after symptom onset. Viral load and chest radiograph scores were significantly different between the severe and mild groups of COVID-19 patients.

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Running Title: Viral kinetics of SARS-CoV-2

Da Young Kim, M.D.[1] Ju-Hyung Lee, M.D.,Ph.D.[2,] Hyeon Jeong Oh, B.S.[1],Jun-Won Seo, M.D.[1] Na Ra Yoon, M.D.[1] Choon-Mee Kim, Ph.D.[3], Dong-Min Kim, M.D.,Ph.D.[1]

Affiliations:

[1]Department of Internal Medicine, College of Medicine, Chosun University, Gwangju, Republic of Korea

[2]Department of Preventive Medicine, Jeonbuk National University Medical School, Jeonju, Republic of Korea

[3]Department of Premedical Science, College of Medicine, Chosun University, Gwangju, Republic of Korea

Da Young Kim and Ju-Hyung Lee contributed equally to this paper.

Address of correspondence for Dong-Min Kim

Department of Internal Medicine, School of Medicine, Chosun University,

365, Pilmun-daero, Dong-gu, Gwangju Metropolitan City, 61453, Republic of Korea

Tel: 82-62-220-3108 Fax: 82-62-234-9653

E-mail address:*drongkim@chosun.ac.kr*

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Summary

As only few studies have analyzed viral kinetics between the incubation and symptomatic periods of COVID-19 patients, we investigated the viral kinetics and compared viral loads between patients with mild and severe COVID-19.

We determined the viral kinetics of 10 patients diagnosed with COVID-19 at Chosun University Hospital. Six patients were classified into the "mild" group and 4 into the "severe" group according to supplemental oxygen use during admission. Samples were collected via nasopharyngeal swabs and sputum specimens. SARS-CoV-2 was detected using real-time reverse transcription-polymerase chain reaction (RT-PCR). Chest radiograph scores during hospitalization were obtained.

Ct values of the upper respiratory tract specimens were low during the early stages after symptom onset but gradually increased over time in both groups. The severe group had lower Ct values than the mild group. The Ct values of the *RdRP* and *E* genes on day 6 after symptom onset were significantly lower in the severe group than in the mild group ($p < 0.05$). Three of 6 patients had positive results on RT-PCR even before symptom onset; 2 of them had the lowest Ct values. The chest radiograph scores were higher in the severe group than in the mild group, and the score in the severe group was the highest at approximately 3 weeks after symptom onset.

Viral load and chest radiograph scores were significantly different between the severe and mild groups of COVID-19 patients.

Introduction

The World Health Organization declared a pandemic in March 2020 after the emergence of an infectious disease (COVID-19) in Wuhan, Hubei Province, China, in December 2019 that was caused by a novel coronavirus (SARS-CoV-2) (Coronavirus disease 2019 (COVID-19) Situation Report – 89. Geneva: World Health Organization, 2020). In Korea, as of May 15, 2020, there were a cumulative total of 10,991 confirmed COVID-19 cases and 260 deaths (Center for Systems Science and Engineering. Coronavirus COVID-19 global cases. 2019). The relationship between the viral load of SARS-CoV-2 and the severity of COVID-19 has not been studied. Only a few studies have compared the viral kinetics and chest radiograph score between patients with mild and those with severe COVID-19.

Therefore, this study was conducted to investigate the viral kinetics of COVID-19 patients diagnosed at Chosun University Hospital, Gwangju, Korea to compare the viral load between patients with mild and those with severe COVID-19.

Materials and Methods

We investigated the viral kinetics of 10 COVID-19 patients diagnosed at Chosun University Hospital, Gwangju, Korea. Of the 10 patients, 6 were classified into the "mild" group and 4 into the "severe" group on the basis of their supplemental oxygen use during admission.

Chest radiograph scores were calculated by using 121 chest radiographs obtained for the 10 patients during admission at their bedside. The scores were obtained by dividing each lung into the upper, middle, and lower zones and scoring each zone from 0 to 4 points on the basis of the degree of infiltration. The scores of each lung were then added considering a total of 6 zones, yielding a total score ranging from 0 to 24 (Conway et al., 1994).

Ribonucleic acid (RNA) was extracted using a nasopharyngeal swab to detect SARS-CoV-2. The sputum specimens were quantified by using the real-time reverse transcription-polymerase chain reaction (RT-PCR) assay, targeting the *E* and *RdRP* genes. A SARS-CoV-2 test kit manufactured by Kogene Biotech (Seoul, Korea) was used, with a cut-off cycle threshold (Ct) value of higher than 35 cycles. Moreover, we compared the positive rate of SARS-CoV-2 on the PCR test when the cut-off value was 38 cycles and 35 cycles.

The Ct value of the test results performed on the day of symptom onset and incubation period or asymptomatic phase were included in the results for day 0 since symptom onset. The test results from day 2 to day 4 since the day of symptom onset were included in the results for day 3 since symptom onset. The test results from day 5 to day 7 day since the day of symptom onset were included in the results for day 6 since symptom onset.

Moreover, the Ct values between the severe and mild groups were compared with the Wilcoxon rank sum test by using Stata 14.2 (Stata LLC, TX, USA). P-values less than 0.05 were considered significant.

Results

The median age of the 10 patients (6 men and 4 women) with COVID-19 was 44.95 years (range, 29–79 years). Of the 10 patients, 4 had hypertension, 2 were taking antihypertensive medications, 1 was receiving an angiotensin-converting enzyme inhibitor, and 1 was receiving an angiotensin II receptor blocker. On the basis of their supplemental oxygen use, the patients were divided into the mild and severe groups. Of the 4 patients in the severe group, 2 were intubated and were on mechanical ventilators, including 1 patient supported with a high-flow nasal cannula via nasal prongs and 1 with nasal prongs only. Four patients in the severe group received lopinavir/ritonavir. In the mild group, 1 elderly patient with pneumonia received lopinavir/ritonavir and 1 with consistently low Ct values received hydroxychloroquine. The remaining patients in the mild group did not receive antiviral medications (Table 1).

Among the patients with COVID-19 observed in this study, the Ct values of the upper respiratory tract specimens were low during the early stages after symptom onset but gradually increased over time in both the severe and mild groups. Moreover, patients in the severe group had lower Ct values than those in the mild group did. The Ct values of the*RdRP* and *E* genes on day 6 after symptom onset were significantly lower in the severe group than in the mild group ($p < 0.05$). The values increased at a slower rate in the severe group than those in the mild group did during the first 3–6 days after symptom onset. Moreover, virus shedding lasted longer in the severe group (Figure 1).

The viral load in the lower respiratory tract specimens remained undetectable in the mild group 7 days after symptom onset. However, virus shedding persisted for more than 3 weeks in the severe group (Figure 2; see Appendix Figure 1 and Appendix Figure 2).

The positive rate of the SARS-CoV-2 on the PCR test—performed with a nasopharyngeal swab targeting the*RdRP* gene during the admission period—was 48.3% when the cut-off value was 35 and 74.1% when the cut-off value was 38. When the*E* gene was targeted, the positive rate of the SARS-CoV-2 on the PCR test performed with the nasopharyngeal swab was confirmed to be 55.2% when the cut-off value was 35 and 74.1% when the cut-off value was 38. The positive rate of the SARS-CoV-2 on the PCR test—performed with a sputum specimen targeting the *RdRP* gene—was 43.2% when the cut-off value was 35 and 50.0% when the cut-off value was 38. When the *E* gene was targeted, the positive rate of the SARS-CoV-2 on the PCR test performed with the sputum specimen was confirmed to be 46.3% when the cut-off value was 35 and 50.0% when the cut-off value was 38.

During hospitalization, Ct values for both the mild and severe group tended to be lower at symptom onset and tended to increase with time. In particular, 3 of 6 patients showed positive results on PCR even before symptom onset, and 2 of them had the lowest Ct values.

Of the 3 patients, one had a Ct value of 16.19 (nasopharyngeal swab,*E* gene) 2 days before symptom onset and the other had a Ct value of 13.75 (sputum specimen, *E* gene) 1 day before symptom onset. The Ct value of the test results during the asymptomatic phase of these 3 patients (patient E, patient G, and patient J) was included in the result of day 0 after symptom onset, as shown in Figure 3.

The mean ± standard error of the mean (SEM) Ct value obtained using the nasopharyngeal swab during the incubation period was 22.20 ± 4.0 when the *RdRP* gene was targeted and 21.28 ± 3.88 when the *E*gene was targeted. The mean Ct value obtained using the sputum specimen was 22.46 ± 6.02 when the *RdRP* gene was targeted and 23.33 ± 7.18 when the *E* gene was targeted during the incubation period. The lowest Ct

value was observed on day 3 after symptom onset, and the mean Ct value obtained using the nasopharyngeal swab was 21.49 ± 1.91 when the *RdRP* gene was targeted and 21.19 ± 1.94 when the *E*gene was targeted. The mean Ct value obtained using the sputum specimen was 19.76 ± 1.61 when the *RdRP* gene was targeted and 18.88 ± 1.01 when the *E* gene was targeted (Figure 3; Table 2).

The chest radiograph scores were higher in the severe group than in the mild group and were significantly different on days 9, 12, and 15 after the onset of symptoms. Moreover, the chest radiograph scores in the severe group were the highest at approximately 3 weeks after symptom onset (Figure 4; see Appendix Figure 3).

Discussion

In a previous analysis of 76 confirmed COVID-19 patients in China, the number of viral copies was higher in the lower respiratory tract than in throat swabs and in nasal swabs when the *ORF1ab* and *N*genes were targeted by using RT-PCR (Yu et al., 2020).

In addition, in sputum specimens, the number of viral copies was higher in the early and progressive stages than in the recovery stage (Yu et al., 2020).

In another study of 18 confirmed COVID-19 patients in China, PCR was performed for the *ORF1ab* and *N* genes by using nasal and throat swabs. A high number of viral copies was confirmed in the test that was performed not long after the onset of symptoms, and the number of copies was higher in the nasopharyngeal sample than in the oropharyngeal sample. In addition, the viral load was detected in both symptomatic and non-symptomatic patients (Zou et al., 2020).

Considering the information in the literature about reported cases of severe acute respiratory syndrome (SARS), the viral load increased from the early phase of the disease after symptom onset and peaked after approximately 10 days. The viral load then gradually decreased, and virus shedding was observed from 10 to 21 days (Peiris et al., 2003). In another study on SARS, the viral load in nasopharyngeal swabs peaked on day 10 after the onset of symptoms. The serum viral load showed a proportional relationship with oxygen saturation reduction, mechanical breathing, and death (Hung et al., 2004).

Only a few studies have compared the number of viral copies of SARS-COV-2 during the incubation period and symptomatic phase.

Studies involving patients with Middle East respiratory syndrome (MERS) have also shown that the mortality rate in MERS patients increased with a high viral load (Min et al., 2016). In addition, in studies on the viral load and severity of MERS-CoV, a lower Ct value in the sample taken from the upper respiratory tract was associated with a higher mortality rate and higher ICU hospitalization rate (Feikin et al., 2015). Another study showed that the peak and mean viral loads tended to be similar between MERS patients and SARS patients (Corman et al., 2016).

In other respiratory viruses (e.g., respiratory syncytial virus [RSV]), a high viral load in the upper respiratory tract is associated with disease severity (Fuller et al., 2013).

However, in the current study, both the severe and mild groups of patients with COVID-19 showed low Ct values at symptom onset. One patient in the mild group even had a very low Ct value in the incubation period. The Ct values of the lower respiratory tract specimens were also low in the early phase after the onset of symptoms in the patients in the mild group, exhibiting a different trend from that of patients with other respiratory viruses such as MERS, SARS, and RSV.

In a study on SARS patients, 60 of 75 patients (80%) showed radiological worsening after a mean of 7.4 days (Fuller et al., 2013). In another study of 17 patients with MERS, the chest radiograph scores peaked at approximately 2 weeks after the onset of symptoms (Oh MD et al.,2016).

However, in the current study, the chest radiograph scores peaked in the severe group at approximately 3 weeks after the onset of symptoms.

The viral load of COVID-19 patients was high at the onset of symptoms. The patients in the mild group also showed a high number of virus copies in the early stages of the disease. Even in the incubation period, very low Ct values were observed.

Therefore, it is necessary to evaluate whether an asymptomatic infected person can transmit the virus even in the incubation period.

Studies are currently underway to confirm the contagiousness of the virus even during the incubation period by determining whether the virus can be cultured during the incubation period.

Acknowledgments

None.

Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article.

Conflict of Interest

None.

Ethical statement

Ethical approval for this study was granted by the Institutional Review Board of the Chosun University Hospital (CHOSUN 2020-02-011).

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Table 1. Patient characteristics

Group	Patient	Age/Sex	Underlying disease	ACEi/ARB	Treatment (day
Severe group (O_2 inhalation needed)	A	79/F	DM	None	Lopinavir/ritonav
	B	79/M	HTN/DM	None	Lopinavir/ritonav
	C	75/F	HTN/CVA	None	Lopinavir/ritonav
	D	61/F	None	None	Lopinavir/ritonav
Mild group (O_2 inhalation not needed)	E	46/M	HTN/Dyslipidemia	None	None
	F	30/M	HTN	Azilsartan	None
	G	30/M	None	None	None
	H	29/F	None	None	None
	I	74/M	HTN/DM	Perindopril	Lopinavir/ritonav
	J	36/M	None	None	Hydroxychloroqui

Abbreviation: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; DM, diabetes mellitus; HTN, hypertension; CVA, cerebrovascular accident; MV, mechanical ventilator; NP, nasal prong; HF, high-flow nasal cannula

The incubation period (days)$^{+}$is the period from the date of symptom onset to confirmation

A negative value, -2^{++} and -1^{++}, indicates the number of days before symptom onset

Table 2. Mean Ct values when the *E* gene and *RdRP*gene were targeted to detect SARS-CoV-2 on the basis of the days after symptom onset

Days after symptom onset	Ct value (Mean ± SEM)	Ct value (Mean ± SEM)	Ct value (Mean ± SEM)	Ct value (Mean ± SEM)	Ct value (Mean ± SEM)	Ct value (Mean ± SEM)	Ct value (Mean ± SEM)	Ct value (Mean ± SEM)
	Upper airway swab (*RdRP* gene)	Upper airway swab (*RdRP* gene)	Upper airway swab (*E* gene)	Upper airway swab (*E* gene)	Sputum specimen (*RdRP* gene)	Sputum specimen (*RdRP* gene)	Sputum specimen (*E* gene)	Sputum specimen (*E* gene)
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0+	22.20	4.00	21.28	3.88	22.46	6.02	23.33	7.18
3	21.49	1.91	21.19	1.94	19.76	1.60	18.88	1.01
6	31.16	3.02	32.11	3.26	29.07	3.92	32.30	4.79

Days after symptom onset	Ct value (Mean ± SEM)	Ct value (Mean ± SEM)	Ct value (Mean ± SEM)	Ct value (Mean ± SEM)	Ct value (Mean ± SEM)	Ct value (Mean ± SEM)	Ct value (Mean ± SEM)	Ct value (Mean ± SEM)
9	32.83	2.25	32.46	2.24	33.86	2.72	29.86	3.30
12	35.25	0.98	34.17	1.16	35.55	1.79	34.86	2.04
15	37.62	1.06	37.73	1.22	36.72	2.22	37.20	1.96
18	34.00	1.68	33.32	1.75	38.79	1.21	37.71	1.58
21	38.64	1.37	38.72	1.29	37.18	2.82	36.62	3.39
24	36.22	NA	36.50	NA	35.637	4.33	33.28	NA
27	35.80	0.45	36.25	0.17	40.00	NA	40.00	0.00
30	40.00	NA	40.00	NA	40.00	NA	40.00	NA

Abbreviation: SEM, standard error of the mean; NA, not available

$^{+}$Asymptomatic phase or incubation period was included in day 0

Figure legends

Figure 1. Ct values when the *RdRP* gene (A) and *E*gene (B) were targeted by using nasopharyngeal swabs to detect SARS-CoV-2 on the basis of the days after symptom onset

The Ct values between the severe and mild groups were compared with the Wilcoxon rank sum test.

Figure 2. Ct values when the *RdRP* gene (A) and *E*gene (B) were targeted by using sputum specimens to detect SARS-CoV-2 on the basis of the days after symptom onset

The Ct values between the severe and mild groups were compared with the Wilcoxon rank sum test.

Figure 3. Ct values when the *RdRP* gene and *E* gene were targeted to detect SARS-CoV-2 on the basis of the days after symptom onset in all the patients

Figure 4. Chest radiograph scores according to the days after symptom onset

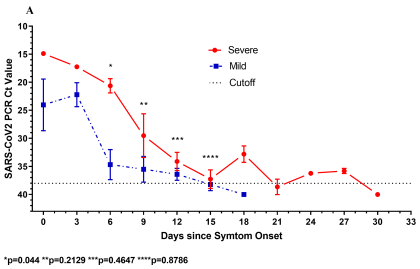
The chest radiograph scores between the severe and mild groups were compared with the Wilcoxon rank sum test.

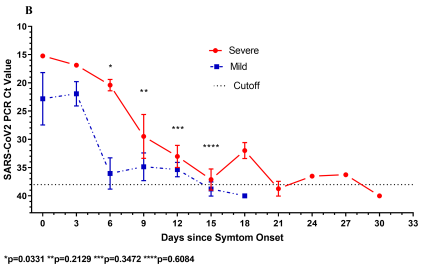
Appendix figure

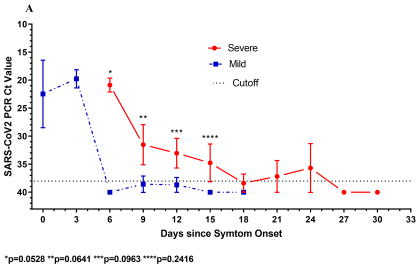
Appendix Figure 1. Ct values when the *RdRP* gene and*E* gene were targeted by using nasopharyngeal swabs and sputum specimens to detect SARS-CoV-2 on the basis of the days after symptom onset in the severe group

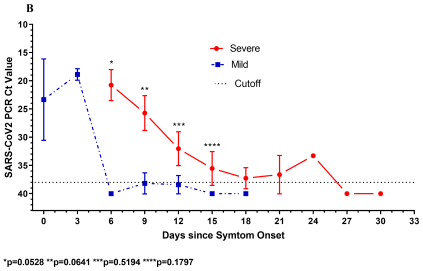
Appendix Figure 2. Ct values targeting the *RdRP* gene and*E* gene by using nasopharyngeal swabs and sputum specimens to detect SARS-CoV-2 on the basis of the days after symptom onset in the mild group

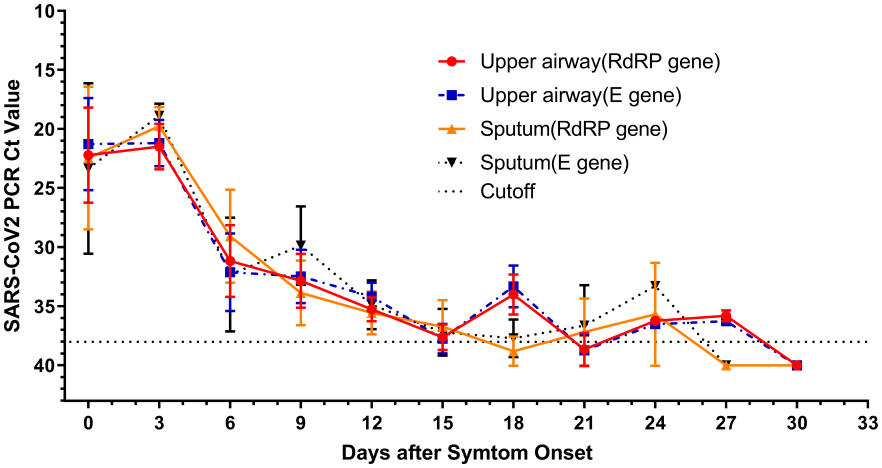
Appendix Figure 3. Chest radiograph scores according to the days after symptom onset

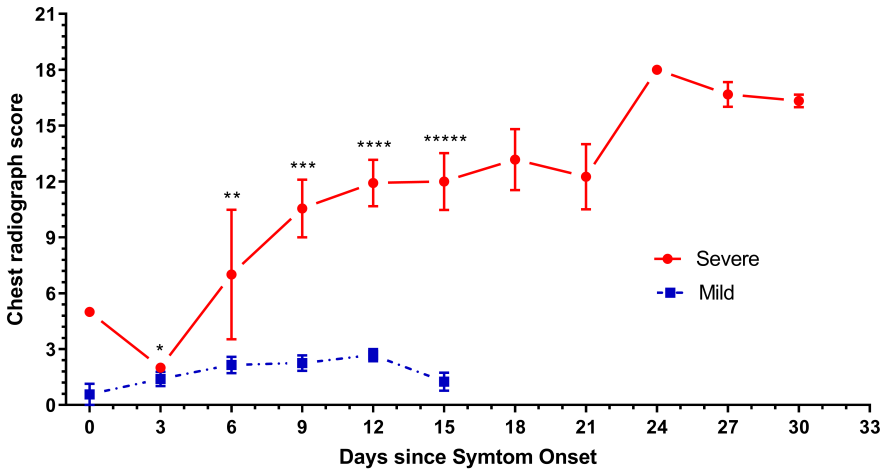












*p=0.4414 **p=0.0741 ***p=0.0005 ****p=0.0069 *****p=0.0035