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Real-time RT-PCR Ct value is not associated with COVID-19 disease severity: an observational study in tertiary COVID-19 referral hospital of West Java, Indonesia

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Abstract. – OBJECTIVE: Real-time reverse transcription-polymerase chain reaction (RT-PCR) is the gold standard examination to confirm coronavirus disease 2019 (COVID-19). This study aimed to determine the association between RT-PCR Ct value and COVID-19 clinical severity in the second week of illness.

PATIENTS AND METHODS: This study was a cross-sectional study. Data were collected from medical records of COVID-19 patients at the tertiary COVID-19 referral hospital of West Java, Dr. Hasan Sadikin Hospital Bandung, from January to May 2021. A total of 207 patients who met inclusion criteria were divided into four severity groups. The data were analyzed with One Way ANOVA Test.

RESULTS: There was no significant difference in RT-PCR Ct value among mild, moderate, severe and critical groups measured in the second week of illness, with *p*=0.825 for Ct Helicase/ORF1b gene, *p*=0.821 for Ct RdRp gene and *p*=0.870 for the lowest Ct gene.

CONCLUSIONS: Although Ct value reflects viral load, its role is concluded to be clinically insignificant in terms of association with the severity of COVID-19 in the second week of illness.

Key Words:
Reverse transcription-polymerase chain reaction (RT-PCR), Ct value, COVID-19 severity.

Introduction

Coronavirus Disease 2019 (COVID-19) has become a global problem since its first identification in Wuhan, China in December 2019[1]. The disease severity varies from asymptomatic to multi-organ disorders, leading to death[2]. The gold standard examination for COVID-19 diagnosis is a real-time reverse transcription-polymerase chain reaction (RT-PCR) conducted using bronchoalveolar fluid, sputum, nasal swab, pharyngeal swab, stool, blood, and urine specimens, each of which has different accuracy values[3-5]. The RT-PCR examination provides Ct value, defined as the number of amplification cycles required for a target gene to exceed the threshold level[6].

The Ct value is influenced by many factors, such as days of illness since symptom onset, technician competence, tool calibration, reagents used, and the analytical ability of technicians who perform interpretations[4]. As the Ct value reflects the amount of genetic material (RNA) in each sample, a low Ct value correlates with a high viral load depending on the COVID-19 severity degree[7]. Some studies[8-10] suggested that the viral load in the respiratory tract could be the predictor of disease severity. Disease worsening occurs in the second week of illness if there is no resolution of the disease[11]. Few studies[11-13] reported a high viral load in more severe patients during the second week. Concurrently, hyperactivity of inflammatory mediators occurred, leading to cytokine storm and disease deterioration[14]. This condition is often referred as the second week crash[15].

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We investigated the association between the Ct value of SARS-CoV-2 RT-PCR and the COVID-19 clinical severity in the second week of illness.

Patients and Methods

This retrospective study was conducted at Dr. Hasan Sadikin General Hospital Bandung, the tertiary COVID-19 referral hospital in West Java province, Indonesia. All patients with an RT-PCR confirmed COVID-19 between January 1st and May 31st 2021 were enrolled as participants. This study has been approved by Dr. Hasan Sadikin General Hospital Ethics Committee, as stated in letter No. LB.02.02 /X.6.5/151/2021, in compliance with the Declaration of Helsinki.

RT-PCR Kit with a mBioCoV-19 reagent was used in this study. Ct values were obtained based on the RdRp, Helicase/ORF1b genes, as well as the lowest gene values between RdRp and Helicase/ORF1b. COVID-19 clinical severity was assessed during the second week of illness, as previous studies[16,17] found that the course of this disease improves or worsens to a severe or critical stage at that time. This was classified according to the National Institutes of Health (NIH) criteria into mild, moderate, severe, and critical cases. The mild case was defined as uncomplicated upper respiratory tract viral infections in patients with non-specific symptoms and no evidence of viral pneumonia or hypoxia. The moderate case was described as oxygen saturation > 94% with clinical and imaging evidence of lower respiratory tract disease, while severe case was referred to oxygen saturation < 94%, PaO_2/FiO_2 < 300, respiratory rate > 30 x/minute, or lung infiltrates > 50%. The critical case was defined as respiratory failure, septic shock, and multi-organ dysfunction[18]. Moreover, mean Ct values were compared among all groups of COVID-19 clinical severity, but asymptomatic cases were not included because the exact day of illness was difficult to be determined.

Patients aged ≥ 18 years old which were confirmed to be COVID-19 positive based on nasopharyngeal and oropharyngeal swab tests performed by Dr. Hasan Sadikin General Hospital technician with the Ct value of RdRp and Helicase/ORF1b genes in second week of illness were included in this study. Those with secondary bacterial pneumonia during the second week of illness (defined as procalcitonin > 0.5 ng/ml) and pregnant women were excluded.

All data were collected using Research Electronic Data Capture (REDCap). Furthermore, among the 784 confirmed COVID-19 adult patients between January 1st and May 31st 2021, only 207 patients met the inclusion criteria. The basic characteristics, history taking, comorbid disease, physical examination, and laboratory results were obtained on the same day that the PCR swab test was conducted.

Data were analysed using bivariate analysis with one-way ANOVA. Variables that were analysed Ct values difference among Helicase/ORF1b, RdRp and the lowest gene groups based on the clinical COVID-19 severity. A *p*-value less than 0.05 was considered statistically significant with a two-tailed hypothesis. Data normality was assessed using Shapiro-Wilk. Statistical analysis was performed using Statistical Product and Service Solution (SPSS) 18.0 version for Windows (Chicago, IL, USA).

Results

Baseline characteristics of the patients are presented in Table I.

Between January 1st and May 31st 2021, a total of 207 patients were admitted to the study site based on an RT-PCR confirmed diagnosis of COVID-19 that matched the inclusion and exclusion criteria. Females and males were distributed evenly (51.7% *vs.* 48.3%), with the mean age of 55 ± 14 years old. A total of 39 patients (18.8%) had mild disease, followed by 64 (30.9%) moderate disease, 61 (29.4%) severe disease, and 43 (20.7%) patients with critical disease. The most common symptoms are cough (74.9%), fever (69.1%), and dyspnea (62.8%). Hypertension and diabetes mellitus were found to be the most frequent comorbidity in 83 (40.1%) and 57 (27.5%) patients, respectively. Laboratory parameters of the patients are also shown in Table I.

The distribution of the Ct values and clinical severity of illness are shown in Table II. Table II showed there was no significant difference in Ct values among mild, moderate, severe and critical cases, with *p*-value = 0.825 for Ct Helicase/ORF1b gene, *p*-value = 0.821 for Ct RdRp gene and *p*-value = 0.870 for the lowest Ct gene, hence the Ct values were not associated with the COVID-19 clinical severity. The mean, minimum and maximum values for each Ct gene value are presented in Figures 1-3.

Table I. Baseline characteristics.

Variables	Total (n = 207)
Age (years)[a]	55 ± 14
Age[c]	
18-39 years	39 (18.8)
40-60 years	86 (41.5)
> 60 years	82 (39.6)
Sex[c]	
Male	100 (48.3)
Female	107 (51.7)
Clinical severity[c]	
Mild	39 (18.8)
Moderate	64 (30.9)
Severe	61 (29.5)
Critical	43 (20.8)
Symptoms[c]	
Fever	143 (69.1)
Cough	155 (74.9)
Headache	32 (15.5)
Runny nose	24 (11.6)
Anosmia	20 (9.7)
Dysphagia	23 (11.1)
Dyspnea	130 (62.8)
Weakness	43 (20.8)
Myalgia	26 (12.6)
Diarrhea	20 (9.7)
Vomit	24 (11.6)
Physical examination	
Consciousness[c]	
Compos mentis	184 (88.9)
Somnolen	13 (6.3)
Sopor	10 (4.8)
Blood pressure[b]	
Systole	120 (80-180)
Diastole	80 (60-100)
Pulse rate[b]	88 (56-129)
Respiration rate[b]	22 (13-45)
Temperature (°C)[b]	36.7 (35.9-39.0)
Oxygen saturation (room air)	93 (40-100)
Mechanical ventilator[c]	25 (12.08)
Comorbid[c]	
Hypertension	83 (40.1)
Diabetes mellitus	57 (27.5)
Heart disease	38 (18.4)
Kidney disorder	29 (14.0)
Laboratory examination[b]	
Haemoglobin (gr/dL)	13 (6.3-18)
Hematocrit (%)	38.9 (15-51.5)
Leukocytes (10^3/µL)	7.77 (0.09-43.2)
Platelets (10^3/µL)	273.5 (16-829)
Lymphocytes (%)	17 (2-65)
Total lymphocyte count (TLC) (10^3/µL)	1.13 (0.04-4.03)
Neutrophil lymphocyte ratio (NLR)	4.62 (0.37-49)
Lactate dehydrogenase (LDH) (IU/L)	326 (145-1475)
Creatinine (mg/dL)	0.88 (0.05-11.53)
Procalcitonin (ng/mL)	0.07 (0.01-0.5)

Note: [a]Mean ± SD, [b]Median (min-max), [c]n (%), otherwise presented. *Abbreviations:* TLC, total lymphocyte count; NLR, neutrophil-to-lymphocyte ratio; LDH, lactate dehydrogenase.

Discussion

This study elucidated the relationship between Ct values and COVID-19 severity in the second week of symptom onset, during which deterioration of the disease usually occurs[11]. A previous study[19] showed that acute respiratory distress syndrome (ARDS) may occur on the ninth day of onset. Ragab et al[20] also reported that disease worsening often occurs 1-2 weeks after the symptom onset, as documented by radiological findings. Cytokine storm which is an underlying mechanism of the mortality and poor outcome was also reported at the beginning of the second week[21].

Several factors may affect the severity outcome of COVID-19, therefore both viral and host factors ought to be considered. The quantity, virulence factor, and host immunity are crucial, as seen in the patients' characteristics. Obesity, smoking history, older age, and male gender were previously reported to worsen the disease[22]. In our study, the median age of the patients was 55 ± 14 years old, with most patients being in the 40-60 years old group, followed by the greater than 60 years old group. Older age was associated with abnormal innate and adaptive immune responses. The elderly exhibit continuous production of inflammatory mediators and cytokines, aberrant ciliary function, and ciliary ultrastructural anomalies which tends to decrease the clearance of SARS-CoV-2 virus particles[23].

Hypertension (40.1%) was found as the most frequent comorbidity, followed by diabetes mellitus (27.5%), heart disease (18.4%), and kidney disorder (14.0%). This was in accordance with previous studies[22,24-27] which stated hypertension, diabetes mellitus, cerebrovascular disease, chronic obstructive pulmonary disease, renal disease, or tuberculosis contribute to the poor COVID-19 outcome. A meta-analysis conducted by Chang et al[28] showed hypertension, diabetes mellitus, and kidney disorders to be strongly correlated with COVID-19 severity. Diabetic patients have weakened and impaired immune responses, which potentially exacerbate the COVID-19 condition. Pneumonia was also seen more frequently in diabetic patients, and it is detrimental to the prognosis of COVID-19[29]. Chronic kidney disease (CKD) can be a risk factor for acute kidney injury (AKI), which is strongly associated with increased mortality in COVID-19 patients. Renal disorder can complicate the assessment and management of fluid status in patients with respi-

Table II. Ct values differences based on the COVID-19 severity measured in the second week of illness.

Variable	Total n = 207	Clinical Severity COVID-19				*p*-value
		Mild n = 39	Moderate n = 64	Severe n = 61	Critical n = 43	
Ct Helicase/ORF1b gene						0.825
Mean ± SD	28.95 ± 4.89	29.13 ± 4.61	29.34 ± 4.54	28.71 ± 5.09	28.54 ± 5.43	
Min-Max	(16.4-39.06)	(18.27-35.86)	(18.88-39.06)	(16.4-38.84)	(19.9-38.85)	
Ct RdRp gene						0.821
Mean ± SD	30.65 ± 4.99	31.01 ± 4.71	30.97 ± 5.22	30.32 ± 4.96	30.32 ± 5.07	
Min-Max	(18.09-53.36)	(19.67-38.75)	(20.79-53.36)	(18.09-38.83)	(21.37-39.49)	
The Lowest Ct gene						0.870
Mean ± SD	28.78 ± 4.81	29.13 ± 4.62	29.01 ± 4.43	28.57 ± 5.08	28.41 ± 5.25	
Min-Max	(16.4-38.83)	(18.27-35.86)	(18.88-38.6)	(16.4-38.83)	(19.9-38.78)	

Abbreviations: COVID-19, coronavirus disease; Ct, cycle threshold; ORF, open reading frame; RdRp, RNA-dependent RNA polymerase.

ratory failure[30]. The risk for stage 4 and 5 CKD was higher than diabetes mellitus or chronic heart disease[31]. Therefore, CKD can be a risk factor for death in COVID-19 patients. The most common causes of death from COVID-19 are respiratory and kidney failure, as well as septic shock[32]. The laboratory result could also indicate the severity of the disease, such as lymphopenia, increased NLR, elevated LDH and procalcitonin, which were reported to be associated with more severe COVID-19 disease[33-36].

Previous studies[6,37] showed that the ability of Ct values to reflect a different viral load is still being questioned. Ct value for a single specimen varies between several kits and techniques including target genes, primers, and fluorescence threshold values, and it tends to also vary significantly between different sequence processes of the same kit[38]. Our study used RT-PCR as the diagnostic tool. To reduce the study bias, we used the same laboratory sampling technician, sampling locations (both nasopharynx and oro-

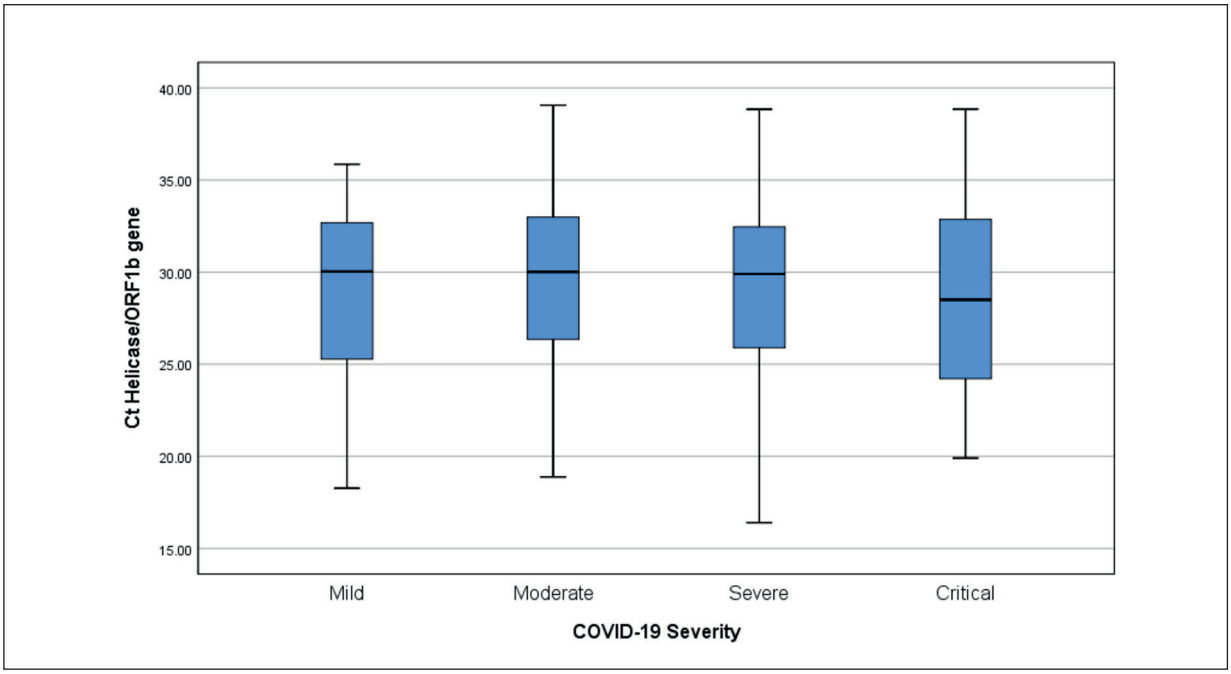

Figure 1. Ct Helicase/ORF1b gene value based on COVID-19 severity measured in the second week of illness.

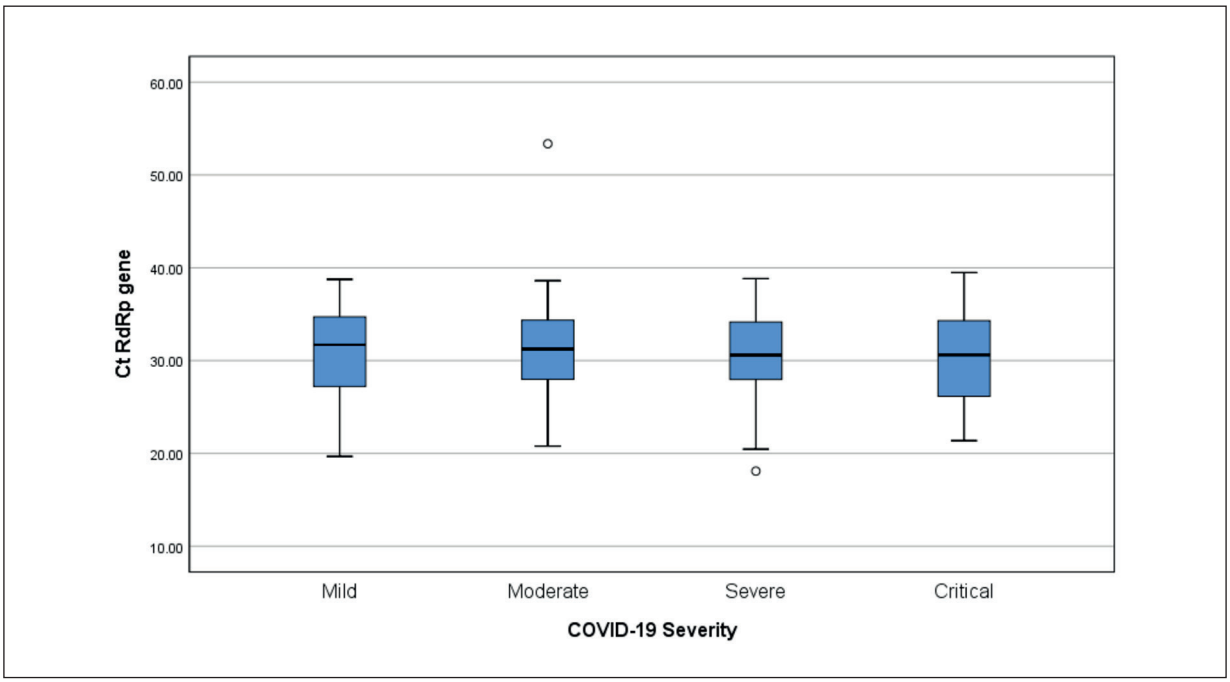

Figure 2. Ct RdRp gene value based on COVID-19 severity measured in the second week of illness.

pharynx), target genes, PCR tools, and reagents. The samples examined were collected from the upper respiratory tract (URT). This may partly explain the result of the study, as the previous studies[39,40] stated that the viral load in the URT reaches its highest point in the first week before deteriorating. Moreover, a higher viral load was found in the lower respiratory tract (LRT) and

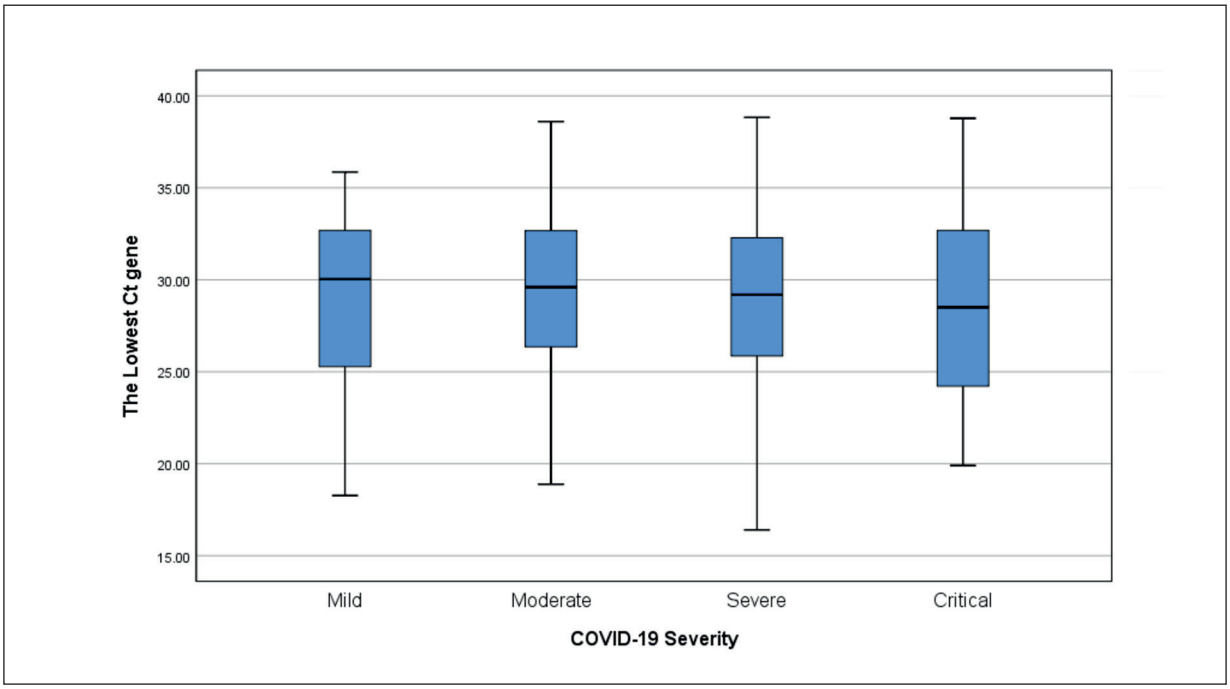

Figure 3. Lowest Ct gene value based on COVID-19 severity measured in the second week of illness.

feces in the second week of illness[13]. According to Wang et al[3], viral RNA through Bronchoalveolar Lavage (BAL) in severe COVID-19 patients is potentially beneficial in diagnosing and monitoring the disease. Other studies[7,8,11,41] discovered the viral load in LRT to be at its peak in the second week of illness, whereas the peak in the URT occurred earlier and began to shed by then. Since our study samples were collected from nasopharyngeal and oropharyngeal swabs, they were assumed to partly affect the result. Additionally, viral load dynamics are closely related to the time of symptom onset and can be a potential bias since symptom onset is based on patient's subjectivity[41,42].

Yagci et al[43] showed viral loads obtained from nasopharyngeal swabs were low in COVID-19 with severe chest Computed Tomography (CT) images. This result led to speculation that high viral load in early-phase nasopharyngeal swab specimens is not always associated with changes in chest CT severity. A lower viral load is obtained from nasopharyngeal swabs of many patients with a worsened chest CT scan, while the LRT samples show a higher viral load. Therefore, samples from LRT were more valid in later phase of disease[43].

McEllistrem et al[44] reported that symptomatic COVID-19 health workers had higher Ct values than their asymptomatic counterparts. However, the intensity and duration of virus exposure to each patient were not assessable, which tended to influence the variation of the result[44]. Patients with the asymptomatic and mild diseases had efficient innate immune responses allowing enough time to enhance the response for T-cells, therefore, the viral activity could be controlled[45]. The clinical deterioration that occurs in severe patients is related to the viral tropism expansion, involving both direct infection of respiratory lining cells and alveolar epithelium, as well as inflammatory activation of monocytes and macrophages[46].

Many factors affect Ct values and can be classified into pre-analytic, analytic and post-analytic. The pre-analytic includes specimen type, sampling time, and collection. Analytic factors are type of RT-PCR and contamination of the reagents and sample, while post-analytical factors include the result interpretation. In addition, high Ct values are similarly found in different infection states[47], and a positive value in the RT-PCR test could only detect RNA virus, but is unable to distinguish the viable ones[48]. Osman et al[49] found that the RT-PCR test detected the genetic materials but failed to differentiate between live and dead virus. The disease progressivity, patients' immune response, and the viral clearance may also contribute to this difference[50]. Therefore, a single swab could not determine the proceeding stage of the disease[47,51].

Based on authors' knowledge, this is the first study that compared Ct value with–COVID-19 severity during the second week of illness. This study has several limitations. Mean Ct values obtained with the target Helicase/ORF1ab and RdRp genes using the m-BioCov-19 reagent cannot be extrapolated to other target genes and/or platforms. The symptom onset cannot be determined precisely since it is based on patients' subjectivity. Moreover, the Ct values in new COVID-19 variants such as Beta or Delta which are lower than historical variants cannot be differentiated. This is in line with Teissou et al study which showed that COVID-19 viral load value obtained from the nasopharyngeal swabs in the Delta variant was significantly higher compared to Beta variant and previous variants[52].

Conclusions

There was no association between the Ct value from oropharyngeal and nasopharyngeal swabs and the COVID-19 severity in the second week of illness. Many factors potentially contribute to Ct value, therefore the number of viruses reflected by the viral load is possibly not the only factor affecting the disease severity.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

All authors contributed to the conception and design of this study. BA and HS acquired the data, while AYS, RA and NNA carried out data collection, analysis and interpretation. All authors contributed to the drafting and revising the article.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for Publication

The authors declare that this manuscript is original, has not been published before, and is not currently being considered for publication.

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