ORIGINAL ARTICLE

Implication Between RT-PCR's Cycle Threshold (CT) Value and Clinical Insight in COVID-19

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ABSTRACT

Introduction: Coronavirus disease 2019 (COVID-19) is a contagious with speed transmission and cause pandemic around the globe. A real-time polymerase chain reaction (RT-PCR) has become the major diagnostic method for COVID-19. Some believe that releasing patient from isolation or evaluating clinical progression could be made based on cycle threshold (CT) values. Here, we aimed to compare CT-value to the clinical insight using three different PCR's kit. **Method:** We collected 48 patients with confirmed COVID19 positive, then we divided into three groups that were (1) pneumonia, (2) non-pneumonia and (3) asymptomatic. The specimens came from nasopharyngeal and oropharyngeal swabs, were extracted using the same matrix column method and then detected by RT-PCR using different kit. The kits were commercially that detect Orf1ab, E gene (kit A); Orf1ab, N, E gene (kit B) and Orf1ab, N gene (kit C). Thus, we compared the result using comparation analysis based on CT-value and clinical groups by using SPSS 20.0 **Result:** From those patients there were 23 asymptomatic (48%), 9 symptomatic non-pneumonia (19%) and 16 pneumonia cases (33%) respectively. The mean difference of CT-values within three kits were wide and convergence. There were also significantly different (Kruskal-Wallis Test) between clinical course and CT-value in three PCR's kit even from the same detected gene ($p < 0.005$). **Conclusion:** This study conclude that CT-value cannot be the only determination to exclude patient from the isolation or to predict the clinical manifestation in COVID-19 since it has wide variation within same sample in different PCR kits.

Keywords: COVID19, CT-value, Isolation release, Clinical course

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INTRODUCTION

Coronavirus diseases 2019 (COVID-19) has become a pandemic since in the beginning of 2020. In Indonesia only, the total case in the beginning of May 2022 are 6,05 million cases, with the death rate around 3-4% (1,2). Thus, a real-time polymerase chain reaction (RT-PCR) is the golden standard for the diagnosis of COVID-19. Within RT-PCR process, there will be a cycle threshold's value (CT-value) that is used to determine whether the results become positive or negative. CT-value is the value of minimal cycle that can pass the 'threshold' to detect amplification reaction (3) Companies worldwide are currently focusing in developing SARS CoV-2 RNA detection kits with multiple different target gene and CT-value's cut off. CT value is quite different from the viral load number, since the method and intention to measure

CT and viral load is different each other's (4–6) The viral load of SARS CoV-2 can be detected by measuring the N-gene specific quantitative RT-PCR. A study by Pan Y, et al indicated that viral load is increased in day 5th to 6th after the onset, and the viral load in sputum is higher than nasopharyngeal swab (7). The number of viral load can reflect active replication of the virus in the respiratory tract, might be useful to predict the severity of clinical manifestation (8,9).

Some study shows that CT-value higher than 34 cannot be cultured, as might be the viable viral was too low.4 An article published by Tom MR and Mina MJ, 2020 suggested that by reporting CT value and or calculated viral load could help interpretation and clinical decisions (5). Thus, based on our preliminary study, many laboratories in Indonesia put CT value number in their RT-PCR reports that make people thinks CT value is a predictor for COVID19 clinical progression or evaluation of infectious phase that help doctor to release patient from isolation. By doing this study, we want to analyze the impact of CT value and clinical presentation of COVID-19 patients.

MATERIALS AND METHODS

We had already collected around 14.000 nasopharyngeal and oropharyngeal swabs specimen for COVID19 molecular diagnostic test using real-time PCR from year 2020-2022. However, we only chose randomly 48 patients with positive COVID19 and those who had a complete both epidemiological/clinical data. We use 48 patients based on our statistic calculation that meet the minimal quantity for the design. Our study had already passed the ethical clearance from Ethical Committee of Faculty of Medicine UIN Syarif Hidayatullah Jakarta with the number B-005/F12/KPK/TL00/02/2021. All samples were gathered from nine different hospitals around Tangerang and Tangerang Selatan, Banten Province, Indonesia. We compared CT values from different PCR kit reagents within same subjects. Those subjects were divided into three groups based on clinical symptoms as classified in Indonesian guidance for COVID19 diagnosis.10 The samples included in this study were both from diagnostic (day-1 or day-2 of onset) or screening condition of COVID19 so we could include the symptomatic and asymptomatic patient.

RNA extraction and RNA quantification

The first step after gathering the patient's data, we did manual extraction using spin column method (Geneaid Viral Nucleaid Acid Extraction Kit II). From 200µL of viral transport media we got 50 µL RNA elution. Before running for RT-PCR we examined the purity of RNA using NanoDropTM spectrophotometer to ensure the ratio was around 2.0 (260/230 nm absorbance). We only use extraction result with high purity RNA to make condition similar to each other before PCR.

Real-time PCR procedures

After getting RNA elution for each sample with pure RNA, we conducted COVID19 PCR test with three different reagents. Those were commercially provided and already validated by the Indonesian Ministry of Health, each kit has different gene target which are *Orf1ab, E* gene (kit A, cut off CT-value > 36, consider as negative); *Orf1ab, N, E* gene (kit B, cut off CT-value > 41, consider as negative) and *Orf1ab, N* gene (kit C, cut off CT-value > 40, consider as negative). We used Roche LC 480 for real-time PCR machine with thermocycler condition as described in each manual protocol of the PCR reagents kit. Each kit's protocol was conducted differently and blind without the staff knows the clinical course of the patients.

Based on cycle threshold (CT)-value we conducted a statistical analysis to compare between clinical presence with CT-value number. The clinical presentations were categorized into pneumonia (fever, shortness of breath, and another respiratory symptoms); a non-pneumonia (anosmia, diarrhea, fatigue, and others non-respiratory symptoms) and asymptomatic condition. The criteria were based on description of COVID19 clinical presentation within Guidance of COVID19 5th edition issued by Indonesia Ministry of Health (10). In this study we only compare the CT value of *Orf1ab* and *E* gene. The statistical analyzes was using Kruskal-Wallis by SPSS 20.0 version.

RESULTS

There were 54% female and 46% male in this study with average age was 40,02 years old (y.o), from those patients there were 23 asymptomatic (48%), 9 symptomatic without pneumonia (19%) and 16 pneumonia cases (33%) respectively (Table I). Most subject were within 30-39 years old (35%). From those patients we compared the CT-value number within three different PCR kit at the same gene target which is *Orf1ab* as we want to make the comparation equal. Based on the result in table II we can conclude that the interval difference of CT-value within three kits were wide and convergence since the CT-value cut off was difference each other (table II). We can see in table II, the minimum number of CT-value in asymptomatic group only can be very diverse, from 16 for kit A, 10 for kit B and 22 for kit C, although the CT-value cut off nearly around 40 for kit B and C. Those condition found in all group, asymptomatic, symptomatic without pneumonia and pneumonia respectively.

For an example in table II, we can see that for *Orf1ab* gene the maximum value for pneumonia can be vary from 32, 37, 39 in kit A, B and C, respectively. Thus, in table III we can assume that between kit in same clinical course and gene target (*Orf1ab*) the CT value number is different significantly, such as in asymptomatic

Table I: Patients' characterization and their clinical presentation

Characteristic/ variable	n	%
Age group (years old; ± SD = 40,02 ± 14,53)		
0-9	2	4
10-19	0	-
20-29	8	17
30-39	17	35
40-49	8	17
50-59	7	15
60-69	5	10
70-79	1	2
Sex		
Female	26	54
Male	22	46
PCR aims		
Diagnostic	25	52
Screening	23	48
Group based on clinical presentation		
Asymptomatic	23	48
Pneumonia	16	33
Non-pneumonia	9	19

Table II: The comparison between minimum and maximum CT-value number in three different PCR COVID19's kits within similar clinical presentation group

PCR's Kit	**Kit A** lot number : MNCO0120023 cut off CT : 36		**Kit B** lot number : P20200502 cut off CT : 41		**Kit C** lot number : 20200901 cut off CT : 40	
clinical presentation group						
CT gene *Orf1ab*	**min**	**max**	**min**	**max**	**min**	**max**
Asymptomatic	16	33	10	41	22	38
Non pneumonia	11	27	18	33	15	30
pneumonia	7	32	0[1]	37	12	39
CT gene *E*						
Asymptomatic	17	34	10	41		N/A
Non pneumonia	11	27	18	33		
pneumonia	7	34	0[2]	37		

Note: [1]In some condition, Kit B which is multiplex PCR with three genes target, the *Orf1ab* gene could not be amplified while the other genes (*E* gene and *N* gene) were amplified nicely.[2] Similar to the previous explanation, the *E* gene could not be amplified while the other genes (*Orf1ab* gene and *N* gene) were amplified.

condition, the mean is varied from 25,61 to 31.

Based on result in table III, there were also significantly different (Kruskal-Wallis Test) between clinical course and CT value in three PCR's kit even from the same detected gene (*Orf1ab* gene, p< 0.05). From this result we can assume that CT value number can be diverse between different kit and clinical course. From the table 2 and 3, the minimum, mean and standard deviation of CT value number in same clinical course is vary in each kit, for example, in table III, the kit A, B and C have 25,61±5,28; 31,00±6,78 and 31,04±4,85 within asymptomatic group respectively. It is also seen that i.e kit C for target gene *Orf1ab* have almost similar number for asymptomatic, non-pneumonia and pneumonia

Table III: The Statistical analysis between different kits in same gene target

Target gene	± SD			P
	Asymptomatic	Non pneumonia	pneumonia	
CT gene *Orf1ab*				
Kit A	25,61±5,28	19,56±5,83	17,75±8,02	0.004*
Kit B	31,00±6,78	25,89±5,18	21,13±11,14	
Kit C	31,04±4,85	24,89±5,20	24,63±8,53	
CT gene *E*				
Kit A	26,52±5,31	20,11±6,10	18,19±8,59	0.003*
Kit B	31±6,78	25,89±5,18	21,13±11,14	

*Analyzed by using Kruskal Wallis test

group which are 31,04±4,85; 24,89±5,20; 24,63±8,53, respectively.

DISCUSSION

A RT-PCR of COVID19 considered positive when the amplification curve shows a significant exponential result within a 'S' shape curve. The curve that passes the threshold represent the cycle threshold (CT) value. A greater number of genetic material's target available within the sample, the amplification time process will be shorter, and the CT-value number will be smaller (11–13). In this study we had used the same sample, same RNA elution and same RNA quantity from same positive subject to be assessed by three different PCR kits, however as explained previously in the result, we concluded that the CT-value number are different between kits. This result can lead to the conclusion that CT-value number might not be correlated to the RNA quantity only but also how the reaction setting during the PCR. Each kit that we used in this study have different PCR reaction setting, such as the denaturation, annealing and elongation phase are different in temperature and cycling time. The enzymatic reaction might also different, that can also impact on limit of detection and cycle threshold graph formation (14,15).

Today, we can find whole genome sequence of the SARS CoV-2 and easy access in GISAID database to identify the multiple-sequence alignment, mutation, and variation analysis in its genome. In this study we used kits with *Orf1ab* as gene target, because the *Orf1ab* was a part of ORF regions that have a low variation, around 13 sites of variation recently found. As we can assume from table II and III, the *Orf1ab* can be detected in all study groups, it is important to use kits that have primer/ probe with low mutation rate, so it can be used to detect all variant of SARS CoV-2 as we understand that SARS CoV-2 is easy mutated (16–18).

Based on many evidences, all individuals are susceptible to COVID19 (19,20). An asymptomatic patient revealed can be source of infection, although the antibody neutralization seems low (21,22). Based on this study (table II), we can still detect the gene target (*Orf1ab* and *E*) in asymptomatic subject as low CT-value as pneumonia

subject, the smallest CT-value between both groups is almost the same in each kit. This condition might be supporting the evidence that both asymptomatic and symptomatic can transmit the virus to susceptible person, as both have similar viral loads (6,23). Another study also shown a similar result, that asymptomatic person can have high viral load (24). Although, CT-value is not viral load, however the CT-value number might useful to predict the viral load (14,9,22). Some studies have been conducted to assess the correlation between CT-value number and COVID19 infectivity, unfortunately it is not yet established that high number of CT-values in condition cannot be cultured are not infectious.

From table II and III, we can learn that CT-value number are vary and have wide of variation even in the same gene target. The result of this study is similar with the result of Walker et al (2021)(14). They found that CT value varied widely in positive cases, including in subjects without symptoms. Walker et al (2021) also found that there were consistent lower CT values in first positive detection than second detection in days after positive. In our study, we found that no matter the aim of test, whether diagnostic or screening a low CT-value was not represent the clinical course condition. As clearly described in table III, a significant different is found in each clinical course and CT-values number from different PCR kits. Our result also shown a similar range on CT-value between asymptomatic and symptomatic person can also support the evidence that CT-value can't predict or determine the clinical manifestation in COVID19. Thus, it is maybe unproper to release patient from isolation only by using CT-value determination.

CONCLUSION

CT value cannot be the only determination to exclude patient from the isolation or to predict the clinical manifestation in COVID-19 since the number vary between different kits or PCR reagent.

REFERENCES

1. Update Covid-19 Di Indonesia Analisa Data. 2020;(April).
2. Khifzhon Azwar M, Setiati S. COVID-19 and Indonesia. Acta Med Indones. 2020;52(1):84-89. https://www.researchgate.net/publication/340645813
3. World Health Organization (WHO). Laboratory testing of human suspected cases of novel coronavirus (nCoV) infection: interim guidance. WHO. 2020;(January):1-8.
4. Bullard J, Dust K, Funk D, et al. Predicting infectious severe acute respiratory syndrome coronavirus 2 from diagnostic samples. Clin Infect Dis. 2020;71(10):2663-2666. doi:10.1093/cid/ciaa638
5. Tom MR, Mina MJ. To Interpret the SARS-CoV-2 Test, Consider the Cycle Threshold Value. Clin Infect Dis. 2020;71(16):2252-2254. doi:10.1093/cid/ciaa619
6. Bhat TA, Kalathil SG, Bogner PN, Blount BC, Goniewicz ML, Thanavala YM. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. N Engl J Med. 2020;382(12):1175-1177. doi:10.1056/NEJMc2000231
7. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis. 2020;20(4):411-412. doi:10.1016/S1473-3099(20)30113-4
8. Zheng S, Fan J, Yu F, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: Retrospective cohort study. BMJ. 2020;369(March):1-8. doi:10.1136/bmj.m1443
9. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. The Lancet Microbe. 2021;2(1):e13-e22. doi:10.1016/S2666-5247(20)30172-5
10. Kementrian Kesehatan Republik Indonesia. Pedoman Pencegahan Dan Pengendalian Coronavirus Disease (COVID19).; 2020. doi:10.29239/j.agrikan.9.2.i-iii
11. US Centers for Disease Control and Prevention RVBD of VD. 2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes. 2020;(Cdc):2-3.
12. zhou yunying, Pei F, Wang L, et al. Sensitivity evaluation of 2019 novel coronavirus (SARS-CoV-2) RT-PCR detection kits and strategy to reduce false negative. medRxiv. 2020;(105):2020.04.28.20083956. doi:10.1101/2020.04.28.20083956
13. AdvaMedDx. Introduction to Molecular Diagnostics: The Essential of Diagnostic Series. Published online 2013:1-19.
14. Walker AS, Pritchard E, House T, et al. CT threshold values, a proxy for viral load in community sars-cov-2 cases, demonstrate wide variation across populations and over time. Elife. 2021;10. doi:10.7554/eLife.64683
15. Kanamoto M, Tobe M, Takazawa T, Saito S. COVID-19 with repeated positive test results for SARS-CoV-2 by PCR and then negative test results twice during intensive care: A case report. J Med Case Rep. 2020;14(1):4-7. doi:10.1186/s13256-020-02534-2
16. van Kasteren PB, van der Veer B, van den Brink S, et al. Comparison of seven commercial RT-PCR diagnostic kits for COVID-19. J Clin Virol. 2020;128(April):104412. doi:10.1016/j.jcv.2020.104412
17. Sixto-Lypez Y, Correa-Basurto J, Bello M, Landeros-Rivera B, Garzyn-Tiznado JA, Montaco S. Structural insights into SARS-CoV-2 spike protein and its

natural mutants found in Mexican population. Sci Rep. 2021;11(1):1-16. doi:10.1038/s41598-021-84053-8
18. Pachetti M, Marini B, Benedetti F, et al. Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant. J Transl Med. 2020;18(1):1-9. doi:10.1186/s12967-020-02344-6
19. Singh R, Kang A, Luo X, et al. COVID-19: Current knowledge in clinical features, immunological responses, and vaccine development. FASEB J. 2021;35(3):1-23. doi:10.1096/fj.202002662r
20. Li H, Zhou Y, Zhang M, Wang H, Zhao Q, Liu J. Updated approaches against SARS-CoV-2. Antimicrob Agents Chemother. 2020;64(6):1-7. doi:10.1128/AAC.00483-20
21. Long QX, Liu BZ, Deng HJ, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med. 2020;26(6):845-848. doi:10.1038/s41591-020-0897-1
22. Tan W, Lu Y, Zhang J, et al. Viral Kinetics and Antibody Responses in Patients with COVID-19. medRxiv. Published online 2020:2020.03.24.20042382. doi:10.1101/2020.03.24.20042382
23. Yu F, Yan L, Wang N, et al. Quantitative Detection and Viral Load Analysis of SARS-CoV-2 in Infected Patients. Clin Infect Dis. 2020;(Xx Xxxx):2-7. doi:10.1093/cid/ciaa345
24. Kam KQ, Yung CF, Cui L, et al. A well infant with coronavirus disease 2019 with high viral load. Clin Infect Dis. 2020;71(15):847-849. doi:10.1093/cid/ciaa201