INVESTIGATION OF FACTORS RELATING TO THE CHANGE OF CYCLE THRESHOLD (CT) INDEX IN RT-PCR TESTING AMONG COVID-19 PATIENTS, AT MILITARY HOSPITAL 7

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ABSTRACT

Purpose: *We aimed to evaluate some factors related to changes of CT values in RT-PCR testing among patients infected with COVID-19 treated at Military Hosptial 7, Military Zone 3. This contributes to diagnosis and prognosis of disease.*

Methods: *Prospective and cross-sectional descriptive study.*

Results: *The mean age of the patients was 40.06 ± 14.34 years. 80.46% were males. 97.39% of the patients were vaccinated against COVID-19. 60.58% of them experienced post-COVID-19 syndrome.*

- On day 5 of COVID-19, the significantly lower CT values found in individuals reporting receipt less than 3 doses with respect to those who received completely 3 doses of COVID-19 vaccines (OR = 1.59; p < 0.05). Patients developing post-COVID-19 syndrome had a lower CT value than those without manifestations of this syndrome (OR = 1.73; p < 0.05); subjects treated with antiretroviral drugs (ARV) had a higher CT value than those nontreated (with OR = 5.06; p < 0.001).

- On day 10 of COVID-19, subjects without antiretroviral drugs (ARV) use had a lower CT values than those on antiretroviral medications (OR = 3.91; p < 0.001).

Keywords: Cycle threshold value, RT-PCR testing, Post-COVID-19 syndrome, Military Hospital 7.

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1. INTRODUCTIONS

In March, 2020, WHO (World Health Organization) declared the coronavirus disease (COVID-19) as a global pandemic. This is an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Since its emergence and rapid spread, SARS-CoV-2 has had many variants causing more than 600 million cases and roughly 7 million deaths globally [1]. By August, 2022, Viet Nam had recorded more than 9.7 million COVID-19 infections and 45,000 deaths. The clinical manifestations can be seen in COVID-19 patients ranging from asymptomatic or transient infections to severe disease (severe pneumonia, ARDS, septic shock, multi-organ function), even resulting in mortality (particularly in older adults, immunocompromised patients, underlying diseases, the bacterial and fungal co-infections/ superinfections...). To reduce mortality among COVID-19 patients, prevention as well as early and strong interventions to prevent the progression of disease.

Since non-specific clinical manifestation, laboratory tests plays a vital role in early detection of SARS-CoV-2 infection, of which Real-time PCR technique is used as a rapid and sensitive method for the detection of influenza viruses. This is a widely used molecular biology technique for detecting SARS-CoV-2 infection with the highest sensitivity and specificity (> 95%). The cycle threshold (CT) value in RT-PCR varies over stage of disease. Duration of viral load measured through the CT value differs in each patient. CT value is not only of great significance in diagnosis, treatment and post-discharge follow-up but also serves as an assessment tool of infectivity. In Vietnam, to date, there have been scarce studies evaluating associations between CT values and clinical outcomes for COVID-19 patients.

From above-mentioned reasons, we conducted this study with a view to evaluating some factors related to changes of CT values in RT-PCR testing among patients infected with COVID-19 treated at Military Hosptial 7, Military Zone 3. This contributes to diagnosis and prognosis of disease.

2. SUBJECTS AND METHODS

2.1. Subjects

307 patients performed RT-PCR for the diagnosis of COVID-19 [1] were treated at Military Hosptial 7, between December, 2021 and September, 2022.

2.2. Methods

- Study design: Prospective and cross-sectional descriptive study.

- Study criteria:

+ Patient characteristics: Age, gender, condition of obesity, dose of COVID-19 vaccine, history of underlying disease, clinical symptoms (fever, cough, fatigue, muscle pain, sorethroat, runny nose, nasal congestion), chest X-ray lung lesions, CT value in RT-PCR; patients on antiviral treatment.

+ Association of CT values with patient features at timepoints: Day 1 (D1), day 5 (D5) and day 10 (D10) after admission.

- Evaluation of CT value in RT-PCR testing: detection of ORF1a gene region for SARS-CoV-2 (Higene™ Viral DNA RNA Prep Kit, Korea)

- Dignosis of post-COVID-19 syndrome [2]: Patients were followed-up after three months of recovery. If clinical signs and symptoms apprear persistently after suffering COVID-19 and cannot be explained by other alternative diagnosis, patients will be confirmed post-COVID-19 syndrome. The most common symptoms include fatigue persist for more than 24 hours, sleep disorders, impaired attention and memory, fever, cough and chest pain...).

- Research ethics: The study was approved by Hospital Ethics Committee. Participants were given complete information about the study in the informed consent document. All information about patients were kept confidential and used for research purpose.

- Data processing: By using SPSS.22.0 software. Continuous quantitative variables can be described by mean (X), standard deviation (SD), Min-Max value and compared with student's t-test. Compare qualitative variables using χ^2 test. Risk was estimated using odds ratio OR with 95% confidence interval (95%CI).

3. RESULTS

3.1. Patient characteristics (n = 307)

- Gender:

+ Males were found in 247 patients (80.5%).

+ Females in 60 patients (19.5%).

There was a statistically significnant difference in gender with $p < 0.05$.

- Age distribution was commonly seen in 16-80 years of age, ranging 40.06 ± 14.34 years.

- BMI categories were mainly observed in 17.6-24.1 kg/m^2, averaged 20.85 ± 1.44 kg/m^2.

- Status of COVID-19 vaccines:

+ No injection: 8 patients (2.6%).

+ One injection: 10 patients (3.3%).

+ Two injection: 128 patients (41.7%).

+ Three injection: 161 patients (52.4%).

- History of underlying disease:

+ Present: 79 patients (25.7%).

+ Absent: 228 patients (74.3%).

- Clinical manifestation:

+ Present: 290 patients (94.5%).

+ Absent: 17 patients (5.5%).

- Status of chest X-ray lesions:

+ Present: 28 patients (9.1%).

+ Absent: 279 patients (90.9%).

- Utility of antiviral medication during treatment:

+ Use: 267 patients (87.0%).

+ No use: 40 patients (13.0%).

- Signs of post-COVID-19 syndrome:

+ Present: 186 patients (60.6%).

+ Absent: 121 patients (39.4%).

- CT values during treatment:

Table 1. Changes of CT value over time (n = 307)

CT value	Mean	Min-Max
D1	20.47 ± 4.34	13.99-37
D5	30.05 ± 7.03	15.03-40
D10	37.84 ± 3.82	21.44-40

3.2. Some factors related to changes of CT value in RT-PCR testing

Since there are currently no similarity studies for reference, cut-off value of 7 and 15 has been selected for identification of change in CT value of D5, D10 and D1 of hospital admission as compared to mean CT value of D5 and D10.

Table 2. Association of CT value with gender (n = 307)

Changes of CT value		Gender		OR (95%CI); p
Day	Value	Male (n, %)	Female (n, %)	
D5	≤ 7	98 (31.9%)	19 (6.2%)	OR = 1.42 (0.79-2.59); p > 0.05
	> 7	149 (48.5%)	41 (13.4%)	
D10	≤15	105 (34.2%)	23 (7.5%)	OR = 1.19 (0.67-2.12); p > 0.05
	>15	142 (80.5%)	37 (12.1%)	

Table 3. Association of CT value with number of doses of COVID-19 vaccine (n = 307)

Changes of CT value		Number of vaccinated doses		OR (95%CI); p
Day	Value	≤ 2	> 2	
D5	≤ 7	64 (20.8%)	53 (17.3%)	OR = 1.59 (1.00-2.53); p = 0.049
	> 7	82 (26.7%)	108 (35.2%)	
D10	≤15	67 (21.8%)	61 (19.9%)	OR = 1.39 (0.88-2.19); p > 0.05
	>15	79 (25.7%)	100 (32.6%)	

Table 4. Association of CT value with BMI (n = 307)

Changes of CT value		BMI		OR (95%CI); p
Day	Value	Male	Female	
D5	≤ 7	15 (4.9%)	102 (33.2%)	OR = 1.59 (1.00-2.53); p = 0.049
	> 7	18 (5.9%)	172 (56.0%)	
D10	≤15	16 (5.2%)	112 (36.5%)	OR = 1.39 (0.88-2.19); p > 0.05
	>15	17 (5.5%)	162 (52.8%)	

Table 5. Association of CT value with underlying disease (n = 307)

Changes of CT value		Underlying diseases		OR (95%CI); p
Day	Value	Present (n, %)	Absent (n, %)	
D5	≤ 7	34 (11.1%)	83 (27.0%)	OR = 1.32 (0,78-2.22); p > 0.05
	> 7	45 (14.7%)	145 (47.2%)	
D10	≤ 15	38 (12.4%)	90 (29.3%)	OR = 1.42 (0.85-2.38); p > 0.05
	>15	41 (13.4%)	138 (45.0%)	

Table 6. Association of CT value with COVID-19 clinical symptoms (n = 307)

Changes of CT value		Clinical symptoms		OR (95%CI); p
Day	Value	Present (n, %)	Absent (n, %)	
D5	≤ 7	113 (36.8%)	4 (1.3%)	OR = 2.08 (0.66-6.52); p > 0.05
	> 7	177 (57.7%)	13 (4.2%)	
D10	≤15	122 (39.7%)	6 (2.0%)	OR = 1.33 (0.48-3.70); p > 0.05
	>15	168 (54.7%)	11 (3.6%)	

Table 7. Association of CT value with lung lesions on chest X-ray at admission (n = 307)

Changes of CT value		Lung lesions		OR (95%CI); p
Day	Value	Present (n, %)	Absent (n, %)	
D5	≤ 7	12 (3.9%)	105 (34.2%)	OR = 1.24 (0.57-2.73); p > 0.05
	> 7	16 (5.2%)	174 (56.7%)	
D10	≤15	10 (3.3%)	118 (38.4%)	OR = 0.76 (0.34-1.7); p > 0,05
	>15	18 (5.9%)	161 (52.4%)	

Table 8. Association of CT value with antiviral drug (n = 307)

Changes of CT value		Utility of antiviral drug		OR (95%CI); p
Day	Value	Use (n, %)	Not use (n, %)	
D5	≤ 7	112 (36.5%)	5 (1.6%)	OR = 5.06 (1.92-3.32); p < 0.001
	> 7	155 (50.5%)	35 (11.4%)	
D10	≤15	121 (39.4%)	6 (2.0%)	OR = 3.91 (1.67-9.15); p < 0.001
	>15	146 (47.6%)	34 (10.7%)	

Table 9. Association of CT value with post-COVID-19 syndrome (n = 307)

Changes of CT value		Post-COVID-19 syndrome		OR (95%CI); p
Day	Value	Present (n, %)	Absent (n, %)	
D5	≤ 7	80 (26.1%)	37 (12.1%)	OR = 1.73 (1.06-2.78); p = 0.28
	> 7	106 (34.5%)	84 (27.4%)	
D10	≤15	83 (27.0%)	45 (14.7%)	OR = 1.36 (0.85-2.17); p > 0.05
	>15	103 (33.6%)	76 (24.8%)	

4. DISCUSSIONS

4.1. Patient characteristics

The study consisted of 307 patients diagnosed with COVID-19 [1]. Subjects aged 16-80 years, with mean age of 40.06 ± 14.34 years. Male (80.5%) outnumbered females (19.5%) with a statistically significant difference p < 0.05. This discrepancy was due to our study subjects who were service members.

Our patient charateristics differ from Guan et al's study on 1.099 COVID-19 patients in Wuhan city, China where this epidemic first broke out. In his study, patients averaged 35-58 years of age, mean age was 47 years. 58.1% of the patients were male and 41.9% were female [6]). Similarly, research by Hoang Van Hung et al in 370 COVID-19 patients at Technical University of Health Hai Duong showed that patients had mean age of 34.74 years; 42.4% were male and 57.6% were female) [3]). Our findings were lower than others because most participants in the current study were military personnels, 259 of whom (n=307) were performing military service.

In the present study, 97.4% of participants were vaccinated against COVID-19. 161 patients 52.4%) had complete 3 doses. 128 (41,7%) had 2 doses and 10 (3.3%) had one dose. This finding is different from Hoang Van Ly et al on 1.168 COVID-19 patients at Field Hospital 5D, Di An City, Binh Duong Province (the number of patients with one and two doses of COVID-19

vaccination was 140 (12.0%), 10 (0.9%) [4]). This discrepancy lies in duration of our collection data between the end of 2021 and September, 2022 when people had national access to COVID-19 vaccination. Moreover, awareness, knowledge and acceptance of COVID-19 pandemic were greatly improved.

94.46% of patients had clinical symptoms; 60.58% had post-COVID-19 syndrome. 86.97% were administered antiviral medications. On a chest X-ray, lung abnormalities were detected in 9.2% of patients. This suggests COVID-19 patients in the current study are mainly at a slight-to-moderate level.

4.2. Some factors related to changes of CT value in RT-PCR testing for COVID-19 patients

CT value in male patients was lower than in females (OR = 1.42 on D5 and OR = 1.19 on D10), which is statistically significant difference with $p > 0.05$. There are growing evidence that females are thus less susceptible to viral infection than males, possible due to protection of X chromosome and sex hormones which play an important role in innate and adaptive immune response. Additionally, males often have bad habits (smoking, alcoholism) are attributed to increased severity of many viral infections [9].

The significantly lower CT values found in individuals reporting receipt less than 3 doses with respect to those who received completely 3 doses of COVID-19 vaccines (OR = 1.59; $p < 0.05$ on D5). This disparity can be explained that antibodies developed to fight off infection in individuals receiving 3 doses of vaccination are better than unvaccinated or 2 doses.

Lower CT values were reported in overweight compared to non-overweight patients(OR = 1.40 on D5 and OR = 1.36 on D10; $p > 0.05$). Obesity is a critical risk factor for severe COVID-19 illness. This association is attributed to systemic inflammatory response [10].

Patients with underlying diseases had lower CT value than those without underlying diseases (OR = 1.32 on D5 and OR = 1.42 on D10; $p > 0.05$). Research by Le Tien Dung et al showed more pronounced clinical symptoms and more severe lung injury in subjects with underlying medical conditions than those without underlying diseases.

CT values of symptomatic patients with COVID-19 were significantly lower than those without clinical symptoms (OR = 2.08 on D5 and OR = 1.33 on D10; $p > 0.05$). Various studies show a lower CT value in symptomatic patients with COVID-19. A research by Singanayagam et al on 324 COVID-19 patients demonstrated the greatest CT values occurred during the symptomatic phase, followed by a steady decrease in the first 10 days post-infection.

Patients with lung lesions on chest X ray had lower CT value than those without lung injury. với OR = 1.24 on D5, $p > 0.05$. This finding is different from Hoang Van Hung et al's report [3] in which no lung abnormalities were found on X-ray in the majority of patients (> 75%). This disparity can be due to our subjects who were vaccinated many doses of COVID-19, therefore, having a small precentage of lung lesions (9.13%).

In the current study, the proportion of patients with post-COVID-19 syndrome was higher than those without signs of post-COVID-19 syndrome (60.58% vs. 39.42%). This finding is different from Peghin M et al's study, of which the prevalence of post-COVID-19 syndrome was 40.2% [8]. The presence of IgG antibodies was substantially associated with the occurrence of post-COVID-19 syndrome (OR = 2.56, 95%CI: 1.48-4.38, $p = 0.001$). Our research showed lower CT value in patients with post-COVID-19 syndrome than those free of this syndrome. (OR = 1.73; $p < 0.05$). Clinical symptoms are diverse (more than 200 various symptoms). The common symptoms reported in this study included persistent fatigue more than 24 hours, sleep disorder, impaired attention and memory, fever, cough and chest pain. Post COVID-19 condition is not a typical feature of critically ill patients. However, it also represents a significant proportion of those that presented with only mild to moderate symptoms after the acute infection [11].

Patients administered antiviral medication had greater CT value than those nontreated (OR 5.06, $p < 0.001$ on D5 and OR 3.91 on D10). This finding is in line with study by Joo E.J et al evaluating 86 severe COVID-19 patients (48 receiving Remdesivir [12]). His study showed substantially increased CT value during hospitalization of 1-5 to 11-15 days in treated-patients with respect to nontreated patients $p = 0.007$). Although we have not yet assessed adverse effects of Molnupiravir, we recorded no severe side effects in 276 out of 307 treated patients.

5. CONCLUSIONS

The study on 307 COVID-19 patients treated at Military Hospital 7 between December, 2021 and September, 2022 obtained some following results:

- RT-PCR CT value was associated with number of dose of COVID-19 vaccination, post-COVID-19 syndrome and utitily of antiviral medication during treatment.

- We found no statistically significant association between CT value and features of gender, obesity, underlying diseases, symptomatic clinical manifestation or chest Xray lung lesions at admission.

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