Original Article

Correlation of the cycle threshold value of SARS-CoV-2 by RT-PCR with biomarker levels in the prognosis of patients hospitalized with COVID-19

Shweta Singhal[1,2], Geetika Rana[2], Atul K. Singh[3], Shekhar Pal[2], Shweta Thaledi[2]

[1]Department of Microbiology, Sarojini Naidu Medical College, Agra, Uttar Pradesh, India, [2]Department of Microbiology, Government Doon Medical College, Dehradun, Uttarakhand, India, [3]Department of Anesthesia, Government Doon Medical College, Dehradun, Uttarakhand, India

Abstract

Background and Objective: The COVID-19 disease caused by SARS-CoV-2 was declared as pandemic by WHO soon after its emergence in 2019. This virus was known to cause serious clinical symptoms and severe illness. By using RT-PCR, which reports the cycle threshold value, the disease is diagnosed, whereas for the severity of the disease, biomarker levels, like IL-6, CRP, D-dimer, serum ferritin and serum procalcitonin, can be measured. We, thus, aimed to explore any potential correlation of the cycle threshold value and biomarker level with the outcome of COVID-19-positive hospitalized patients. **Method:** Patients with the cycle threshold (Ct) value <35 were included in the study and their initial Ct values were noted. Different biochemical parameters, such as C-reactive protein (CRP), serum ferritin, D-dimer, Interleukin-6 and serum procalcitonin, were assessed for severity. They were classified according to Ct value into three groups: Group 1 >30.0, Group 2 20.0–30.0 and Group 3 <20.0. **Results:** The study included 370 hospitalized COVID-19 patients with a mean age (mean ± SD) of 51.08 (16.58%) years and 250 (67.5%) males and 120 (32.4%) females. Comparison of data with outcome shows that IL-6, CRP amongst the biomarker and Ct value (deduced by RT-PCR test) were significantly correlated with the mortality (*P* value < 0.05). The ROC curve was also plotted for these parameters, which shows that IL-6, CRP, PCT and Ct value were better prognostic marker. Poor prognosis was found in Group 2 (Ct value 20.0–30.0) patients compared to Group 1 and Group 3. There was significant correlation (*P* value < 0.05) between Ct value and outcome of the patient. **Interpretation and Conclusion:** This study depicts that low Ct value and elevated levels of IL-6 and CRP can be used as a screening tool to detect the mortality in COVID-19 patients as they are significantly correlated with the mortality.

Keywords: Biomarker, COVID-19, outcome, sensitivity, severity, specificity

Introduction

Coronavirus (SARS-CoV), a recently discovered acute respiratory illness that resembled the severe acute respiratory syndrome was first identified in Wuhan, China, in December 2019. It quickly spread to most of the country worldwide, including India.[1] World Health Organization (WHO) first came to know about this virus on 12 January 2020 after a cluster of cases of viral pneumonia and officially named the disease as coronavirus disease 2019 (COVID-19) on 11 February 2020.[2] Later on WHO declared this disease as global pandemic on 12 March 2020.

Address for correspondence: Dr. Shekhar Pal, Department of Microbiology, Government Doon Medical College, Dehradun - 248 001, Uttarakhand, India.
E-mail: drshekharpal@gmail.com

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The COVID-19 disease has a variable clinical presentation ranging from asymptomatic to milder symptoms, including fever to other complications requiring intensive care unit (ICU) admission and mechanical ventilation. Numerous studies have been reported on the predictors of disease severity and mortality in COVID-19 patients.[3] Self/spontaneous recovery usually starts in second or third week of infection.[4] The real-time RT-PCR test is the gold standard method for SARS CoV-2 detection and it is the laboratory test of choice for the diagnosis of symptomatic patients in the acute phase.[5] Reporting of qualitative SARS-CoV-2 result as positive or negative is sufficient for diagnosis, but the cycle threshold (CT) value may offer benefit to clinicians in making clinical and patient management decision for patients with COVID-19 as well as guide to infection control, public health and occupational health decision.[6] Since laboratory medicine has always supported clinical decision-making in various infectious diseases, therefore, it is important to evaluate the ability of laboratory biomarkers (which is less expensive, faster and easier to obtain) to facilitate risk stratification of the COVID-19 disease.[7] However, these biomarkers are time-sensitive as the disease progress and therefore can be variable in different studies or areas. Therefore, keeping this in view, in this study we aim to assess the cycle threshold value and biomarker level in the prognosis of COVID-19 patients.

Materials and Methods

This was a descriptive analytical study conducted in the Department of Microbiology, Govt. Doon Medical College, Dehradun, from September 2020 to February 2022.

All RT-PCR test COVID-19 positive non-duplicate chronological cases that were admitted in Doon Hospital were included and all symptomatic cases but real-time RT-PCR test negative or patients or their attendant's refusal to be enrolled in the study or transferred to other hospitals were excluded.

The study was approved by the Institutional Ethics Committee with ref no. GDMC/IEC/2021/02.

Sample collection and processing

Sample collection was done according to the guidelines given by ICMR for COVID-19.[8] Dacron swabs were collected from nasopharyngeal and oropharyngeal sites and placed in viral transport media (VTM), which were packed in triple-layered packing, kept in a cool box immediately and transported to the microbiology laboratory.

The viral RNA was extracted manually using QIAamp Viral RNA Kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The extracted RNA was then amplified by real-time PCR BIO-RAD CFX96 system. TRUPCR SARS-CoV-2 KIT (3B BlackBio Biotech India Ltd) was used for the detection of three genes, namely, E gene (screening), RdRp +N (confirmatory gene) and RNase P (internal control) as per standard protocol by the manufacturer. Samples with Ct values <35.0 were considered positive, and samples with >35.0 values or undetectable Ct values were considered negative.

Serum biomarker analysis

Blood samples were collected for biomarker analysis from all the patients who tested positive and serum was separated for quantitative detection of biomarker level. It was done on the COBAS e411 automated ELISA system using different kits for the biomarkers (IL-6, CRP, D-dimer, serum ferritin and serum procalcitonin).

The COVID-19 patients with different severity of illness were classified into three groups according to the guidelines of diagnosis and treatment for SARS-CoV-2 pneumonia as follows: mild disease who had upper respiratory tract symptoms (and/or fever) without shortness of breath or hypoxia; moderate disease who had any one of: respiratory rate >24/min, breathlessness and SpO2: 90% to <93% on room air; and severe disease with any one of: respiratory rate >30/min, breathlessness and SpO2 <90% on room air.[9]

Statistical analysis

The categorical data in the form of frequency and percentage, continuous and normal distributed variables in the form of mean ± SD, and continuous and skewed data were presented in the form of median (IQR). The data were analysed using the SPSS Software version 22. A *P* value less than 0.05 was defined as statistical significance.

Results

A total of 538 nasopharyngeal and oropharyngeal samples were collected from patients who presented in the Department of Medicine in Govt. Doon Medical College, Dehradun, out of which 142 tested negative. Twenty-six patients who tested positive were referred to other hospitals; therefore, these patients were excluded from the study and hence 370 patients were enrolled for the study.

The descriptive statistics of confirmed COVID-19 patients are summarized in Table 1. The clinical and laboratory profile of

Table 1: Descriptive statistics of study variables

Parameter	*n* (%)
Mean age of presentation	51.08±16.58 years
Age group (years)	
0–20	07 (1.89%)
21–40	94 (25.4%)
41–60	156 (42.1%)
61–80	101 (27.2%)
>80	12 (3.24%)
Gender	
Male	250 (67.20%)
Female	120 (32.25%)
Mean days of hospitalization	11.89±7.07

patients is summarized in Table 2. According to the table, most patients had deranged parameters, amongst which maximum derangement was seen in CRP levels (74.32%), followed by D-dimer level (62.16%). The distribution of the patients enrolled in the study was done into three groups (as per cycle threshold values): with a low Ct value (value <20) 7.56% of patients, intermediate Ct value (value between 21 and 30) 55% of patients and a high Ct value (value >30) 37% of patients. Out of all the patients infected with COVID-19, 300 patients were discharged from the hospital and 70 patients died as shown in Table 2. The ROC curve was plotted for various parameters, such as IL-6, CRP, D-dimer, S. ferritin, S. procalcitonin and Ct value, to determine which marker can be used as a screening tool in COVID-19. Table 3 shows AUROC of different parameters that ranged between 0.6 and 0.7. The sensitivity and specificity were also calculated with cut-off value for these parameters as shown in Table 3. The area under the curve was highest for IL-6 as plotted on the graph, followed by PCT as shown in Figure 1.

Discussion

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) responsible for the pandemic causing COVID-19 infection has affected many countries, including India; therefore, this study was conducted in a tertiary care hospital for a period of 18 months and samples of patients from all age groups were included. The mean age of presentation of patients in this study was 51.08 ± 16.58 years and majority of the patients admitted in the hospital were aged between 41 and 60 years, followed by 61–80 years of age. Mehra *et al.* from Gwalior and Devang *et al.* from South India had observed the similar findings. However, the findings did not correlate with the finding of Kumar A *et al.* and Singh J *et al.*[10-13] Males were affected more than females in this study and this finding was similar to the study done by Agarwal *et al.* from North India.[14] The reason for the infection to be present in adolescent and elderly could be explained with the fact that because of lockdown and schools and colleges being closed, there was limited travelling done by very young and elderly due to which there was a less risk of exposure of these populations with the infected persons.

This study was undertaken during various waves of COVID-19. The first wave of COVID-19 was observed in 2020, where cases slowly increased during the latter part of 2020, and the second wave's peak occurred in May 2021 as described by Garg *et al.* in their study.[15] These COVID-19 waves varied in clinical presentation and prognosis; hence, the criteria for hospitalization as well as discharge were also different in these waves. The number of days of hospitalization of patients in our hospital ranged from 01 to 38 days with a mean of 11.89 ± 7.07 days.

The main clinical manifestations of COVID-19 were fever, cough, breathlessness and sore throat. The present study also reported fever (30.81%) as the major symptom, followed by cough (19.72%). This finding was similar to the Indian study done by Bhandari *et al.*[16] from Jaipur. Some international studies also reported these similar findings, such as by Huang *et al.* and Chen

Table 2: Clinical and laboratory profile

Variable	*n* (%)
Symptoms	
Fever	114 (30.81%)
Cough	73 (19.72%)
Myalgia	54 (14.59%)
Shortness of breath	49 (13.2%)
Headache	39 (10.54%)
Diarrhoea	27 (7.29%)
Asymptomatic	14 (3.78%)
Admission profile	
Admitted in ward	188 (50.8%)
Admitted in intensive care unit	182 (49.2%)
Biochemical parameter	
Interleukin-6	
Normal: 0–5.9 pg/ml	55 (14.86%)
Moderate: 6–60.0	215 (58.1%)
Severe: >60	100 (27.01%)
C-reactive protein	
Normal: 0.3–1.0 mg/dl	28 (7.56%)
Moderate: 1.0–10	69 (18.64%)
Severe: >10	275 (74.32%)
D-dimer	
Normal: <0.50 mg/dl	140 (37.83%)
Increase: >0.50	230 (62.16%)
Serum ferritin	
Males: N: 17.9–464	58 (23.2%)
Increase: >464	192 (76.8%)
Female: N: 6.24–264	41 (34.16%)
Increase: 264	79 (65.83%)
Serum procalcitonin	
Normal: 0.1–0.5 ng/dl	311 (84.05%)
Increase: >0.5	59 (15.94%)
Outcome of patient	
Discharged	300 (81%)
Died	70 (18.9%)

Table 3: Area under the receiver operating characteristic curve (AUROC) and optimal cut-off values

Parameter	AUROC	Optimal cut-off value	Sensitivity	Specificity
IL-6	0.71	37.38	70	66
CRP	0.683	46	70	57
PCT	0.71	0.08	70	63
Ct value	0.607	25.43	70	51

X *et al.*[17,18] We have also observed a subset of patients who were asymptomatic (3.78%) but tested positive on the RT-PCR test.

In our study, lower Ct values were associated with more signs and symptoms at diagnosis (severe disease). There was a significant correlation between Ct value versus disease severity and outcome ($P < 0.05$). The mortality rate was high amongst patients with intermediate Ct values [N = 44 (62.8%)], which was similar to studies done by Zheng *et al.* and Liu *et al.*[19,20]

Laboratory parameters, such as CRP, PCT, D-dimer, Interleukin-6 and serum ferritin, are the indicators of a severe stage of the disease

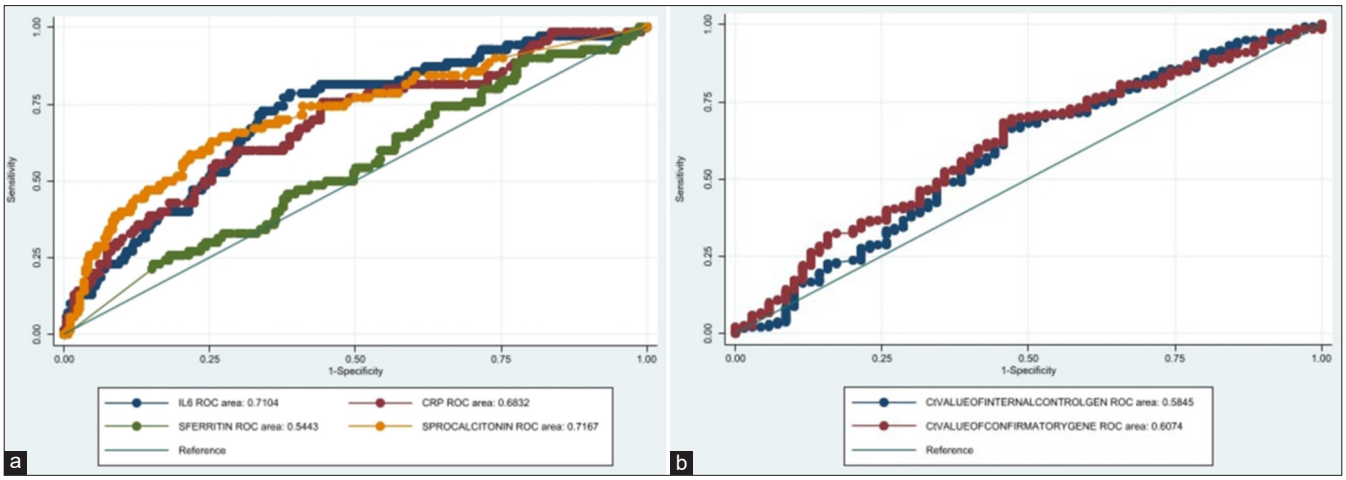

Figure 1: ROC curve analysis for biochemical parameters (a) and Ct value (b) for detecting the mortality of COVID-19. ROC: Receiver operating characteristic

since the host inflammatory response to the virus in severe disease may lead to cytokine storm. These laboratory test abnormalities are associated with the severity of disease presentation. High level of D-dimer (62.16%), serum ferritin (73.2%), and CRP levels (74.32%), as shown in Table 2, were observed in many of the admitted patients in the study. C-reactive protein is a type of non-specific acute phase reactant that is produced as a result of IL-6 in the liver and increased level of these markers is linked to adverse disease outcomes. The present research also observed that poor disease outcome was associated with increased level of CRP and IL-6. This was similarly shown in the study done by Asha K.S. from AIIMS, Raipur.[21] Based on these findings, it may be possible to avert the unfavourable consequences by taking CRP level and IL-6 measurement at admission.

A greater proportion of patients (62.16%) in the severe group of the current study had elevated D-dimer levels than the mild group, implying that coagulopathy is the cause of significant COVID-19 morbidity and death. The levels of serum ferritin, as observed in our study, were also significantly raised in many patients (73.2%) with no significant gender variation. These results seem to indicate that measuring CRP and IL-6 levels upon admission could help prevent adverse outcomes.

In our study, it was observed that IL-6 and PCT have the highest AUROC value, which suggests that these markers can be used as screening tool for COVID-19, followed by CRP and Ct values (deduced by RT-PCR test). This finding was in coherence with a study done by Singh J *et al.*[13] However, Devang *et al.*[11] in their study reported that LDH and ferritin can be predictors of the mortality in severe COVID-19 patients.

Conclusion

COVID-19 is an acute respiratory disease affecting people of all age groups and affecting every system of the body. Therefore, to aid in the prognosis of the disease, disease pathophysiology can be understood by clinical and biochemical parameters. The results of this study illustrated a substantial link between the cycle threshold value and the outcome, indicating that the primary care physician may find it beneficial to report the Ct value while making decision about COVID-19 patient care. According to the sensitivity and specificity of the biomarker test result, a biomarker may be utilized as a screening tool to identify COVID-19 patient mortality.

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Conflicts of interest

There are no conflicts of interest.

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