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Association of Cycle Threshold (Ct) Values of SARS-CoV-2 RT-PCR with Disease Severity and Symptoms among COVID-19 Patients in Ghadamis, Libya

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ABSTRACT

Background: This study aimed to assess the relationship between cycle threshold (Ct) values and disease severity, symptoms, and comorbidities in COVID-19-positive individuals.
Materials & Methods: This cross-sectional study was conducted on COVID-19 patients who were admitted to General Ghadamis hospital and diagnosed with COVID-19 from August 2020 to 2021. The association between Ct values and symptoms, demographic characteristics, and clinical characteristics was analyzed by SPSS analysis.
Findings: A total of 286 patients were included in this study, of whom 53.5% were female, and 46.5% were male, with a median age of 53 years (range: 1–90 years). Among symptomatic individuals, 51.4% had severe COVID-19 symptoms, and 26.6% had mild symptoms. Severe systemic symptoms were significantly associated with older age groups. The majority of elderly patients (66%) exhibited low Ct values (Ct ≤ 24), indicating a high viral load. Additionally, 64.6 and 40.8% of patients with severe and mild symptoms had low Ct values. Symptoms varied significantly across patients with low, medium, and high Ct values. There was a strong correlation between lower Ct values and the presence of comorbidities such as hypertension, diabetes mellitus, ischemic heart disease (IHD), cerebrovascular accident, and cancer.
Conclusion: Compared to asymptomatic individuals, most symptomatic patients with severe and mild symptoms had significantly lower Ct values. These findings highlight the critical role of viral load in SARS-CoV-2 progression and suggest that Ct values could be used to predict the spread of infection in the community.

Keywords: SARS-CoV-2, COVID-19, COVID-19 severity, Comorbidities, Viral load, Coronavirus infections, Infection severity

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Introduction

Since the onset of the COVID-19 (coronavirus disease 2019) pandemic, the identification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA gene sequences by reverse transcription polymerase chain reaction (rRT-PCR) has been the primary method for SARS-CoV-2 detection [1]. Real-time RT-PCR, a molecular diagnostic technique that uses thermocycling to amplify viral nucleic acid, is considered the gold standard method for COVID-19 diagnosis [2].

While this analysis typically provides a qualitative result for viral presence, it also yields cycle threshold (Ct) values to end users. The Ct value refers to the number of amplification cycles required for the fluorescence of a specific gene target to exceed a predetermined detection threshold level [3].

Ct values offer an indirect measure of the number of viral ribonucleic acid (RNA) copies present in a sample [2]. rRT-PCR tests generally inform clinicians only of the presence or absence of SARS-CoV-2. However, these assays also provide quantitative data on Ct values, which are inversely related to viral load and carry significant clinical implications [4].

Several studies have reported a correlation between lower Ct values and increased illness severity, with Ct values in SARS-CoV-2 RT-PCR serving as a marker for COVID-19 severity [5, 6]. Ct values could encourage decisions about patient care, infection prevention, and public health [7].

Lower Ct values are strongly associated with symptomatic COVID-19, indicating higher viral load and longer duration of viral shedding, both of which may affect the spread and transmissibility of the disease [8]. It is now evident that Ct values are an important marker for the development and incidence of local SARS-CoV-2 outbreaks. Studies have shown that trends in Ct values reflect epidemic trajectories and could predict COVID-19-related hospitalizations [9, 10]. In addition to these significant epidemiological insights, accumulating evidence suggests that regular monitoring of SARS-CoV-2 Ct values using precise and standardized molecular assays could have significant clinical implications [11].

Despite the proven association of Ct values with COVID-19 infection outcomes, there is currently no consensus on the clinical utility of SARS-CoV-2 Ct values. The role of Ct levels in understanding the spectrum of symptoms remain unclear.

Objectives: This study aimed to evaluate the accuracy of Ct values and determine the association between Ct values and symptoms, disease severity, and comorbidities among COVID-19-positive cases in Libya.

Materials and Methods

This cross-sectional study was conducted in the Molecular Biology Department of Ghadamis hospital in Ghadamis city

Table 1) shows the primer and probe sequences used in real-time RT-PCR to detect SARS-CoV-2.

Assay/ Use	Oligonucleotide ID	Sequence (5`-3`)
RdRP	RdRP_SARSr-F2	GTGARATGGTCATGTGTGGCGG
	RdRP_SARSr-R1	CARATGTTAAASACACTATTAGCATA
	RdRP_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGCBBQ
	RdRP_SARSr-P1	FAMCCAGGTGGWACRTCATCMGGTGATGCBBQ
E gene	E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT
	E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCGBBQ

(western Libya) from August 2020 to August 2021. Samples from all patients admitted to various clinical departments of General Ghadamis hospital and confirmed as COVID-19 cases by Cepheid® GeneXpert real-time PCR instrument were included in this study.

Data of all COVID-19 patients were collected from their hospital records, including information on gender, age, diagnosis, and clinical data. Ct values of COVID-19-positive samples collected during the one-year study period were evaluated. For detection, reverse transcription polymerase chain reaction (RT-PCR) was employed. The collected samples were subjected to Phoenix Dx 2019-nCoV assay, a real-time RT-PCR-based test designed for the in vitro qualitative detection of 2019-novel coronavirus (2019-nCoV) in respiratory and serum samples (Rotor-gene – QIAGENE). Specific primer and probe sets were designed for 2019-nCoV detection following the latest CDC (Centers for Disease Control and Prevention) and WHO (World Health Organization) guidelines.

A 25-μL reaction mixture was prepared, containing 5 μL of RNA, 12.5 μL of 2X reaction buffer from the Superscript III One-Step RT-PCR System with Platinum Taq Polymerase (Invitrogen; containing 0.4 mM of each deoxyribonucleotide triphosphates and 3.2 mM magnesium sulfate), 1 μL of reverse transcriptase/Taq mixture from the kit, 0.4 μL of 50 mM magnesium sulfate solution (Invitrogen – not provided with the kit), and 1 μg of nonacetylated bovine serum albumin (Roche). All oligonucleotides were synthesized and provided by Tib-Molbiol, Berlin. Thermal cycling was carried out at 55 °C for 10 min for reverse transcription, followed by one cycle of 95 °C for 3 min and then 45 cycles of 95 °C for 15 seconds and 58 °C for 30 seconds.

Ct values were evaluated based on different age groups, gender, history of chronic diseases, and clinical symptoms of COVID-19 patients. Ct values above 30, between 25 and 30, and below 25 were grouped as low, moderate, and high viral load, respectively. The COVID-19 disease was classified based on its symptoms and severity according to the WHO as follows: mild cases typically involve fever, cough, and fatigue; moderate cases may include difficulty breathing or mild pneumonia; and severe cases may have severe pneumonia, other organ failure, and possibly death.

Statistical analysis: The collected data were tabulated and analyzed using IBM SPSS Statistics for Windows, Version 19 software (IBM Corporation, Somers, NY). Significant differences in the association between variables were assessed using Pearson's Chi-square test, with the level of statistical significance set at $p < .05$.

Findings

This study included a total of 286 COVID-19-positive patients, of whom 153 (53.5%) were female, and 133 (46.5%) were male. The age of the studied patients ranged from one year to 90 years with a median age of 53 years. Most of the patients were in the age group of 41-60 years (n=166, 58.1%), followed by the age groups of 18-40 (n=61, 21.3%) and ≥61 (n=53, 18.5%) years, respectively. The pediatric group was the least affected group as shown in Table 2.

The majority of examined patients (78%) were symptomatic, 51.4% had severe symptoms, and 26.6% had moderate (mild) symptoms. Meanwhile, 22% of the patients were asymptomatic (Figure 1).

Among the 286 COVID-19 patients included in this study, 72.7% (n=208) had comorbidities, with asthma, diabetes mellitus, and hypertension being the most prevalent underlying conditions (Table 3). Two-thirds of the pediatric population were asymptomatic, while around 70-85%

of patients in the other age groups were symptomatic. Severe systemic symptoms were the most common in the age groups of ≥61 (75.5%) and 41-60 (50%) years, respectively. These differences were statistically significant (p< .000) (Figure 2). By comparing the severity of symptoms between patients with chronic comorbidities, it was found that the majority of severe cases occurred in patients with chronic conditions. Conversely, a large proportion of patients without chronic conditions (66.7%) were asymptomatic, and these differences were statistically significant (p<.000) (Figure 3). To assess the association between Ct values and risk factors, the studied patients were categorized into three Ct value groups: low (≤24), medium (25-30), and high (>30) groups. The frequency of low, medium, and high Ct values was 45.1% (n=129), 30.8% (n=88), and 24.1% (n=69), respectively.

Table 2) Frequency distribution of COVID-19 patients by age

Age Groups	No. of Cases	Percent (%)
Pediatrics: ≤17	6	2.1
Adults: 18-40	61	21.3
Middle-aged: 41-60	166	58.1
Elderly: ≥61	53	18.5
Total	286	100

The correlation between Ct values and gender was not significant (p= .093). However, the majority of elderly patients (66%) were in the low Ct value group, while patients in the other age groups were equally distributed across the three Ct value categories. A statistically significant relationship was found between Ct values and age groups (p= .023).

This study demonstrated a significant association between symptomatic presentation and lower Ct values, with

Table 3) Comorbidities of the COVID-19 patients

Comorbidities diseases	No. of patients	Percentage (%)
Asthma	55	19.2
Cancer	14	4.9
Cerebrovascular accident (CVA)	3	1.0
Diabetes mellitus (DM)	44	15.4
Diabetes mellitus + hypertension (DM+HTN)	33	11.5
Hypertension (HTN)	37	12.9
Ischemic heart disease (IHD)	17	5.9
Kidney failure	5	1.7
No	78	27.3
Total	286	100.0

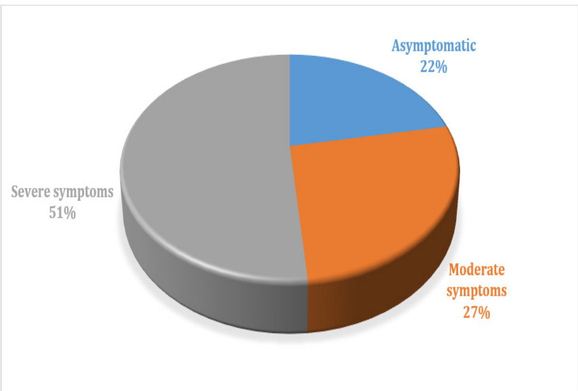

Figure 1) Frequency distribution of COVID-19 patients based on symptoms

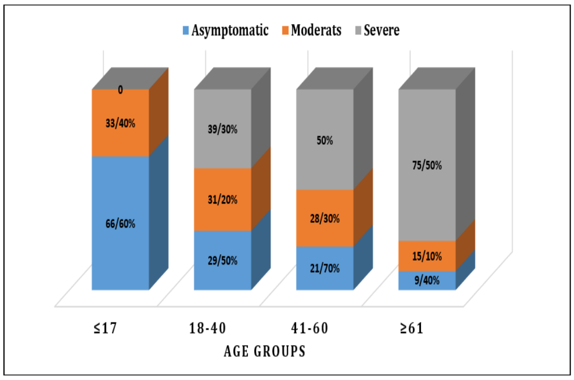

Figure 2) Distribution of symptoms in different age groups

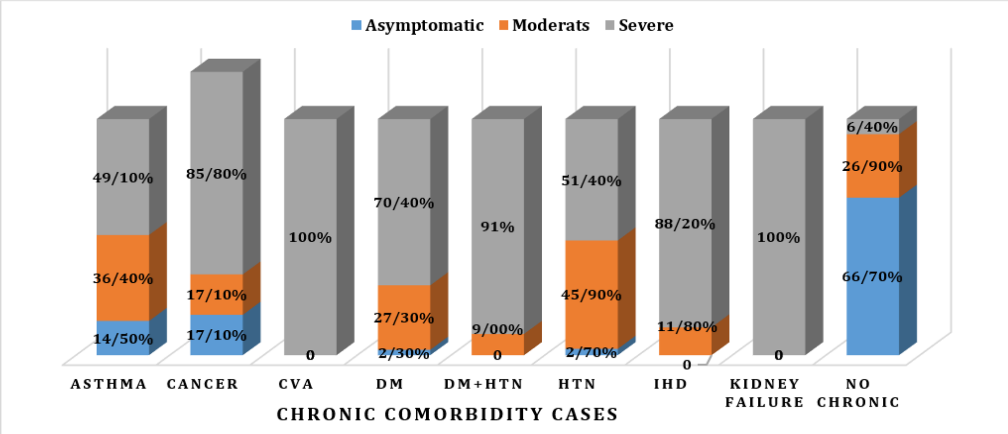

Figure 3) Distribution of symptoms based of different chronic diseases

Table 4) Association of Ct values with age, gender, blood groups, and comorbidities of the study participants

Risk Factors	Categories	Low Ct Value (≤24) N (%)	Medium Ct Value (25-30) N (%)	High Ct Value (>30) N (%)	Total Cases	*P*-Value
Age groups	≤17	2 (33.3)	2 (33.3)	2 (33.3)	6	0.023
	18-40	20 (32.8)	21 (34.4)	20 (32.8)	61	
	41-60	72 (34.4)	53 (31.9)	41 (24.7)	166	
	≥61	35 (66.0)	12 (22.4)	6 (11.3)	53	
Gender	Male	69 (51.9)	37 (27.8)	27 (20.3)	133	0.093
	Female	60 (39.2)	51 (33.3)	42 (27.5)	153	
Underlying medical condition	Asthma	19 (34.5)	25 (45.5)	11 (20)	55	0.000
	Cancer	9 (64.3)	4 (28.6)	1 (7.1)	14	
	Cerebrovascular accident (CVA)	3 (100)	0	0	3	
	Diabetes mellitus (DM)	27 (61.4)	14 (31.8)	3 (6.8)	44	
	Diabetes mellitus + Hypertension (DM+HTN)	24 (72.7)	7 (21.2)	2 (6.1)	33	
	Hypertension (HTN)	22 (59.5)	11 (29.7)	4 (10.8)	37	
	Ischemic heart disease (IHD)	9 (52.9)	6 (35.3)	2 (11.8)	17	
	Kidney failure	3 (60)	2 (40)	0	5	
	No	13 (16.7)	19 (24.3)	46 (59)	78	
Symptom status	Severe symptoms	95 (64.6)	42 (28.6)	10 (6.8)	147	0.000
	Moderate symptoms	31 (40.8)	33 (43.4)	12 (15.8)	76	
	Asymptomatic	3 (4.8)	13 (20.6)	47 (74.6)	63	
Blood groups	A	53 (64.6)	24 (29.3)	5 (6.1)	82	0.000
	B	39 (47)	37 (44.6)	7 (8.4)	83	
	AB	31 (62)	16 (32)	3 (6)	50	
	O	6 (8.5)	11 (1.4)	54 (76.1)	71	

severe disease found to be significantly correlated with lower Ct values (*p*= .000). Regarding the association between Ct values and blood groups, the majority of patients with blood groups A (64.6%) and AB (62%) were in the low Ct value category, whereas most of the patients with blood group O (76.1%) were in the high Ct value category, and this difference was statistically significant (*p*= .000) (Table 4).

This study also found a significant association between lower Ct values and the presence of comorbidities such as hypertension, diabetes mellitus, ischemic heart disease (IHD), cerebrovascular accident, and cancer (*p*= .000).

Discussion

Ghadamis hospital is located in Ghadames, a city in the western part of Libya, connected to the capital, Tripoli, where approximately 3 million people live. The prevalence rate of SARS-CoV-2 (COVID-19) in this region has been documented as 18.6% per 100,000 inhabitants. Additionally, the testing rate during the COVID-19 pandemic was 5 per 100,000 people per week, which is considered inadequate testing at the national level [12].

In this study, the association between rRT-PCR Ct values and demographic and clinical characteristics of COVID-19 patients was investigated to determine whether Ct values could serve as an important marker for predicting the severity of COVID-19 disease in hospitalized COVID-19 individuals.

There are limited studies using RT-PCR Ct values to quantify SARS-CoV-2 RNA in clinical samples in our region. This is the first study conducted to examine the relationship between RT-PCR Ct values and severity of COVID-19 in Libya.

A total of 286 COVID-19 patients were included in this investigation. Most of the COVID-19 patients were female (53.5%). This female predominance has also been reported in other studies [11]. These results are in contradiction with the results of a similar study in India [13], where males were more likely to be infected with COVID-19 than females.

In this study, the majority of positive cases (58%) were in the middle-aged group (41-60 years), followed by adults (18-40 years, 21.3%) and the elderly population (≥61 years, 18.5%). In an Indian study, adults aged 18-40 years comprised the largest proportion of positive patients (45%), followed by the elderly (41%), while pediatric and geriatric populations were the least affected groups [13].

Regarding symptoms, 78% of the patients were symptomatic, with 66% experiencing severe symptoms, while 22% were asymptomatic. These finding are in agreement with the results reported in England (19%) [14] and China (29.4%) [15], showing a lower percentage of asymptomatic cases. In contrast, the percentage of asymptomatic cases (43%) was reported to be high in the United Arab Emirates (UAE) [16]. The increased number of asymptomatic COVID-19 cases in the UAE has been attributed to extensive active tracing and screening efforts implemented by health authorities.

A significant portion of the population may be asymptomatic, which could contribute to the effective spread of the infection without being detected by testing or requiring medical intervention.

Regarding the significant association between the severity of COVID-19 and older age groups (41-60 and ≥61 years) as well as individuals with chronic comorbidities, older individuals are generally less healthy and often have underlying conditions such as diabetes mellitus and hypertension. Additionally, older populations are less likely to be engaged in physical activities (e.g., sports, walking) and may be retired or unemployed. These suggest that age, chronic comorbidities, and overall health status of populations play a critical role in the incidence of moderate and severe forms of COVID-19.

The median Ct value of SARS-CoV-2 RNA in this study was 25 (range: 12-40) among COVID-19 patients. Also, a study in Turkey reported a median Ct value of 28.16 (range: 24.5-31.6) for hospitalized patients [17]. Another study by Kleiboeker et al. (2020) Kleiboeker, Cowden [18] studied the RT-PCR results of more than 4000 COVID-19 samples and reported Ct values in the range of 6.16–37.92.

This study findings revealed that the frequency of low, medium, and high Ct values (indicating high, moderate, and low viral loads) was 45.1, 30.8, and 24.1%, respectively. Ct values inversely serve as a representative marker of viral load. A higher viral load is indicated by a lower Ct value and vice versa [19]. Li, Zeng [20] documented that higher viral loads were associated with severe disease, with viral loads being higher during the initial stages of the illness. Magleby [21] confirmed that higher viral load was linked with older age, existing medical conditions, smoking, and recently administered chemotherapy.

In this study, the majority of middle-aged and elderly subjects (40-60 and ≥61 years) exhibited low Ct values compared to the adult and pediatric populations. These findings are consistent with other research indicating lower Ct values in older COVID-19 patients compared to younger individuals, suggesting higher viral loads [22, 23]. However, a study by George, Murugan [13] found that the majority of pediatric, adult (18-40 years), and middle-aged patients (40-60 years) exhibited low Ct values, while the older group primarily displayed median Ct value. This study also identified a significant association between lower Ct values and symptomatic presentation and disease severity. Symptomatic COVID-19 cases were notably linked with lower Ct values, indicating elevated viral load and prolonged viral shedding, which may influence the disease spread and contagiousness [24]. Similar studies have also revealed that symptomatic patients tend to have lower Ct values (higher viral loads) [24-26].

In this study, the results showed that low Ct values were the most prevalent, followed by medium and high Ct values. The findings indicated that a significant proportion of non-severe patients with mild symptoms (41%, 31 of 76) also had low Ct values. These results indicate the important role of non-severe COVID-19 patients in the spread of the disease regardless of their symptomatic status.

Interestingly, a significant association was found between lower Ct values (indicating high viral loads) and the presence of comorbidities such as hypertension, diabetes mellitus, cerebrovascular accident, and cancer. The existence of comorbidities such as hypertension, asthma, diabetes mellitus, and cancer is known to be linked with worse outcomes in COVID-19 patients [27, 28]. These individuals might have a reduction in cardiopulmonary capacity, making them vulnerable to the physiologic challenges caused by COVID-19 [4]. Several factors affect the Ct values of SARS-CoV-2 positive samples, including pre-analytical and analytical factors such as sample collection, transportation, and storage, RNA extraction, gene targets, PCR sensitivity, amplification range, and techniques used.

Conclusion

The majority of COVID-19 cases identified in Libya between August 2020 and August 2021 were symptomatic, with a significant portion of asymptomatic cases being children. In this study, the majority of older individuals showed lower Ct values (indicating a high viral load) compared to the adult and pediatric populations. Symptomatic patients exhibited notably low Ct values, indicating their important role in the spread of COVID-19. A significant association was found between high viral loads and the presence of comorbidities in patients. Low Ct values were also observed in non-severe patients with mild symptoms (moderate), suggesting their crucial role in SARS-CoV-2 spread regardless of their symptomatic status. Identifying low Ct values and quarantining patients with mild symptoms are essential measures to prevent COVID-19 transmission. These findings emphasize the crucial role of viral load in SARS-CoV-2 pathogenesis and indicate that Ct values in positive COVID-19 cases could be used to predict the spread of infection in the community.

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