

Association between Cyclic Threshold Values and Clinical Symptoms in Patients with COVID-19 at Teaching Hospital, Jumla, Karnali, Nepal

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ABSTRACT

Introduction: The cyclic threshold (CT) value obtained in the reverse transcriptase polymerase chain reaction (RT-PCR) has been used as the surrogate marker of viral load. Therefore, to understand viral kinetics and evaluate the disease severity with clinical symptoms, it is necessary to evaluate cyclic threshold value in COVID-19-infected patients. **Aims:** To assess association between cyclic threshold value and clinical symptoms in patients with COVID-19. **Methods:** A descriptive cross-sectional study was conducted on PCR-positive patients (n=208) for six months at Karnali Academy of Health Science, Teaching Hospital Jumla. The RT-PCR was performed and data of the cyclic threshold value of PCR-positive patients was taken from the laboratory in predesigned study proforma. Data entry and analysis were done in SPSS version 20.0. **Results:** A larger proportion of females (65.9%) than males (43.1%) had a COVID-19 infection symptoms of COVID-19 were present in about equal numbers of infected males (61%) and females (68.7%). Most of the individuals were in the age range of 19 to 39 years. A cyclic threshold value of less than 30 was present in the majority (49%) of patients. Symptoms were present in 65.9% of individuals. Symptoms were present in a large proportion of subjects with cyclic threshold values of 1–19 and 20–29, at 89.5% and 77.9%, respectively. **Conclusion:** This study provides valuable insights into the association between cyclic threshold values and clinical symptoms of COVID-19 in patients. It also explores the underlying factors like gender disparities and age-specific disease severity. Patients showing symptoms of COVID-19 typically had a cyclic threshold value below 30 and carry a higher amount of the virus compared to those who don't show symptoms. The association between signs and symptoms and the cyclic threshold is highly significant.

Keywords: Covid-19, Cyclic threshold value, Symptoms

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INTRODUCTION

An atypical case of pneumonia was reported in Wuhan, China on December 2019¹ and January 7. World Health Organization (WHO) named the virus a novel coronavirus (2019-nCoV). Later on, it was renamed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and disease named Coronavirus disease 2019 (COVID-19) by WHO on 11th Feb 2020.² They are enveloped non-segmented positive-sense single stranded ribonucleic acid (ssRNA) viruses.³

On 11th March 2020 WHO, declared it as a pandemic disease.⁴ Infection occurs through close contact or droplet-expelling with an infected person. Indirect contact through fomites may also be a source of infection.⁵ The most common clinical manifestations included fever, cough, fatigue, shortness of breath, sore throat headache and gastrointestinal symptoms with diarrhea and vomiting. The elderly and persons with underlying disorders developed rapidly into acute respiratory distress syndrome, septic shock, metabolic acidosis and coagulation dysfunction, even leading to death.⁶

Reverse-transcription polymerase chain reaction (RT-PCR) from nasopharyngeal swabs is the most common test for detecting acute COVID-19 infection. The results of the RT-PCR are reported as positive or negative but the cyclic threshold (CT) value has not commonly been reported to patients. The CT values are inversely related to viral loads-the lower the CT value, the higher the viral load. A correlation between high viral load and disease severity of SARS-CoV-2 is seen.⁷ This study aims to find the association between CT values and clinical symptoms in COVID-19 patients that help the clinician to differentiate the symptomatic patient with infective concentration of viruses

METHODS

A descriptive cross-sectional study was conducted on PCR-positive patients (n=208) from January to June 2022 at Karnali Academy of Health Science (KAHS) Teaching Hospital, Jumla, Nepal. The Sample size was determined based on RT-PCR tests done and positive results in the previous months. All the patients meeting the inclusion criteria within six months were included in this study. Ethical Approval was taken from the Institutional Review Committee, Karnali Academy of Health Sciences "IRC-KAHS" The written informed consent was obtained from the participants before taking the sample. Nasopharyngeal swab was taken as a sample from all the participants in an aseptic condition. RT-PCR was performed in the molecular level 2 Laboratory of KAHS as mentioned below. All data were recorded in a predesigned study proforma. Data entry and analysis were done in Statistical Package for Social Science (SPSS) version 20.0.

Inclusion criteria: Confirmed PCR-positive patients (patients, who have a CT value <40 for COVID-19, with the following criteria, were included in this study:

1. Only Nasopharyngeal (NP) swabs were processed for testing
2. Only properly labeled and barcoded vials with intact sample quality were processed for testing.

Exclusion criteria: The following samples were not included in this study:

1. Suspected Covid-19 patients with PCR-negative reports
2. Improperly labeled vials and samples with thick mucus
3. Sample with compromised integrity, e.g., viral transport medium (VTM) or swab missing in the sample vial, leaked VTM vial, hemolyzed samples.

Clinical manifestations (fever, headache, myalgia, sore throat, loss of taste, loss of smell, diarrhea, and loss of sensation), clinical findings along with disease condition changes were recorded after interviewing patients and from patients medical documents. Laboratory confirmation of SARS-CoV-2 was performed by RT-PCR in the Department of Microbiology at the Molecular setup, which is a Bio-Safety Level (BSL-II) Laboratory at KAHS. Viral RNA was isolated from NP swabs from VTM by using the Maccura kitt (Siemens, Munich, Germany). The amplification and detection of viral RNA were performed on Quant Studio 5DX molecular system (Applied Bio systems, Waltham, Massachusetts). The amplification

or detection is based on the principle of RT-PCR containing specific primers and various fluorescent probes targeting specific genes such as ORF1ab, N and E gene of SARS-CoV2. Viral nucleic acid is detected by monitoring the intensity of fluorescence in real time which is a vitro quantification, to control or minimize the PCR inhibitors within the specimens to effectively prevent the presence of false negative results the internal control is added. Simultaneously it amplifies and detects several genes: Open reading frame 1ab (ORF-1ab), Envelop (E) gene, and Nucleocapsid protein (N) genes. The fluorescent channels FAM, ROX and Cy5 were used to detect the ORF-1ab, E gene and N genes respectively, whereas the HEX or VIC channel was used to detect the fluorescent signal of internal control (IC). An RT-PCR assay was performed using the following thermo cycler protocol: Reverse transcription at 50°C for 15 minutes for 1 cycle, cDNA pre-denaturation at 95°C for 2 minutes for 1 cycle whereas denaturation at 95°C for 15 seconds and annealing, extension fluorescence acquisitions at 58°C for 35 sec for 40 cycles, at the end instrument cooling at 40°C for 10 sec. Data on CT value and clinical symptoms (fever, headache, myalgia, sore throat, loss of taste, loss of smell, diarrhea and loss of sensation) were recorded. The data were analyzed using descriptive and inferential statistics by using SPSS version 20.0.

RESULTS

A total of 208 Covid-19 positive cases were included in this study out of which 77 were male(34.2%) and 131 female (65.8). The mean age of the participants was 37.1±18.5 years.

Sex		Sign and symptoms		Total	P value
		Absent	Present		
Male	N	30	47	77	0.291
	%	39.0%	61.0%	100.0%	
Female	N	41	90	131	100.0%
	%	31.3%	68.7%	100.0%	
Total	N	71	137	208	100.0%
	%	34.1%	65.9%	100.0%	

Table I: Signs and Symptoms According to Sex

Table I illustrates a similar percentage of infected males(61%) and females (68.7%) based on signs and symptoms. There was no significant association was found between the signs and symptoms and the sex of the participants.

Sex		Cyclic Threshold value			Total	P value
		1-19	20-29	≥30		
Male	N	11	25	41	77	0.471
	%	14.3%	32.5%	53.2%	100.0%	
Female	N	27	43	61	131	100.0%
	%	20.6%	32.8%	46.6%	100.0%	
Total	N	38	68	102	208	100.0%
	%	18.3%	32.7%	49.0%	100.0%	

Table II: Cyclic Threshold Value According to Sex

Table II shows that half (49%) of the infected participants had a cyclic threshold value ≥ 30 . The percentage of males (53.2%) and females (46.6%) with cyclic threshold value ≥ 30 were slightly above and below 50% respectively. A similar percentage of males and females had cyclic threshold values 20-29. The lowest percentage of males and females had cyclic threshold value 1-19. Also there is no association between cyclic threshold value and Sex.

Age		Sign and symptoms		Total	P Value
		Present	Absent		
Upto 18 years	Frequency (f)	20	13	33	0.05
	Percentage (%)	60.6%	39.4%	100.0%	
19-39 years	Frequency (f)	24	67	91	100.0%
	Percentage (%)	26.4%	73.6%	100.0%	
40-59 years	Frequency (f)	23	34	57	100.0%
	Percentage (%)	40.4%	59.6%	100.0%	
60 and above	Frequency (f)	4	23	27	100.0%
	Percentage (%)	14.8%	85.2%	100.0%	
Total	Frequency (f)	71	137	208	100.0%
	Percentage (%)	34.1%	65.9%	100.0%	

Table III: Sign and Symptoms According to Age Group

Table III shows that about half of the infected participants belong to the age group 19-39 years followed by the age group 40-59, and the least participants infected with COVID-19 belonged to the age group 60 and above. The majority of the participants among total participants had the presence of COVID-19 symptoms. The majority of infected participants (73.6%) of the age group 19-39 years showed symptoms of infection. Though very few participants in the age group 60 were infected, the majority (85.2 %) of them showed symptoms of infection. Furthermore, the table shows a significant ($p < 0.05$) association between signs and symptoms in different age groups.

Age		Cyclic Threshold value			Total	P value
		1-19	20-29	≥ 30		
Up to 18 years	N	3	6	24	33	0.045
	%	9.1%	18.2%	72.7%	100.0%	
19-39 years	N	21	36	34	91	100.0%
	%	23.1%	39.6%	37.4%	100.0%	
40-59 years	N	9	18	30	57	100.0%
	%	15.8%	31.6%	52.6%	100.0%	
60 and above	N	5	8	14	27	100%
	%	18.5%	29.6%	51.9%	100%	
Total	N	38	68	102	208	100.0%
	%	18.3	32.7%	49.0%	100.0%	

Table IV: Distribution of Cyclic Threshold Value According to Age Group

Table IV shows, the majority of the infected participants (49%) had cyclic threshold value ≥ 30 . Slightly more than half of the participants of the age groups 40-59 years and 60 and above had cyclic threshold value ≥ 30 . The lower percentage of infected participants had cyclic threshold values 1-19 which is 18.3% of total participants and the lowest percentage i.e. 9.1% of the age group up to 18 years had cyclic threshold values 1-19. The association between the cyclic threshold value and age group shows a significant P value (0.045).

Cyclic Threshold value		Sign and symptoms		Total	P Value
		No	Yes		
1-19	N	4	34	38	0.001
	%	10.5%	89.5%	100.0%	
20-29	N	15	53	68	100.0%
	%	22.1%	77.9%	100.0%	
≥ 30	N	52	50	102	100.0%
	%	51.0%	49.0%	100.0%	
Total	N	71	137	208	100.0%
	%	34.1%	65.9%	100.0%	

Table V: Sign and symptoms according to Cyclic Threshold value

Table V illustrates the Cyclic Threshold value compared to signs and symptoms. Among the participants with Cyclic Threshold value ≥ 30 , there were almost equal participants with the presence and absence of the signs and symptoms of infection. However, the majority of the participants with cyclic threshold values 1-19 and 20-29 had the presence of signs and symptoms of infection at 89.5% and 77.9% respectively and the association between sign and symptom and the cyclic threshold is highly significant (0.001).

DISCUSSION

COVID-19 encloses a diverse spectrum of disease courses. The prognosis of patients at the time of diagnosis will greatly contribute to nonspecific symptomatic pharmacotherapy and patient management decisions. Our study explores the association between cyclic threshold (CT) values and clinical symptoms in patients with COVID-19 at a teaching hospital in Jumla Nepal. Our study provides valuable insights into the gender distribution of COVID-19 patients and the presence of symptoms. Notably, a larger proportion of females were infected compared to males. Similar findings were observed in the study of Dorre A et al.⁸ This observation might be attributed to multiple factors such as differences in social behaviors, occupational exposure, or health-seeking behavior.⁹ Interestingly despite higher infection rates in females, the presence of symptoms was similar between the two genders. This raises the important question about the biological, immunological, or genetic factors that may influence the manifestation of symptoms in COVID-19 patients. Further research could delve into these gender specific differences to better understand the disease's impact. In our study, a significant proportion of COVID-19 patients (49%) had

CT values below 30. This finding suggests that a substantial number of individuals with COVID-19 had high viral load, which might be associated with increased transmissibility. Similar findings were demonstrated in the study of Kurzeder L et.al.^{10,11}

The data further shows that both males and females exhibited similar patterns in terms of CT values with a slightly higher percentage of males having CT values below 30. This could be due to variations in viral shedding dynamics between genders, but more research is needed to confirm this hypothesis. We categorized patients into different age groups. The age range of 19-39 had the highest number of infections, followed by the 40-59 age group, similar result was seen in the study of Doerre A et al⁸ and Liu Y et al.¹² Interestingly the 60+ age group had the smallest percentage of infections. However, a striking finding is that a significant majority of individuals in the 60+ age group displayed symptoms. Similar results were seen in the study of Fathi M et al.¹³ This suggests older individuals, despite being less frequently infected, may experience more severe clinical outcomes when infected, possibly due to age-related co-morbidities.¹⁸ A similar pattern is observed in the age 19-39 group, with a substantial percentage of symptomatic cases. This raises concern about the potential for younger individuals to experience severe illness emphasizing the importance of vaccination and preventive measures across all age groups.¹⁴

Our study explored the relationship between age groups and CT values. It reveals that a substantial proportion of infected patients, across various age groups, had CT values below 30. The lowest proportion was observed in the age group under 18 years, where CT values of 1-19 was found in a mere 9.1% of cases. Similar findings were demonstrated in the study of Mishra B et.al.¹⁵ These findings highlight the need for age-specific strategies for COVID-19 management, as the viral load, as indicated by CT values, varies across different age categories. The younger age group may have lower viral loads on average, which could influence transmission dynamics.¹⁶ Our study established a correlation between CT values and clinical symptoms. Interestingly, symptoms were present in a considerable proportion of subjects with CT values below 30. A high percentage of individuals with CT values in the range of 1-19 and 20-29 also displayed symptoms. Results demonstrated in our study were quite similar to the findings of Walker AS et al.¹⁷ These findings suggest that symptom severity may depend on the viral load, as individuals with lower CT values still exhibited more clinical symptoms.¹⁷

CONCLUSION

Symptomatic individuals diagnosed with COVID-19 tend to exhibit more pronounced symptoms and harbor a higher viral load compared to their asymptomatic counterparts. Their viral load, assessed through CT values in RT-PCR tests, is notably elevated. However, the direct clinical significance of these CT values in predicting the severity and ultimate outcomes of COVID-19 requires deeper investigation. Foretelling the course of COVID-19 effectively relies on promptly accessible, objective, and universally applicable measures

during the initial clinical assessment. These insights hold promise in shaping timely and efficient healthcare strategies, particularly in regions bracing for further waves of the pandemic.

LIMITATIONS

There are some limitations of this study. Small sample size and single centre based study are the main limitations. Also, though close monitoring of signs and symptoms in potential COVID-19 cases arriving for testing at hospital was done, there's insufficient solid evidence regarding the importance of assessing CT values from RT-PCR tests, which indicate viral loads and predict outcomes. Consequently, further investigations are recommended to better understand the significance of these values. Additional research is crucial to crafting protocols that leverage clinical and diagnostic tools for foreseeing COVID-19 outcomes.

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