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SARS-CoV-2 RT-PCR assessment in asymptomatic versus symptomatic patients from a tertiary care center with recovery monitoring: A study from Kathmandu, Nepal

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ABSTRACT

Background
The SARS-CoV-2 infection led to a global health crisis with both symptomatic and asymptomatic cases emerging. This study aimed to study the prevalence of SARS-CoV-2 among both symptomatic and asymptomatic patients visiting a tertiary care hospital in Nepal and to assess the viral load variations in COVID-19 positive cases throughout the recovery period.

Material and methods
A cross-sectional study was conducted on symptomatic and asymptomatic patients visiting Shahid Gangalal National Heart Centre, Kathmandu, Nepal, over a five-month study period from December 2021 to March 2022. The real-time (RT) PCR was performed to confirm the presence of SARS-CoV-2, and the reporting was done in terms of the cycle threshold (Ct) value.

Results
A total of 3738 samples were tested for SARS-CoV-2 during the study. Among 526(14.1%) cases that tested positive, 423(80.4%) were symptomatic and 103(19.6%) were asymptomatic. The common symptoms observed in the COVID-19 patients in our study were cough and fever. Additionally, by tracking the PCR reports of available patients concerning Ct value, we found that 81.5% of patients recovered by the third week of infection, with individuals aged 60 years or older taking the longest to recover.

Conclusion
The findings highlight the prevalence of asymptomatic cases that were widespread during the pandemic. It also implies that the use of reliable diagnostic techniques like RT-PCR with its specific Ct value is important in managing and controlling pandemics like COVID-19.

Keywords
COVID-19, cycle Threshold-Ct, Nepal, SARS-CoV-2, pandemic

Background

Wuhan city of China reported the first human case of an infectious disease termed COVID-19 in December 2019, which later emerged as a pandemic worldwide [1]. The cause of the pandemic was a virus identified as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). In Nepal, the first COVID-19 case was reported on January 13, 2020, in a student returning from Wuhan, China, and the country experienced the first wave between July 2020 and February 2021[2, 3].

The mode of transmission of the SARS-COV-2 virus is primarily respiratory droplets, which are produced by symptomatic individuals while sneezing, coughing, and talking. Surprisingly, it was found that asymptomatic COVID-19 cases also exist where individuals without symptoms of the disease could transmit the virus to others, hence serving as a silent source of transmission [4].

Among different testing methods available, Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was used worldwide as a gold standard molecular diagnostic tool for the confirmation of infection with SARS-CoV-2. This technique is efficient because it can detect genetic material as little as ~1,000 copies of viral RNA per milliliter [5]. Our study aimed to assess the prevalence of COVID-19 in both symptomatic and asymptomatic cases using RT-PCR.

Moreover, although not properly utilized in the country, the Ct value is equally important to determine the severity of COVID-19 cases. The Ct value denotes the number of amplification cycles required in RT-PCR for viral RNA to be detected in a sample [6]. Therefore, we also evaluated the Ct values of COVID-19 patients during their recovery period, where the threshold of ≥40 cycles was an indicator of viral clearance.

Material and methods

Study design and participants

This hospital-based cross-sectional study was conducted from December 2021 to March 2022 at the molecular laboratory of Shahid Gangalal National Heart Centre (SGNHC), Kathmandu, Nepal. The samples were collected from all the patients visiting the hospital, and the samples were tested for the SARS-CoV-2 virus.

Clinical and demographic information of the patients was recorded with the laboratory requisition form and questionnaire filled in by patients or medical personnel. The symptomatic and asymptomatic cases were separated based on the criterion defined by previous studies [7, 8].

Sample size calculation

The sample size was calculated using the formula:

$n= Z\ 2\times p\times q/\ e^2$

$= (1.96)^2 \times 0.463\times (1-0.46)/ (0.05)^2$

= 384 (n= minimum number of samples required for the study); Z= 1.96 (for 95% confidence interval)

p= prevalence of COVID-19 infection in previous study, 46.3% [9]

q= 1-p

e= allowable error, 5%

The minimum required sample size hence was calculated as 384. However, all the 3738 samples obtained from mass screening during the study period were included in the study.

Sample collection

The oropharyngeal and nasopharyngeal specimens were collected from a total of 3738 patients visiting the tertiary care center. The collected samples were placed in viral transport medium (VTM) and immediately transported to the COVID-19 lab for further analysis.

RNA extraction and RT-PCR

The viral RNA was extracted using a viral extraction kit (PureLink, Thermo Fisher Scientific Inc.). Extracted viral RNA was purified on a Viral Spin Column (GenElute™, Thermo Fisher Scientific Inc.).

RT-PCR was performed using a PCR kit (TaqPath™, Thermo Fisher Scientific Inc.) according to the manufacturer's instructions. In brief, RT-qPCR was performed in a 20 µl reaction mixture containing 5 µl Master Mix reaction buffer, 1 µl of Forward primer, 1 µl of Reverse primer, and 13 µl of RNA template.

The reaction was executed on PCR thermocycler (QuantStudio™ 5 Real-Time PCR System, Thermo Fisher Scientific Inc.) with the following conditions: UNG incubation at 25°C for 2 min, reverse transcription at 53°C for 10 min, Taq activation with pre-denaturation at 95°C for 2 min, denaturation at 95°C for 3 s, followed by 40 cycles of amplification with a final annealing, extension and fluorescence acquisition at 60°C for 30 s.

Assessment of Ct value during the recovery period

The Ct value was reported during the detection for the first time and in follow-up weeks until recovery.

Interpretation and Analysis

The samples with a Ct value lower than 40 of all ORF1ab/S/N genes were considered positive for SARS-CoV-2 in our study, while the cases with a Ct value of 40 or greater than 40 as negative for SARS-CoV-2.

The data collected was retrieved into MS Excel 2016, and statistical analysis (chi-square test) was performed using Statistical Package for Social Science (SPSS) software for Windows (version 25).

Ethical considerations

Ethical approval was obtained from the Institutional Review Committee SGNHC/IRC no. 53-2021.

Results

Among 3738 clinical samples collected, the majority were obtained from the asymptomatic individuals, 3278(87.7%), visiting the center. A total of 526(14%) samples tested

positive for SARS-CoV-2, out of which most positive cases were symptomatic 423 (80.4%).

More than half of the positive cases were from males 295 (56%) and the age group 15-59 was seen to be most affected with 435 (21.7%) of the total cases falling within this range (Table 1).

Table 1: Demographic and clinical characteristics of collected samples and positive COVID-19 cases

Variables	Total Tests (%) N=3738	Positive Tests (%) N=526	Chi-square	P value
Age Group				
0-14	358(9.6)	21(5.9)	208.04	<0.001*
15-59	2005(53.6)	435(21.7)		
≥60	1375(36.8)	70(5.1)		
Gender				
Male	2134(57.1)	295(56)	0.253	0.615×
Female	1604(42.9)	231(44)		
Clinical Symptoms				
Symptomatic	460(12.3)	423(80.4)	2631.5	<0.001*
Asymptomatic	3278(87.7)	103(19.6)		

*p>0.05, statistically not significant, *p<0.05, statistically significant

Clinical symptoms of symptomatic patients were recorded, and patients with at least one of the following symptoms were classified as being symptomatic, as presented in Figure 1.

The prime symptoms shown by patients were a cough followed by fever. Nasal obstruction and loss of taste and smell were other important symptoms in SARS-CoV-2-positive patients.

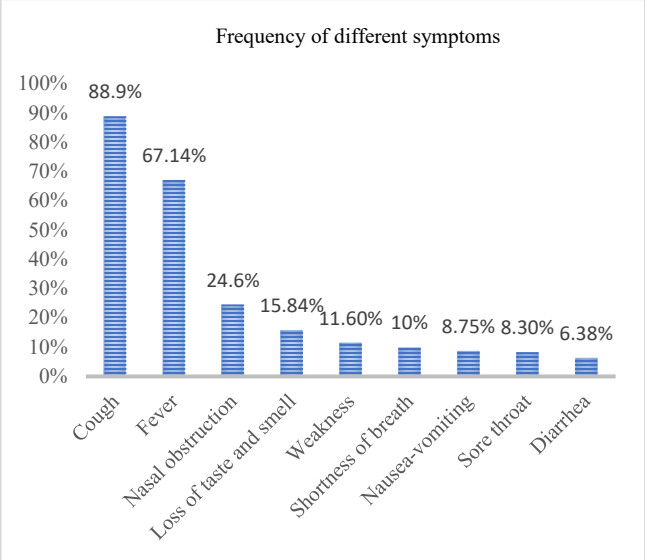

Figure 1: Clinical symptoms in symptomatic COVID-19 positive patients.

Only 65/526 COVID-19 positive patients who provided samples until the RT-PCR test was negative were included in the study. 8(12.3%) samples were available from the patients aged 0-14, 46(70.8%) from 15-59, and the remaining 11(16.9%) from patients aged ≥60. All these cases were symptomatic.

The average days required for the viral clearance for the age group ≥60 was the longest with a median of 25.45 days followed by the age group 0-14 recovered in a median of 19.25 while those in the 15-59 age group had the shortest median duration of 17.04 days. Also, around 80% of patients recovered by the third week of the initial detection of COVID-19 (Figure 2).

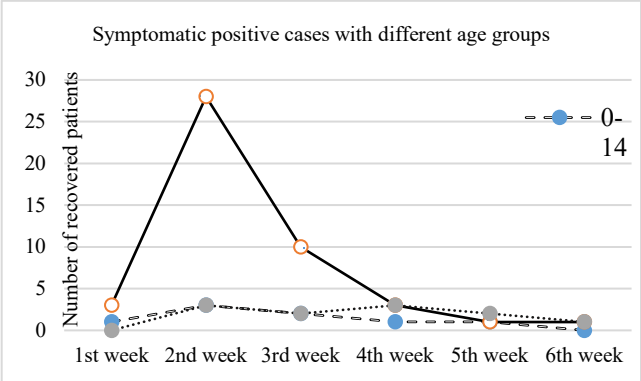

Figure 2: Recovery time among different age groups

When first tested, 100% of the patients had a Ct value below 40. The value then increased over 40 in most cases (81.5%) by the third week of recovery, indicating a negative result (Figure 3).

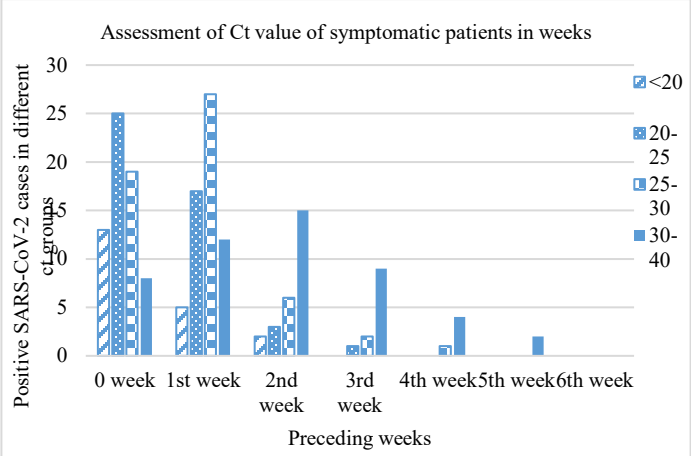

Figure 3: Ct values in different weeks of recovery

Discussion

Both symptomatic and asymptomatic cases have a role in the transmissibility of COVID-19; however, asymptomatic cases are most likely to be missed in detection [10]. In our study using RT-PCR as the diagnostic method, the prevalence of asymptomatic cases was 19.6% which is similar to that reported by a meta-analysis [11]. The identified positive asymptomatic cases highlight the importance of appropriate detection strategies.

As compared to females, males were more infected, and the age group 15-59 showed the highest rate (82.7%) of infection. The common symptoms among the patients were cough and fever, which are similar to other studies [12, 13]. Furthermore, among 65 symptomatic cases that provided samples regularly until recovery, the recovery rate varied among different age groups, with older people ≥60 taking the longest to recover, as seen in other studies [14, 15]. The delayed recovery in this age group is generally due to the presence of comorbidities and immunosenescence [16, 17]. Overall, 81.5% of the patients recovered by three weeks, which was similar to a study done by Shaikh et al. (2023) and Ibitoye et al. (2022) [18, 19].

The rising Ct values in different weeks of recovery can be used to interpret different stages of infection as well as viral clearance [20]. Moreover, tracking the Ct values is helpful in recognizing asymptomatic positive cases, which is helpful to reduce the risk of disease transmission [21].

Conclusion

The prevalence of asymptomatic COVID-19 cases was comparatively high (80.4%) in our study. Cough and fever were the most common symptoms in symptomatic positive cases. The Ct value was used to assess the viral load and to track the recovery, with most of the COVID-19 cases resolving by the third week of the infection.

Abbreviations

Severe Acute Respiratory Syndrome-Corona Virus-2 (SARS-CoV-2), Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), Corona Virus Disease-19 (COVID-19), Cyclic threshold value (Ct value), Ribonucleic acid (RNA)

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Authors' contribution

a. Study planning: GR, UK, DP
b. Data collection: GR, UK
c. Data analysis/ interpretation: GR, DP, SA, DK
d. Manuscript writing: GR, DP
e. Manuscript revision: UK, SA, DK
f. Final approval: GR, UK
g. Agreement to be accountable for all aspects of the work: GR, UK, DP, SA, DK

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Availability of data and materials

All data underlying the results are available as part of the article.

Competing interests

The authors declare that there are no conflicts of interest to disclose in relation to this manuscript.

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