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Clinica Chimica Acta 533 (2022) 42–47

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Elucidation of correlation between SARS-CoV-2 *RdRp* and *N* gene cycle threshold (C*t*) by RT-PCR with age and gender

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ARTICLE INFO

Keywords:
Coronavirus
Age
Gender
Cycle Threshold
RdRp gene
N gene
rRT-PCR

ABSTRACT

Background: Coronavirus disease 2019 (COVID19) caused by the new severe acute respiratory syndrome coronavirus 2 (SARSCoV2) is a global public health emergency. Age and gender are two important factors related to the risk and outcome of various diseases. Cycle threshold (C*t*) value is believed to have relation with age and gender.

Objective: This study has been conducted to investigates the association between SARS-CoV-2 cycle threshold to age and gender of COVID-19 patients, to investigate whether the population-wide change of SARSCoV2 RTPCR C*t* value over time is corelated to the number of new COVID19 cases and to investigate the dynamic of *RdRp* and *N* genes.

Methods: 72,811 individuals from second wave of COVID19, were observed in current study at Pure Health Lab, Mafraq Hospital, Abu Dhabi, UAE.

Results: 15,201/72,811 (21 %) positivity was observed. COVID-19 were more prevalent in males (59.35%) as compared to female (40.65%). The Positivity rate were significantly higher in Male than in Female cases (p-Value = 0.04). The C*t* values for both targets of all the samples were ranged from 4.57 to 29.73. Longitudinal analysis showed significant increased during the study period from starting to end as were hypothesized. Interestingly, both the targets (*RdRp* and *N*) were present in age < 1 year. Which may indicate that mutated strains are not prevalent in children's < 1 year.

Conclusion: There was no statistically significant difference in viral loads in between age-groups. Males were tending to higher viral load compared to females. The findings have implications for preventive strategies.

1. Introduction

SARS-CoV-2, is a non-segmented + -ive sense ssRNA virus, belongs to the genus of β-coronavirus and family coronaviridae. The size of genome is ranging 26–32 kb and is comprising of a ORF-1ab which coded for RNA replication proteins, leader sequence and for nonstructural proteins (nps) genes and structural-proteins including envelope (E), spike (S), nucleoprotein (N) and membrane protein (M) [1–3].

The coronavirus disease COVID19 caused by SARSCoV2 was first reported in Dec 2019 at Wuhan city China. The COVID-19 has spread to more than 226 territories and countries with about 507,035,403 cases and 6,232,220 fatalities by April 21, 2022 [4] and become an emergency for public health globally. On March 11, 2020 WHO declared this a pandemic globally [5]. After declaration of pandemic, quarantines, social distancing, and travel restrictions put the world economy on hold. Furthermore, there is no effective vaccine neither specific medicine for COVID19 at this stage. The global economy to reopen is totally based on effective diagnostics, contact tracing, patient isolation and quarantine.

For this reason, understanding viral load dynamics and covariates is critical to determining protective measures for individuals and the public. Studying the dynamics of viruses and their changes in population subgroups can help to understand the role of age, gender, and other

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https://doi.org/10.1016/j.cca.2022.06.008
Received 24 April 2022; Accepted 5 June 2022
Available online 14 June 2022
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factors in the epidemiology of the disease. Age and gender are two important factors related to the risk and outcome of COVID19 disease [6]. The morbidity COVID19 is also seems to be affected by patient gender and age. It has been observed that elder age-groups have more severe sign and symptoms of COVID19 with high fatality rate than in children's [7]. Preliminary reports also showed that gender has a role in the epidemiology of a disease. Study conducted in China revealed that males are at high risk of disease and mortality as compared to females [8].

In recent guideline Real-time reverse-transcriptase PCR (rRT-PCR) is recommend by WHO for the detection of SARSCoV2 [9]. Various studies interrelated the cycle threshold values (C*t*) with viral load and severity of the disease [10,11]. Tom et al. [12] proposed C*t* values of targets for considering in clinical decision-making. The concept that C*t* value is negatively correlated with viral load is very tempting, especially when it comes to managing patients' hospital stays during a pandemic. It is always recommended to use two molecular targets at the same time to avoid possible cross reactions with other endemic coronaviruses and a possible genetic drift of SARSCoV2 [13,14]. The knowledge of viral dynamics is crucial for making strategies for epidemiological control, management, and treatment of COVID19 [15]. Several reports on viral dynamics of COVID-19 positive cases, showed that C*t* value of the target is inversely-proportional to the viral loads [16–20]. It is important to distinguish between viral load and infectious dose and the related effects of the two. The infectious dose of the virus refers to the dose that is sufficient to cause infection if a person is exposed to the virus. On the other hand, viral load is the amount of virus particles that an infected patient carries in their system. The higher the viral load of a patient, the more virus is released, which makes it more likely that other people will be exposed to the infectious dose needed to get the disease. Like general viruses, the viral load of COVID19 patients can be derived from the cycle threshold (C*t*) value of the RTPCR test on the obtained samples. The RTPCR test amplifies viral RNA from patient samples until it reaches a detectable concentration above a threshold. The number of cycles required for this to happen is called the C*t* value. Therefore, the lower the C*t* value of the patient sample, the higher the viral load, and the higher the C*t* value, the lower the viral load. [21].

Recently, a mutation (C29200T) in *N* gene is affecting its detection in COVID19 patients. Interestingly, the mutation type is C: T, a common single nucleotide polymorphism (SNP) which may be related with strong host-cell mRNA-editing mechanisms, known as apolipoprotein-B mRNA-editing enzyme, a catalytic polypeptide-like cytidine deaminase (APOBEC) [22–24]. Another report showed, G: U substitution in position 29140, which also affect the detection of *N* gene [25].

In present cohort, we are exploring the dynamics of N and *RdRp* gene from 15,201 COVID-19 positive cases. There is limited literature exploring the association of C*t* values with gender and age and association of higher viral load with a higher degree of transmissibility. First objective of the study is to investigates the correlation between SARSCoV2 cycle threshold to age and gender of COVID19 patients. And the second purpose is to investigate whether the population-wide change in the SARSCoV2 RTPCR C*t* value over time is related to the number of new COVID19 cases. Our hypothesis is based on a higher viral load indicating greater viral shedding, a higher risk of exposure to infectious doses, and therefore a higher degree of transmission.

2. Methodology:

2.1. Design of study:

This study was conducted on 72,811 individuals from second wave of COVID19, in Pure Health Lab, Mafraq Hospital in UAE. The Pure Health is one among the main and largest in COVID-19 screening center in UAE. Data on the total number of tests per day and the newly diagnosed cases were attained from the TrakCare, a centralized database. The highest number of cases (8839, 12.13%) were observed on May 26, 2021, followed by 8717, 11.97% cases on May 31, 2021. Data collected included age, sex, and date wise positivity. Out of 72,811 individuals 15201, 21% were positive for SARSCoV2 (Table 1). Only two parameters; age and gender in combination with C*t* values of the positive samples were considered for the study. Other parameters such as patient conditions, infection source, other complications and treatment were not taken for the current study because of retrospective cohort. The SARS-CoV-2 positive test confirmation was made according to kit manufacturer instructions and the guidelines. The consent was waived from the positive individuals due to retrospective cohort nature of study.

2.2. Collection, processing of samples and extraction of RNA:

For the detection of SARS-CoV-2 nasopharyngeal specimens were collected from 72,811 individuals by using Disposable Virus Specimen Collection Tube (SUNGO Europe B.V. Amsterdam, Netherlands) and transported to Pure Health laboratory, Mafraq Hospital for testing. The specimens were properly vortexed and RNA extraction was done with automation (TianLong, GeneRotex, China) following manufacturer guidelines, Briefly, specimens were properly vortexed for 10–14 s followed by extraction from 200-µl of sample, and all the specimens were subjected to automation extraction with 50-µl elution volume.

2.3. SARS-CoV-2 detection using rRT-PCR:

The qualitative rRT-PCR was done for the detection of COVID19 positive individuals and C*t* values for *RdRp* and *N* genes were recorded. The rRT-PCR assay was performed on the CFX96 Real-Time PCR-Detection-System (BioRad Laboratories, Inc.). The master mix preparation and amplification profile were used as per manufacturer instructions (LabGun^{TM} COVID-19 ExoFast RT-PCR Kit, LabGenomics Co., Ltd. Korea). Briefly, master mix contains 4 µl of 5x ExoFast 1step Buffer, 2 µl of ExoFast 1step Enzyme, 4 µl of Assay, 5 µl of RNase Free Water and 5 µl of Template RNA. 20 µl is the total volume/sample. The rRT-PCR were run under the following profile: reverse transcription at 50 °C for 5 min, pre- denaturation at 95 °C for 1 min, 32 cycles of denaturation at 95 °C for 1 s and extending and collecting fluorescence signal at 60 °C for 1 s. The C*t* values for *N* and *RdRp* genes under 30 were considered as positive result. Confirmatory RT-PCR was done if first reaction showed the C*t* values in between 28 and 30. Positive, negative, and internal controls were included for IQC purpose.

2.4. Statistical analyses

IBM-SPSS 25.0 (SPSS. Inc. Chicago. IL. USA) and Microsoft Excel 2016 were used for the data statistical analysis. The data was arranged into a daily group comprising of 11 days starting from May 22nd and ending on May 31st, 2021, and an age group, consisting of 11 age groups starting from < 1 year to 100 years. Data were presented as median, mean, stander error (ER), standard deviation (SD), Interquartile-range (IQR), Chi-square (x^2) and *p*-Value. One-way and Two-way ANOVA

Table 1
Date-wise Positivity of the COVID19 patients.

Date	Total Cases	Positive	%	Negative	%
22/05/2021	6175	1212	8%	4963	9%
23/05/2021	7833	1595	10%	6238	11%
24/05/2021	7129	1037	7%	6092	11%
25/05/2021	7192	2064	14%	5128	9%
26/05/2021	8839	1769	12%	7070	12%
27/05/2021	7634	1531	10%	6103	11%
28/05/2021	6160	1520	10%	4640	8%
29/05/2021	6216	1246	8%	4970	9%
30/05/2021	6916	1560	10%	5356	9%
31/05/2021	8717	1667	11%	7050	12%
	72,811	**15,201**	**21%**	**57,610**	**79%**

were used in a Univariate Post Hoc Multiple Comparison for Observed Means (Tukey, Duncan, Heteroskedasticity Tests; F-test and White's test) and multivariable-analysis to show the association between Ct values of *RdRp* and *N* genes, with gender and age. For all the tests, *p*-Values < 0.05 were considered as statistically significant.

3. Results

During this study period 72,811 patients were tested for COVID-19 using specimens taken from nasopharyngeal, 15,201 (21 %) of which showed positive result for COVID-19. In positive cases approximately 9022/15,201, 59.35% of the study population were males and most of them (2669/9022, 29.58%) were from 31 to 40 years old age group. In case of females, 6179/15,201, 40.65% of the study population were females and most of them (1625/6179, 26.29%) were from 31 to 40 years old age group (Table 3). The C*t* values for both targets of all the samples were ranged from 4.57 to 29.73, with a mean of 15.60 for *RdRp* gene, SD: 5.83; median: 15.23; SE: 0.047, and mean for *N* gene: 15.29, SD: 5.96; median: 14.97; SE: 0.048) (Table 2). The means of.

C*t* values for *RdRp* gene varying between age groups from 7.16 to 16.98 and for *N* gene varied between age groups from 7.10 to 16.34.

3.1. Univariate Post Hoc Multiple comparison analysis

Analysis between *RdRp* gene and age-groups using one-way ANOVA showed no significance in all age groups (*p*-Value = 0.28) also no significance was observed between *N* gene and age groups (*p*-Value = 0.19). A comparison of mean C*t* values of males (n = 9022/15,201, 59.35%) and females (n = 6179/15,201, 40.65%) revealed that men had a statistically significant higher mean than females. The Positivity rate were significantly higher in Male than in Female cases (*p*-Value = 0.04) (Table 2). When positive rate according to age were analyzed, it was observed that positive rate was increased from 11.84% (age 1–10) to 28.24% (age 31–40) (Table 3). Longitudinal analysis showed significant increased during the study period from starting to end as were hypothesized, from 22 to 24th May 2021, 3844/15,201, 25.28% positivity was observed followed by 25-27th May 2021, 5364/15,201, 35.28% and

Table 3
15,201 samples baseline characteristics by age and gender.

Age Group	Positivity		Gender			
			Male		**Female**	
	N	**%**	N	%	N	%
<1	80	0.53	41	0.45	39	0.63
1–10	1801	11.84	910	10.08	891	14.41
11–20	1862	12.24	959	10.62	903	14.61
21–30	3227	21.22	1937	21.46	1290	20.87
31–40	4294	28.24	2669	29.58	1625	26.29
41–50	2394	15.74	1550	17.18	844	13.65
51–60	1007	6.62	675	7.48	332	5.37
61–70	391	2.57	215	2.38	176	2.84
71–80	109	0.71	58	0.64	51	0.82
81–90	34	0.22	8	0.08	26	0.42
91–100	2	0.013	0	0	2	0.03
	15,201		9022	59.35	6179	40.65

Table 2
Statistical analytics of C*t* values of 15,201 samples Positive for SARS CoV-2.

No. of Specimens		Target 1 (FAM, *RdRp*)							Target 2 (Cy5, *N*)						
		Mean CT	**Median CT**	**SD**	**SE**	**IQR**	x^2	***p* Value**	**Mean CT**	**Median CT**	**SD**	**SE**	**IQR**	x^2	***p* Value**
Overall		15.60	15.23	5.84	0.05	20.68–10.45	75.65	0.28	15.30	14.97	5.96	0.05	20.53–10.12	84.36	0.19
Sex															
Male	9022	15.64	15.24	5.84	0.06	20.74–10.56	76.74	**0.04**	15.34	15.04	5.97	0.06	20.59–10.19	78.67	0.06
Female	6179	15.54	15.20	5.83	0.07	20.6–10.34	70.71	0.46	15.23	14.87	5.95	0.08	20.44–10.01	77.72	0.75
Age Groups															
< 1 Years	80	16.20	15.57	5.66	0.63	20.84–11.35	7.50	0.69	15.86	15.67	5.94	0.66	20.68–10.6	7.59	0.80
Male	41	16.08	15.27	5.45	0.85	20.74–12.79	5.79	0.58	15.75	15.53	5.50	0.86	20.51–11.77	6.94	0.49
Female	39	16.32	16.99	5.93	0.95	21.48–10.3	2.74	0.99	15.95	16.09	6.44	1.03	21.13–9.4	4.17	0.72
1–10	1801	15.55	15.24	5.86	0.14	20.66–10.42	13.27	0.88	15.28	15.03	5.94	0.14	20.45–10.06	9.48	0.74
Male	910	15.31	14.86	5.82	0.19	20.15–10.22	14.28	0.49	14.96	14.49	6.03	0.20	20.17–10	10.57	0.40
Female	891	15.80	15.51	5.88	0.20	21.06–10.58	5.26	0.63	15.34	15.37	6.15	0.21	20.76–10.01	6.12	0.71
11–20	1862	15.43	14.83	5.89	0.14	20.66–10.24	15.28	0.70	15.09	14.54	6.07	0.14	20.54–9.82	9.85	0.78
Male	959	15.55	15.07	5.82	0.19	20.94–10.42	11.94	0.43	15.14	14.76	6.08	0.20	20.6–9.95	10.03	0.43
Female	903	15.30	14.63	5.95	0.20	20.61–10.1	8.07	0.77	14.83	14.22	6.27	0.21	20.44–9.61	5.78	0.67
21–30	3227	15.56	15.20	5.86	0.10	20.71–10.36	5.95	0.64	15.23	14.94	5.99	0.11	20.49–10.01	7.41	0.92
Male	1937	15.43	15.07	5.89	0.13	20.51–10.24	13.75	0.88	15.04	14.78	6.06	0.14	20.31–9.84	16.87	0.80
Female	1290	15.76	15.46	5.81	0.16	21.11–10.57	6.54	0.52	15.35	15.07	6.04	0.17	20.89–10.09	6.90	0.84
31–40	4294	15.67	15.34	5.82	0.09	20.63–10.54	4.21	0.54	15.39	15.11	5.93	0.09	20.47–10.24	5.92	0.59
Male	2669	15.82	15.49	5.83	0.11	20.93–10.77	4.39	0.75	15.48	15.26	6.02	0.12	20.70–10.34	6.81	0.70
Female	1625	15.44	15.17	5.79	0.14	20.36–10.21	6.51	0.64	15.09	14.69	5.93	0.15	20.12–9.98	4.70	0.80
41–50	2394	15.68	15.37	5.82	0.12	20.74–10.67	3.32	0.35	15.38	14.98	5.95	0.12	20.73–10.28	2.51	0.25
Male	1550	15.88	15.59	5.86	0.15	21.14–10.87	3.01	0.11	15.51	15.17	6.06	0.15	20.95–10.4	2.43	0.06
Female	844	15.29	14.91	5.72	0.20	20.13–10.3	8.81	0.55	14.96	14.55	5.92	0.20	20.11–9.96	8.88	0.56
51–60	1007	15.48	14.88	5.83	0.18	20.87–10.27	4.30	0.44	15.14	14.50	5.93	0.19	20.53–10.08	4.80	0.45
Male	675	15.49	15.05	5.84	0.22	20.67–10.23	3.86	0.16	15.11	14.56	6.05	0.23	20.53–9.9	3.87	0.14
Female	332	15.44	14.64	5.80	0.32	21.16–10.54	5.90	0.51	14.92	14.18	6.00	0.33	20.42–10.05	5.22	0.43
61–70	391	15.88	15.58	5.69	0.29	20.67–11.2	8.54	0.66	15.69	15.37	5.76	0.29	20.74–10.97	12.47	0.68
Male	215	15.82	15.50	5.60	0.38	20.48–11.42	7.34	0.47	15.41	15.32	5.94	0.41	20.43–10.58	11.38	0.63
Female	176	15.94	15.90	5.80	0.44	21.13–11.01	6.11	0.95	15.76	15.36	5.85	0.44	21.08–10.87	9.19	0.86
71–80	109	15.54	15.01	5.82	0.56	20.71–10.03	5.44	0.75	14.98	14.13	5.85	0.56	19.75–9.50	6.62	0.79
Male	58	15.42	14.55	5.77	0.76	20.46–10.8	8.10	0.84	14.80	13.91	5.90	0.78	19.72–10.33	8.47	0.91
Female	51	15.68	16.47	5.94	0.83	20.92–9.49	5.18	0.79	15.19	15.69	5.84	0.82	19.95–8.99	5.74	0.75
81–90	34	16.98	17.07	5.91	1.01	22.45–12.1	9.42	0.84	16.34	17.09	5.62	0.96	21.32–11.58	11.19	0.94
Male	8	18.04	16.91	5.89	2.08	23.27–12.55	3.11	0.56	17.51	17.18	5.40	1.91	22.14–12.3	2.70	0.68
Female	26	16.65	17.78	6.00	1.18	22.45–11.29	9.59	0.61	15.98	16.94	5.73	1.12	21.32–10.67	11.35	0.76
91–100	2	7.16	7.16	3.66	2.59	9.75–4.57	–	0.26	7.10	7.10	5.48	3.88	10.97–3.22	2.00	0.09
Female	2	7.16	7.16	3.66	2.59	9.75–4.57	–	0.26	7.10	7.10	5.48	3.88	10.97–3.22	2.00	0.09

28-31st May 2021, 5993/15,201, 39.42% (Table 5).

3.2. Multivariable analysis

A two-way analysis of variance was performed to discover the effects of age and gender on the C*t* values of the *RdRp* and *N* genes. There is no significant interaction between age and sex and the C*t* value. The main effect indicates that after adjusting for age groups, the statistically significant difference between men and women still exists (p-Value = 0.04). The tow-way analysis of variance also did not show statistically significant differences between the age groups (Table 2).

3.3. SARS-CoV-2 Gene's dynamic

The RT-PCR results interpretation for the two genes *RdRp* and *N* revealed that: 15,126/15,201 (99.55%) of cases were positive for 2 genes *RdRp* and *N*. All cases 15,201/15,201 (100%) were positive for *RdRp* gene while 15,126/15,201 (99.55%) of cases were positive for *N* gene. (Table 4). The median, mean and interquartile-range (IQR) of the CTs values for FAM: *RdRp* (Target 1) were all numerically higher than comparative CTs values for Cy5: *N* (Target 2) (mean, 15.60 vs 15.30; median, 15.23 vs 14.97; IQR, 20.68–10.45 vs 20.53–10.12). (Table 2) When stratified by age, the mean C*t* values for each age-group were statistically equivalent to the entire data set for each target. Interestingly, both the targets (*RdRp* and *N*) were present in age < 1 year. Which may indicate that mutated strains are not prevalent in children's < 1 year. Further study is necessary to find the actual cause. Table 2 showed the details of statistical analysis for all the data.

4. Discussion

The current SARSCoV2 pandemic is the 3rd outbreak related to coronavirus in 21st century, and extremely the rate of positive COVID19 cases have surpassed both MERS and SARS [4,26]. In present cohort, we exploring the dynamics of *RdRp* and *N* gene from the 15,201 COVID-19 positive cases. There is limited literature exploring the association of C*t* values with gender and age and correlation of higher viral load with a higher degree of transmissibility.

In current study we report 15,201 (21%) COVID-19 positive cases from 72,811 samples. In contrast to this, one of hospital in Wuhan City, China, reporting 38.42% positivity which is higher than our study [27]. We also revealed that the COVID-19 infection was prone to affect men more than women with different percentages of 59.35% and 40.65% respectively. Study form Morocco showed the same trends for male and females, 55.76% and 44.24% respectively [28]. Our results are consistent with many conclusions from different studies of Wuhan, China [18,29]. This increased prevalence in male is mainly due the fact that their high expression of *ACE-2* receptors in men, high ratio of drinking and smoking among men [30]. Furthermore, this can also be driven by cultural norms. Men are more socialize outdoors and thus could be infected [31].

The current study compared COVID19 viral load, as indicated by C*t* values, across eleven age groups, and between males and females. It found that viral load in patients did not differ by age group but was higher among males as compared to females (p-Value = 0.04). Similarly, significance was observed in the study conducted by Mahallawi et al. which revealed the significance in case of male (p-value = 0.002) [32].

Table 5
SARS-CoV-2 Nucleic Acid Targets Positive rate in different date periods.

NA	22-24th May (n = 3844, 25.28%)		25-27th May (n = 5364, 35.28%)		28-31th May (n = 5993, 39.42%)	
	n	**Positive rate**	**n**	**Positive rate**	**n**	**Positive rate**
FAM, RdRp	3844	100	5364	100	5993	100
Cy5, N	3829	99.6	5315	99.08	5982	99.81
Double Positive	3829	99.6	5315	99.08	5982	99.81

In current study the C*t* values for both targets were ranged from 4.57 to 29.73, with a mean and SD, 15.60, 5.83 respectively for *RdRp* gene and 15.29 and 5.96 for *N* gene. In contrast to our study, study conducted by Waleed et al. revealed that the C*t* values were ranged 15.08–35, with a mean of 27.44 (SD: 5.23) [32]. In our study the means of C*t* values for *RdRp* gene (target 1) were varied between the age groups 7.16–6.98 and for *N* gene 7.10–16.34. The study by Mahallawi et al. showed that means of C*t* values varied between age groups from 27.05 to 27.82. Analysis between *RdRp* gene and age groups using one-way ANOVA indicated no statistically significant in all age groups (p-Value = 0.28) also no significance was observed between *N* gene and age groups (p-Value = 0.19). Study by Mahallawi et al. revealed the same, no significant difference was observed (p-value = 0.135) [32].

To date, the literature has not more highlighted the correlation between the RTPCR C*t* value in samples from infected people and the total number of positive cases over time. In this study, the C*t* value was used as an indicator of viral load and transmissibility. Considering that positive cases with a low C*t* value have a higher viral load than cases with a high C*t* value, and a higher viral load will lead to an increase in the spread of the virus, which leads to an increase in infectivity in the population, so the population can reasonably be expected to be affected. Decreasing the C*t* value will be associated with an increase in cases [31]. The argument of this study is that the viral load is directly proportional to the infectivity of the virus infection. The relationship between viral load and risk of transmission has been established in other viral diseases [33,34]; COVID19 seemed likely following the similar pattern of transmission [35]. Therefore, the identification of factors related to viral load can help preventive strategies and identify the groups that are at higher risk of transmission. According to our findings, the lower the C*t* value, the higher the proportion of new positive tests in the population. Longitudinal analysis showed significant increased during the study period from starting to end, from 22 to 24th May 2021, 3844/15,201, 25.28% positivity was observed followed by 25-27th May 2021, 5364/15,201, 35.28% and 28-31st May 2021, 5993/15,201, 39.42% the highest. Similarly, a recent population-based study (preprint) from Massachusetts, USA has determined the relationship between the population-level cross-sectional distribution of C*t* values and the growth rate of the epidemic. These data were used to successfully develop an accurate inference model of the outbreak trajectory [36]. A surveillance study in Italy also reported that the C*t* value increased significantly during three different consecutive epidemic periods, indicating that it may be related to the underlying epidemiological dynamics [37].

To best of our knowledge, the dynamic of the different targets for SARS-CoV-2 were not analyzed before. According to our results 15,126/15,201 (99.55%) of cases were positive for both the targets while 15,201

Table 4
SARS-CoV-2 Nucliec Acid Targets Positive rate in Male and Female groups.

NA	Male (n = 9022)		Female (n = 6179)		Total (n = 15201)		x^2	p Value
	n	**Positive rate**	**n**	**Positive rate**	**n**	**Positive rate**		
FAM, RdRp	9022	100	6179	100	15,201	100	3.00	0.08
Cy5, N	8982	99.55	6144	99.43	15,126	99.5		
Double Positive	8982	99.55	6144	99.43	15,126	99.5		

(100%) were positive for target 1 while 15,126 (99.55%) of cases were positive for target 2. In contrast to this study conducted by Benrahma et al. revealed that 4% of cases were positive for 3 genes *RdRp*, *N*, and *E*. 31% cases were positive only for *RdRp* gene, 3% of cases were positive for both *RdRp* and *N* gene [28]. In current study the median, mean and interquartile-range (IQR) of the CTs values for *RdRp* (Target 1) were all numerically higher than comparative CTs values for *N* (Target 2) (mean, 15.60 vs 15.30; median, 15.23 vs 14.97; IQR, 20.68–10.45 vs 20.53–10.12). In contrast to this study by Buchan et al. revealed that the mean, median, and interquartile-range (IQR) of CTs for Target 1 were all numerically lower than comparative values for Target 2 (median, 25.02 vs 25.93; mean, 25.26 vs 26.29; IQR, 20.35–20.99 vs 21.14–21.73) [38]. When stratified by age, the mean C*t* values for each age group were statistically equivalent to the entire data set for each target. Interestingly, both the targets (*RdRp* and *N*) were present in age < 1 year. Which may indicate that mutated strains are not prevalent/ not affecting the children's < 1 year. Further study is necessary to find the actual cause.

The limitations of this study are the lack of clinical data and therefore the inability to correlate laboratory values with the stage or severity of the disease. In addition, considering only respiratory samples, the study does not include alternative elimination pathways, which may represent the future development of the study.

The spread of viral diseases like COVID19 among the population is multi-factorial. Public health measures, testing protocols, population compliance, and viral factors all play a role in the degree of transmission, the rate of positive cases, and the epidemiological trajectory. In summary, the study did not find statistically significant differences in viral load between age groups. The results showed that males have a higher viral load of SARSCoV2 compared to females. Longitudinal analysis showed that the study period increased significantly from baseline to end, as assumed. These findings have implications for prevention strategies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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