SARS-CoV2 Viral Ct Values Correlation with Some Patients’ Factors and Disease Outcome

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Abstract:

Viral load of COVID-19 can be concluded from the cycle threshold (Ct) values measured by RT-PCR test performed on suspected samples. SARS-CoV2 viral load has not been standardized yet to reflect the outcome of COVID-19 infection. This cross-sectional study was performed to detect the frequency of SARS-CoV2 among suspected patients and the correlation of viral RNA load represented by cycle threshold (Ct) values with patients’ clinical presentation (disease severity), comorbidities and demographic data. A total of 520 participants suspected to have SARS-CoV2 infection were enrolled. After filling a questionnaire, a nasopharyngeal swab was collected from each patient and subjected to RT-PCR with the use of specific primers. Ct values of positive cases were obtained. Results revealed that 277 (53.3%) of samples were positive by RT-PCR, of which 160 (57.8 %) were males and 117 (42.2 %) females, with mean Ct value being 27.43. There was no significant correlation of Ct values with different age groups, gender and comorbidities. However, higher Ct values (lower viral RNA load) were recorded in severe and critical patients, which was an interesting result.

Key words: SARS-CoV2, Ct value, Disease severity, Comorbidities.

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Introduction:

SARS-CoV-2 is a member of the beta Corona viruses genus and order Nidovirales [1]. It has a single-stranded positive RNA genome of 26–32 kb and four structural proteins, including membrane (M), spike (S), protein nucleocapsid (N), and glycoprotein envelope (E), along with six accessory proteins that are encoded by ORF6, ORF3a, ORF7b, and ORF7a and ORF8 genes [2]. Human corona viruses such as the beta-corona viruses HCoVs-OC43, HCoVs-HKU1, the SARS-CoV, and the MERS-CoV the alpha-corona viruses HCoVs-NL63 and HCoVs-229E have all been identified [3]. The major cell surface receptor for both SARS-CoV-2 and SARS-CoV is ACE-2, according to genome sequence analysis utilizing the spike protein which identifies the entry of the receptor binding site [4]. Whereas, the dominant receptor for MERS-CoV is dipeptidyl peptidase 4 (DPP4) [5]. Similar to SARS-CoV, SARS-CoV 2 was proven by the evidence that it can be spread through contact and inanimate objects as well as directly from person to person through respiratory droplets. According to estimates, SARS-CoV-2 infection can cause asymptomatic incubation to last anywhere from 1 to 14 days (most often 3–10 days) [6]. Since its discovery in patients with severe pneumonia in Wuhan, SARS-CoV-2 has spread quickly [7]. A large majority of documented instances of COVID-19 disease had a mild course, despite the disease's known severity range being from mild upper respiratory infection symptoms to severe pneumonia [8].

The basic reproduction number (R0) which gives the average number of individuals infected from a person infected with COVID-19, ranges from 2.2 to 2.6 with a doubling time of 6.4 days. If R0 is greater than 1, continuous transmission is envisaged. The R0 value of SARS-CoV and MERS-CoV was determined as less than 1 and 1.4 - 2.5 respectively. The higher R0 of SARS-CoV-2 indicates more infectious nature regarding the virus and the potential of SARS-CoV-2 as a pandemic [9]. SARS-CoV-2 RNA might be identified 1–3 days prior to the beginning of symptoms. In addition, the viral concentration is high surrounding the day of symptom onset and then gradually decreased by the time. Asymptomatic infected individuals may require 1-2 weeks for RT-PCR results to be negative, while individuals with mild-to-moderate disease may need up to 3 weeks [10]. Furthermore, several studies have examined the relationship between high viral loads and the severity of COVID-19. The findings have shown that there is either a strong, mild or no statistical relationship with COVID-19 disease severity [11]. Diabetes mellitus and hypertension have been the most prevalent comorbidities identified [12]. A worse prognosis has been seen in older adults and individuals of any age who had underlying comorbidities, including diabetes and hypertension. [13]. Infected people typically have major illness and breathing problems in 1 out of 6 cases, particularly the elderly and those with underlying medical disorders. [14]

The viral load of COVID-19 of infected people can be concluded from the cycle threshold (Ct) values regarding RT-PCR test performed on the received samples. The RT-PCR test magnifies the viral RNA from the infected person's sample until it is at a measurable
concentration that surpasses the threshold value. The number of cycles essential for that to take place is referred to as the Ct value. The lesser the Ct value of an infected person's sample, the greater the viral load and vice versa \[15\].

The goal of this study was to identify the incidence of COVID19 infection among examined patients and to investigate a correlation between cycle threshold (CT) of viral RNA with patients’ clinical presentation, comorbidities and demographic data.

**Patients and Methods**

**Ethical approval:**

This research was approved by Research Ethics Committee at the Directorate General of Health No. 24102021-10-16 at 24/10/2021. Informed consents were taken from all participants.

**Study design:**

This cross-sectional study was conducted in Duhok, Iraq at Duhok Central Lab, COVID-19 center from September 2021 to May 2022.

**Study population:**

A total of 520 individuals suffering from symptoms of upper or lower respiratory tract infections and suspected of SARS-CoV2 infection living in Duhok governorate, participated in this study.

**Questionnaire:**

A pretest questionnaire was filled by all participants to answer questions about demographic criteria, age, gender, any comorbidities, history, duration and timing of symptoms. According to National Institute of Health (NIH) COVID-19 guidelines for symptoms, we classified them into several groups such as asymptomatic (individual's without symptoms), mild (who had any of these symptoms including; cough, fever, malaise, sore throat, muscle pain, headache, vomiting, nausea, loss of smell and taste, diarrhea, yet without shortness of breath & dyspnea), moderate (as mild symptoms but with oxygen saturation (SpO2) ≥94% on room air at sea level), severe (those with a blood oxygen saturation (SpO2) < 94% at room air at sea level) and critical (patients with multiple organ dysfunction, septic shock and/or respiratory failure).

**Sample processing:**

All participants were subjected to nasopharyngeal and oropharyngeal swabs. The specimens then transported in viral transport medium (VTM) to the lab and extraction of viral RNA was done immediately by Add Prep Viral Nucleic Acid Extraction kit and Revere transcription of viral RNA and amplification by real-time RT PCR by rotor-gene Q cycler using SARS-COV-2 Nucleic acid detection kit (Zybio), according to manufacturer's instructions.

**Specific probe and primers of SARS-COV-2 were used for RT PCR:**

Reverse transcription and subsequent amplification of a particular target sequence were conducted in the same reaction well in a one-step real-time RT-PCR format for the detection. Utilizing specific primers and a fluorescent-labeled probe, reverse transcriptase was utilized to convert the extracted RNA target into complementary DNA, which was later on utilized for amplifying a conserved section of the ORF1ab and N genes for SARS-CoV-2. When both of the target genes' Ct values were below 40, the result was deemed positive; and when both values were above 40, it was considered as negative. It was considered a single-gene positive if only one of the target genes had a Ct value less than 40 and the other had a value over 40. The corresponding sequences for open reading frame 1ab were
5'-CCCTGTGGGTTTTA CACTTAA-3' (F), 5'-ACGATTGTGCATCAGCT GA-3' (R), and 5'-CY3-CCGTCCTCGGTATGG-BHQ1-3' (probe), and those for N were 5'-GGGGAACCTCCTGCTAGAAT-3'(F), 5'-CAGACATTITGCTCTCAAG CTG-39(R), and 5'-FAM-TTGCTGCTGCTTG ACAGATT-TAMRA-3'(probe) [16].

Statistical analysis:
All data obtained was analyzed through SPSS version 26. The variables were described by their range, mean and standard deviation. The level of statistical significance was set at p< 0.05.

Results:
This study included 520 subjects suspected of SARS-CoV2 infection living in Duhok city. Total of 270 (51.9 %) were males and 250 (48.1 %) females, with their ages ranging between 18 - 83 years. The results revealed that 277 (53.3%) subjects were positive and 243 (46.7 %) negative by real-time RT-PCR (Rotor gene Q). 160 (57.8 %) were males and 117 (42.2 %) females. Their mean age was 45.6±SD (16.7). There was no significant correlation when comparing Ct values with gender (p> 0.05) (Table 1). There was also no significant correlation when Ct values were compared with different age groups where overall p.value > 0.05 was detected (Table 2).

Table 1: CT by gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>No. (%)</th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>117 (42.2)</td>
<td>17.38 - 38.12</td>
<td>27.65</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>160 (57.8)</td>
<td>16.38 - 38.11</td>
<td>27.26</td>
<td>4.5</td>
<td>0.4816</td>
</tr>
<tr>
<td>Total</td>
<td>277 (100)</td>
<td>16.38 - 38.12</td>
<td>27.43</td>
<td>4.62</td>
<td></td>
</tr>
</tbody>
</table>

*Ct value is inversely related to the viral load
**No significant correlation was found between male and female p > 0.05
SD: Standard deviation; Ct value: cycle threshold value

Table 2: Ct values comparison with different age groups.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. (%)</th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
<th>Significantly Different from*</th>
</tr>
</thead>
<tbody>
<tr>
<td>a- 18-27 yrs</td>
<td>43 (15.5)</td>
<td>17.38 -37.79</td>
<td>27.57</td>
<td>5.65</td>
<td>**</td>
</tr>
<tr>
<td>b- 28-37 yrs</td>
<td>66 (23.8)</td>
<td>17.15-38.12</td>
<td>26.97</td>
<td>4.4</td>
<td>**</td>
</tr>
<tr>
<td>c- 38-47 yrs</td>
<td>53 (19.1)</td>
<td>17.93-34.82</td>
<td>27.25</td>
<td>4.44</td>
<td>**</td>
</tr>
<tr>
<td>d- 48-57 yrs</td>
<td>41 (14.8)</td>
<td>17.7-35.47</td>
<td>27.47</td>
<td>4.41</td>
<td>**</td>
</tr>
<tr>
<td>e- 58-67 yrs</td>
<td>41 (14.8)</td>
<td>16.38-38.11</td>
<td>27.49</td>
<td>4.71</td>
<td>**</td>
</tr>
<tr>
<td>f- &gt;68 yrs</td>
<td>33 (11.9)</td>
<td>19.8-34.5</td>
<td>28.23</td>
<td>4.15</td>
<td>**</td>
</tr>
<tr>
<td>Total</td>
<td>277 (100)</td>
<td>16.38 - 38.12</td>
<td>27.43</td>
<td>4.62</td>
<td></td>
</tr>
</tbody>
</table>

*Ct value is inversely related to the viral load
** No significant differences were detected among different age groups, overall (P > 0.05)
P > 0.05 based on LSD (least squared difference) test for multiple comparisons.
SD: Standard deviation; Ct value: cycle threshold value
Table 3 shows no significant correlation of Ct levels with different comorbidities and even those without comorbidities, overall p > 0.05.

**Table 3: Ct values with different comorbidities and without comorbidities.**

<table>
<thead>
<tr>
<th>Diseases</th>
<th>No. (%)</th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
<th>(Without comorbidities) Significantly Different from</th>
</tr>
</thead>
<tbody>
<tr>
<td>a- HT</td>
<td>25 (32.9)</td>
<td>17.87 – 34</td>
<td>27.34</td>
<td>4.37</td>
<td>**</td>
</tr>
<tr>
<td>b- DM</td>
<td>13 (17.1)</td>
<td>20 - 32.25</td>
<td>27.54</td>
<td>3.96</td>
<td>**</td>
</tr>
<tr>
<td>c- HT and DM</td>
<td>15 (19.7)</td>
<td>20 - 31.6</td>
<td>26.69</td>
<td>3.42</td>
<td>**</td>
</tr>
<tr>
<td>d- HT and DM and CVD &amp; others</td>
<td>8 (10.5)</td>
<td>23.32 - 34.3</td>
<td>29.86</td>
<td>3.59</td>
<td>**</td>
</tr>
<tr>
<td>e- Others</td>
<td>15 (19.7)</td>
<td>23 - 37.26</td>
<td>29.14</td>
<td>4.51</td>
<td>**</td>
</tr>
<tr>
<td>Without comorbidities</td>
<td>201(72.6)</td>
<td>16.38 - 38.12</td>
<td>27.26</td>
<td>4.79</td>
<td>**</td>
</tr>
<tr>
<td>Total</td>
<td>277 (100)</td>
<td>16.38 - 38.12</td>
<td>27.43</td>
<td>4.62</td>
<td></td>
</tr>
</tbody>
</table>

* Ct value is inverse related to the viral load.
** No significant correlation was observed between different comorbidities with & without comorbidities.

d- Five individuals had (HT and DM and CVD) with two other who had HT, DM, CVD with asthma and other with renal failure.


Table 4 shows patients’ symptoms correlation with Ct values showed that there were significant differences between severe with asymptomatic and moderate symptoms presentation (p<0.05). Highly significant correlation was detected between severe with mild symptoms p. value (p < 0.001), and a significant correlation between critical with mild and moderate symptoms (p<0.05).

**Table 4: Ct value with severity of symptoms**

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>No. (%)</th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
<th>Significantly Different from**</th>
</tr>
</thead>
<tbody>
<tr>
<td>a- Asymptomatic</td>
<td>29 (10.5)</td>
<td>17.87 - 35.12</td>
<td>27.62</td>
<td>4.54</td>
<td>d</td>
</tr>
<tr>
<td>b- Mild</td>
<td>155 (56.0)</td>
<td>16.38 - 38.11</td>
<td>26.72</td>
<td>4.63</td>
<td>d, e</td>
</tr>
<tr>
<td>c- Moderate</td>
<td>53 (19.1)</td>
<td>17.38 - 38.12</td>
<td>27.34</td>
<td>4.98</td>
<td>d, e</td>
</tr>
<tr>
<td>d- Severe</td>
<td>23 (8.3)</td>
<td>27 – 35</td>
<td>30.37</td>
<td>2.13</td>
<td>a, b, c</td>
</tr>
<tr>
<td>e- Critical</td>
<td>17 (6.1)</td>
<td>19.8 - 34.5</td>
<td>30.03</td>
<td>3.79</td>
<td>b, c</td>
</tr>
<tr>
<td>Total</td>
<td>277 (100)</td>
<td>16.38 - 38.12</td>
<td>27.43</td>
<td>4.62</td>
<td></td>
</tr>
</tbody>
</table>

*Ct value is inversely related to the viral load.
** One of them p < 0.001 (b with d) (based on one way analysis of variance)

SD: Standard deviation; Ct value: cycle threshold value.

Most cases of asymptomatic presentation were below 47 years old, while most severe and critical cases were detected among over 68 years olds (Table 5).
Table 5: Severity of disease and age groups

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>Asymptomatic</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Critical</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-27 yrs</td>
<td>9</td>
<td>24</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>43</td>
</tr>
<tr>
<td>28-37 yrs</td>
<td>7</td>
<td>47</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>66</td>
</tr>
<tr>
<td>38-47 yrs</td>
<td>10</td>
<td>29</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>53</td>
</tr>
<tr>
<td>48-57 yrs</td>
<td>1</td>
<td>23</td>
<td>8</td>
<td>5</td>
<td>4</td>
<td>41</td>
</tr>
<tr>
<td>58-67</td>
<td>1</td>
<td>25</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>41</td>
</tr>
<tr>
<td>&gt;68</td>
<td>1</td>
<td>7</td>
<td>9</td>
<td>9</td>
<td>7</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>155</td>
<td>53</td>
<td>23</td>
<td>17</td>
<td>277</td>
</tr>
</tbody>
</table>

Discussion:
SARS_CoV2 emerged more than two years ago with millions of infections and mortality cases which necessitated a lot of work and researches to reveal different aspects of impact of epidemiological, virological and clinical criteria associated with infections. This study concentrated on individuals suspected to have COVID-19 disease in different age groups and with symptoms presentation, in addition to record any comorbidity, for which nasopharyngeal swab and RT-PCR were applied. Although mean of Ct values in females were slightly higher than those of males involved which meant higher viral load for males. There was no significant correlation between Ct values of males and females involved. Other studies revealed similar results to this study with no significant correlation of Ct values with demographic data, including gender [17]. Ibrahim et al. 2021, [18] found higher viral load in males, meaning higher infectious potential. In another article with 1077 patients, females who were significantly older than the males, had somewhat higher mean Ct values [19].

Males were shown to have much higher disease mortality and severity compared to the females, with older men being the most at risk [20]. Whereas, Bwire. 2020 [21] demonstrated that males had a higher rate of SARS-CoV-2 infection compared to the females. Differences in obtained results could be attributed to different number of both genders involved in each study and the usual daily activity and social habits accomplished by each gender mainly in developing countries which may have enhanced exposure to the virus.

In those cases where Ct levels were put to comparison with the various age groups included in this work, no significant correlation was detected. According to Ade et al. 2021 [19], only the group older than 80 years showed differences in Ct values when compared with other age groups [19]. Older people had lower Ct levels at the time of diagnosis compared to younger people, according to a prior observational study carried out in Greece during the pandemic's initial phase [22].

Patients' symptoms correlation with Ct values showed that there were significant differences between severity with asymptomatic and moderate symptoms presentation. However, highly significant correlation was detected between severity with mild symptoms and significant correlation between critical with mild and moderate symptoms. Since then, more research has also been published to substantiate the link between disease severity and viral load. Based on the severity of the infection, a univariate analysis revealed statistically significant variations in viral load values. [23], [24], [25]. In particular, the severe group's average CT values were considerably lower than those of the mild and moderate disease groups and between the mild and moderate clinical categories [23].
In contrast to our findings, another earlier study covering severe and mild cases by Liu et al. 2021 [26], found that the Ct value was low in severe than mild diseases. The impact of viral load on illness points to a correlation between viral load and COVID-19 disease severity that is often favorable. This conclusion is supported by a number of other recent investigations [24], [27], [28], [29]. Yet, it is unknown how viral load dynamics in tissue samples from the lower respiratory tract and other areas of the body relate to the severity of the disease [30]. The longer the time interval between the onset of symptoms and the Ct values estimation, the higher the Ct values. Higher levels of Ct were related with more severe disease, suggesting lower viral loads in sicker patients [17] who also showed similar findings to ours.

Our investigation found that high Ct levels (lower viral load) correlated with the disease's severity, in contrast to recent studies which found that lower Ct findings were related to an increased likelihood of severe COVID-19 [22], [31], [32].

This could be explained by the sample size of patients enrolled in this study, it could be due to the time elapsed from beginning of illness till appearance of severe symptoms which makes patients look for medical care and diagnosis, and therefore at this stage, taking a swab from nasopharynx that is part of upper respiratory tract may detect a lower virus titer because it spread downwards to the lower respiratory tract.

Results similar to ours were found in a prior observational study carried out in New York City via Argyropoulos et al. 2020 [33], during the early stages of the pandemic which suggested that this could reflect the correlation between the interval from onset of infection and increased severity of disease [17]. This is due to the fact that the viral load peaks during the pre-symptomatic phase of the disease course and then drops to become invisible by 18–21 days [34].

A medical facility in New York City studied a large cohort of 4254 patients and found that inpatients had much higher Ct values compared to outpatients [29]. Between out- and inpatients, the Ct values did not differ statistically [19]. No correlation between admission Ct levels and the disease severity was discovered by Saglik et al. 2022 [35].

This could be particularly true for the elderly in whom even low viral loads might result in serious infection, particularly in the presence of comorbidities like diabetes, hypertension, or pulmonary disorders [36]. We also found that most asymptomatic cases were in lower age groups while severe and critical cases were detected in old age group. Many factors impact on Ct values and one of them is the time of sample collection post symptoms appearance which is very sensitive regarding the viral load obtained from nasopharyngeal swab, in addition to efficiency of sampling and primers supplied with the used kits for RT-PCR.

Table 4 demonstrates that there were no significant relationships between Ct levels for individuals with various co-morbidities or even those who had no such illnesses. According to a study on comorbidities, type 2DM patients had a low Ct value of N gene that was 1.324 times higher, hypertensive patients had a low Ct value which was (1.871 times higher) and hospitalized patients were 2.480 times more vulnerable to shifts in ICU [37]. Using various instruments with different primers and the sensitivity regarding those instruments could have also contributed to the contradictory results.

**Conclusion:**

During COVID-19 pandemic, high incidence of SARS-CoV2 infection was recorded among patients with respiratory symptoms. Although non-significant, higher viral loads in males mean
higher infectious potential. Viral load was not significantly correlated with different age groups. On the contrary, significant variation was detected when Ct values were compared with clinical presentation of patients but higher Ct values (lower viral RNA load) was recorded in severe and critical patients which was an interesting result, supporting the fact that the longer the time interval between the onset of symptoms and the Ct value estimation, the higher the Ct values.

References