

Bio-molecular Features of COVID-19 in Hodeidah, Yemen

ABSTRACT

Background: The bio-molecular technique namely Real-Time Polymerase Chain Reaction (RT-PCR) is very important in confirmation of coronavirus disease 2019 (COVID-19) but in progressive infection and predication of the illness severity is not used.

Objective: Therefore, the study aimed to determine the relationship between the viral load of COVID-19 infection and the severity of illness based on cycle threshold (Ct).

Methodology: The research was designed in a case series study. The study included 60 patients that were confirmed by the RT-PCR test with COVID-19 and divided into two major groups. The first major group was mild and moderate cases that were treated at home (outpatient) and the second major group was severe and critical cases that were treated in the isolation center (inpatient).

Results: The results showed no relationship between the viral load and severity of illness, where the minimum Ct was 14, and the maximum Ct was 35. The mean of Ct was 22 ± 7 . On the other hand, the low Ct was reported in early detection cases. On the other hand, kinetic of viral load-based Ct where the viral load was reported in the first week as very high (low Ct 21) with a longitudinal assessment of RT-PCR test results in individuals requiring third –fourth weeks to clear COVID-19 RNA showed a significant reduction of the viral load in samples (Ct values > 30).

Conclusion: The study concluded that several factors can affect the Ct of RT-PCR (onset date, collection technique, type of swab, sampling method). Briefly, the COVID-19 RT-PCR test cannot be used as a predictor of the severity of illness.

Keywords: COVID -19, Bio-molecular, Viral Load, Cycling Threshold, Hodeidah, Yemen

1. INTRODUCTION

“Coronavirus disease 2019 (COVID-19), is a severe acute respiratory syndrome and a new infectious disease that first emerged in Hubei province, China, in December 2019, which was found to be associated with a large seafood and animal market in Wuhan. One of the most important ways of virus transmission is a human-to-human transmission which is primarily achieved through close contact with respiratory droplets, direct contact with the infected individuals, or contact with contaminated objects and surfaces” [1]. “Coronaviruses belong to the order Nidovirales, family Coronaviridae, and subfamily Orthocoronavirinae, are spherical (125nm diameter) and enveloped with club-shaped spikes on the surface giving the appearance of a solar corona. Within the helically symmetrical nucleocapsid is the large positive sense, single-stranded RNA. The coronaviral genome contains four major structural proteins: the spike (S), membrane (M), envelope (E), and the nucleocapsid (N) protein, all of which are encoded within the 3' end of the genome. The S protein mediates attachment of the virus to the host cell surface receptors resulting in fusion and subsequent viral entry. The M protein is the most abundant and defines the shape of the viral envelope. The E protein is the smallest of the major structural proteins and participates in viral assembly and budding. The N protein is the only one that binds to the RNA genome and is also involved in viral assembly and budding” [2,3]. “A novel coronavirus is the seventh member of the coronavirus family to infect humans. Phylogenetic analysis of full-length genome sequences obtained from infected patients showed that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is similar to severe acute respiratory syndrome coronavirus (SARS-CoV) and uses the same cell entry receptor, angiotensin-converting enzyme 2 (ACE2), as SARS-CoV” [4,5]. “Many infected individuals develop COVID-19 with fever, cough, and shortness of breath that can progress to pneumonia. Disease progression promotes the activation of immune cells, platelets, and coagulation pathways that can lead to multiple organ failures and death” [6].

“An effective strategy for controlling the COVID-19 pandemic is to develop highly accurate methods for the rapid identification of COVID-19 infected patients. Many companies and institutes are therefore striving to develop effective methods for the rapid

detection of COVID-19 RNA” [7]. “Clinically, nucleic acid-based methods are sensitive but prone to false-positive” [8]. “The primers and probe were designed based on the nucleocapsid protein gene (N gene) sequence of COVID-19. The detection limit was 10 copies per reaction in this assay, which could be conducted within 15 min at a constant temperature (39 °C), without any cross-reaction with other respiratory tract pathogens, such as other coronaviruses” [9]. “The diagnosis of COVID-19 is critically dependent on the detection of COVID-19 RNA from clinical specimens (e.g., nasopharyngeal swabs). While laboratory-developed testing for COVID-19 is an essential component of diagnostic testing for this virus, the majority of clinical microbiology laboratories are dependent on commercially available COVID-19 molecular assays. In contrast to assays approved or cleared by the U.S. Food and Drug Administration (FDA) for in vitro diagnostic use, assays for the detection of COVID-19 nucleic acids have emergency use authorization (EUA) from the FDA. Outside of highly specialized academic and commercial laboratory settings, clinical microbiology laboratories are likely unfamiliar with the EUA classification, and thus, assay verification can be daunting” [10].

“In clinical practice, the SARS-CoV-2 real-time RT-PCR test reports as negative or positive based on a specific Ct value threshold” [11]. “This cut-off is determined by an algorithm that automatically interprets various amplification process parameters. However, there are some uncertainties, such as whether Ct values should be used to predict the level of infectivity and disease severity in SARS CoV-2 patients. RT-PCR and Ct values have been linked to a more complete picture of viral load in COVID-19 deceased. A lower viral RNA load and a lower risk of infection transmission are represented by higher Ct values” [12].

In our study, we aimed to answer this question, Can the bio-molecular techniques (RT-PCR) determine and predict the severity of the illness of COVID-19.

2. METHODOLOGY

2.1. Study area

The study was carried out in the COVID-19 isolation department, Molecular Biological Unit, Center of Tropical Medicine and Infectious Diseases (CTMID), AL Thawara Public Hospital Authority, Hodeidah Yemen. In this study area 2020, AL Kamarany et al reported 505 COVID-19 patients in 2020 . A total of 49/505 cases (9.70 %) were confirmed and admitted to the isolation department. On the other mean, 386 patients (76.43%) with mild and moderate cases, and 70 patients (13.86%) with severe illnesses were treated at home. 21 patients with severe illness (4.16%) and 28 patients (5.54%) with critical illness were treated in the isolation department. 49 patients (9.7 %; severe and critical) needed admission to an intensive care unit (ICU) that were confirmed based on RT-PCR. Other cases (mild and moderate) were confirmed epidemiologically (epidemiologically linked case: a case in which the patient has/ had contact with one or more persons who have/had the disease, and transmission of the agent by the usual modes of transmission is plausible. A case may be considered epidemiologically linked to a laboratory-confirmed case if at least one case in the chain of transmission is laboratory confirmed [13-15]. The study area is high endemic of vector – borne diseases (VBDs) such as dengue , malaria , Chikungunya , west – Nile virus [16-20].

2.2. Study design

The research was designed in a pilot study and one-time cross-sectional COVID-19 Ct-based viral load measurement that included 60 participants, divided into three groups: the first group included 20 mild and moderate patients of COVID-19 who were isolated and treated at home. The second group was severe cases (n:20) and the third group was critical cases (n:20) that treated at the COVID-19 isolation department, CTMID, AL Thawara Public Hospital Authority, Hodeidah Yemen (Figure 1).

- **Mild and moderate cases:** Mild Cases: Symptoms of respiratory infection (fever, cough, pharyngitis, headache, ... etc.) Symptomatic, meeting the case definition for COVID-19, without evidence of viral pneumonia or hypoxia. Moderate Cases: Clinical signs of non-severe pneumonia (cough or difficulty breathing and fast breathing and/or chest indrawing) and no signs of severe pneumonia (n:20)
- **Critical cases:** The multi-organ failure (n:20)
- **Severe cases:** Clinical signs of pneumonia (fever, cough, dyspnea, fast breathing) plus one of the following: respiratory rate > 30 breaths/min; severe respiratory distress; or $SpO_2 < 90\%$ on room air (n:20)

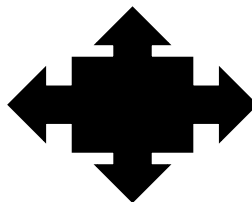


Figure 1. Study design to determine the relationship between the viral load of COVID-19 infection and severity of illness based on cycle threshold (Ct) in Hodeidah, Yemen [21-23]

2.3. Real-Time Polymerase Chain Reaction (RT-PCR) for Detection of the COVID-19 infection

The RT- PCR of COVID-19 detection was re-validated partially in the Molecular Biological Unit of CTMID, AL Thawara Public Hospital Authority of Hodeidah, Yemen. The assay for molecular detection of COVID-19 on nasopharyngeal swabs was performed using the RT-PCR Bio-System. Norgen's COVID-19 TaqMan RT-PCR kit was designed for the detection of COVID-19-specific RNA [24].

2.4. Data Management

A simple statistical process was used, firstly, the RT-PCR was validated to assure the results and secondly, data were collected ,checked ,and entered in Excel Software 2013, and then the data was analyzed using tables, graphs, percentages, median, range, and average were the main descriptive tools.

3. RESULTS

3.1. Viral load in mild , moderate , severe and critical cases

At symptom onset, the mean Ct - based viral load was 25 cycles. The viral load change was 1 Ct /day. The highest viral load was 14 Ct on day 2 and the lowest viral load was Ct 36.8 on day 41. The earliest and latest positive results were on day 1 and day 42. In addition, 60 % (36 cases) of patients had low Ct (high viral load) of more than 15 and less than 25 cycles and 40 % of patients (24 cases) had high Ct (low viral load) that was more than 25 and less than 39 cycles (Table 1 and Figure 2).

Table 1: Viral load in mild, moderate, severe, and critical cases

| Ct values | Mild and Moderate n:20 | | Severe Cases n: 20 | | Critical Cases n: 20 | |
|-----------|------------------------|---|--------------------|---|----------------------|---|
| | n | % | n | % | n | % |
| 15 – 25 | 19 | | 11 | | 6 | |
| 26 – 39 | 1 | | 9 | | 14 | |
| Total | 20 | | 20 | | 20 | |

- The chi-square statistic is 18.6678 , the p -value is .000088 that is significant at $p < .05$.

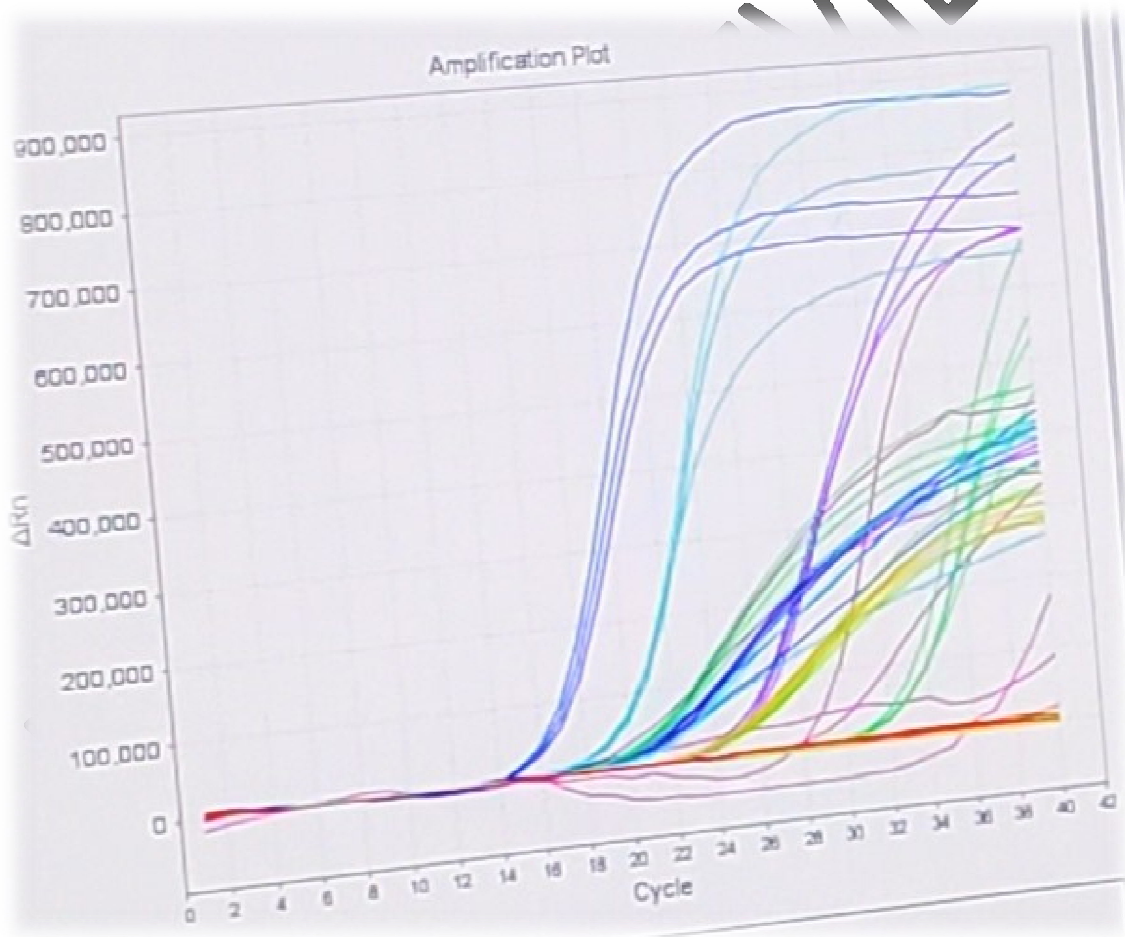
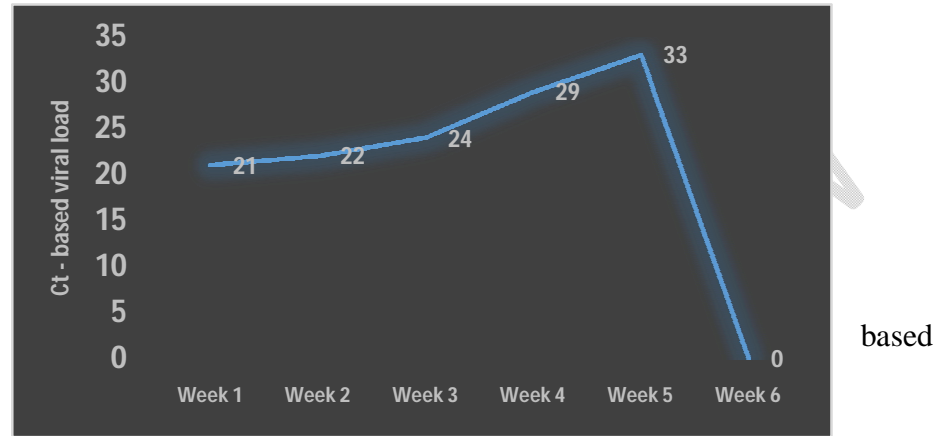


Figure 2: Amplification plot of Ct-based viral load in COVID-19 patients

3.2. Kinetic of Ct-based viral load in COVID-19 patients

Figure 3 showed the kinetics of viral load–based Ct where the viral load was reported in the first week as very high (low Ct 21) with a longitudinal assessment of RT-PCR test results in individuals requiring fifth - sixth weeks to clear COVID-19 RNA showed a significant reduction of the viral load in samples (Ct values > 30).

Figure 3:
Kinetic of Ct-
viral load in
COVID-19



patient

3.3. Other factors in mild , moderate , severe and critical cases

30/40 patients (65.30%) with severe and critical COVID-19 infection were affected significantly by one or more co-morbidity caused by an underlying chronic disease ($X^2 = 6.36$ and $p = 0.0066$), the most common of which was diabetes mellitus namely 13 patients (32.5%). 5 (12.5%) of these patients had diabetes alongside other chronic conditions. 5 patients (12.5%) had underlying cardiac disorders and hypertension, of which 2 patients (5.0 %) (also concurrently had respiratory disorders (bronchial asthma). 4 patients (10.0 %) had bronchial asthma without other co-morbidities, and a further 2 patients (5.0 %) suffered from renal failure. 2 patients (5.0%) were identified with coinfections of an infectious disease namely hepatitis C virus (HCV) and tuberculosis (TB). 12 of the patients (30.0 %) did not present with any underlying chronic or infectious disease or related co-morbidity (Table. 2).

Table 2: Other factors in in mild, moderate, severe, and critical cases

| Risk Factors | Mild and Moderate | Severe Cases n: 20 | Critical Cases n: 20 |
|--------------|-------------------|--------------------|----------------------|
|--------------|-------------------|--------------------|----------------------|

| | n:20 | | | | | |
|---------------------------------|------|---|----|---|----|---|
| | n | % | n | % | n | % |
| Co-morbidity | | | | | | |
| Diabetes | 1 | | 5 | | 3 | |
| Diabetes with chronic diseases | 0 | | 2 | | 3 | |
| Heart diseases and hypertension | 0 | | 2 | | 3 | |
| Heart diseases and asthma | 0 | | 1 | | 1 | |
| Asthma | 1 | | 2 | | 2 | |
| Renal Failure | 1 | | 1 | | 1 | |
| Coinfection | | | | | | |
| - Tuberculosis | 0 | | 1 | | 1 | |
| - Hepatitis C | 0 | | 0 | | 1 | |
| Non | 17 | | 6 | | 6 | |
| Total | 20 | | 20 | | 20 | |

4. DISCUSSION

“RT-PCR is considered the gold standard confirmatory test for COVID-19. The Ct values are being utilized to diagnose COVID-19 infection” [25,26]. “On the other mean, most RT-PCRs are designed for qualitative COVID-19 reporting (COVID-19 detected or not detected) but have been used for semi-quantitative estimation of viral load based on Ct value” [27,28]. The results showed a significant difference in viral load between mild (outpatients) and severe patients (inpatients needed oxygen therapy) among Hodeidah patients, Yemen. Our results agreed with a previous study by Abdulrahman et al reported that “the viral load, as indicated by Ct values, did not seem to be associated with the requirement for oxygenation on admission” [29]. Another previous study by Shah et al reported that “the patients with mild disease had significantly lower Ct values than patients with severe disease but had also been tested significantly earlier in the illness than those with severe disease. The patients who died had significantly lower Ct values than patients who survived but here again they had a significantly shorter duration of symptoms before testing” [30].

Some authors have demonstrated “an independent relationship between the Ct value (indicative of viral load) of an individual's first positive COVID-19 PCR test, and overall mortality, in which a lower Ct value is associated with greater hazards of death. Ct values may allow for early risk stratification in patients with COVID-19. Further work is required to confirm this association and to assess the utility of including Ct values in early risk stratification” [31].

Al Dossary et al reported that age, sex, and ethnicity are important predictors of COVID-19 severity. The Ct of the COVID-19 RT-PCR test cannot be used as a predictor of the criticality of illness [32]. Research published by AL Kamarany et al concluded that old age, chronic diseases, and co-infection may be contributing factors to excess morbidity and mortality among COVID-19 patients [13,14].

Our study proved that viral load is reduced with the recovery phase (third week to the fourth week) and the duration of isolation may complete at home. These results agreed with the study of Aranha et al that reported “in the longitudinal assessment of RT-PCR test results in individuals requiring 15-30 days to clear COVID-19 RNA showed a significant reduction of the viral load in samples with high or intermediate viral loads (Ct values ≤ 25 and between 26 and 30, respectively) but the follow-up group with low viral RNA (Ct values ≥ 31) exhibited a stable viral load. Together, these results suggest that COVID-19 positive cases with Ct values more than or equal to 31 require reduced duration to clear COVID-19, and thus, a shorter isolation period for this group might be considered to facilitate adequate space in the COVID Care Centres and reduce the burden on healthcare infrastructure” [33]. The author reported that the viral load reduces with the time of symptoms onset and these results agreed with the study of Boan et al that reported that “ COVID-19 viral load declines from the time of symptom onset; at symptom onset, the mean viral load was 4.34 \log_{10} IU/mL with Ct 28.9 cycles and viral load at symptom onset was higher for that reporting fever compared to those not reporting fever [34]. The COVID-19 RT-PCR test and Ct values cannot be used to predict the severity of illness because Ct values are commonly affected by pre-analytic factors such as collection technique, specimen type, time of sample collection, viral

kinetics, the difference in viral load between upper respiratory tract (URT) and lower respiratory tract (LRT) samples, transport and storage conditions, and so on. Analytical variables include nucleic acid extraction efficacy, viral RNA load in collected samples, primer design, nature of the target RNA, real-time PCR efficiency, and Ct value determination method, and post-analytical variables include result interpretation and reporting [35]. “Other techniques can predicate for severity illness of COVID-19 and previous studies were published from Yemen’s authors, the radiological techniques have good predictive values on severity and mortality. Also can support the clinicians staff in early detection, early triage, criteria admission, and effective management of COVID-19 infection” [14]. In addition, the hematological parameters namely WBCs, neutrophil and lymphocytes are early indicators in COVID-19 diagnosis and severity illness [36].

On the other hand, several studies reported the fatal outcomes that are particularly associated with certain social determinants, chronic diseases or other communicable disease co-infections [37-38]. The risk of mortality from COVID-19 increases dramatically with age, as well as in those who have underlying comorbidities with diabetes, hypertension and coronary heart disease [39-43].

5. CONCLUSION

Several factors influence the diagnosis of SARS-CoV-2 infection or COVID19, including the selection of appropriate tools and techniques, the most appropriate sample form, appropriate sampling techniques, and the duration of the infection. The RT-PCR technique has been widely used, and Ct values are regarded as indicators of viral load. In addition, study aligns with other epidemiological and clinical studies in highlighting old age and comorbidity with non-communicable diseases and coinfection as key potential contributing factors to excess the severity among COVID-19 patients.

Limitations of the study: There are some limitations in this study that needs to be considered. The small sample size in this study and other bio-molecular features are not included.

CONSENT

As per international standards or university standards, Participants' written consent has been collected and preserved in Medical File. The raw data are secured in CTMES – HU and CTMID, Hodeidah, Yemen.

ETHICAL APPROVAL

The studies involving human participants were reviewed and approved by the Ethics Committee of CTMES – HU, Hodeidah, Yemen.

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