International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Sciences, 2022; 10 (2): 121-130
ISSN:2320-7817(p) | 2320-964X(o)

Research Article Open Access

Correlation of COVID-19 RT-PCR CT values and symptoms, duration and medication of patients.

Sayed Chandini

Garden City University, Bangalore, Department of Molecular and Human Genetics.
Email: Chandinisayed15@gmail.com

Manuscript details:

Received: 13.04.2022
Accepted: 25.05.2022
Published: 29.05.2022

Cite this article as:
Sayed Chandini (2022) Correlation of COVID-19 RT-PCR CT values and symptoms, duration and medication of patients, *Int. J. of Life Sciences*, 10 (2): 121-130.

Available online on http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

Coronaviruses (CoVs) belong to the subfamily Orthocoronavirinae in the family Coronaviridae, Order Nidovirales. There are 4 genera inside the subfamily Orthocoronavirinae, like Alphacoronavirus (α-CoV), Betacoronavirus (β-CoV), Gammacoronavirus (γ-CoV) and Deltacoronavirus (δ-CoV). The CoV genome is an enveloped, positive-sense, single-stranded RNA with a dimension various between 26 kb and 32 kb, the biggest genome of recognised RNA viruses. Real Time Reverse Transcription Polymerase Chain Reaction (Real Time RT-PCR) is the gold widespread test for detection of SARS-CoV-2. This test allows early detection of viral genome in medical samples. A positive test permits the clinicians and public health experts to rapidly isolate the affected person and stop progression of the disease. The cycle threshold or Ct value of a RT-PCR response is the number of cycles at which fluorescence of the PCR product is detectable over and above the background signal. Theoretically, the Ct value is inversely proportional to the quantity of genetic material (RNA) in the beginning sample and decrease Ct values usually correlate with excessive viral load. It is being assumed with the aid of some researchers /clinicians that excessive viral load immediately correlates with elevated infectiousness and severity of disease. ICMR has mentioned the problem of correlating COVID-19 disease severity with Ct values and consequently figuring out on affected person management protocol, with a panel of esteemed laboratory experts. Real-time RT-PCR protocols for detecting SARS-CoV-2 in different countries. Three fluorescent indicators from FAM, ROX, and CY5 channels detected the viral replica DNA (cDNA). We selected the nucleocapsid protein gene N and the RNA-dependent RNA polymerase gene (RdRP) [also stated as Open Reading Frame 1ab (ORF1ab)]. Internal control helped to check for biases of the complete procedure. We used a positive control such as plasmids containing the whole SARS-CoV- two N gene in our study. In this study the reports that has been taken for the survey has selected these markers as control.

Keyword: COVID-19, corona virus, viral load, SARS-COV 2, Co-morbidities, Medications, Severity, Ct- values.

INTRODUCTION

Coronaviruses (CoVs) belong to the subfamily Orthocoronavirinae in the family Coronaviridae, Order Nidovirales. There are 4 genera inside the subfamily Orthocoronavirinae, particularly Alphacoronavirus (α-CoV), Betacoronavirus (β-CoV), Gammacoronavirus (γ-CoV) and Deltacoronavirus (δ-CoV). The CoV genome is an enveloped, positive-sense, single-stranded RNA with a size varying between 26 kb and 32 kb, the biggest genome of recognized RNA viruses.

Since the outbreak of novel coronavirus pneumonia in Wuhan, Hubei Province, China, in December 2019, the epidemic swept the complete country and different countries in the world and has been lasting till now. At the early stage of the epidemic, China's public health system and medical and scientific circles acted rapidly in order to well-timed perceive the novel virus and publicize the gene sequence of the virus to the world. On February 12, 2020, the disease was officially named coronavirus disease 2019 (COVID-19) by World Health Organization (WHO). The most frequent signs and symptoms of COVID-19 are fever, cough, myalgia, fatigue, pneumonia, and dyspnea, whilst a few sufferers have headache, diarrhea, hemoptysis, runny nose, expectoration, etc. Besides, the prognosis of asymptomatic COVID-19 sufferers is additionally a hard issue. For suspected cases, they are typically proven upon positive nucleic acid testing for the 2019 novel coronavirus (SARS-CoV-2) in sputum, throat swab, and decrease breathing secretion by means of real-time fluorescence PCR (RT-PCR). RT-PCR can successfully and rapidly finish detecting virus samples, with excessive sensitivity and specificity. Hence, it has grow to be the first detection approach endorsed in the Guidelines on the Novel Coronavirus-Infected Pneumonia Diagnosis and Treatment (6th edition).

SARS-CoV-2 is a positive-stranded RNA (+RNA) virus and belongs to Coronaviridae family, Nidovirales order. Coronaviruses can infect the respiratory system, gastrointestinal tract, liver, and central nervous system of humans, mammals, birds, bats, etc. The positive single-stranded RNA genome of the SARS-CoV-2 is about 30 k nucleotides in size, encoding 9,860 amino acids. Traditionally, the desired aims for RT-PCR detection of coronavirus encompass conversed or mostly expressed genes such as structural Spike glycoprotein (S) and Nucleocapsid protein (N) genes and unstructural RdRp and replicase open reading frame (ORF) 1ab genes. Fluorescence RT-PCR can be carried out to detect the cycle threshold (Ct) values of these genes, which ought to be used to signify viral load, as there is a negative correlation between Ct value and virus RNA replication number.

Recently, some researchers have mentioned the correlation between the viral load and disease development and spread. A retrospective study by (Xu et al. 2006) cautioned that Ct values detected from the tertiary patients at the time of admission have been comparable to these from the Wuhan imported and secondary cases (both). For the tertiary group, the viral load was undetectable for all patients on day. For 1/3 of the patients in the imported and secondary groups, the viral load remained positive on day 14 after the admission, which indicated that the tertiary patients have been gradually much less prone to SARS-CoV-2 infection. Another research unveiled that the viral load of COVID-19 patients' sputum peaked in the 1st week after the appearance of symptoms, however that diminished with time going by. Besides, the older people tended to have a greater viral load. Different from the extreme acute respiratory syndrome (SARS), patients with COVID-19 have a greater viral load, which can also account for the quickly spread of the epidemic. The contamination mode of SARS-CoV-2 is totally distinctive from SARS. For SARS, the incubation duration is not contagious. Generally, the virus RNA level in vivo reaches its peak 7-10 d after the onset of symptoms, while for SARS-CoV-2, the RNA level reaches its height within 5 d of the onset of signs and symptoms and replicates actively in the upper respiratory tract tissue, indicating that people infected with COVID-19 shed or spread the virus in large quantities within the first 5 d of the onset of symptoms. Therefore, infected patients with moderate signs or even asymptomatic stages are specifically vital for epidemic prevention and control. Currently, the relationship between the viral load and severity of sickness of COVID-19 patients has not been utterly understood. Although there is research suggesting that COVID-19 patients with extreme disease prerequisites need to be handled in intensive care unit (ICU), and they have a relatively greater viral load, but the time of exacerbation for these patients in the pathogenic process remains unclear. As a result, it is essential to proceed exploring the correlation between the viral load and development of COVID-19, which helps to better monitor disease development and is of sure guiding significance for the cure and prevention of COVID-19.

In this study a survey is conducted to understand the relation between COVID-19 Ct values that we obtain from RT-PCR and the symptoms, duration of these signs and symptoms and medications took by the patients suffered from COVID -19. This is just an approach to understand the change in Ct values and the signs and symptoms confronted by the patients, even while using the medications. There are few objectives, that are mentioned below. COVID -19 RT-PCR Ct values and viral load.Relation between Ct values and other blood routine & biochemical tests,severity of the disease, duration & time of recovery of disease, stages of disease & medication.

METHODOLOGY

An Online survey has been conducted from July - September 2021 using advanced features and tools of MICROSOFT FORMS. The above survey link was circulated to all age groups via social media (i.e, face-book, Instagram, what's app etc). This survey form contains a questionnaire, which contains questions regarding covid-19 test reports details and severities & consequences both pre and post COVID-19 infection , co-morbidities details also have been involved , few details have collected from the patients reports from diagnostic centers and also contacted them over call for further details.

RESULTS

A small population of 75 people has taken for the survey by people's responses via survey form and reports from few diagnostic centres. An age group of 18-60 years has been taken for the survey conducted. Comorbidities faced by the patients were diabetes type 2, hyper tension, PCOD, Pregnancy, Bipolar disorder, Asthma, cigarette addiction, renal problem, hypothyroidism.

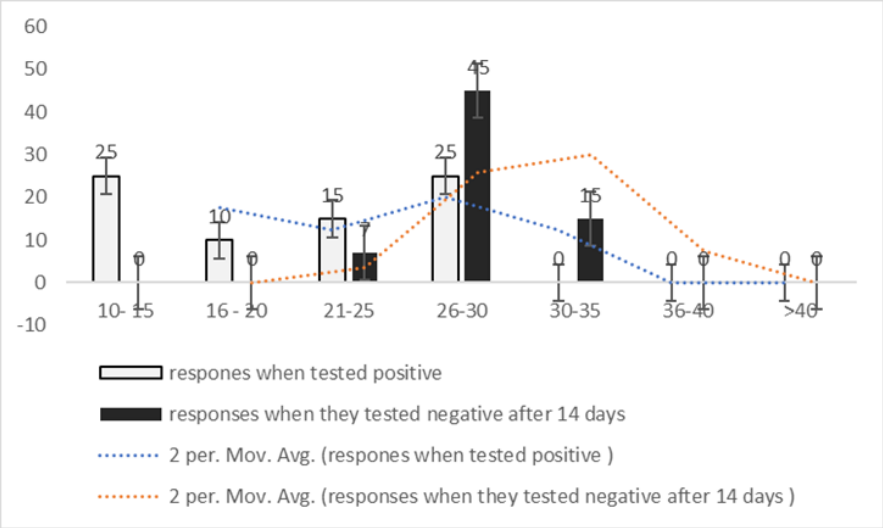

Fig 1: This chart depicts the Ct value range responses, that has been gathered from the survey both when patients got detected for COVID-19 and later at the time when they were declared as negative for the infection.

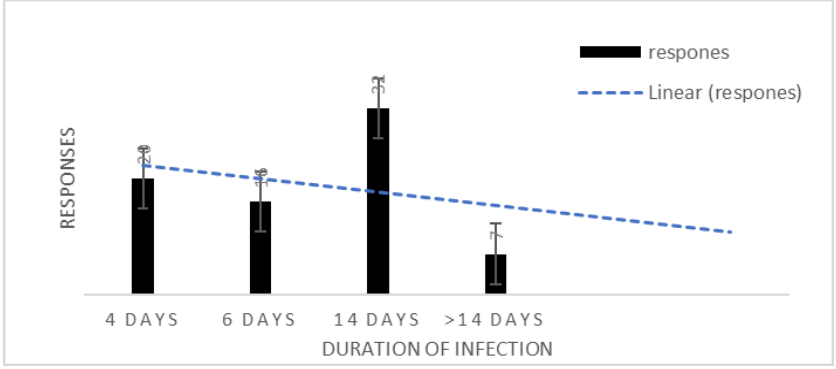

Fig 2: This chart shows the duration of the infection in the patients

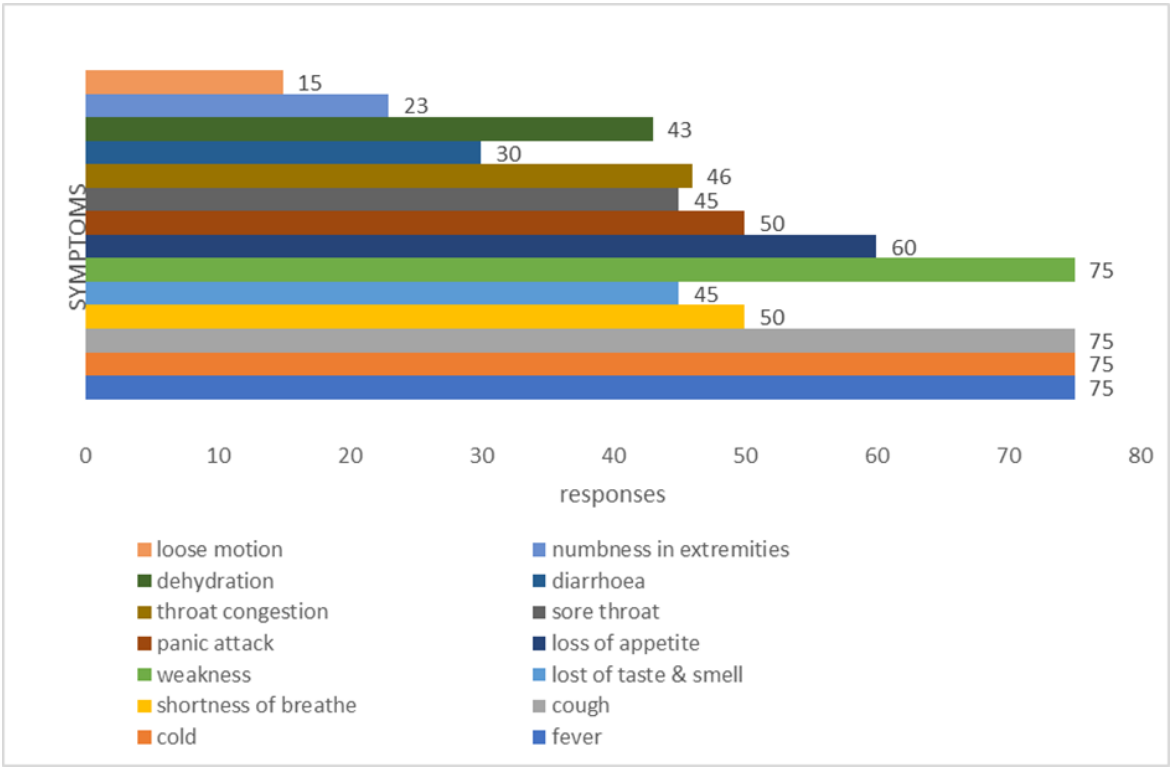

Fig 3: This chart depicts the symptoms faced by the patients during the duration of the infection or during progression of the disease. Infection first starts with fever and cold& cough, sore throat, shortness in breathe, weakness. Though few of these symptoms might prolong even after the patient gets negative for the disease. Low energy, weakness and dehydration and cramps are few of those.

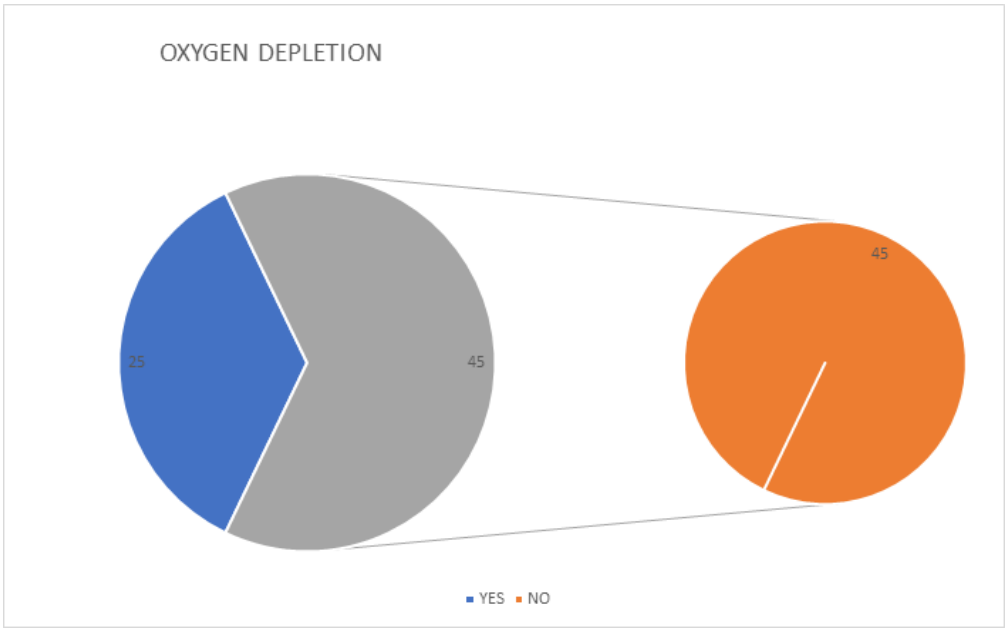

Fig 4: This chart shows the number responses from patients who went through oxygen depletion at-least once in their infection progression period.

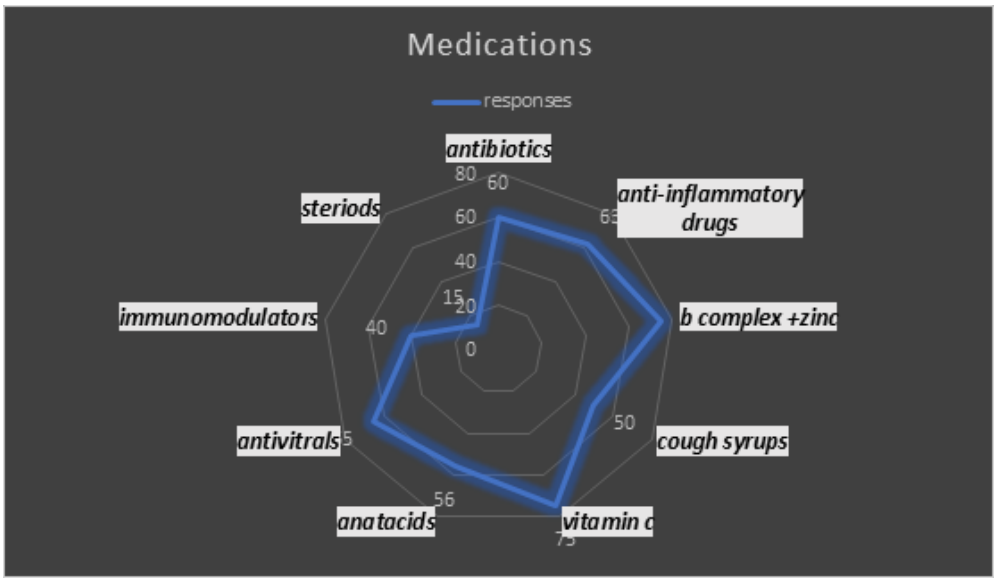

Fig 5: This chart shows the responses for the medications taken by or prescribed for the patients during the infection

Table 1: This table depicts the increment or decrement of the indices of the above biological and haematological tests during the infection

CT values & Biochemical Markers + Haematological Indices

Increased in most (COVID +VE) patients	Increased in few (COVID +VE) patients	Decreased in most (COVID +VE) patients	Normal in most (COVID +VE) patients
CRP (C-reactive protein)	D-dimer	Lymphocyte count	Procalcitonin
LDH (lactate dehydrogenase)	Procalcitonin	albumin	
ALT (alanine aminotransferase)	Urea	WBC	
TOTAL BILURIBIN	Myo-haemoglobin		
CREATININE	CK (creatinine kinase)		
CARDIAC TROPONIN	Ferritin		
ESR (erythrocyte sedimentation rate)	Blood Glucose		
CYTOKINES (IL-6, IL10, IL-2,IL-7)			
Neutrophiles			

DISCUSSION

Based on this survey conducted, we can make few interpretations.

RT-PCR CT values and viral loads

In a real-time PCR assay a positive reaction is detected by accumulation of a fluorescent signal. The Ct (cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold (i.e exceeds background level). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (i.e the lower the Ct level the greater the amount of target nucleic acid in the sample). WVDL real time assays undergo 40 cycles of amplification.

- Ct value of < 25 are strong positive reactions indicative of abundant target nucleic acid in the sample
- Ct value of 26-37 are positive reactions indicative of moderate amounts of target nucleic acid
- Ct value of 38-40 are weak reactions indicative of minimal amounts of target nucleic acid which could represent an infection state or environmental contamination.

Relation between CT values and other blood routine & biochemical tests.

(1) Blood Routine Indexes- lymphocyte count, neutrophil count, ESR range

(2) Biochemical Indexes. CRP, LDH, Creatinine

The lower Ct values normally signify the greater viral load. Consequently, we speculated that patients with a greater viral load tended to have increased CRP and neutrophil count but reduced lymphocyte count. Hypersensitive CRP (hs-CRP) is a nonspecific marker in the acute phase of systemic inflammatory response. Body injury induced via increased viral load triggers the sharp upward push in CRP level, and CRP elevation may want to promote the action of neutrophil and macrophages, inhibit mixed lymphocyte reaction, and induce inflammatory response in physique to withstand virus invasion. In addition, more than a few researches have published that the variety of lymphocytes is negatively correlated with degree of inflammation. Therefore, increased CRP and lowered lymphocyte both indicated worsening of inflammation, demonstrating that elevated viral load would be accompanied by immoderate inflammation, which is exceedingly likely to be the key factor contributing to the induction of cytokine storm and progression of disease.

The result of which uncovered that they have been markedly positively correlated, that means that patients' lymphocyte count number had a falling tendency with the upward shove of the viral load in respiratory tracts. This might also partially give an explanation for the motive why most COVID-19 patients had a decreased lymphocyte count.

CRP is a kind of sharply risen protein (acute protein) in plasma when physique is infected or tissue is injured, which leads to opsonization through activating complement and promotion the phagocytosis of phagocyte and is in a position to clear away the pathogenic microorganism that invades body. Research has discovered that CRP level is positively correlated with the diameter of lung lesions and could mirror disease severity of COVID-19 patients. In addition, (Liu et al.) discovered that the expand of neutrophil, SAA, PCT, CRP, cTnI, D-dimer, LDH, and lactate ranges could indicate COVID-19 progression and reduced lymphocyte count. These researches have revealed that expanded CRP stage is related with ailment progression. Besides, we found that a greater viral load (lower Ct value) corresponded with a greater CRP level via the reports we collected n survey we've done.

Neutrophils are one of the essential immune cells which can be rapidly accrued to sites of contamination to play their function in host protection and immunoregulation. Some retrospective research have explored the relationship between physique internal change of neutrophils and COVID-19. For instance, research unveiled that, patients who had ocular signs had been extra probably to have greater leukocyte and neutrophil count compared with these who had no ocular symptoms.

Relation between CT values and severity of disease

In mild and severe COVID-19 cases, analysis of respiratory samples detected a minimum level of almost 10 copies of vRNA/μL. Júnior et al. (n = 43) reported an average Ct value of 34.92 from asymptomatic individuals in Brazil.A Chinese study found that severe cases have higher viral load and longer virus persistence. In severe and critical diseases, the viral load remained consistently high over the disease course, with persistent low Ct values. Although some studies have reported an association between disease severity and Ct values another study reported no difference in mean viral loads among patients with and without pneumonia. Pneumonia patients had high viral load only in the female subgroup, and the elevated CRP and serum amyloid A positive subgroups.

To study the association between virus persistence and disease severity, the method of detecting viral RNA in non-survivors until death was used. RNA detected via RT-PCR does not provide information on its infectious capacity. Furthermore, no live virus was cultured from a specimen obtained eight days post-symptoms onset. In other words, viral RNA's persistence indicates the immune response status (RNA clearance) and not the disease severity. Recent research has revealed that there could be a relationship between Ct values and disease severity or associated morbidity with COVID 19, as patients who died had substantially lower Ct values compared to patients who recovered, but these patients had a considerably shorter period with symptoms before testing. The sample size of the study was too small to show a strong positive or negative association between Ct value and disease severity and fatality rate using statistical analysis. On the other hand, a

retrospective cohort analysis in New York by Zacharioudakis et al. (n = 42) showed that a three-fold increase in Ct value denoted a correlation with a 0.15 improvement in terms of genomic load on Sequential Organ Failure Assessment (SOFA) score. Another larger study with 192 patients revealed that patients who died in hospital had significantly lower Ct values than those who were discharged alive.

The same was true for patients who needed admission to the ICU or developed shock, having significantly lower Ct values than those who did not. Ct values had a statistically significant negative correlation with the length of ICU stay.

Relation between CT values and duration & time of recovery of disease.

Studies from semiquantitative RT-PCR the use of Ct values provide a valuable proxy for infectious virus detection and can also assist to inform decision-making on infection control. This study adds on to the proof base on duration of infectiousness following mild-to-moderate COVID-19, demonstrating that infectious virus can persist for a week or extra after symptom onset, declining over time. At 10 days after symptom onset, in line with current instruction from the World Health Organization and the UK on release from isolation, likelihood of culturing virus declines to 6%. The findings concur with smaller research that recognized infectious virus shed for 8 or 9 days and others demonstrating correlation between Ct value/viral load and cultivable virus. Strengths consist of the comparatively giant size of this dataset, inclusion of a large percentage (> 50%) of samples taken more than 7 days after symptom onset and that all evaluation was carried out in a single laboratory (Van Kampen et al.) stated more prolonged detection of cultivable virus from 23 hospitalised cases, for up to 20 days after symptom onset. However, their cohort covered in most cases decrease respiratory tract samples from patients with greater severe disorder which include almost one in 5 who have been immunocompromised, which is not going to be consultant of the general population. Taken together with information presented here, the results of (Van Kampen et al.) point out that more prolonged excretion of infectious virus could be related with extreme disorder or an immunocompromised state.

This study recognized that Ct values and the presence of infectious virus had been similar in samples from asymptomatic and pre-symptomatic persons, compared with those who had been symptomatic, and is one of the first reports of virus isolation from cases who stay absolutely asymptomatic. The findings advise that asymptomatic and pre-symptomatic persons do symbolize a supply of potentially transmissible virus. Extensive records on cultivable virus from asymptomatic or pre-symptomatic men and women are lacking, with one outbreak investigation in a care home reporting detection of cultivable virus in one asymptomatic and pre-symptomatic cases. Although we noticed a greater proportion of asymptomatic cases in the age crew 81–100 years, the reasons and importance of this are unclear. It may additionally mirror sampling bias from care home outbreaks. However, it could additionally replicate actual variations in response to infection in this age group (e.g. lower response to fever, lower reporting of subjective signs and symptoms in this age group).

Of note, recall bias may additionally affect the interpretation of timing of virus detection in relation to symptom onset, especially in aged patients and those presenting with peculiar symptoms. Duration and cessation of signs and symptoms is additionally not properly recorded. For asymptomatic cases, the time when infection was obtained is not known. A similarly limitation is that this dataset comprises real-world information and subjects have been not sampled systematically; therefore, there may additionally be bias in the timing of sampling associated to the clinical scenario. Finally, the sensitivity of virus propagation from medical samples is dependent on laboratory expertise, cell lines and protocols used, and can also be affected by sample quality, storage and transport conditions, which means it is difficult to immediately evaluate data between laboratories described in different literature.

On an common we conclude that it takes 10-14days get better from the signs and symptoms of COVID-19. The RT-PCR cycle threshold (Ct) values as a measure of SARS-CoV-2 viral load confirmed that the level of SARS-CoV-2 RNA in the Upper Respiratory tract was greatest around symptom onset, gradually lowered at some point of the first 10 days after sickness onset and then plateaued.

- In days −2 to 7 since symptom onset geometric mean (GM) Ct was 28 (95% CI 27.8 to 28.6).
- In the second week (days 8 to 14), GM Ct was 31 (95% CI: 9.8 to 32; $p < 0.001$ compared with week 1)

- After 14 days, GM Ct was 32 (95% CI: 31.6–34.5; p = 0.01 compared with week 1 (there was no significant difference in Ct values between days 8–14 and after 14 days).

Relation between CT values and degrees of disease & medication

Based on the Ct values the degrees of the disease and medication are mentioned in the below table.

Table 2: This table depicts the medication based on the Ct values of the COVID-19 test

Degree of disease	Ct value of disease	Medications
Mild	35-31 < (till 40)	Antibiotic Anti-inflammatory Multivitamin (zine + B Complex) Vitamin C Antacid
Moderate	26-30	Immune modulators Cough syrup Nasal spray Antibiotic Anti-inflammatory Multivitamin (zine +B Complex) Vitamin C Antacid
Severe (strong positive)	<17-25	Antiviral Immunomodulators Corticosteroids (required) Cough syrup(required) Nasal spray (required) Antibiotic Anti-inflammatory Multivitamin (zine + B Complex) Vitamin C Antacid

CONCLUSION

This study concludes the relation between COVID-19 Ct values that we obtain from RT-PCR and the symptoms, viral load, other co-morbidities, severity, medications and duration of the infection.

The relation between COVID-19 Ct values and the viral load is inversely proportional. That is as the Ct value is lower, the higher the viral load.

The relation between Ct values and symptoms, progression, duration of the infection depends on the person's health history i.e co-morbidities and viral load, which sums up that Ct values are not related to the symptoms, Duration and progression of the infection.

The relation between Ct values and the medications, in this topic we can say that medications are related to the Ct values as we can predict viral load of patients via Ct values, medications can be provided accordingly, though considering co-morbidities.

The relation between Ct values and biological tests and haematological indices, these can be related as the viral load in patient's body will differ the ranges of all the biology markers, this can also happen due to co-morbidities, which again need to be considered before prescribing medications.

Acknowledgment

I want thank my university, garden city university, for providing me an opportunity to perform this study and also helping me to improve my knowledge. I want to thank my professor Dr. Proma Chakraborty, assistant professor, Garden City University for helping me improving quality of my study documentation and also helping me out to get reports from many patients. I also want to show my gratitude to my family, friends,

colleagues and professors for helping me out in sharing the survey as many as possible and giving their genuine responses for the study.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

Arentz M, Yim E, Klaff L, Lokhandwala S, Riedo FX, Chong M, Lee M. Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington state. JAMA. 2020; 323:1612. doi: 10.1001/jama.2020.4326.

BCCDC. COVID-19 vulnerable populations. British Columbia Centre for Disease Control. 2020. [Accessed April 18, 2020, http://www.bccdc.ca/health-info/diseases-conditions/covid-19/vulnerable-populations].

Boddington NL, Charlett A, Elgohari S, Walker JL, Mcdonald H, Byers C, et al. COVID-19 in Great Britain: epidemiological and clinical characteristics of the first few hundred (FF100) cases: a descriptive case series and case control analysis. medRxiv. 2020.05.18.20086157.

CDC Severe outcomes among patients with coronavirus disease 2019 (COVID-19) — United States, February 12–March 16, 2020. Centers for Disease Control and Prevention: MMWR. 2020;69(12):343–346. [PMC free article] [PubMed] [Google Scholar]

CDC. Coronavirus (COVID-19): symptoms of coronavirus. Centers for Disease Control and Prevention. 2020. [Accessed April 18, 2020, https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html].

Chang D, Mo G, Yuan X, Tao Y, Peng X, Wang FS, Xie L, Sharma L, dela Cruz CS, Qin E. Time kinetics of viral clearance and resolution of symptoms in novel coronavirus infection. AJRCCM. 2020;201:1150–1152. doi: 10.1164/rccm.202003-0524LE.

Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3)

Ebrahim Abbasi-Oshaghi, Fatemeh Mirzaei, Farhad Farahani, Iraj Khodadadi, Heidar Tayebinia Int J Surg. Diagnosis and treatment of coronavirus disease 2019 (COVID-19): Laboratory, PCR, and chest CT imaging findings.NCBI ,2020 Jul; 79: 143–153. 2020 May 15.

Franki R. Comorbidities the rule in New York's COVID-19 deaths. Hospitalist. 2020; [Accessed June 1, 2020, https://www.the-hospitalist.org/hospitalist/article/220457/coronavirus-updates/comorbidities-rule-new-yorks-covid-19-deaths].

Garg S, Kim L, Whitaker M, O'Halloran A, Cummings C, et al. Hospitalization rates and characteristics of patients hospitalized with laboratory-confirmed coronavirus disease 2019 — COVID-NET, 14 states, March 1–30, 2020. Centers for Disease Control and Prevention: MMWR. 2020;69(15):458–464. [PMC free article] [PubMed] [Google Scholar]

Huang C, Wang Y, Li X, Ren L, Zhao J, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497–506. doi: 10.1016/S0140-6736(20)30183-5.

IAS. COVID-19 and HIV: what you need to know. The International AIDS Society. 2020. [Accessed April 20, 2020, https://www.iasociety.org/covid-19-hiv].

Jefferson T, Heneghan C. COVID-19: Clinical Utility of Cycle Threshold Values.The centre of evidence-based medicine, August 12, 2020.

Li B, Yang J, Zhao F, Zhi L, Wang X, et al. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. Clin Res Cardiol. 2020;109:531–538. doi: 10.1007/s00392-020-01626-9. .

Li Q, Med M, Guan X, Wu P, Wang X, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. N Engl J Med. 2020; 382:1199–1207. doi: 10.1056/NEJMoa2001316.

Ludvigsson JF. Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. Acta Paediatr. 2020;109:1088–1095. doi: 10.1111/apa.15270.

Maragakis LL. Coronavirus symptoms: frequently asked questions. Johns Hopkins Medicine. 2020; [Accessed April 18, 2020, https://www.hopkinsmedicine.org/health/conditions-and-diseases/coronavirus/coronavirus-symptoms-frequently-asked-questions].

Meo SA, Alhowikan AM, Al-Khlaiwi T, Meo IM, Halepoto DM, et al. Novel coronavirus 2019-nCoV: prevalence, biological and clinical characteristics comparison with SARS-CoV and MERS-CoV. Eur Rev Med Pharmacol Sci. 2020;24(4):2012–2019. doi: 10.26355/eurrev_202002_20379. [PubMed] [CrossRef] [Google Scholar]

Pan, Y.; Zhang, D.; Yang, P.; Poon, L.L.M.; Wang, Q. Viral load of SARS-CoV-2 in clinical samples. Lancet Infect. Dis. 2020, 20, 411–412.

Paudel SS. A meta-analysis of 2019 novel coronavirus patient clinical characteristics and comorbidities. Research Square. 2020. 10.21203/rs.3.rs-21831/v1 [Accessed April 18, 2020, https://www.researchsquare.com/article/rs-21831/v1].

Perera, R.A.P.M.; Tso, E.; Tsang, O.T.Y.; Tsang, D.N.C.; Fung, K.; Leung, Y.W.Y.; Chin, A.W.H.; Chu, D.K.W.; Cheng, S.M.S.; Poon, L.L.M.; et al. SARS-CoV-2 virus culture and subgenomic RNA for respiratory specimens from patients with mild Coronavirus disease. Emerg. Infect. Dis. 2020, 26, 2701–2704.

Rabaan, A.A.; Tirupathi, R.;Sule, A.A; Aldali, J.; Mutair, A.A.; Alhumaid, S.; Muzaheed; Gupta, N.; Koritala, T.; Adhikari, R.; et al. Viral Dynamics and Real-Time RT-PCR Ct Values Correlation with Disease Severity in COVID-19. MDPI DIAGNOSTICS, 15 June 2021.

Scott J Bergman, PharmD, FCCP, FIDSA, BCPS, BCIDP; COVID-19 Treatment: Investigational Drugs and Other Therapies, The Medscape, Aug 05, 2021.

Shuva Das, Department of Microbiology, Chittagong Medical College, Chattogram, Bangladesh, Comorbidities of

COVID-19 Patients with Low Cycle Threshold (Ct) Value of Nucleocapsid (N) Gene: An Application to Cluster-Based Logistic Model, Journal of Antivirals & Antiretrovirals,Feb 19, 2021.

van Kampen, J.J.A.; van de Vijver, D.A.M.C.; Fraaij, P.L.A.; Haagmans, B.L.; Lamers, M.M.; Okba, N.; van den Akker, J.P.C.; Endeman, H.; Gommers, D.A.M.P.J.; Cornelissen, J.J.; et al. Shedding of infectious virus in hospitalized patients with coronavirus disease-2019 (COVID-19): Duration and key determinants. medRxiv 2020.

Wang B, Li R, Lu Z, Huang Y. Does comorbidity increase the risk of patients with COVID-19: Evidence from meta-analysis. Aging (Albany NY) 2020;12(7):6049–6057. doi: 10.18632/aging.103000.

Wang J, Hajizadeh N, Moore EE, McIntyre RC, Moore PK, et al. Tissue plasminogen activator (tPA) treatment for COVID-19 associated acute respiratory distress syndrome (ARDS): a case series. J Thromb Haemost. 2020. 10.1111/jth.14828 [Accessed May 30, 2020, https://onlinelibrary.wiley.com/doi/full/10.1111/jth.14828]. [PMC free article] [PubMed]

WHO. Coronavirus COVID-19. World Health Organization. 2020. [Accessed April 29, 2020, https://covid19.who.int/].

WHO. Q&A on coronaviruses (COVID-19). World Health Organization. 2020. [Accessed April 18, 2020, https://www.who.int/news-room/q-a-detail/q-a-coronaviruses].

WHO. Q&A on COVID-19, HIV, and antiretrovirals. World Health Organization. 2020. [Accessed April 20, 2020, https://www.who.int/news-room/q-a-detail/q-a-on-covid-19-hiv-and-antiretrovirals].

W-Jie G, Liang W-H, He J-X, Zhong N-S. Cardiovascular comorbidity and its impact on patients with COVID-19. Eur Respir J. 2020;55:2001227. doi: 10.1183/13993003.01227-2020.

Wu C, Chen X, Cai Y, Xia J, Zhou Z, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med. 2020:e200994. 10.1001/jamainternmed.2020.0994 [Accessed April 20, 2020, https://jamanetwork.com/journals/jamainternalmedicine/fullarticle/2763184]. [PMC free article] [PubMed]

Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, Ji R, Wang H, Wang Y, Zhou Y. Prevalence of comorbidities in the novel Wuhan coronavirus (COVID-19) infection: a systematic review and meta-analysis. Int J Infect Dis. 2020; S1201–9712(20):30136–30133. doi: 10.1016/j.ijid.2020.03.017.

Yousefzadegan S, Rezaei N. Case report: death due to novel coronavirus disease (COVID-19) in three brothers. Am J Trop Med Hyg. 2020;102:1203–1204. doi: 10.4269/ajtmh.20-0240.

Yu, J.; Kang, M.; Song, Y.; Xia, J.; Guo, Q.; Song, T.; He, J.; Yen, H.-L.; Peiris, M.; Wu, J. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. N. Engl. J. Med. 2020, 382, 1177–1179.

Zhao Q, Meng M, Kumar R, Wu Y, Huang J, et al. The impact of COPD and smoking history on the severity of COVID-19: A systemic review and meta-analysis. J Med Virol. 2020. 10.1002/jmv.25889 [Accessed May 30, 2020, https://pubmed.ncbi.nlm.nih.gov/32293753/]. [PMC free article] [PubMed]

Zhou F, Yu T, Du R, Fan G, Liu F, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054–1062. doi: 10.1016/S0140-6736(20)30566-3.

Zhu L, She Z-G, Cheng X, Qin J-J, Zhang X-J, et al. Association of blood glucose control and outcomes in patients with COVID-19 and pre-existing type 2 diabetes. Cell Metab. 2020; [Accessed May 30, 2020, https://www.sciencedirect.com/science/article/pii/S1550413120302382]. [PMC free article] [PubMed]