Virus Load and Incidence Olfactory, Gustatory, Respiratory, Gastrointestinal Disorders in COVID-19 Patients: A Retrospective Cohort Study

Abstract

Objectives: This study investigated the relationship between viral load and the incidence and recovery of olfactory and gustatory dysfunction (OD and GD), incidence of respiratory and gastrointestinal symptoms in COVID-19 patients.

Design: A Retrospective Cohort Study.

Setting and Participants: In total 599 outpatients' cases polymerase chain reaction (PCR)–confirmed COVID-19-positive patients in Golestan province were included in the study.

Main Outcome Measures: The incidence of OD, GD, their severity and the time of recovery was determined. The association of these variables with cycle threshold (CT) values of SARS-CoV-2 polymerase chain reaction was assessed.

Results: The mean age of patients was 38.27 ± 13.62 years. The incidence of general symptoms included myalgia 70.1%, headache 51.8%, fever 47.7%, and dyspnea 21.4%. 41.9% of patients had gastrointestinal symptoms, including abdominal pain 26.5%, diarrhea 25.2%, nausea 20.5%, and vomiting 12.9%. 12.2% of patients had comorbidity. The trimester recovery rates of OD and GD were 93.94% and 94.74%, respectively. The mean recovery time of OD and GD was 14.56±13.37 and 13.8±3.77 days, respectively. The mean CT value in all patients was 27.45±4.55. There were significant associations between CT value with headache (P=0.04), GD (P=0.002) and OD (P=0.001).

Conclusions: The intensity and the recovery of OD and GD in Covid-19 patients may be affected by initial viral load. Unlike to respiratory and gastrointestinal symptoms, the OD and GD were associated with lower viral load. Therefore, it may be recommended to use these clinical symptoms as an indicator in the initial screening of patients during pandemics.

Key points

- A retrospective analysis of 599 COVID-19 patients was performed to determine the incidence of olfactory and gustatory dysfunction and its recovery.

- The association of OD, GD, its recovery and time of recovery were assessed with cycle threshold (CT) values of SARS-CoV-2.
- The mean CT in patients with complete OD was significantly higher than patients with partial OD. Also, the mean of CT in patients with GD and without GD showed a significant difference.
- Correlation between OD and GD recovery time with CT value was not significant.
- There was a strong correlation between the recovery time of OD and GD.

INTRODUCTION

COVID-19 is caused by severe acute respiratory syndrome, SARS-CoV-2 and the cumulative incidence and its morbidity are still growing (1). Gastrointestinal symptoms such as diarrhea, abdominal pain, nausea, and vomiting are also common manifestations in COVID-19 (2).

The symptoms of the disease are nonspecific in the early stages and are indistinguishable from the symptoms of the common cold. Even asymptomatic carriers of SARS-CoV-2 can spread the virus and be contagious (3). Although classic respiratory symptoms such as fever, dry cough, sputum, shortness of breath, sore throat, and myalgia, along with a history of suspected contact with infected individuals or travel to an infected area, have been used as screening symptoms for patients, olfactory and taste dysfunction (OD, GD) received special attention to be used to screen the community (4). Following numerous reports of the high prevalence of OD and GD due to COVID-19 in European and American countries, the American Academy of Otolaryngology (Head and Neck Surgery) recommended adding anosmia and hypoxemia to screen for possible COVID-19 infection (5). This has led to many research questions about the pathophysiology and prognosis of the association between OD and GD and COVID-19.

Detection of COVID-19 is performed by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) test (6). This test shows the nucleic acid of the virus in the saliva-nasal secretions of the patient (7). In this test, the value of cycle threshold value (CT) means the number of amplification cycles required to reach the detection threshold of virus nucleic acids. The value of CT is inversely related to the load of the virus in the test sample and indirectly indicates the level of virus replication (8). Sampling for RT-PCR test can be obtained from nasopharynx,

oropharynx and lower respiratory track. Some studies reported SARS-CoV-2 virus loads in the nasal samples were more than the pharyngeal samples (9). Viral loads have also been reported to typically increase during the first week after the onset of symptoms and remain high for subsequent weeks (10). It has also been reported that the amount of virus in samples prepared from the lower respiratory tract is very high, but due to the problems of this sampling and its limited implementation in all health centers, this method has not been approved by the FDA (11).

There are few studies on virus-related factors that may affect the outcome, and therefore the American Infectious Diseases Association has emphasized quantitative testing as a prognostic factor for the diagnosis of SARS-CoV-2 (12). In this regard, the CT value of the RT-PCR test has been considered as an indicator of virus load in determining the prognosis and outcome of patients and the occurrence of some disorders.

Due to the prevalence of COVID-19 and a large percentage of patients with OD and GD and the importance of determining the predictors of some complications and outcome of patients, this study was performed to determine the relationship between CT value of RT-PCR test and OD and GD in patients with COVID-19 in Golestan province in Iran.

Methods

A cohort study was conducted between February and June 2020 in Golestan province on the southern shore of the Caspian Sea in northern Iran. The strengthening the reporting of observational studies in epidemiology (STROBE) statement was used for reporting the study. Patients who were suspected of having COVID-19 based on clinical signs were evaluated by a family physician and then the COVID-19 disease was confirmed by RT-PCR test. The sampling of patients was performed by trained experts using swaps. For each patient, a nasopharyngeal sample was taken through the nose and a nasopharyngeal sample was taken orally. Samples were transferred to the Department of Virology of the School of Medicine in Golestan University of Medical Sciences. RT-PCR was performed by ABI Step One Plus, and CT values were extracted. Demographic characteristics of patients including age, sex, and time of infection were collected from patients' electronic records. As well as, the respiratory symptoms including fever, headache, myalgia, cough, and gastrointestinal symptoms including diarrhea, vomiting, abdominal pain and nausea and OD and GD status were collected and completed via telephone call and patients were interviewed by the specialist, if necessary. According to the severity of

self-reported collection of OD and GD, they are divided into complete or partial forms. Patients were classified according to age and their geographical location in the province, three regions of East, Center, and West.

Statistical analysis

Statistical analysis was performed using STATA, version 14.1 (Stata Corp, College Station, Tx: Stata Corp LLC). Data are presented as mean values with the standard deviation (SD) and frequency. Concerning quantitative variables, after variance equality and normal distribution of the values were checked, we used The Mann-Whitney U test to compare the CT values in binary groups as well as to compare the CT values in categorical variable we used the Kruskal–Wallis test. The correlation between time of recovery of olfactory and gustatory dysfunctions and CT values was assessed using Spearman's rank correlation coefficient. A P value of <0.05 was considered statistically significant.

Results

In this study, 599 Covid-19 cases that approved by RT-PCR were included. Of the total samples included in this study, 313 (52.2%) were female and 286 (47.74%) were male. The mean age of the whole sample was 38.27±13.62 years and most (50.75%) of these people were in the age group of 20-40 years. The clinical characteristics of patients was shown in table 1.

In general, the mean of CT value in Covid-19 patients was 27.45±4.55. The study showed that the mean of CT value of women was 27.34±4.63, and that of men was 27.56±4.46. There was no significant difference between men and women ($P = 0.54$). The mean of CT value increases slightly with age, so the mean of CT value of the age group under 20 is 27.35±5.17, and the mean of CT value of the age group 60 and above is 27.68±4.85, but the difference is not significant ($P = 0.94$).

In addition, there were no significant differences in the mean of CT value of patients from different ethnicity and regions (eastern, western, and central provinces). The mean CT was 28.13±4.55 in patients with OD and 26.91±4.49 in patients without OD. The mean of CT value between the two groups was significantly different ($P = 0.001$).

The mean CT in patients with complete OD was significantly higher than patients with partial OD (P=0.02). Also, the mean of CT was 28.16±4.52 in patients with GD and 27.01±4.52 in patients without GD, which showed a significant difference (P=0.002). However, the difference in mean CT in patients with complete and partial GD was not significant (P=0.32).

Generally, when comparing the mean of CT value of Covid-19 patients with at least one of OD or GD with patients without this dysfunction, the mean of CT value of patients with OD or GD is significantly higher. (P=0.001). However, regarding OD patients, a comparison of the mean CT value of recovered patients and unrecovered patients did not show a significant difference (P = 0.62). Also, there was no significant difference between people who had partial recovery, and those who had complete recovery (P=0.38).

These comparisons were carried out for the GD recovery, which was similar to the OD recovery, and there was no significant difference in the mean of CT value. Mean of CT value, in Covid-19 patients, did not show a significant difference in terms of having or not having symptoms of fever, myalgia, and shortness of breath. However, the mean of CT value of patients with headache symptoms was significantly higher than that of patients without headache symptoms (P = 0.04).

Mean of CT value was not significantly different in patients with and without gastrointestinal disorders (P=0.48). Similarly, in the study of each gastrointestinal disease (abdominal pain, diarrhea, nausea and vomiting), there was no significant difference in the mean of CT value in terms of having or not having these disorders. Also, the co-occurrence of diseases such as diabetes and hypertension did not have a significant effect on the mean CT for Covid-19 patients.

Research on the correlation between OD and GD recovery time and CT value showed that there was no significant correlation between OD recovery time and CT and between GD recovery time and CT. However, there was a strong, direct and significant correlation between the recovery time of OD and GD ($r = 0.97$, $P < 0.001$) (Figure 1).

Figure 1: Matrix correlation between the recovery time of OD, GD, and CT

Discussion

In this study, 599 patients were included in the study, of which 52.2% were female and the mean age of patients was 38.27±3.62 years and the maximum age distribution was in the age group of 20-40 years (50.75%). Comparison of age and sex distribution of our patients who were followed up on an outpatient basis was similar to studies by Zhong-Hu et al. (13). That is, it is slightly higher in women. But it was a little lower in terms of age. The lower age of our patients may be because these patients have been followed up on an outpatient basis; however, in other studies, patients were hospitalized, most of whom were older (14).

The CT value in our patients was 27.45±4.55 and this number was 27.34±4.63 in women and 27.56±4.64 in men, which was not significantly different. In this study, the mean CT value showed a slight increase with age, so that in the age group under 20 years 27.35±5.7 and the age group 60 years and above this number was 27.68±4.85 years. That is, the load of the virus was lower in old age. In the study of Buchan et al., The Ct value was slightly lower only in the age group of 90-80 and did not differ between other groups (15). Virus load was different in the two age ranges of our study with the Buchan study. In the Buchan report, the virus load was higher with age. In our study, the virus load decreased with age.

These observations show that the virus load is lower in people with severe OD or GD, which is similar to the results of the study by Cho et al. (16). In that study, it was also reported that the more severe the OD or GD, the higher the CT value, i.e., the lower the virus load. At first glance, it may have been thought that people with OD or GD had a higher number of viruses, but the results show the opposite. What is certain is that the development of anosmia in coronavirus infection has been proven, but its mechanism or pathophysiology is still unclear (17) . One possibility is that the Sars-Cov-2 virus attacks ACE2 receptors in the basal and sustentacular cells of the olfactory epithelium. Another possibility is the ability of the virus to invade the central nervous system through the olfactory bulb (18).

Although it is difficult to explain why Covid-9 patients with a lower load of the virus develop OD or GD, the possible reasons for this can be summarized as follows. First, during the Covid-19 pandemic, the amount of detergent used in the community to maintain hygiene was very high, and OD may be caused by stimulation of the olfactory epithelium with a minimal number of viruses. Another reason may be due to the unknown nature of the pathogenicity of the virus or the proximity of the virus to the olfactory epithelium, which increases its vulnerability with

minimal virus load. The last point that is more epidemiologically important is that people with OD experience more unexpected symptoms, and because of the anxiety it causes them, they refer to health centers at the beginning of the disease when the load of the virus has not yet risen or have not seen more severe systemic symptoms. This finding could be due to one of the important symptoms in Covid-19, which can be used to screen and isolate the virus in a phase where the virus load is not yet high. Although the above is a hypothesis, it is better to perform PCR in these patients to measure the CT value in the following days to judge more acceptably whether to accept or reject this information.

In our study, among the general symptoms, only a significant relationship was found between headache and CT value. This means that patients with headaches had fewer viruses at the time of referral and testing. Although no significant relationship was found between CT value and many variables in this study, in many studies CT value has shown valuable results. In some studies, a significant relationship was observed between low CT value and an increase in LDH and a decrease in lymphocytes, indicating a worse prognosis (19, 20). SARS-CoV-2-induced pneumonia has also been reported that lower CT values accompanied more tissue damage in the patient's lungs and a worse prognosis (21). In the study of Zacharioudakis et al., Which was performed on hospitalized patients with COVID 19 pneumonia, a change in CT value was reported in accordance with the general clinical condition of the patient. This means that with the improvement of the patient's general condition, the CT value also increased and this index was used to predict the patient's outcome (22). In the Aquino-Jarquin study, it was reported that Ct value and clinical outcome were directly and non-linearly related (23). In the meta-analysis of Sonia et al., Low CT value was associated with increased disease severity and was reported to be consistent with the results of other studies (24). The study by Huang et al. Also reported an increased risk of mortality with lower CT values (25). This observation is consistent with the results of previous epidemiological studies on coronaviruses.

Although many studies have linked the relationship between Ct value and prognosis and clinical severity, some other studies have not found such a relationship. In asymptomatic but infected individuals, the viral load was similar to that in symptomatic patients, and it was concluded that virus load alone was not a reliable indicator of predicting disease outcome. According to the

results obtained on CT value in OD and GD, it seems that the exact pathophysiology of this phenomenon needs further study.

Strength and limitation

The following limitations should be considered in generalizing the results of this study. Although RT-PCR tests were performed centrally in the Department of Virology of Golestan University of Medical Sciences under the supervision of faculty members, sampling was performed by trained experts throughout the province, and nasopharyngeal sampling may be in some cases be inaccurate.

Conclusions

Although no significant relationship was found between CT value and many variables in this study. A significant relationship was found between CT value and headache, OD, and GD. This means that patients with these symptoms are less likely to develop the virus. therefore, in screening patients, if patients who have these symptoms, especially in the case of OD and GD, which are clearer and more transparent to the patient and are reported in a high percentage of Covid-19 patients, these indicators can be used as Important symptoms to isolating patients in the early stages with low virus count. Due to the high prevalence of OD and GD in Covid-19 patients and the fact that these symptoms are more pronounced and that the number of viruses is still low at this stage, it is recommended to use these clinical symptoms as indicators in the initial screening of patients during pandemics.

References

1. World Health Organization. Coronavirus disease 2019 (COVID): situation report 124. Available at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200523-covid-19-sitrep-124.pdf?sfvrsn=9626d639_2.
2. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. nature. 2020;579(7798):270-3.
3. Nishiura H, Kobayashi T, Miyama T, Suzuki A, Jung S-m, Hayashi K, et al. Estimation of the asymptomatic ratio of novel coronavirus infections (COVID-19). International journal of infectious diseases. 2020;94:154.
4. Hopkins C, Surda P, Kumar N. Presentation of new onset anosmia during the COVID-19 pandemic. Rhinology. 2020;10.
5. Lechien JR, Chiesa-Estomba CM, De Siati DR, Horoi M, Le Bon SD, Rodriguez A, et al. Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. European Archives of Oto-Rhino-Laryngology. 2020:1-11.

6. Tang Y-W, Schmitz JE, Persing DH, Stratton CW. Laboratory diagnosis of COVID-19: current issues and challenges. Journal of clinical microbiology. 2020;58(6).
7. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virological assessment of hospitalized patients with COVID-2019. Nature. 2020;581(7809):465-9.
8. Joynt GM, Wu WK. Understanding COVID-19: what does viral RNA load really mean? The Lancet Infectious Diseases. 2020;20(6):635-6.
9. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. New England Journal of Medicine. 2020;382(12):1177-9.
10. Pan Y, Zhang D, Yang P, Poon LL, Wang Q. Viral load of SARS-CoV-2 in clinical samples. The Lancet Infectious Diseases. 2020;20(4):411-2.
11. Huang Y, Chen S, Yang Z, Guan W, Liu D, Lin Z, et al. SARS-CoV-2 viral load in clinical samples of critically ill patients. American journal of respiratory and critical care medicine. 2020(ja).
12. IDSA Outlines Key COVID-19 Research Questions and Recommendations [cited 2020 May 18]. Available from: https://www.idsociety.org/globalassets/idsa/public-health/covid-19/idsa-covid-19-key-research-qs.pdf.
13. Zhang H, Liao Y-S, Gong J, Liu J, Xia X, Zhang H. Clinical characteristics of Coronavirus Disease (COVID-19) patients with gastrointestinal symptoms: A report of 164 cases. Digestive and Liver Disease. 2020.
14. Taziki Balajelini MH, Vakili MA, Saeidi M, Tabarraei A, Hosseini SM. Using Anti-SARS-CoV-2 IgG and IgM Antibodies to Detect Outpatient Cases with Olfactory and Taste Disorders Suspected as Mild Form of COVID-19: a Retrospective Survey. SN comprehensive clinical medicine. 2020:1-7.
15. Buchan BW, Hoff JS, Gmehlin CG, Perez A, Faron ML, Munoz-Price LS, et al. Distribution of SARS-CoV-2 PCR cycle threshold values provide practical insight into overall and target-specific sensitivity among symptomatic patients. American Journal of Clinical Pathology. 2020;154(4):479-85.
16. Cho RH, To ZW, Yeung ZW, Tso EY, Fung KS, Chau SK, et al. COVID−19 Viral Load in the Severity of and Recovery from Olfactory and Gustatory Dysfunction. The Laryngoscope. 2020;130(11):2680-5.
17. Potter MR, Chen JH, Lobban NS, Doty RL, editors. Olfactory dysfunction from acute upper respiratory infections: relationship to season of onset. International Forum of Allergy & Rhinology; 2020: Wiley Online Library.
18. Netland J, Meyerholz DK, Moore S, Cassell M, Perlman S. Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. Journal of virology. 2008;82(15):7264-75.
19. Yan L, Zhang H-T, Goncalves J, Xiao Y, Wang M, Guo Y, et al. An interpretable mortality prediction model for COVID-19 patients. Nature Machine Intelligence. 2020:1-6.
20. Kermali M, Khalsa RK, Pillai K, Ismail Z, Harky A. The role of biomarkers in diagnosis of COVID-19–A systematic review. Life Sciences. 2020:117788.
21. Kishaba T, Tamaki H, Shimaoka Y, Fukuyama H, Yamashiro S. Staging of acute exacerbation in patients with idiopathic pulmonary fibrosis. Lung. 2014;192(1):141-9.
22. Zacharioudakis IM, Zervou FN, Prasad PJ, Shao Y, Basu A, Inglima K, et al. Association of SARS-CoV-2 genomic load trends with clinical status in COVID-19: A retrospective analysis from an academic hospital center in New York City. Plos one. 2020;15(11):e0242399.
23. Aquino-Jarquin G. The raw Ct values from RT-PCR detection are not viral load quantitation units. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2020.
24. Rao SN, Manissero D, Steele VR, Pareja J. A narrative systematic review of the clinical utility of cycle threshold values in the context of COVID-19. Infectious diseases and therapy. 2020:1-14.
25. Huang J-T, Ran R-X, Lv Z-H, Feng L-N, Ran C-Y, Tong Y-Q, et al. Chronological Changes of Viral Shedding in Adult Inpatients with COVID-19 in Wuhan, China. Clinical Infectious Diseases. 2020.

Table 1: Characteristics, presenting symptoms of patients with SARS-CoV-2 infection and Ct Values from SARS-CoV-2 Polymerase Chain Reaction Assays in included cases in the study

Variables		N (%)/Mean±SD	CT Values From SARS-CoV-2 PCR Mean±SD	P-value
Total (N, %)		599 (100)	27.45±4.55	
Sex (N, %)	Men	286 (47.75)	27.56±4.46	0.54*
	Women	313 (52.25)	27.34±4.63	
Age (Mean±SD)		38.27±13.62		
Age group (N, %))	<20	40 (6.68)	27.35±5.17	0.94**
	20-40	304 (50.75)	27.38±4.48	
	40-60	210 (35.06)	27.51±4.50	
	≥60	45 (7.51)	27.68±4.85	
Ethnicity (N, %)	Fars	123 (34.6)	27.21±4.71	0.87
	Sistani-Persian	32 (9)	27.13 ±4.68	
	Turkmen	200 (56.3)	27.48 ±4.35	

Location	West	69 (11.58)	27.31±4.02	0.54
	Center	101 (16.95)	27.91±4.59	
	East	426 (71.48)	27.38±4.59	
Olfactory dysfunction (OD)	Yes	263 (43.9)	28.13±4.55	0.001
	No	336 (56.1)	26.91±4.49	
The severity of olfactory dysfunction	Partial	60 (10)	26.87±4.93	
	Complete	203 (33.9)	28.50±4.38	0.02
	None	336 (56.1)	26.91±4.49	
Gustatory dysfunction (GD)	Yes	228 (38.1)	28.16±4.52	0.002
	No	371 (61.9)	27.01±4.52	
The severity of Gustatory dysfunction	Partial	64 (10.7)	27.57±5.09	0.32
	Complete	164 (27.4)	28.39±4.27	
	None	371 (61.9)	27.01±4.52	
OD and GD	Yes	264 (44.1)	28.13±4.54	0.001
	No	335 (55.9)	26.91±4.49	
Recovery of OD	Yes	248 (93.94)	28.15±4.55	0.62
	No	16 (6.06)	27.57±4.63	
Rate of recovery of OD	Complete	213 (80.68)	28.30±4.57	0.38
	Partial	35 (13.26)	27.23±4.39	
	Non-recovery	16 (6.06)	27.57±4.63	
Recovery of GD	Yes	216 (94.74)	28.11±4.49	0.95
	No	12 (5.26)	28.19±4.93	
Rate of recovery of GD	Complete	182 (79.82)	28.22±4.52	0.70
	Partial	34 (14.91)	27.52±4.33	
	Non-recovery	12 (5.26)	28.19±4.93	
Fever	Yes	286 (47.7)	27.70±4.52	0.15
	No	313 (52.3)	27.73±4.62	
Myalgia	Yes	420 (70.1)	27.32±4.52	0.38
	No	179 (29.9)	27.73±4.62	
Headache	Yes	310 (51.8)	27.80±4.51	**0.04**
	No	289 (48.2)	27.07±4.57	
	Yes	128 (21.4)	27.47±4.83	0.86

Shortness of breath	No	471 (78.6)	27.44±4.48	
Abdominal pain	Yes	159 (26.5)	27.34±4.57	0.74
	No	440 (73.5)	27.49±4.55	
Diarrhea	Yes	151 (25.2)	27.04±4.60	0.24
	No	448 (74.8)	27.58±4.53	
Nausea	Yes	123 (20.5)	27.51±4.57	0.76
	No	476 (79.5)	27.43±4.55	
Vomit	Yes	77 (12.9)	27.56±4.62	0.80
	No	522 (87.1)	27.43±4.54	
Gastrointestinal disorders (GI)	Yes	251 (41.9)	27.27±4.56	0.48
	No	348 (58.1)	27.57±4.55	
History of diabetes	Yes	31 (5.2)	28.23±5.66	0.21
	No	568 (94.8)	27.40±4.48	
History of hypertension	Yes	57 (9.5)	27.92±5.08	0.33
	No	542 (90.5)	27.40±4.49	
Comorbidity	Yes	72 (12.02)	28.08±5.01	0.21
	No	527 (87.98)	27.36±4.48	
Pregnant	Yes	11 (3.9)	29.47±3.59	0.10
	No	271 (96.1)	27.17±4.64	

* For binary variable used the Mann-Whitney Test

** For categorical variable used the Kruskal-Wallis Test