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ORIGINAL ARTICLE

Evaluation of the consistency of RT-PCR test results in nasopharyngeal and fecal specimens in patients with Covid-19 disease with gastrointestinal symptoms

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Addition to respiratory manifestations, gastrointestinal symptoms have been observed in some patients with COVID-19. The aim of the present study was to investigate the simultaneous diagnosis of SARS-CoV-2 RNA by RT-PCR in nasopharyngeal and fecal specimens of patients with covid-19 with gastrointestinal symptoms. In the present study, 100 patients with COVID-19 disease with gastrointestinal symptoms were included. Isolation of viral RNA in nasopharyngeal and fecal samples performed using specific laboratory kits. Detection of COVID-19 in clinical samples conducted using TaqMan-probe Real Time PCR method with specific primers and probes (Pishtaz Teb RT-PCR kit, Iran). PCR test results showed that in 21% of patients with positive nasopharyngeal test, SARS - CoV - 2 RNA was detected. As a result, the compatibility of PCR test in the diagnosis of Covid-19 virus between nasopharyngeal and fecal results was 21%.

Keywords: Covid-19, nasopharyngeal, fecal specimens, RT-PCR

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The first case was identified in Wuhan, China, in December 2019. The disease has since spread around the world, leading to a persistent pandemic. The clinical spectrum of COVID-19 varies from asymptomatic or mild respiratory problems to severe pneumonia and respiratory distress syndrome (1-3).

Most patients infected with COVID-19 show symptoms of fever, cough, myalgia, fatigue, and shortness of breath. It is generally believed that airway exposure to respiratory droplets from an infected patient is the main route of transmission for the disease (4). However, apart from fever and respiratory manifestations, gastrointestinal symptoms such as diarrhea, nausea and vomiting have been observed in some patients with COVID-19. Previous studies have shown that viruses from the coronavirus family can progress in the gastrointestinal tract. As a result, there is a hypothesis that the COVID-19 virus can be detected in gastrointestinal-related specimens, including fecal specimens (5). Wang et al. showed that the virus could be detected in the feces and sewage samples of two hospitals that treated COVID-19 patients in Beijing, China (6). Evidence from previous SARS studies suggests that gastrointestinal manifestations of SARS have been confirmed by viral diagnosis in patients' biopsy and fecal specimens, which may partly explain gastrointestinal symptoms, potential recurrence, and even transmission of SARS through feces (7, 8).

COVID-19 can be temporarily diagnosed based on symptoms and confirmed by polymerase chain reaction (RT-PCR) or other nucleic acid testing of infected secretions. Along with a laboratory diagnosis, a chest CT scan may be helpful in diagnosing COVID-19 in people who suspect high clinical signs of infection. Diagnosis of past infection by serological tests, antibodies produced by the body in response to infection can be made (9).

The standard test methods for the presence of SARS-CoV-2 are nucleic acid assays that detect the presence of viral RNA fragments. Because these tests detect RNA but do not detect an infectious virus, "its ability to determine the duration of infection in patients is limited." This test is usually done on respiratory samples from the nasopharyngeal swab. But nasal swabs or sputum may also be used. Results are generally available within a few hours. WHO has published several test methods (9, 10)

Considering the importance of early detection of people with COVID-19 in quarantine and prevention of disease transmission through patients without classical symptoms, the aim of the present study was to investigate the simultaneous diagnosis of SARS-CoV-2 RNA by RT-PCR in nasopharyngeal and fecal specimens of patients with covid-19 with gastrointestinal symptoms in order to evaluate a new diagnostic method with non-invasive sampling in patients without respiratory and classic symptoms of the disease.

MATERIALS AND METHODS

The aim of this cross-sectional study was to determine the simultaneous diagnosis of SARS-CoV-2 RNA by RT-PCR method in nasopharyngeal and fecal samples of covid-19 patients of gastrointestinal clinic of Baqyatallah Hospital in Tehran during June to November 2020. In this study, patients of gastroenterology clinic of Baqiyatallah Hospital in Tehran who showed gastrointestinal manifestations and suspected to have covid-19 were examined by a gastroenterologist. Then, patients who were suspected of having a high-risk of the disease, were referred to the laboratory for COVID-19 RT-PCR test for nasopharyngeal and fecal samples and complete blood count (CBC) test.

RNA Extraction

Isolation of viral RNA in nasopharyngeal samples performed using specific laboratory kit, RNJia virus kit (ROJE TECHNOLOGIES-Iran) and in fecal specimens was performed using NORGEN Stool Total RNA Purification Kit (Cat. 49500- Canada). After RNA isolation, samples were kept in -70 °C until PCR procedure.

RT-PCR

Detection of COVID-19 in clinical samples conducted using TaqMan-probe Real Time PCR method with specific primers and probes (Pishtaz Teb RT-PCR kit, Iran).

RESULTS

In the present study, 100 patients with COVID-19 disease with gastrointestinal symptoms were included. The results of gender distribution showed that 63% of patients were male and 37% were female. The results related to the age of the patients showed that the mean age was 42.93±1.94 years. The minimum age of patients was 15 years and the maximum age was 87 years. In the present study, information on five main and important gastrointestinal symptoms of patients with COVID-19 including diarrhea, abdominal pain, nausea, anorexia and fecal blood was collected through an oral questionnaire from patients. The results have summarized in Table 1 (Table 2 and Figure 1). Based on these results, the symptom with the highest frequency was anorexia (81%) and the lowest symptom was blood in the stool (7%).

Table 2 Descriptive information about patients' gastrointestinal symptoms

Gastrointestinal symptoms	Frequency (person)	Frequency (%)
Diarrhea	53	53%
stomach ache	19	19%
Nausea	33	33%
Anorexia	81	81%
Blood in the stool	7	7%

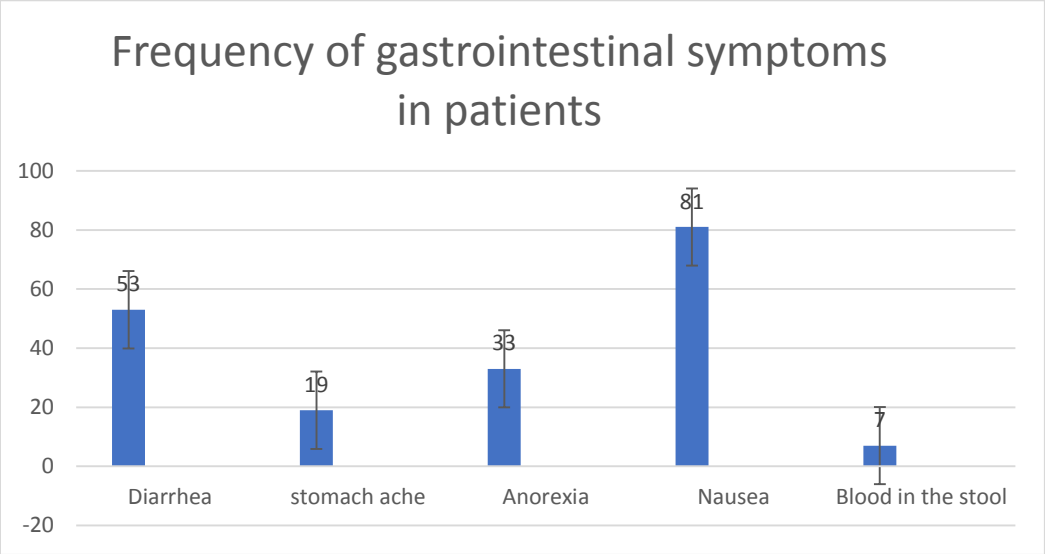

Figure 1 Frequency of gastrointestinal symptoms in patients

Due to the changes in the parameters of the CBC test, CBC test was performed on all patients studied. Three main parameters including white blood cell count (WBC), lymphocyte percentage (Lymph%) and hemoglobin (Hb) were examined in patients. The results have summarized in Table 3.

Table 2 Results of patients' blood tests.

Parameter	Average	Maximum	Minimum
White blood cell count (WBC)	3871 in microlitre	8000 in microlitre	1500 in microlitre
Lymphocyte percentage (Lymph%)	25.18 %	10%	65%
Hemoglobin (Hb)	13/7 gram in decilitre	10 gram in decilitre	18 gram in decilitre

In patients with suspected symptoms of gastrointestinal disease, patients who tested positive for nasopharyngeal specimens were tested for PCR testing in fecal specimens. After RNA extraction from fecal samples, PCR test results showed that in 21% of patients with positive nasopharyngeal test, SARS - CoV - 2 RNA was detected. As a result, the compatibility of PCR test in the diagnosis of Covid-19 virus between nasopharyngeal and fecal results was 21%.

DISCUSSION

Isolation of virus from infected specimens is the gold standard as well as detection of viral genome confirmatory diagnostic methods for viral infections. It should be noted that the diagnosis and accuracy of PCR for human coronaviruses is low compared to other respiratory infections; As a result, more accurate methods based on reverse transcription of the virus genome, such as RT-PCR or multiplex Real Time PCR, can be more effective for diagnosis. Using TaqMan probes in RT-PCR method, the reported sensitivity for coronavirus detection is 71% - 78%. There are reasons for the low efficiency of this method, such as low levels of viral load in patients, Variation in the diagnostic rate of laboratory kits and some errors in sampling, alteration of viral RNA sequence, and limitations in nucleic acid detection technology. Due to the high rate of genetic modification in the corona virus, there is a possibility of false negative results. The three main genes in the diagnosis of coronavirus are the cover gene (E), the RNA-dependent RNA polymerase gene (RdRp), the nucleoprotein (N) gene, and the ORF-1b gene from the 2019-nCov genome (11, 12). In the present study we used multiplex Real Time PCR to detect N and RdRp genes of COVID-19.

In patients with suspected symptoms of gastrointestinal disease, patients who tested positive for nasopharyngeal specimens were tested for PCR testing in fecal specimens. After RNA extraction from fecal samples, PCR test results showed that in 21% of patients with positive nasopharyngeal test, SARS - CoV - 2 RNA was detected. As a result, the compatibility of PCR test in the diagnosis of Covid-19 virus between nasopharyngeal and fecal results was 21%. In the study of Chen et al., nasopharyngeal swabs, feces and urine were collected from people suspected of having COVID-19 and RT-PCR was tested for SARS-CoV-2. A total of 42 laboratory-approved patients with COVID-19 disease were included in the study, of which 8 (19.05%) had gastrointestinal symptoms. 28 (66.67%) of patients had a positive stool test for SARS-CoV-2 RNA while the gastrointestinal symptoms were absent (13). In the study of Xi Jin et al., Out of 651 patients with suspected COVID-19 disease, 74 (11.4%) had at least one gastrointestinal symptom (nausea, vomiting or diarrhea) with a mean age of 46.14 years. In patients with COVID-19 with gastrointestinal symptoms, 29 (39%), 23 (31%), 8 (10%) and 16 (21%) with fever 38 °C, fatigue, shortness of breath and headache, respectively (14). In a study by Jiaxi Chen et al., It was shown that the virus test in the respiratory tract is positive earlier than in the intestinal tract, so that 106 patients (77%) tested positive for the first swab throat virus and only in 22 patients (16%) the first stool virus test was positive in the first 10 days. However, the course of their disease lasts for 10-14 days in both respiratory tract and intestinal tract, respectively. In addition, many patients have a negative throat swab test when they have positive stool RNA. This study suggests that both throat swab and stool samples are required to completely confirm the removal of SARS-CoV-2 (15).

In general, it can be concluded that due to the importance and frequency of gastrointestinal complications in patients with Covid-19, it is useful and necessary to perform tests related to these symptoms in patients. It seems that by developing and improving methods of extracting RNA from fecal samples, and conducting a study on a larger statistical population, more reliable results can be obtained and PCR testing in non-invasive fecal samples can be used as a rapid diagnostic tool in patients with Covid-19 disease with gastrointestinal symptoms.

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Conflict of interests: The authors declare that there are no conflict of interests.

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