

# No sign of Rotavirus co-infection in COVID-19 patients with gastrointestinal symptoms

Milad Zandi<sup>1+</sup>, Saber Soltani<sup>1+</sup>, Riam Sadooni<sup>2</sup>, Shokrollah Salmanzadeh<sup>3</sup>, Yousef Erfani<sup>4</sup>, Ramin Shahbahrani<sup>4</sup>, Maghsud Piri<sup>2</sup>, Reza Pakzad<sup>5</sup>, Nasrin Ghodratifard<sup>2</sup>, Alireza Eftekhari Moghadam<sup>2</sup>, Samaneh Abbasi<sup>6\*</sup>

1. Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

2. Abadan University of Medical Sciences, Abadan, Iran

3. Infectious and tropical diseases research center, Health research institute, Faculty of medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

4. Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Tehran University Medical Sciences, Tehran, Iran

5. Department of Epidemiology, Faculty of Health, Ilam University Medical Sciences, Ilam, Iran

6. Department of Microbiology, School of Medicine, Abadan University of Medical sciences, Abadan, Iran

+ Dr. Milad Zandi and Dr. Saber Soltani contributed equally

\*Corresponding Author: Samaneh Abbasi; E-mail: s\_abbasi80@yahoo.com

## Abstract

### Background and aims

The main goal of the present study is to investigate the incidence of Rotavirus co-infection in COVID-19 patients.

### Methods and Results

Fecal samples of COVID-19 patients with gastrointestinal symptoms which had positive PCR- were collected from Abadan's hospital, Iran during the period December 2020 to January 2021. Samples were analyzed by RT-PCR to determine the presence of Rotavirus. Finally, the total samples size of 37 were included in this study. The mean age of patients was 48.22 years. Abdominal pain alone was detected in 48.65% of the patients. At least one gastrointestinal symptom was detected in all of the patients. Diarrhea and fever were seen in 13.51% and 59.46% of patients, respectively. Nausea and vomiting were seen in 5.41% of the patients. RT-PCR showed no infection of Rotavirus among the patients.

### Conclusion

Gastrointestinal symptoms related to COVID-19 are common. More studies is need among these patients groups for investigate co-infection with other fecal viral shedding carries, due to a worse prognosis and its association with disease severity.

**Keywords:** SARS-CoV-2, COVID-19, Rotavirus, Gastrointestinal symptoms

## Introduction

Since December 2019, global public health facing to a great challenge by emerging COVID-19<sup>1-3</sup> As of July 29, 2022, more than 572 million confirmed cases and more than 6 million deaths had been reported globally. As of July 29 2022 more than 141,795 deaths were reported from Iran to WHO<sup>4</sup>. For diagnosis SARS-CoV-2, molecular methods such as PCR is admitted<sup>5,6</sup>. However, nanotechnology based strategies that previously used for other viruses<sup>7</sup> can increase sensitivity of SARS-CoV-2 detection<sup>5</sup>. The most commonly reported symptoms in patients affected by coronavirus are respiratory tract manifestations such as fever and cough, so the disease known as respiratory disease<sup>8</sup>; But as evidence increased, serious systemic consequences such as digestive system problems identified<sup>9,10</sup>. Recently, evidence of digestive system involvement in patients with COVID-19 was reported by Xiao and colleagues<sup>11</sup>. As data shows, due to the expression of angiotensin-converting enzyme 2 (ACE2), the major receptor of SARS-CoV-2, in the gastrointestinal tract, it could be a target organ of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)<sup>12</sup>. A variety of manifestations such as loss of appetite, diarrhea, vomiting,

and abdominal pain were seen in patients with COVID-19<sup>13</sup>. Based on a meta-analysis study reports, 7.8 and 5.5 percent of patients infected by COVID-19 experienced diarrhea and nausea, and/or vomiting respectively<sup>14</sup>. In the aforementioned study, the pooled analyses of studies revealed patients with severe disease are more likely to have abdominal pain in comparison with patients with non-severe disease, so it is suggested that this could be an indicator of severity, and the risk of acute respiratory distress syndrome (ARDS) may increase for them<sup>14</sup>.

The rotavirus belongs to the genus Rotavirus and from the family of *Reoviridae* in the subfamily *Sedoreovirinae*<sup>15</sup>. This non-enveloped virus is constituted by different species, although more than 90 percent of rotavirus infections in humans are caused by rotavirus A species<sup>16</sup>. Rotavirus infection can occur any time of the year, but most of the cases are typically seen in the winter months. The incubation period is 1 to 3 days and infection can last for 10 days<sup>17</sup>. Patients may experience asymptomatic mild watery diarrhea or severe dehydrating diarrhea with vomiting, fever, and abdominal pain<sup>16</sup>. Rotavirus can be caused by a single infection or by a

combination of viral and bacterial pathogens<sup>18</sup>. This study aimed to determine viral shedding of Rotavirus in stool samples in patients with confirmed SARS-CoV-2 infection with gastrointestinal problems.

**Materials and Methods**

*Study site*

This cross-sectional study was done in Abadan, the city in the Southwest of Iran. COVID-19 positive Patients admitted to the Taleghani hospital of the city were considered to conduct the research. Sampling was done from December 2020 to January 2021

**Table 1: Specific primers for VP6 region**

	Sequence (5'→3')	Length
Forward primer	TGTGCCACTCCGAAGAGTGTT	21
Reverse primer	CGGCCTGAGAATCACTGGGT	20
Product length	136	

*Patient enrolment and sample collection*

37 fecal samples were collected from Real-Time RT-PCR confirmed COVID-19 patients that had gastrointestinal symptoms. It should be mentioned that after giving the patients the necessary information about the project, their written informed consent was received before enrolment. A questionnaire recording all of the essential socio-demographic information was filled for each involved patient. Then a stool sample was collected from each patient in a sterile VTM container (Viral Transport Medium) and samples were transported on a cool box to the laboratory.

*Detection of rotavirus in COVID-19 patients by RT-PCR*

We used Reverse Transcription Polymerase Chain Reaction (RT-PCR) to detect rotavirus presence in the stool collected samples. Rotavirus viral genome (RNA) was extracted based on the manufacturer’s protocol (RNJia Virus Kit). Subsequently, cDNA was synthesized based on the kit manufacturer’s instruction (RT-ROSET Kit). To conduct RT-PCR Specific primers were designated for the VP6 region of the rotavirus A genome (Table 1).

*Statistical analysis*

Data were analyzed with Stata version 11. Quantitative and qualitative data are presented as mean and percentages,

**Table 2: Comorbidity, Clinical symptoms and Demographic characteristics of COVID-19 patients**

Variables		N of cases	Percent (95% CI)
Comorbidity	Blood pressure	12	32.43 (16.61 to 48.26)
	Diabetes	7	18.92 (5.68 to 32.16)
	Chronic renal disease	2	5.41 (0.66 to 18.19)
	Hypothyroidism	2	5.41 (0.66 to 18.19)
	Allergy	2	5.41 (0.66 to 18.19)
Clinical symptoms	Fever	22	59.46 (42.86 to 76.06)
	Nausea	2	5.41 (0.66 to 18.19)
	Diarrhea	5	13.51 (1.96 to 25.07)
	Vomiting	2	5.41 (0.66 to 18.19)
	Headache	15	40.54 (23.94 to 57.14)
	Muscle pain (Myalgia)	3	8.11 (1.70 to 21.91)
	Dry cough	11	29.73 (14.28 to 45.18)
	Lack of taste (Ageusia)	3	8.11 (1.70 to 21.91)
	Lack of smell (Anosmia)	3	8.11 (1.70 to 21.91)
	Chills	7	18.92 (5.68 to 32.16)
	Any GI symptoms	37	100
	Dysphagia	17	45.95 (29.1 to 62.79)
	Abdominal pain	18	48.65 (31.75 to 65.54)
Demographic	Gender	Male	12 32.43 (18.01 to 49.78)
		Female	25 67.57 (50.21 to 81.98)
Age*		48.22 (42.49 to 53.94)	

N: numbers; CI: Confidence Interval; GI: Gastrointestinal symptoms.  
 \*: Age Present by Mean (95% CI)

respectively, with their corresponding 95% confidence intervals (95%CI).

## Results

### *Comorbidity, Clinical symptoms and Demographic characteristics of COVID-19 patients*

37 cases with COVID-19 patients were included in the present study, the mean age (95% CI) was 48.22 (42.49 to 53.94) years old and 12 (32.43%) cases were male. Table 2 shows the comorbidity and clinical symptoms data of COVID-19 patients. 100% of patient were inpatient, 32.43% (16.61 to 48.26) had blood pressure, also 18.92% (5.68 to 32.16) had diabetes and 5.41% (0.66 to 18.19) had Allergy. It is worth mentioning that 59.46% (42.86 to 76.06) had a fever, 8.11% (1.70 to 21.91) experienced lack of taste, 8.11% (1.70 to 21.91) developed a lack of smell. Other comorbidities and clinical symptoms have shown in Table 2. Based on RT-PCR results none of the COVID-19 patients had rotavirus in their stool samples.

## Discussion

The hospital environmental microorganisms are important risk factors among hospitalized patients<sup>19</sup>. Different microbial agents such as bacterial infections could develop gastrointestinal symptoms<sup>20</sup>. Numerous studies reported gastrointestinal symptoms in patients with COVID-19<sup>13,21,22</sup>. The most common reported symptom was diarrhea (3 to 34%) and then nausea (1 to 17%), GI bleeding (0 to 13%) and abdominal pain (2 to 5%)<sup>14</sup>. Rotavirus has been recognized as the major pathogen of acute diarrhea<sup>23</sup>. The aim of this study was to determine viral shedding of Rotavirus in stool samples in patients with confirmed SARS-CoV-2 infection with gastrointestinal problems; based on RT-PCR results none of the COVID-19 patients had rotavirus in their stool samples. Recently, no study determined the co-existence of SARS-CoV-2 and rotavirus in COVID-19 patients with gastrointestinal problems. In 2009, Barry et al. examined the co-infection of bovine coronavirus and group A rotaviruses in stool samples of calves. They find that 15.9% of the samples were infected concomitantly with bovine coronavirus and group A rotaviruses<sup>24</sup>. In 1998, Fuente et al. found that 5.1% of the total samples of calves feces were infected with rotavirus and coronavirus concomitantly<sup>25</sup>. Another study reported 2.43% of samples calves feces were infected with both rotavirus and coronavirus<sup>26</sup>. Also, Brandão et al reported co-infection of rotavirus and bovine coronavirus in beef calf feces samples<sup>27</sup>. These investigators suggested that there may be a synergistic effect between rotavirus and coronavirus in a mixed infection<sup>27</sup>. It is claimed that the existence of bovine coronavirus may reduce intestinal immunity and make it easier to infect by rotavirus<sup>24</sup>. Finally, the imitations of the current study were the small sample size due to the small population of the investigated city. In future studies, it is also possible to investigate the infection rate of other gastrointestinal viruses among Covid-19 patients.

## Conclusion

In the current study, 13.51 percent of participants had diarrhea, but none of the participants had rotavirus in their sample. Further high-quality study with more sample size and other methodological approaches and younger age may determine certain results.

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