ORIGINAL RESEARCH



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

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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 coinfection 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-19¹⁻³ 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 WHO⁴. For diagnosis SARS-CoV-2, molecular methods such as PCR is admitted^{5,6}. However, nanotechnology based strategies that previously used for other viruses⁷ can increase sensitivity of SARS-CoV-2 detection⁵. 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 disease⁸; But as evidence increased, serious systemic consequences such as digestive system problems identified9,10. Recently, evidence of digestive system involvement in patients with COVID-19 was reported by Xiao and colleagues¹¹. 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)12. A variety of manifestations such as loss of appetite, diarrhea, vomiting,

and abdominal pain were seen in patients with COVID-19¹³. 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 respectively¹⁴. 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 them¹⁴.

The rotavirus belongs to the genus Rotavirus and from the family of *Reoviridae* in the subfamily *Sedoreovirinae*¹⁵. This nonenveloped virus is constituted by different species, although more than 90 percent of rotavirus infections in humans are caused by rotavirus A species¹⁶. 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 days¹⁷. Patients may experience asymptomatic mild watery diarrhea or severe dehydrating diarrhea with vomiting, fever, and abdominal pain¹⁶. Rotavirus can be caused by a single infection or by a

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combination of viral and bacterial pathogens¹⁸. 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 contest 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)
	Blood pressure		12	32.43 (16.61 to 48.26)
Comorbidity	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)
	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 (My	/algia)	3	8.11 (1.70 to 21.91)
	Dry cough		11	29.73 (14.28 to 45.18)
	Lack of taste (Ag	jeusia)	3	8.11 (1.70 to 21.91)
	Lack of smell (Ar	iosmia)	3	8.11 (1.70 to 21.91)
	Chills		7	18.92 (5.68 to 32.16)
	Any GI symptoms		37	100
Clinical symptoms	Dysphagia	1	17	45.95 (29.1 to 62.79)
	Abdominal p	ain	18	48.65 (31.75 to 65.54)
	Oandan	Male	12	32.43 (18.01 to 49.78)
Demographic	Gender	Female	25	67.57 (50.21 to 81.98)
	Age*		48.22 (42.49 to 53.94)	

*: 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 patients¹⁹. Different microbial agents such as bacterial infections could develop gastrointestinal symptoms²⁰. Numerous studies reported gastrointestinal symptoms in patients with COVID-1913,21, 22. 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%)14. Rotavirus has been recognized as the major pathogen of acute diarrhea²³. 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 rotaviruses²⁴. In 1998, Fuente et al. found that 5.1% of the total samples of calves feces were infected with rotavirus and coronavirus concomitantly²⁵. Another study reported 2.43% of samples calves feces were infected with both rotavirus and coronavirus26. Also, Brandão et al reported co-infection of rotavirus and bovine coronavirus in beef calf feces samples²⁷. These investigators suggested that there may be a synergistic effect between rotavirus and coronavirus in a mixed infection²⁷. It is claimed that the existence of bovine coronavirus may reduce intestinal immunity and make it easier to infect by rotavirus²⁴. 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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