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Original Article

Prevalence of Human Coronaviruses NL63, HKU1, 229E, OC43, MERS, and SARS-CoV-2 among Hospitalized Patients with Acute Respiratory Infection in Tehran

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Abstract

Background and Aims: Coronaviruses cause upper respiratory ailments and sporadically lower tract sickness in vulnerable populations. This study examined the prevalence of six human coronaviruses, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), OC43, 229E, HKU1, NL63, and Middle East respiratory syndrome coronavirus (MERS-CoV) during the 2019 pandemic caused by SARS-CoV-2 in Tehran, Iran.

Material and methods: Specimens were collected from 204 adult patients with acute respiratory illness. The specimens were examined for the presence of six human coronaviruses using consensus and subtype-specific Real-time reverse-transcription polymerase chain reaction (PCR) assays. The demographic and clinical characteristics associated with coronavirus infection were examined retrospectively.

Results: Coronaviruses were identified in 204 adult patients. The gender ratio was 104/100 male and female, respectively. HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, MERS-CoV, and SARS-CoV-2 were detected in 0.5%, 1.0%, 4.0%, 7.4%, 0.0%, and 22.5% of patients, respectively. The acute clinical features were similar across subtypes. There was no report of fatality incidence among the population during the investigation period.

Conclusions: HCoVs could play a significant role in causing upper respiratory tract infections among adults and older children. Based on the findings of this study and those of others, more extensive studies using other diagnostic methods and higher sample sizes are suggested.

Keywords: Human coronavirus, Pandemic, Iran, Prevalence

Introduction

The seven strains of human coronaviruses (HCoVs) belong to the genus Alphacoronavirus or Beta coronavirus of the Coronavirinae subfamily and include HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), MERS-CoV, and SARS-CoV-2 (1-3). All human coronaviruses, except for SARS-CoV, MERS-CoV, and SARS-CoV-2, are frequently linked to minor gastrointestinal and respiratory illnesses (4,5). The highly deadly

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viruses, SARS-CoV, MERS-CoV, and SARS-CoV-2, have resulted in severe illnesses or death in humans (6, 7).

Poor growth and poverty of cytopathic effects in cell cultures have been the main limits for HCoVs research. However, with the development of polymerase chain reaction (PCR) technology, there has been a broad and rapid expansion in the field of corona virology (8,9). According to early studies, HCoV-229E and HCoV-OC43 were known to account for 5 to 30% of human respiratory tract infections (10), and HCoV-NL63 was found to exist in 2 to 3.6% of respiratory samples in several recent studies (11-16) Despite various studies during the last few years on the etiology of coronaviruses (15, 17), our understanding of the status of these viruses in Iran is limited.

However, during the recent pandemic caused by SARS-CoV-2, we conducted a survey study on the prevalence of six human coronaviruses, including HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, MERS-CoV, and SARS-CoV-2 in patients clinically confirmed for severe acute respiratory syndrome presented with fever, nausea, cough, and sore throat, who were admitted to hospitals affiliated with Iran University of Medical Sciences in Tehran, Iran.

Methods and Materials

Patients and Methods

Between October 2020 and June 2021, clinical samples (i.e., throat swabs and nasopharyngeal aspirates) from 204 patients with acute respiratory infections admitted to three general hospitals, including Firroozgar, Rasool Akram, and Ali Asghar in Tehran, were collected. The epidemiology and clinical spectrum of the disease caused by HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, MERS-CoV, and SARS-CoV-2 infection in patients hospitalized for acute respiratory illness were examined during one year.

RNA Extraction

Extraction of viral RNA from samples was carried out using an RNA extraction kit (Yekta Tajhiz Kit, Tehran, Iran). The extracted RNAs (templates for RT-PCR) were immediately stored at -70°C until use.

RT-PCR for Coronaviruses

After RNA extraction to screen for six coronaviruses, PCR was performed using six sets of primers and probes precisely designed as described previously to amplify HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, MERS-CoV, and SARS-CoV-2 (Table 1). Each PCR mixture contained TaqMan one-step PCR 2X Master Mix (10 ul), forward primer (1 ul), reverse primer (1 ul), probe (0.5 ul), double-distilled water (2.5 ul), sample (5 ul), in a total volume of 20 ul. The cycling program consisted of an initial denaturation at 94°C for 3 min, followed by 45 cycles of 94°C for 10s and 60°C for 30s (Table 2).

Results

A total of 204 patients with ages ranging from 1 to 90 years old (mean 42.69 years) were enrolled in this study. Specimens were collected from both female (100) and male (104) patients. HCoV RNA was detected in 72 (30.3%) of the 204 patients. Of the 72 specimens positive for HCoV RNA, 1.0%, 4.0%, 0.5%, 7.4%, and 22.5% were positive for HCoV-NL63, HCoV-OC43, HCoV-229E, HCoV-HKU1, and SARS-CoV-2, respectively, and no positive case was found for MERS-CoV (Table 3).

Additionally, we found no significant associations between the gender and age of the patients infected with these six serotypes of human coronaviruses ($P>0.05$). Both HCoV-NL63 positive cases had fever, vertigo, and malaise. The only patient infected by HCoV-229E presented symptoms including fever, cough, sore throat, vertigo, and malaise. Among the eight HCoV-OC43-positive cases, the most common clinical symptoms were fever (n=5) and body aches (n=4). Likewise, the most common clinical symptoms observed among the 15 patients infected with HCoV-HKU1 were body aches (n=8), fever (n=8), sore throat (n=6), vertigo (n=6), and malaise (n=6).

Table 1. Primers and probes used in this study

Coronavirus	Primer direction	sequence (5'–3')
HCoV-229E	Forward primer	5'- CAGTCAAATGGGCTGATGCA -3'
	Reverse primer	5'- AAAGGGCTATAAAGAGAATAAGGTATTCT -3'
	Probe	5'-FAM CCCTGACGACCACGTTGTGGTTCA-BHQ1 -3'
HCoV-OC43	Forward primer	5'-CGATGAGGCTATTCCGACTAGGT-3'
	Reverse primer	5'-CCTTCCTGAGCCTTCAATATAGTAACC-3'
	Probe	5'-FAM TCCGCCTGGCACGGTACTCCCT-BHQ1 -3'
HCoV-NL63	Forward primer	5'- GACCAAAGCACTGAATAACATTTTCC -3'
	Reverse primer	5'- ACCTAATAAGCCTCTTTCTCAACCC -3'
	Probe	5'-FAM ATGTTATTCAGTGCTTTGGTCCTCGTGAT-BHQ1 3'
HCoV-HKU1	Forward primer	5'- CCTTGCGAATGAATGTGCTC -3
	Reverse primer	5'- TTGCATCACCACTGCTAGTACCAC -3
	Probe	5'-FAM TGTGTGGCGGTTGCTATTATGTTAAGCCTG-BHQ1-3'
MERS-CoV	Forward primer	5'- GCAACGCGCGATTCAGTT -3
	Reverse primer	5'- GCCTCTACACGGGACCCATA -3
	Probe	5'-FAM CTCTTCACATAATCGC CCCGAGCTCG-BHQ1 -3'
SARS-CoV-2	Forward primer	5'- GGG GAA CTT CTC CTG CTA GAA T -3
	Reverse primer	5'- CAG ACA TTT TGC TCT CAA GCT G -3
	Probe	5'-FAM TTG CTG CTG CTT GAC AGA TT-BHQ1 -3'

Table 2. The chart for PCR Real-Time

No.	Cycle	Step	Tem.	Time
1	1	Reverse Transcription	50 °C	15 min
2	1	Initial Denaturation	94 °C	3 min
3	45	Denaturation	94 °C	10 sec
		Annealing-Extension	60 °C	30 sec
4	1	Device Cooling	25 °C	1 sec

Table 3. Prevalence of six studied coronaviruses among 204 patients

No.	HCoV	No. of Positive Case (%)
1	NL63	2 (1.0%)
2	229E	1 (0.5%)
3	OC43	8 (4.0%)
4	HKU1	15 (7.4%)
5	MERS	0 (0.0%)
6	SARS-CoV-2	46 (22.5%)
7	Negative	132 (64.6%)

Among the 46 patients infected with SARS-CoV-2, fever (n=42), chills (n=16), and malaise (n=16) were the most prevalent clinical presentations. The other clinical symptoms observed in all patients infected with the six human coronaviruses are shown in Table 4.

Discussion

Our understanding of the epidemiology of six human coronaviruses in Iran is limited to the clinical symptoms of the respiratory system. However, the objective of this study was to survey the number of patients who might be infected with some of these six strains during the 2019 pandemic SARS-CoV-2 in the country. A total of 204 patients with ages ranging from 1 to 90 years old (mean 42.69 years) were enrolled in this study. Specimens were collected from both female (100) and male (104) patients. The genomes of human coronaviruses were detected in 72 out of 204 patients (30.3%). Among the positively confirmed samples, SARS-CoV-2 (22.5%), HCoV-HKU1 (7.4%), HCoV-OC43 (4%), HCoV-NL63 (1%), and HCoV-229E (0.5%) were responseble for the outbreak, while none of these samples showed to be positive for HCoV-MERS (Table 3). Additionally, one sample was positive for both HCoV-NL63 and HCoV-229E.

In 2015, Madhi and colleagues studied 170 samples collected from hospitalized patients

Table 4. Virus-specific general characteristics and clinical symptoms of the study population

	HCoV						
Parameters	**NL63**	**229E**	**OC43**	**HKU1**	**MERS**	**SARS-CoV-2**	**Total**
No. of detected	2 (1.0%)	1 (0.5%)	8 (4.0%)	15 (7.4%)	0 (0%)	46 (22.5%)	72 (30.3%)
Age range (years)	37-47	47	19-39	40-59	0	1-83	1-90
Age Mean	42	47	33.8	45.5	0	47.2	42.69
Gender (M/F)	1/1	1/0	3/5	5/10	0	23/23	104/100
Symptoms							
Loss of taste	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1/62 (1.6%)
Loss of smell	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (4.3%)	2/62 (3.2%)
Fever	2 (100%)	1 (100%)	5 (62.5%)	8 (53.3%)	0 (0%)	42 (91.3%)	58/62 (93.5%)
Cough	1 (50%)	1 (100%)	3 (37.5%)	5 (33.3%)	0 (0%)	13 (28.3%)	23/62 (37.1%)
Sore throat	0 (0%)	1 (100%)	3 (37.5%)	6 (40%)	0 (0%)	6 (13%)	16/62 (25.8%)
Dyspnea	0 (0%)	0 (0%)	2 (25%)	5 (33.3%)	0 (0%)	7 (15.2%)	14/62 (22.6%)
Chest pain	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (8.7%)	4/62 (6.4%)
Chill	1 (50%)	0 (0%)	2 (25%)	5 (33.3%)	0 (0%)	16 (34.8%)	24/62 (38.7%)
Rhinorrhea	1 (50%)	0 (0%)	1 (12.5%)	2 (13.3%)	0 (0%)	3 (6.5%)	7/62 (11.3%)
Sneeze	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.2%)	1/62 (1.6%)
Vertigo	2 (100%)	1(100%)	3 (37.5%)	6 (40%)	0 (0%)	2 (4.3%)	14/62 (22.6%)
Headache	1 (50%)	0 (0%)	2 (25%)	5 (33.3%)	5 (33.3%)	0 (0%)	13/62 (20.9%)
Malaise	2 (100%)	1 (100%)	3 (37.5%)	6 (40%)	6 (40%)	0 (0%)	28/62 (45.1%)
Body pain	1 (50%)	0 (0%)	4 (50%)	8 (53.3%)	8 (53.3%)	0 (0%)	24/62 (38.7%)

who suffered respiratory infections using the RT-PCR technique to determine the presence of four human coronaviruses: HCoV-OC43, HCoV-229E, HCoV-HKU1, and HCo V-NL-63. This study showed that the number of human coronaviruses was 15 (5.5%), and all were HCoV-E229 (18). In 2015, Sultani et al. sur-veyed samples from 172 hospitalized patients due to respiratory infections using the RT-PCR method for the presence of four human corona-viruses: HCoV-NL63, HCoV-229E, HCoV- SARS, and HCoV-OC43, and only HCoV-NL63 was detected in their study (0.58%) (19). These findings might be considered due to two reasons: firstly, sampling in their study was conducted before the occurrence of the SARS-CoV-2 pandemic, while in our study, sampling was carried out after the appearance of the SARS-CoV-2 pandemic; secondly, during that period of time, the strains HCoV-229E and HCoV-NL63 were dominant in Iran among the other strains.

Zeng et al. conducted surveillance on respiratory infections in children using the Real-time PCR technique. Among 11,399 collected samples, 489 samples were found to be positive

(4.3%). Meanwhile, among the 506 positive cases, HCoV-OC43, HCoV-229E, HCoV-NL63, and HCoV-HKU1 serotypes were detected in 346, 65, 60, and 38 patients, respectively (20). Also, we found no significant relationships between the gender and age of the patients infected with these six serotypes of human coronaviruses (P>0.05). The clinical syndrome observations, along with the causative agents of the six human coronaviruses, are confirmed as the etiologic agents shown in Table 5. For example, in two positive cases infected with HCoV-NL63, fever and dizziness were found, and the remaining fifty percent were in the face of cough, headache, and body ache. 15% of infected patients with HKU1 showed symptoms of fever (53.3%), cough (33.3%), dizziness, and sore throat (40%). Four percent of patients confirmed the infection with OC43: fever (62.5%), cough, sore throat, and malaise (37.5%) (Table 4).

Conclusions

We found that the six human coronaviruses (HCoVs) were associated with acute respiratory tract infections in Tehran. Additionally, our data suggests that HCoVs could play a significant role in causing upper respiratory tract infections among adults and older children. Based on the findings of this study and those of others, more extensive studies using other diagnostic methods and a larger sample size are recommended.

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None

Disclosure

None

Conflict of Interest

No conflict of interest is declared.

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Ethics Approval and Consent to Participate

This study was approved by Ethics Committee of the Iran University of Medical Sciences (Approval No. IR.IUMS.REC.1399.022).

Data Availability Statement

All data generated or analyzed during this study are included in this article.

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