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Original Article

Low Prevalence Of SARS-COV-2 And Influenza Virus Coinfection During COVID-19 Disease Pandemic, In Iran

Alborzi E[1], Monavari SHR[1*], Kiani SJ[1], Karbalaie Niya MH[1], Tavakoli A[1]

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

Abstract

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), originated more likely from Wuhan, China and spread across the world. According to WHO reports, the virus is responsible for the death of almost seven million people around the world until now. Considering the same clinical manifestations of SARS-CoV-2 and influenza virus, simultaneous infection of these two viruses may affect the treatment process of the patient. Thus, we investigated coinfection of SARS-CoV-2, influenza A (IAV) and B (IBV) in patients with respiratory syndrome from June to August 2021. Nasopharyngeal samples were obtained from 84 COVID-19 patients and were tested for detection of SARS-CoV-2, IAV and IBV by using Reverse Transcriptase Real-time Polymerase Chain Reaction. Out of 84 patients, we found 46 and three COVID-19 and influenza positive cases, respectively. Coinfection was only found in two cases. Both cases were female and aged above 60 years. Findings of the currents study represent low prevalence of the influenza virus infection in the 2021 influenza season as well as low coinfection rates with SARS-CoV-2. This low prevalence may be due to the preventive measurements against the COVID-19.

Keywords: Influenza A, Influenza B, COVID-19, SARS-CoV-2

Introduction

A respiratory disease with unknown etiology which was appeared in Wuhan, China in December 2019 turned into a global pandemic within a few months. In a short time, the cause of this disease was named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by the World Health Organization due to its 80% homology with the SARS-1 virus. The disease caused by the virus is called coronavirus disease 2019 or COVID-19. The origin of the disease outbreak is considered to be the transmission of a zoonotic disease in the seafood market in Wuhan, China, and later it was found that human-to-human transmission plays the main role in the worldwide spread of the disease (1, 2).

[*] **Corresponding author:**
Seyed Hamidreza Monavari, Department of Virology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran. E-mail: monavari.hr@iums.ac.ir

The most common symptoms are cough and fever. Elderly people and those with comorbidities are more susceptible to the severe form of the disease (3, 4). Most of the people admitted to the hospital need to be referred to the intensive care unit, and the mortality rate up to 50% in these patients (5, 6).

Influenza virus is classified into four genera, A, B, C, and D. Influenza A virus (IAV) is the most common type that can cause fatal respiratory disease and has the potential to bring about regional epidemics or global pandemics (7, 8). IAV is classified to different subtypes based on the characteristics of the hemagglutinin (HA) and neuraminidase (NA) spikes. Eighteen different HA subtypes and 11 different NA subtypes have been identified so far. At least, four influenza A pandemics have been recorded as yet, including H1N1 (1918), H2N2 (1957), H3N2 (1967), and the last pandemic occurred in 2009 (H1N1) (9, 10). Influenza B virus (IBV), which is considered a seasonal type, is the most common type of

influenza after type A. This type includes two circulating subtypes, Yamagata and B/Victoria (7, 8). Influenza viruses usually cause symptoms such as cough, fever, sore throat, fatigue, which can be severe or mild depending on the affected person (11).

Both influenza and corona viruses can be transmitted through similar routes such as transmission through direct contact or respiratory droplets (12-14). In addition, both viruses cause relatively similar clinical symptoms such as fever, cough, rhinitis and dyspnea which makes differential diagnosis more complicated (12). On the one hand, coinfection of these two viruses can interfere with the process of diagnosis, treatment and prevention of the epidemic, and on the other hand, the severity of symptoms and the mortality rate may increase in the coinfection condition (15, 16).

Few cases of coinfection between SARS-CoV-2 and influenza virus have been reported around the world (11, 17-22) and there is inadequate knowledge regarding the interaction between these viruses and the final pathogenicity. The present study was aimed to evaluate the clinical symptoms, laboratory test results, and the outcomes of patients infected with SARS-CoV-2 and influenza viruses.

Materials and Methods

Patients and Sampling. Patients with respiratory symptoms who attended to hospitals affiliated with Iran University of Medical Sciences were enrolled between June to August 2021.

Written consent was obtained from all the patients. Existence of routine symptoms of viral respiratory infections such as fever above thirty-eight degrees of centigrade, sore throat, cough, dyspnea, malaise and other common viral respiratory symptoms were considered as inclusion criteria.

We evaluated 84 patients with common viral respiratory disease symptoms for coinfection of SARS-COV-2 with IAV and IBV. The study approved by the Ethical Committee of Iran University of Medical Sciences (ethical code: IR.IUMS.FMD.REC.1400.134).

The nasopharyngeal (NP) swabs were collected immediately from each patient on admission and transferred to the sterile tubes containing virus transport media (VTM). The sample was preserved in 4°C for nucleic acid extraction.

RNA Extraction

The RNA was extracted from the NP samples by using the High pure viral RNA kit (Roche, Germany). The extraction was preformed based on the manufacturer's manual and the extracted RNA was evaluated by NanoDrop Spectrophotometer (NanoDrop 2000, Thermo Scientific, USA).

Real-time Polymerase Chain Reaction (RT-PCR)

Presence of IAV, IBV and SARS-CoV-2 in NP samples was evaluated using a multiplex real-time PCR assay. Briefly, for each reaction, 5 µl of the CAPITAL™ qPCR Probe Master Mix (Biotechrabbit, Germany) was mixed with 4 µl of primer-probe mixture. Six microliters of distilled water and 5 µl of the extracted viral RNA were also added to the mixture.

The thermal cycling profile of the real-time PCR was as follows: reverse transcription at 50°C for 20 min, initial denaturation at 95°C for 3 min, 40 cycles of denaturation at 95°C for 10 s and annealing/extension at 55°C for 30 s. Data acquisition was scheduled at the end of annealing/extension step for green (IAV), Yellow (SARS-CoV-2), Orange (IBV), and Red (RNase P) channels. Amplification curves with cycle threshold (Ct) of not more than 40 were considered as true amplification (positive).

Amplification of RNase P was considered as the samples internal control. The primers and probes used for the detection of IAV, IBV, SARS-CoV-2 and RNase P are listed in Table 1. The qualitative realtime reverse-transcriptase polymerase chain reaction was performed by QIAquant 96 5plex machine (QIAGEN, Germany). The efficiency, R2 value, and limit of detection (LOD) of the assay were 0.967, 0.9991, and 200 copy/µl, respectively. .

Statistical Analysis. Statistical analysis was performed using SPSS version 22 and the $p<0.05$ was considered as statistically significant. Based on the characteristics of each variable, the Chi-squared test, Pearson correlation coefficient or Spearman correlation was applied.

Table 1. P List of primers and probes used for the detection of IAV, IBV, SARS-CoV-2 and RNase P

SARS-CoV-2 (N gene)	Sequence
Forward Primer	GCCACTAAAGCATACAATGTAACAC
Reverse Primer	GCCAATGTTTGTAATCAGTTCCTTG
Probe	HEX-CGGCAGACGTGGTCCAGAACAAACCC-BHQ1
Influenza A (M gene)	
Forward Primer	CTTCTAACCGAGGTCGAAACGTA
Reverse Primer	GGTGACAGGATTGGTCTTGTCTTTA
Probe	FAM-TCAGGCCCCCTCAAAGCCGAG-BHQ1
Influenza B (H gene)	
Forward Primer	AAATACGGTGGATTAAACAAAAGCAA
Reverse Primer	CCAGCAATAGCTCCGAAGAAA
Probe	ROX-CACCCATATTGGGCAATTTCCTATGGC-BHQ2
Rnase P	
Forward Primer	AGATTTGGACCTGCGAGCG
Reverse Primer	GAGCGGCTGTCTCCACAAGT
Probe	Cy5-TTCTGACCTGAAGGCTCTGCGCG-BHQ2

Results

Demographics of the Patients. The demographic features and clinical manifestations of the included patients were listed in tables 2 and 3. The mean age in 84 recruited patients were 47.9 years and half of them were male. The most common clinical presentations were fever, chill, malaise and cough, respectively.

Prevalence of SARS-CoV-2 and Influenza Viruses: In current study, 84 patients with upper respiratory symptoms were evaluated for the detection of IAV, IBV and SARS-CoV-2 by a multiplex real-time PCR assay. Among 84 patients, 46 patients were diagnosed positive for SARS-CoV-2 and three were positive for IAV. Coinfection between IAV and SARS-CoV-2 was detected in two female cases who aged 66 and 70 years. Clinical symptoms of these two cases were fever, dyspnea and chill. No patient with IBV infection was detected in this study.

Table 2. Demographic features of the studied participants

Characteristic	All cases	Female	Male
Mean Age	47.9	49.1	46.8
Gender (M/F)	42/42	42	42
Virus Detection (+/-)	49/84	26/42	23/42

Table 4. Prevalence of SARS-CoV-2 and Influenza viruses

	N=84	Percent
Influenza	3	3.5%
SARS-CoV-2	46	54.7%
Co-infection	2	2.3%

Table 5. Distribution of viral infections in different age groups

Age groups	Detected Viruses No. (%)		
	Influenza	SARS-CoV-2	Co-infection
<20	0	5	0
20-40	1	17	0
41-60	0	8	0
>60	2	16	2

Discussion

Generally, COVID-19 causes non-specific symptoms such as fever, cough and myalgia in the infected patient (23). In addition to SARS-CoV-2, the existence of other respiratory viruses such as influenza, parainfluenza, respiratory syncytial virus (RSV), and adenovirus should also be suspected.

Since the first announcement of COVID-19 pandemic by the World Health Organization,

Table 3. Demographic data and clinical symptoms of the positive cases

Parameters	Detected Viruses		
	Influenza	SARS-CoV-2	Co-infection
Age range (years)	36-70	1-83	66-70
Age Mean	57.3	47.2	68
Gender (M/F)	0/3	23/23	0/2
Symptoms			
Loss of taste	0 (0%)	0 (0%)	0 (0%)
Loss of smell	1 (33.3%)	2 (4.3%)	0 (0%)
Fever	2 (66.6%)	42 (91.3%)	1 (50%)
Cough	1 (33.3%)	13 (28.2%)	0 (0%)
Sore throat	1 (33.3%)	6 (13.1)	0 (0%)
Dyspnea	2 (66.6%)	7 (15.2%)	1 (50%)
Chest pain	0 (0%)	4 (8.6%)	0 (0%)
Chill	1 (33.3%)	16 (34.7%)	1 (50%)
Rhinorrhea	0 (0%)	3 (6.5%)	0 (0%)
Sneeze	0 (0%)	1 (2.1%)	0 (0%)
Vertigo	1 (33.3%)	2 (4.3%)	0 (0%)
Headache	1 (33.3%)	5 (10.8%)	0 (0%)
Malaise	1 (33.3%)	16 (34.7%)	0 (0%)
Body pain	1 (33.3%)	11 (23.9%)	0 (0%)

there have been increasing concerns about the spread of the coinfection of SARS-CoV-2 and influenza viruses, especially in the winter season (24). The role of coinfection in aggravating symptoms, severity and consequences of the disease is unavoidable in many cases (25). Several studies have reported coinfection of respiratory pathogens along with SARS-CoV-2 (26-29). There are also multiple reports of COVID-19 and influenza coinfections globally (30-33). In our study, the prevalence of influenza A and SARS-CoV-2 coinfection was very low (2.3%). During a sentinel surveillance among 13,467 samples, Aggarwal et al. identified coinfections between SARS-CoV-2 and influenza viruses in only five (0.04%) cases (34). In a study conducted by Hashemi et al. from November 2, 2021 to January 30, 2022 on inpatients and outpatients with COVID-like symptoms, 14,116 samples were screened for the presence of SARS-CoV-2 and influenza viruses by a multiplex real-time PCR assay. They reported 14.19% and 17.11% infections with influenza and SARS-CoV-2, respectively. The coinfection rate was reported in only 191 (1.35%) cases which is in parallel with the results of the present study (19).

In Brazil, Eisen et al. reported that 6 (0.6%) cases out of 987 cases with acute respiratory disease syndrome (ARDS) were coinfected with both viruses (35). The results of a meta-analysis of 11 studies showed that the rate of coinfection with influenza virus in patients with confirmed COVID-19 was 0.8% (15).

However, some other studies have reported radically different findings. Examining the prevalence of influenza and other respiratory infections in Saudi Arabia, Alosaimi et al. found evidence of coinfection in 17(35.4%) out of 48 cases in ICU and non-ICU COVID-19 patients (5). Many factors can affect the results of this study. Competitive or cooperative forms of pathogen–pathogen interactions are one of these factors that can explain why some respiratory infections are less common during COVID-19 pandemic (36).

In addition, the small volume of samples can also affect our results. Coinfection of SARS-Cov2 and influenza may increase disease severity and risk of death in afflicted patients (3, 37). Our findings indicate that people aged >60 years are more likely to be coinfected with SARS-Cov2 and influenza. Dadashi et al. in a meta-analysis of SARS-CoV-2 and Influenza coinfections showed that most of the coinfections were from the age group of >50 years (15).

In India, Ravikumar et al. examined 959 SARS-CoV-2 positive samples collected from six states and three union territories in India from May to December 2022. Their results revealed a significant number of coinfections with H1N1pdm09 and Influenza A (other subtypes) in SARS-CoV-2 positive samples. In 959 cases, they found 168 (17.5%) cases of coinfections by qRT-PCR. Higher prevalence of H1N1pdm09 was found in the age group of 15 to <50 years compared to the other age groups (20).

Our study had some limitations. Limited number of samples and unavailability of vaccination statues of patients may affect analyses of our findings. Moreover, viral loads under

the limit of detection of the realtime PCR assay may lead to under-reported test results.

Conclusion

In conclusion, our findings suggest that coinfection with SARS-CoV-2 and influenza virus was not common in our investigated population. We suggest further studies that investigate the possible causes of significant decrease in influenza virus infections during the COVID-19 pandemic to better understand the relationship between the COVID-19 pandemic and the decrease in influenza virus infections. The most likely explanation is that adherence to personal hygiene guidelines, especially during the pandemic, led to a noticeable decrease in the prevalence of other respiratory infections.

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Conflict of Interest

No conflict of interest is declared.

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