ORIGINAL RESEARCH

Co-infection of *SARS-CoV-2* and *Influenza A*: a report from in Southwestern Iran

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Abstract

Background

Since December 2019, coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (*SARS-CoV-2*) started in China, and quickly spread worldwide. To date, SARS-CoV-2 infection has become a global concern and health problem.

Method

In this study, we evaluated the co-infection of *SARS-CoV-2* and Influenza viruses in confirmed COVID-19 patients in Abadan, Iran. They referred to the centers for COVID-19 detection at Abadan University of Medical Sciences in Southwest Iran. Nasopharyngeal and oropharyngeal throat swabs were collected from each person and tested for Influenza A using a multiplex Real Time-Polymerase Chain Reaction.

Results

In this study, among 40 *SARS-CoV-2*-positive cases, 2 patients (5%) were co-infected with influenza A virus.

Conclusion

The low frequency of influenza in our study could be due to the small sample size, which is one of the main limitations of our study. Also, other respiratory tract infections were not investigated in this study.

Keywords: Co-infection, Coronavirus disease 2019, COVID-19, *SARS-CoV-2*, *Influenza*

Introduction

In December 2019 an outbreak of cases of pneumonia with unknown etiology was reported worldwide, and most patients complained of symptoms including fever, cough, fatigue, headache, chest pain, dyspnea, sore throat, vomiting, and acute respiratory distress syndrome[1].

This disease was named COVID-19. Laboratory tests based on viral genome sequencing led to the confirmation of a novel Coronavirus, which belonged to the *ß-Coronavirus* genus[2]. The genome sequencing of the novel Coronavirus S2 protein confirmed the similarity of 93% with the severe acute respiratory syndrome (*SARS*) pandemic in 2003, therefore, this virus is called *SARS-CoV-2*[3].

SARS-CoV-2 belongs to the *Betacoronavirus* genus in the *Coronaviridae* family that has four structure proteins including a surface glycoprotein (S), nucleocapsid protein (N), matrix protein (M), and envelope protein (E)[4,5]. Protein S is a key protein in the initiation of pathogenesis due to binding to angiotensin-converting enzyme 2 (ACE-2). ACE-2 belongs to the renin-angiotensin system (RAS) and is expressed in the heart, liver, kidney, and intestinal cells in addition to lung epithelial cells. RAS regulates blood pressure in the body, the angiotensin 2 enzyme causes vasoconstriction and blood pressure increase, ACE-2 enzyme causes vasodilation and blood pressure reduction. On the other hand, the angiotensin 2 enzyme plays a role in cell proliferation and hypertrophy, fibrosis, and inflammation, and on the contrary, the ACE-2 enzyme has an antifibrosis and anti-inflammatory role[6]. Therefore, the blocking of the ACE2 enzyme by this virus causes infiltration of inflammatory cells, the release of immune cytokines, increased permeability of the alveolar-capillary membrane, vasoconstriction, increased blood pressure, pulmonary edema, and respiratory distress syndrome (ARDS)[7].

As reported in previous studies, unilateral and bilateral multifocal ground-glass opacities are found in chest computed tomography (CT) images or chest X-rays, which are typical findings in viral pneumonia and patients with COVID-19[8].

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Co-infection in viral respiratory disease is defined as the detection of more than one or multiple viral pathogens in the same sample of patients with an acute respiratory tract infection (ARI)[9,10]. Co-infection of respiratory viral infections such as influenza with the current pandemic can be a larger threat to public health, however, there are no significant symptoms for *SARS-CoV-2* detection from influenza infection, so the accurate and rapid detection of the etiological agent in patients is necessary for survival[11].

In several studies, researchers found that the co-infection of *SARS-CoV-2* with other respiratory viruses such as the Influenza virus can affect the morbidity and mortality ratio[12]. Also, false-negative tests or co-infection with other respiratory viruses can lead to COVID-19 underdiagnosis[3]. Therefore, understanding the interactions between COVID-19 and influenza can aid healthcare professionals in effectively managing and treating individuals with coinfections. These findings encouraged us to evaluate the co-infection of *SARS-CoV-2* with the Influenza virus and compare the clinical and biochemical parameters of coinfected patients.

Material and method

Sample collection

After receiving the ethical number and obtaining consent from patients with COVID-19, 40 respiratory samples (nasopharyngeal and oropharyngeal throat swabs) were collected in VTM (Viral Transport Medium) from 01-Dec-2020 to 31-Jan-2021 from patients living in Abadan. The inclusion criteria for this study were respiratory patients who had a positive result of COVID-19 through Real-Time polymerase chain reaction (Real-Time PCR).

Detection of influenza A in COVID-19 patients

In this study, multiplex real-time PCR was performed to detect the influenza virus. First, the viral genome was extracted according to the manufacturer's instructions for the viral RNA/DNA nucleic acid extraction kit (ROCHE, Mannheim, Germany). Qualitative Multiplex Real-Time PCR was carried out on 5µL of extracted materials using a Flu kit, for the detection of flu A, flu A (H1N1), and flu B (Fast-track diagnostics/SIEMENS, Luxembourg), according to manufacturer's protocols[13]. Briefly, after the transcription of viral RNA to cDNA by reverse transcriptase enzyme and a specific primer, real-time PCR was done as follows: 0.75 µL of probe and primers, 0.5 µL of the replication enzyme, 6.25 µL of the buffer, and 5 µL of each sample (total reaction volume:12.5 µL). The thermal cycler was designed for a primary incubation of 50°C for 15 min, after a second incubation of 95°C for 10 min, and 45 cycles of 10 s at 95°C and 40 s at 60°C.

Statistical analysis

Data were analyzed with Stata version 11. Quantitative and qualitative data are presented as mean ± SD and percentages, respectively. Also, the confidence interval (CI) was calculated using the binomial distribution. P values of less than 0.05 were considered significant.

Results

Demographic and baseline characteristics of COVID-19 patient

In this study, among the 40 patients, 27 were male and 13 were female. The mean age was 52.6(50.6 to 54.7) years old. Fever was observed in all patients, followed by headache (77.5%) and dry cough (65%) were reported as the most frequent symptoms. Also, 37.5% of patients were suffering from shortness of breath. Diarrhea, muscle pain, and chills were other clinical symptoms of COVID-19 patients. Based on the hospitalization status of patients, 57.5% of patients were outpatients. Also, in this study, underlying diseases of COVID-19 patients were investigated. The results showed that 35%, 27.5%, and 35% of patients suffered from cardiovascular disease, diabetes, and blood pressure, respectively. Asthma and allergy were other reported comorbidities.

Table 1: Demographic and baseline characteristics of COVID-19 patients

Variables		N	Prevalence% (95% CI)
Gender	Female	13	32.5 (17.3 to 47.7)
	Male	27	67.5 (52.3 to 82.7)
Hospitalization Status	Outpatient	23	57.5 (41.5 to 73.5)
	Inpatient	17	42.5 (26.5 to 58.5)
Blood Pressure	No	26	65.0 (49.5 to 80.5)
	Yes	14	35.0 (19.55 to 50.5)
Diabetes	No	29	72.5 (58.1 to 86.9)
	Yes	11	27.5 (13.1 to 41.9)
CVD	No	26	65.0 (49.5 to 80.4)
	Yes	14	35.0 (19.5 to 50.4)
Asthma	No	34	85.0 (73.4 to 96.6)
	Yes	6	15.0 (3.4 to 26.6)
Allergy	No	35	87.5 (76.8 to 98.2)
	Yes	5	12.5 (1.8 to 23.2)
Fever	No	0	0
	Yes	40	100
Diarrhea	No	37	92.5 (79.6 to 98.4)
	Yes	3	7.5 (1.6 to 20.4)
Shortness of breath	No	25	62.5 (46.8 to 78.2)
	Yes	15	37.5 (21.8 to 53.2)
Headache	No	9	22.5 (8.9 to 36.1)
	Yes	31	77.5 (63.9 to 91.1)
Muscle pain	No	38	95.0 (83.1 to 99.4)
	Yes	2	5.0 (0.6 to 16.9)
Dry cough	No	14	35.0 (19.5 to 50.4)
	Yes	26	65.0 (49.5 to 80.4)
Chills	No	38	95.0 (83.1 to 99.4)
	Yes	2	5.0 (0.6 to 16.9)
Age (mean and 95% CI)		40	52.6 (50.6 to 54.7)

N: Number; CI: Confidence Interval; CVD: Cardiovascular Disease

CI calculated using binomial distribution

Clinical and demographical characteristics of confirmed COVID-19 patients who are infected by *influenza* A

Case 1 was a 46-year-old man with asthma, allergies, fever, headache, and dry cough. While other signs such as diarrhea, shortness of breath, muscle pain, and chills, comorbidities such as hypertension, diabetes, and cardiovascular

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Table 2: Clinical and demographical characteristics of patients with SARS-CoV-2 and Influenza A infection

Variables	Case 1	Case 2
Age (years)	46	53
Gender	Male	Male
Hospitalization Status	Inpatient	Inpatient
Blood Pressure	No	Yes
Diabetes	No	No
CVD	No	Yes
Asthma	Yes	No
Allergy	Yes	No
Fever	Yes	Yes
Diarrhea	No	No
Shortness of breath	No	No
Headache	Yes	Yes
Muscle pain	No	No
Dry cough	Yes	No
Chills	No	No
Previous COVID-19	No	No
Influenza vaccination	No	No
Variant of SARS-CoV-2	Omicron	Omicron
CVD: Cardiovascular Disease		

disease were not diagnosed. Case 2 was a 53-year-old man with complications such as high blood pressure and cardiovascular disease. Also, fever and headache were other clinical characteristics of this patient. However, this patient did not show symptoms such as chills, dry cough, muscle pain, diarrhea, and shortness of breath. The demographic characteristics and clinical symptoms of these two patients are inserted in Table 2.

Discussion

Co-infection in viral respiratory disease is defined as the detection of more than one or multiple viral pathogens in the same sample of patients with an acute respiratory tract infection (ARI)[9,10]. Several reports showed that co-infection of *SARS-CoV-2* infection with other viral or bacterial respiratory pathogens is associated with more severe disease than mono-infection ones[14, 15]. In co-infection, the mechanisms of disease severity are not well understood. They may result from direct or indirect effects of viral infections on host immune responses[16].

Influenza virus, Respiratory Syncytial Virus (RSV), Human Bocavirus, Parainfluenza Virus, and Human Metapneumovirus have been detected in SARS-CoV-2-positive patients[17,18]. On the other hand, several studies have reported the co-infections of *SARS-CoV-2* infection with Community-Acquired Pneumonia (CAP) infection (Streptococcus pneumoniae, Mycoplasma pneumoniae and Chlamydophila pneumoniae[19]. We also examined the diagnosis of CAP infections in COVID-19 patients in a previous study, the results of our study showed that 10 out of 40 patients with COVID-19 had CAP infections[19]. Therefore, the authors of this study decided to investigate the co-infection of *SARS-CoV-2* and influenza virus A/B infection.

The *influenza* virus, which belongs to the Orthomyxoviridae family, is one of the respiratory viruses and causes infections in the upper and lower respiratory areas[20]. The circulation of the *influenza* virus and SARS-CoV-2 occurs in fall and winter (from October to January)[21]. The overlap of SARS-CoV-2 and *influenza* can complicate the prognosis of COVID-19 disease[22]. The results of a meta-analysis study which was conducted by Dadashi et al. showed that the coinfection of *influenza* and SARS-CoV-2 was 0.8% among 3149 COVID-19 patients. The reason for the low rate of influenza in COVID-19 patients in Dadashi's study may be the lack of data from several countries, however, co-infection of these two viruses in the elderly, children, and immunocompromised individuals can be very complicated[22].

In another meta-analysis study, Musuuza et al. reported that the three most identified viruses among COVID-19 patients were *influenza* A (22.3%), i*nfluenza B* (3.8%), and respiratory syncytial virus (3.8%)[23].

Ozaras et al. in 2020 reported that among 1103 COVID-19 patients, 6 cases (0.54%) were diagnosed co-infected with *influenza*[24]. In 2020, Yue et al. reported that out of a total of 307 patients with COVID-19, 49.8% and 7.5% of them were co-infected with *influenza A* and *influenza B*, respectively[25]. In the study by Kim in the USA, 1217 samples were studied, of which 116 (9.5%) were positive for SARS-CoV-2 and 318 (26.1%) were positive for non-SARS-CoV-2 pathogens. Rhinovirus (6.9%) and respiratory syncytial virus (5.2%) were the most common co-infections among 116 positive samples. Influenza group A was positive in 1 patient (0.9%) and 29 (2.6%) in the SARS-CoV-2 group and the non-SARS-CoV-2 group, respectively. *Influenza B* in the positive group was not positive in any patients but 8 (0. 7 percent) was reported as positive in the non-SARS-CoV-2 group[26].

In a study in Isfahan, Iran, two patients were detected as co-infected with *influenza B* among 1639 patients with COVID-19[27]. Furthermore, in another similar study in Lazio, 7.96% of patients with COVID-19 had co-infection with influenza B[28]. In another study in Shiraz, Iran, 4 cases with co-infection of SARS-CoV-2 and influenza A were found with a co-infection rate of 33% (4 out of 12 patients[14]. In our study, the co-infection of SARS-CoV-2 and *influenza A* virus was at a lower rate (5%).

In a study conducted by Tang et al. between October 2021 and January 2022, a total of 462 individuals were included. Among them, 152 individuals tested positive for influenza, resulting in a monthly co-infection rate ranging from 7.1% to 48%[29]. While, in our study, individuals infected with the Omicron variant exhibited a lower likelihood of co-infection and hospitalization compared to those with the Delta variant. Furthermore, individuals who had received *influenza* vaccines demonstrated a reduced likelihood of co-infection. Key risk factors for co-infection included having comorbidities and being unvaccinated against *influenza.*

Although in the present study co-infected patients displayed symptoms similar to those with either COVID-19 or *influenza* alone, the study revealed that individuals with co-infections faced a heightened risk of adverse outcomes compared to mono-infected COVID-19 patients. Therefore, the study recommends screening for *influenza* in high-risk COVID-19 patients to effectively manage and mitigate the impact of co-infections.

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Due to the small sample size, the authors of this study cannot conclude about the relationship of co-infection of these viral infections with age, sex, and disease progression.

Also, the low frequency of *influenza* in our study may be attributed to the implementation of *SARS-CoV-2* infection prevention measures such as face covering, hand washing, and social distancing. Moreover, other respiratory viruses such as RSV, Boca virus, *Parainfluenza* virus, Human Metapneumovirus, and Adenovirus were not identified. We strongly recommend investigating the increased severity and risk of mortality among patients infected with two or more respiratory viruses.

Conclusion

In conclusion, the coinfection of COVID-19 and influenza presents a complex and challenging scenario in the realm of infectious diseases. The synergistic impact of these two respiratory viruses can lead to heightened severity and complications, posing a significant threat to public health. Our understanding of the clinical manifestations, pathophysiology, and optimal management strategies for such coinfections is still evolving. Although our samples were collected in the cold season, which is the prevalence of viral infections, the co-infection rate of COVID-19 and *influenza* was low, which is due to the small sample size in this study. In our study, two patients with co-infection of *SARS-CoV-2* and *influenza A* were men (Table 2). Although case 2 had underlying diseases including high blood pressure and cardiovascular disease, both cases were discharged.

Authors' Contribution

Design and conception: Saber Soltani and Milad Zandi; data acquisition: Mona Fani, Samaneh Abbasi, Armin Zakeri, Shokrollah Salmanzadeh, Iman Naamipouran, Seyed Mohamad Ali Malaekeh; analysis and interpretation: Reza Pakzad; writing the manuscript Samaneh Abbasi, Saber Soltani; and supervisor Samaneh Abbasi. All authors critically revised the manuscript gave final approval and agreed to be accountable for all aspects of the paper.

Conflict of Interest Disclosures

The authors declare that there is no conflict of interest.

Ethical Statement

The Abadan University of Medical Sciences ethics committee approved the study protocol (Ethics registration code: IR.ABADANUMS.REC.1399.137).

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