Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2) and-influenza virus coinfection during the COVID-19 pandemic

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Research Article

Keywords: SARS-CoV-2, influenza, coinfection, virus

Posted Date: May 17th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1635865/v1

Version of Record: A version of this preprint was published at Archives of Virology on January 7th, 2023. See the published version at https://doi.org/10.1007/s00705-022-05628-y.

Abstract

In covid-19 patients, undetected coinfection may have seriously increased hospitalization, a variety of therapeutic approaches, and mortality; therefore, we examined the proportion of SARS-CoV-2 and influenza virus coinfection among referred cases-patients from November to January 2021 in Hamedan province, Iran. Nasopharyngeal and oropharyngeal samples were obtained from 14116 individuals with COVID-19 symptoms (2216 inpatient and 11903 outpatient) and they screened for respiratory pathogens (SARS-CoV-2 and influenza virus) using multiplex real-time PCR panel assay. Case-patients were with a < = 18, 19–60 and 60 < = age, and 53.56% and 46.44% were male and female, respectively. Among them, 2004 (14.19%) were infected with the influenza virus; 2409 (17.11%) were infected with the SARS-CoV-2, and 191 (1.35%) were co-infected with both viruses. Patients aged 19–60 were more likely to be infected with a coinfection. Also, coinfection 96(50.26) was more common in females than males. The positive and negative cases reported for SARS-CoV-2, influenza virus, and coinfection were more observed in Hamedan city. Our result suggests that the circulating influenza virus was during the COVID-19 pandemic during the peak season, but coinfection with SARS-CoV-2 and influenza virus was not very common.

Introduction

SARS-coronavirus (SARS-CoV) is a new infectious disease, which is found in Wuhan, China, and is widespread around the world now. Coronavirus-2 (SARS-CoV-2), a severe acute respiratory syndrome virus, spreads through droplets expelled through the respiratory tract and can cause pneumonia [1]. In addition to concern with SARS-CoV-2, influenza continues to be a main infectious disease hazard worldwide. With the outbreak of the SARS-CoV-2 epidemic of 2019, the prevalence of influenza season saw a sharp decline in circulation and has decreased in different parts of the world due to the observance of health principles [2]. The seasonal influenza A or B virus continues to cause significant complications and deaths worldwide, even after introducing annual influenza vaccines in recent decades. Coinfections of COVID-19 with other respiratory pathogens can make it difficult to diagnose, treat, and prognosis COVID-19 and cause new concerns. Coinfection may also aggravate symptoms and increase the mortality rate of the disease. Cellular damage to respiratory ciliated cells caused by viral infections can help to be infected with SARS-CoV-2. The overlap between COVID-19 and influenza, with the onset of the seasonal influenza season, can coincide as two epidemics [3, 4]. Influenza and SARS-Cov-2 viruses are transmitted via contact, aerosolized or respiratory droplets, and contaminated surfaces. These viruses have a wide range from mild symptoms to severe illnesses [5, 6]. From the dawn of the history of the COVID-19 pandemic, researchers have been predicting simultaneous SARS-CoV-2 and influenza infections [6]. Influenza is a public concern around the world and has a high prevalence of associated conditions, including cardiovascular conditions, chronic respiratory and malnutrition, which, creates economic hardship and financial burden for individuals [1, 7]. The recognition of COVID-19 is critical, and it is effective in infection control. Potentially beneficial antivirals treatment should be introduced, and the risk of coinfection with COVID-19 should not be ignored. Due to this important issue, the present study evaluated the presence of influenza virus and SARS-CoV-2 coinfection in patients from November to January 2021 in Hamedan province, Iran.

Material And Methods

specimen collection

The study samples were obtained retrospectively from a population (n = 14116) accepted to Hamadan province, Iran. Diagnosed patients were included, inpatient and outpatient individuals. A nasopharyngeal and oropharyngeal samples were taken from each participant by observing the aspects of caution and personal protective equipment during sampling. Patients were enrolled during the COVID-19 pandemic between November to January 2021 in Hamedan. Collected swabs were then stored in Viral Transport Medium (VTM) and placed at room temperature. Participants were grouped into three groups: influenza single positive, coinfection of SARS-CoV-2 with influenza virus, and SARS-CoV-2 single positive.

RNA extraction and SARSCoV2 PCR detection

Under the relevant guidelines and regulations, the treatment of breath samples, aliquots, and viral RNA extraction. RNA was extracted from 200 µL of pooled NP and OP swab samples using the BehGen virus RNA extraction kit (Tehran, Iran). The multiplex RT-PCR assay for detecting influenza virus and SARS-CoV-2 was done by Qia quant96plex, using the RNA Hana gene kit (Tehran, Iran) with internal, positive, and negative controls.

Real-time PCR panel for SARSCoV2, influenza virus, and coinfection

RNA was examined for SARS-CoV-2 and influenza virus using RT-PCR. RT-PCR was performed with specific primers of target genes and probes (RdRp-gene and N-gene-specific primers and probes for SARS-CoV-2, and M2-gene for influenza A and NP-gene for influenza B) following the protocol recommended by the SARS-CoV-2, influenza A and B virus Multiplex RT-qPCR kit (Hana gene, Iran). This multiplex PCR assay incorporated one positive and negative control provided in the kit. Briefly, 8 µl of the extracted RNA was added to an 11 µl PCR Mix of buffer,

and one µl of Enzyme Mix in a final volume of 20 µl. Thermal cycling conditions were 50°C for 10 min, then 95°C for 3 min, following a standard two-step protocol of 42 cycles of 95°C for 10 s, 60°C for 30 s, and lastly, 37°C for 30 s for cooling.

Statistical Analyses

Categorical variables were reported as frequency and percent, and continuous variables as mean and standard deviation (SD). Proportions of influenza and coinfection of influenza and COVID-19 were reported with a 95% confidence interval. The Chi-square test was used to assess the association between categorical variables with influenza and coinfection of influenza and COVID-19. In addition, a logistic regression model was used to evaluate the association of the related factors with influenza and coinfection of influenza and COVID-19. The STATA (version 16.0; Stata Corporation, College Station, TX, USA) was used for statistical analysis. The $P \leq 0.05$ was considered statistically significant.

Result

14116 samples were tested for SARS-CoV-2 and influenza virus between March and December 2020. Case patients were < = 18, 19–60, and > 60 of age, of which 46.44% and 53.56% were females and males, respectively. The demographic and clinical information on the SARS-CoV-2 and influenza virus and coinfection are presented in Tables 1 and 2. There were infected 2409 (17.11%) and 2004 (14.19%) case-patients with SARS-CoV-2 and influenza virus, respectively. Only 191 (1.35%) patients were coinfected with influenza viruses and SARS-CoV-2. Patients aged 19–60 were more likely to be infected with SARS-CoV-2 virus and influenza virus and coinfection. Males were more likely to be infected with influenza (59.78%) than SARS-CoV-2 (49.61) and coinfection (49.74) case-patients. Positive cases were detected chiefly in outpatient cases in the coinfection, SARS-CoV-2, and influenza groups. In other words, among the cities of Hamedan province, the positive and negative instances reported for SARS-CoV-2, influenza, and coinfection were more observed in Hamedan city than in other cities during the study period (Table 2). Based on the results of adjusted logistic regression, the odds of influenza in men were 1.32 times of women. The odds of influenza in outpatients was 1.61 times of inpatient adjusted for age and gender (Table 3). The adjusted odds of coinfection in people with 19–60 and more than 60 years old were 2.68 and 3.7 times more than people lower than 18 years old, respectively (Table 3). The adjusted odds of SARS-CoV-2 in people with 19–60 and more than 60 years old were 2.64 and 3.8 times more than people lower than 18 years old, respectively. The odds of SARS-CoV-2 in men were 15.0% lower than in women adjusted for age and type of the disease. The odds of SARS-CoV-2 in outpatients were 45.0% lower than in inpatients adjusted for age and gender (Table 3).

Table 1
Demographics and clinical characteristics of patients with SARS-CoV-2 and influenza virus infection

Characters	SARS-CoV-2 infection			Influenza virus infection			Coinfection with influenza and SARS-CoV-2		
	Negative n (%)	Positive n (%)	p-value	Negative n (%)	Positive n (%)	p-value	Negative n (%)	Positive n (%)	p-value
Frequency	11668(82.89)	2409(17.11)	-	12117(85.81)	2004(14.19)	-	13930(98.65)	191(1.35)	-
Sex			0.0001			0.0001			0.287
Female	5325(45.66)	1214(50.39)		5750(47.47)	806(40.22)		6460(46.39)	96(50.26)	
Male	6338(54.34)	1195(49.61)		6362(52.53)	1198(59.78)		7465(53.61)	95(49.74)	
Age group			0.0001			0.0001			0.0001
<= 18	1890(16.20)	169(7.02)		1717(14.17)	349(17.42)		2055(14.75)	11(5.76)	
19–60	8472(72.61)	1746(72.48)		8753(72.24)	1497(74.7)		10108(72.56)	142(74.35)	
60<=	1306(11.19)	494(20.51)		1647(13.59)	158(7.88)		1767(12.68)	38(19.90)	
Type of referral			0.0001			0.0001			0.314
Inpatient	1646(14.11)	560(23.27)		2010(16.59)	206(10.28)		2181(15.66)	35(18.32)	
Outpatient	10022(85.89)	1847(76.73)		10105(83.41)	1798(89.72)		11747(84.34)	156(81.68)	

Table 2
Demographic characteristics of patients with SARS-CoV-2 and influenza virus infection in Hamadan province

City	SARS-CoV-2 infection		p-value	Influenza virus infection		p-value	Coinfection with influenza and SARS-CoV-2		p-value
	Positive n (%)	Negative n (%)		Positive n (%)	Negative n (%)		Positive n (%)	Negative n (%)	
Asadabad	88(3.65)	304 (2.61)	0.0001	52(2.59)	343(2.83)	0.0001	10(5.24)	385(2.76)	0.0001
Bahar	56(2.32)	257(2.20)		58(2.89)	263(2.17)		4(2.09)	317(2.28)	
Dargazin	38(1.58)	163(1.40)		39(1.95)	162(1.34)		6(3.14)	195(1.40)	
Famenin	336(2.88)	26(1.08)		122(6.09)	240(1.98)		7(3.66)	355(2.55)	
Hamadan	1,261(52.35)	6,525(55.92)		1141(56.94)	6649(54.87)		115(60.21)	7675(55.1)	
Kabudrahang	109 (4.52)	767(6.57)		199(9.93)	677(5.59)		7(3.66)	869(6.24)	
Malayer	402(16.69)	1,635(14.01)		125(6.24)	1913(15.79)		9(4.71)	2029(14.57)	
Nahavand	226(9.38)	714(6.12)		106(5.29)	836(6.9)		6(3.14)	936(6.72)	
Razan	48(2.97)	347(1.99)		95(4.74)	301(2.48)		6(3.14)	390(2.8)	
Tuyserkan	155(6.43)	620(5.31)		67(3.34)	733(6.05)		21(10.99)	779(5.59)	

Table 3
logistic regression analysis for coinfection with influenza, SARS-CoV-2 and coinfection, age, gender, and type of referral patients

Variables	Odds Ratio	Std. Err.	z	[95% Conf. Interval] Lower Upper	P>|z|
influenza					
age					
19–60 year	0.77	0.05	-3.79	0.68 0.88	< 0.001
60<=	0.52	0.05	-6.30	0.42 0.63	< 0.001
gender	1.32	0.06	5.66	1.19 1.45	< 0.001
type1	1.61	0.131	5.83	1.37 1.89	< 0.001
cons	0.05	0.00	-16.29	0.04 0.07	< 0.001
influenza and SARS-CoV-2					
age					
19–60 year	2.68	0.84	3.11	1.44 4.98	0.002
60<=	3.86	1.33	3.90	1.96 7.62	< 0.001
gender	0.87	0.12	-0.90	0.65 1.16	0.366
type1	0.87	0.18	-0.64	0.58 1.31	0.521
cons	0.00	0.00	-9.32	0.00 0.02	< 0.001
SARS-CoV-2					
age					
19–60 year	2.645699	11.23	0.2292166	2.23 3.135	< 0.001
60<=	3.80826	0.3712183	13.72	3.14 4.60	< 0.001
gender	0.8511123	0.0386452	-3.55	0.77 0.93	< 0.001
type1	0.5539885	0.0340085	-9.62	0.49 0.62	< 0.001
cons	0.3067801	0.0447186	-8.11	0.23 0.40	< 0.001

Discussion

COVID-19 is a highly contagious viral infection leading to many deaths worldwide and has created a challenge to the health care system of countries. Therefore, many studies have been conducted by researchers to identify viral infection, factors involved in the severity of the infection, and its mortality rate, and it continues to this day [6, 8]. The coincidence of other diseases, especially viral infections, could cause the exacerbation of the COVID-19 infection [9]. In this study, we evaluated the rate of influenza virus and SARS-CoV-2 coinfection in people with symptoms of COVID-19 infection. We detected meager coinfection rates among patients (191 of 14,121 samples tested). The studies in China and USA showed a similarly low proportion of coinfection (0.4% and 0.9%, respectively) in their patient samples. The coinfection rate was low in this study, despite the high rate of a single infection, which may be due to competition between the influenza virus and SARS-CoV-2 [10, 11]. In the results of the meta-analysis study, related to Lansbury et al., it has been shown that the coinfections of COVID-19 with viral and bacterial were 3% and 7%, respectively, in patients [9]. Although simultaneous infection with bacterial and viral agents can increase deaths in the SARSCoV2 infection, there is still insufficient evidence [6]. One of the common symptoms that COVID-19 and influenza share is respiratory symptoms. The mechanism of virus transmission, clinical manifestations, and seasonal emergence of the COVID-19 are similar to those of the influenza virus. Therefore, the simultaneous infection of SARS-CoV2 and influenza can intervene with the recognition and therapy of patients. Especially in high-risk patients, this coinfection can increase the severity of the disease and even increase the risk of death [6, 9]. Both influenza virus and SARS-CoV-2 are airborne pathogens. The primary location for the proliferation of influenza virus is alveolar type II cells (AT2 pneumocytes), which the SARS-CoV-2 also preferably infects these cells. A consider a simultaneous infection of influenza and COVID-19; this can increase the side effects of SARS-CoV-2 infection and play a significant role in COVID-19 infection development [12, 13]. For this reason, seasonal influenza and COVID-19 pandemics could include large populations of people at risk of contracting both viruses at the same

time [14]. Regarding demographics, the people aged 19–60 years were more likely to get infected with SARS-CoV-2 and influenza infection. In reports from Bangladesh, the elderly aged > 60 years have been considered a significant risk factor for COVID-19 infection. In our study, males were more likely to be infected with influenza. Also, females were more likely to be infected with SARS-CoV-2 and coinfection pathogens than males. The findings from Italy [15] and Bangladesh [16] research showed males were more infected with SARS-CoV-2 than females. Our result showed the prevalence of influenza and SARS-CoV-2 coinfection was found to be higher in outpatient samples than in inpatients. This study has some limitations; we had no information about the patients' clinical symptoms, and we did not have a history of any underlying disease such as hypertension, diabetes, or use of the seasonal influenza vaccine in the individuals. In conclusion, our data propose that the influenza virus and SARS-CoV-2 were not very common in Hamadan, which shows an apparent absence of influenza virus circulation during the peak influenza season in Hamadan.

Declarations

Acknowledgments

The authors are grateful to thank the Health Department, the Hamada University of Medical Sciences, Hamadan, Iran, for their cooperation.

Funding

This work was supported by the Deputy of Health of Hamadan University of Medical Sciences.

Author contributions

Design and implementation of the research; D R, N A. Contribution to experimental work; D R, SB. Analysis of the results: S B, D RI. Draft manuscript preparation; D R, N A. Funding acquisition; F TA. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Ethical approval

This study was approved by the Research and Ethics Committee of Hamadan University of Medical Sciences (Hamadan, Iran).

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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