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Original Article

Viral respiratory infections and their co-occurrence among patients hospitalized in Ayatollah Rouhani Hospital, Babol, 2021-2024

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Abstract

Background: Vaccination has reduced the viral respiratory diseases in the community, but it has not ended. This study was conducted to determine the distribution of COVID-19, the influenza virus, and the human respiratory syncytial virus (RSV).

Methods: This retrospective, descriptive-analytical study was conducted based on patient file information in Babol City (2021-2024). Clinical examination of the patient, computed tomography (CT), and real-time polymerase chain reaction (PCR) were used to identify the viruses in 10,567 patients. Mean, standard deviation (SD), distribution (%), and logistic regression were used to analyze the data using SPSS-23 ($p<0.05$).

Results: 83.34% and 16.65% of patients suffered from COVID-19 (12.95% $\geq$ 50 years old) and influenza (15.42%, $\leq$ 50 years old), respectively. The most underlying diseases were related to cardiovascular disease (17.47%). Female patients suspected of COVID-19 (females; 14.14% versus (vs) males; 11.87%) and influenza (females; 14.90% vs males; 15.62%) were more than male patients. COVID-19 (12.91%) and influenza (15.27%) in 2022 and 2023 showed a high distribution, respectively. Co-occurrence of COVID-19 and influenza was observed in one female. Gender, season, and year (each with a P-value of P=0.00) was associated in this descriptive analysis with the distribution of COVID-19. A significant relationship between season and year with the distribution of influenza was observed.

Conclusion: A high distribution of COVID-19 was diagnosed among patients. Gender and year of study had a significant effect on COVID-19. Diagnosis and differentiation of the viral agent by molecular methods should be performed in a patient.

Keywords: Viral respiratory infections, Influenza, Coronavirus-2019, Human respiratory syncytial virus.

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Iran was one of the first countries to be affected by the 2019 novel Coronavirus 2019 (2019-nCoV), and the disease remained highly prevalent in the country until a vaccine was developed (1). In Iran, from 20 February 2020 to 20 March 2020, it was reported that patients over 80 years of age (89%) were the most affected by the 2019-nCoV (2). A report in 2020 showed that 2019-nCoV had affected 85,403 people in 57 countries and caused 2,924 deaths in 9 countries (3). The influenza virus also affects the upper and lower respiratory tracts. In Iran, it was reported that there were nine influenza epidemics from 2015 to 2019 (4). Seasonal influenza pandemics result in 290,000 to 650,000 deaths worldwide (5). Like the 2019-nCoV and influenza virus, the human respiratory syncytial virus (RSV) is transmitted through contaminated respiratory secretions and affects the lower respiratory tract in 25% to 40% of primary infections. In Iran, 19.44% (14.72%) and 22.44% (24.107%) of respiratory samples were infected with human RSV in 2010 and 2012, respectively (6).

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In 2024, the frequency of human RSV, coronavirus-2019 (COVID-19), and influenza among hospitalized children was reported to be 27%, 16.5%, and 4.1% in Iran, respectively (7). Influenza co-infection with COVID-19 may interfere with the treatment, diagnosis, and prognosis of COVID-19, and may increase mortality, especially in high-risk populations such as the elderly and children (8). Influenza co-infection among patients with COVID-19 in Iran (0.8%), Asia (5%), and the Americas (0.4%) was observed (9). The spread of viral infections simultaneously in one period or their emergence in one patient is of great importance, as it can harm patients and the healthcare sector. Although strict adherence to the protocols recommended by the World Health Organization (WHO) before vaccination has reduced viral respiratory diseases, it has not yet fully recovered with the presence of many social activities (7-9).

On the other hand, co-infection refers to the emergence of other respiratory pathogens in patients with COVID-19 infection at the time of diagnosis of COVID-19 infection (10). Research in the Netherlands in 2025 showed that 7.7% of patients with positive COVID-19 were co-infected with another respiratory virus, especially (66.1%) with human rhinoviruses (11). A study in 2022 in the United Kingdom found that 0.5% of people (out of 1,240,0009 people) vaccinated with the first dose of the COVID-19 vaccine tested positive for COVID-19, and 0.2% of people (out of 971,504 people) who received the second dose also became infected with this virus. Low immune systems, secondary infections, old age, and underlying diseases were among the factors that contributed to the development of COVID-19 after vaccination (12).

However, secondary infection is defined as the emergence of other respiratory pathogens during the care of patients suffering from COVID-19. Most studies conducted during the COVID-19 epidemic have addressed the diagnosis, interpretation of clinical symptoms, differences in outcomes, and determination of the frequency of secondary bacterial or fungal infections in patients with COVID-19. COVID-19 patients who had secondary infections had longer hospital stays and higher mortality, while patients with co-infections were admitted to the intensive care unit (ICU) more frequently. Also, diagnostic tests and treatment decisions may be influenced by the presence of co-infection or secondary infection with COVID-19 and other respiratory pathogens. Therefore, routine screening and reporting of these types of infections can positively impact patient care (9-11).

As a specific knowledge gap in this area, there is not much information on the simultaneous occurrence of respiratory infections over a given period or the simultaneous occurrence of viral respiratory infections in a patient; there are also local epidemiological uncertainties. Most research has focused on secondary infections, so this study may provide new results in this area, regarding the simultaneous distribution of viral respiratory infections (10, 11), after the vaccination period (when the disease had ceased to be an epidemic) and the emergence of them in a patient with COVID-19 (which has been reported rarely), and also the number of associated risk factors. Also, this study considered patterns of respiratory virus seasonality and demographic associations.

Methods

This retrospective, descriptive-analytical study was conducted based on patient records in the archives of Ayatollah Rouhani Hospital, Babol City, Babol University of Medical Sciences, Iran. In the section related to the descriptive study design, viral respiratory infections (COVID-19, influenza, or human RSV) as public health issues and occurrence (occurrence of COVID-19, influenza, or human RSV in patients during four years in Ayatollah Rouhani Hospital) of these infections were considered. Viral respiratory infection patterns during different seasons, between populations (male and female) in different ages, and according to years were also surveyed. The distribution of it was calculated in Babol city (March 2021 - February 2024). Patient’s data was obtained from the hospital information system (HIS). The records of all 10,567 patients with viral respiratory infections were reviewed cross-sectional. The inclusion criteria were patients with viral respiratory infections hospitalized from 2021 to 2024. These patients were prescribed diagnostic tests for COVID-19, influenza, or human RSV, according to their medical prescription. Some patients were also prescribed diagnostic tests for two or three viruses at the same time. Patients who had respiratory infections but did not have viral respiratory infections, or whose viral respiratory infections were negative, were excluded from the study (12).

All eligible cases during the study period were included; no formal sample size calculation was performed due to the retrospective design. To collect data from all statistical societies with inclusion criteria, all individuals were considered. The results were obtained completely without sampling bias and with high accuracy. Therefore, the findings had a high power of reliability and generalizability (13). Clinical and physical examinations were performed to detect patients. Then, a computed tomography (CT) was done to determine pneumonia, the extent of the infection, and respiratory disorders, the severity of the disease progression, and to detect symptoms of viral or bacterial pneumonia (9, 12). The confirmatory diagnostic test was real-time polymerase chain reaction (PCR) (Geneova, Iran), which can detect the viral ribonucleic acid (RNA) genome of COVID-19, influenza, and human RSV (for molecular confirmation, samples used included nasopharyngeal swabs, sputum, or lower respiratory tract aspirates). RNA processing and sample extractions were performed using the protocols of the viral RNA extraction Kit (Roche Diagnostics, Germany). Real-time PCR was performed on all 10,567 patients according to kit instructions (complementary (c) DNA synthesis; 53°C, 15 min (1 cycle), holding; 95°C, 3 min (1 cycle), denaturation; 94°C, 5 s along with annealing**,** extension and fluorescence measurements; 60°C, 5 s (46 cycles)) and a final volume of 20 microliters (µL) (10 µL Mastermix+10 µL RNA template, negative or positive control (each in a separate tube)) (table 1) (12).

In this study, various values of variables (viral respiratory disease, including positive cases of COVID-19, influenza, and human RSV based on age and gender, during different years and seasons) were examined in patients. This study faced a series of missing data, and it was not possible to eliminate all cases of patients with missing data. Therefore, concerning each variable, data distribution and summarization were performed according to the available information for each dataset. So, the possible values of a variable in the patient population were calculated using the mean±standard deviation (SD) and frequency (%) table. The control of confounding factors was considered in the design phase of the study. The incidence of COVID-19 and influenza was a two-level dependent variable. To examine confounding factors, logistic regression was used to consider the effect of age, gender, years, seasons, and comorbidity on the distribution of COVID-19 and influenza, separately. In the logistic regression, a group of qualitative variables was considered as a reference category or baseline. Subsequent variables were compared to this reference category to obtain odds ratios (OR). Statistical package for the social sciences (SPSS)-23 software was also used for data analysis (p<0.05).

Table 1. Contents of the master-mix, Geneva kit for the detection of viruses

Viruses	Virus gene primers	Human housekeeping gene primer (Internal control)	Probe (Nucleotide sequence-dye)
COVID-19	Nucleocapsid protein (N) of COVID-19, Spike protein (S), and Open reading frame 1a (ORF1a)	Nuclear ribonuclease (RNase) P	Familial sequence similarity (FAM), Hexosaminidase (HEX), Cyanine 5 (Cy5), Texas red
Influenza A and B	Matrix genes M1 and M2	Nuclear ribonuclease (RNase) P	Familial sequence similarity (FAM), Hexosaminidase (HEX), Cyanine 5 (Cy5), Texas red
Human RSV	L protein; RdRp (RNA-dependent RNA polymerase), capping, connector, methyltransferase, and carboxy-terminal domains.	Nuclear ribonuclease (RNase) P	Familial sequence similarity (FAM), Hexosaminidase (HEX), Cyanine 5 (Cy5), Texas red

Results

Out of this total number 10,567 patients (the patients were in the age range of 1 to 100 years old; mean±SD; 55.20±24.50 years old, suspected to viral respiratory infections), 1,465 (13.86%) cases suffered with COVID-19 (83.34%, 1,221 patients) and influenza virus (16.65%, 244 patients) (figure 1). Of 10,567 patients, 49.80% were females (n=5,263) and 50.19% were males (n=5,304). The underlying diseases in patients (n=10,567) were related to cardiovascular disease (17.47%, n=1,847), diabetes (10.93%, n=1,156), malignancies (7.90%, n=835), and other diseases (7.40%, n=782), respectively. Of the 10,567 patients, the age of 9,235 who had tested for COVID-19 was listed in the reviewed records (some cases lacked age information). Most of the patients (n=5,983) were ≥ 50 years old and positive (12.95%) for COVID-19. In the case of influenza**,** 1567 patients had complete information about age (some cases had missing age data). Based on the available age information, most of the patients suspected of viral respiratory infections (n= 1025) showed an age of ≥ 50 years. The most (15.42%) positive influenza cases were detected in patients ≤ 50 years old. According to the complete and available information, among those with respiratory infections (4,710 females and 4,674 males), COVID-19 was confirmed in 14.14% of females (n=666) and 11.87% of males (n=555). There were also more males (n=130, 15.62%) with influenza than females (n=114, 14.90%). Gender showed a significant effect on the distribution of COVID-19 (P=0.00). The distribution of positive COVID-19 (n=630) and influenza (n=170) cases was higher in the fall compared to other seasons. There were no people with suspected or confirmed influenza in the spring and summer. The distribution of positive cases of COVID-19 (n=864) and influenza (n=244) in 2022 and 2023 was higher than in other years, respectively. There were no people with confirmed influenza in 2021 and 2022. A co-occurrence of COVID-19 and influenza was observed in one patient, a 41-year-old female who presented with diabetes. Gender (P=0.00), season (P=0.00), and year (P=0.00) showed a significant effect on the distribution of COVID-19. Also, there was a significant relationship between season (P=0.00) and year (P=0.00) with the distribution of influenza (table 2).

Distribution of human RSV: Human RSV infection was also observed in five (≥ 18 years old) patients. One case was observed in the fall (2023), and four in the winter (2024). There were more females (four cases, 30, 41, 63, and 69 years old) than males (one case, 18 years old). All patients were negative in terms of COVID-19 and influenza. Two of the patients also suffered from malignancy, and one of them from cardiovascular disease.

Multivariable logistic regression: Regarding p-values, COVID-19 was affected by variables of male gender (P=0.0012), Summer and fall (each one p<0.001) seasons, years of 2022 and 2023 (each one p<0.001), cardiovascular disease and malignancies (each one p<0.001) (table 3).

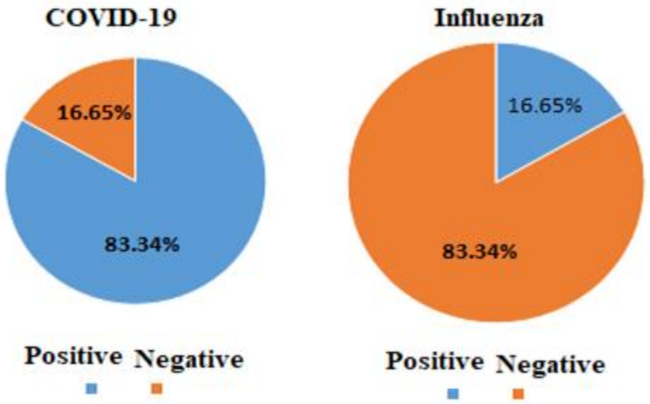

Figure 1. Positivity rate for COVID-19 and influenza among the confirmed patients (n=1,465) with positive viral respiratory infections

Table 2. Demographic/seasonal distribution of COVID-19 and influenza among patients

Variables	Sub-Variables	B	SE	P-value	OR adjusted	95% CI for OR adjusted	
						Lower	Upper
Age	>50 years	0.0143	0.065	0.825	1.0144	0.89	1.153
	≤ 50 (ref)	-	-	-	-	-	-
	Male	-0.201	0.0615	0.0012 *	0.818	0.724	0.923
Gender	Female	-	-	-	-	-	-
	Spring (ref)	-	-	-	-	-	-
Season	Summer	-1.76	0.269	<0.001	0.172	0.097	0.281
	Fall	1.22	0.0819	<0.001	3.39	2.89	3.99
	Winter	0.142	0.088	0.111	1.152	0.968	1.372
	2021 (ref)	-	-	-	-	-	-

Variables	Sub-Variables	B	SE	P-value	OR $_{adjusted}$	95% CI for OR $_{adjusted}$	
						Lower	Upper
Year	2022	-0.403	0.083	<0.001	0.668	0.559	0.801
	2023	-0.654	0.091	<0.001	0.519	0.415	0.651
	2024	-4.06	3.5	0.942	0.0172	-10.9	2.79
	Diabetes	0.002	0.105	0.986	1.002	0.816	1.23
Comorbidity	Cardiovascular disease	-0.865	0.169	<0.001	0.421	0.302	0.587
	Malignancies	-0.507	0.123	<0.001	0.602	0.474	0.766
	Other diseases	-0.047	0.073	0.524	0.954	0.826	1.102
Regression item	Constant	-2.230	0.134	0.000	0.108	-	-

P; Positive, N; negative

Table 3. Results of the logistic regression for COVID-19 disease adjusted for variables

Variables	Sub-Variables	B	SE	P-value	OR $_{adjusted}$	95% CI for OR $_{adjusted}$	
						Lower	Upper
Age	>50 years	0.0143	0.065	0.825	1.0144	0.89	1.153
	≤ 50 (ref)	-	-	-	-	-	-
	Male	-0.201	0.0615	0.0012 *	0.818	0.724	0.923
Gender	Female	-	-	-	-	-	-
	Spring (ref)	-	-	-	-	-	-
Season	Summer	-1.76	0.269	<0.001	0.172	0.097	0.281
	Fall	1.22	0.0819	<0.001	3.39	2.89	3.99
	Winter	0.142	0.088	0.111	1.152	0.968	1.372
	2021 (ref)	-	-	-	-	-	-
Year	2022	-0.403	0.083	<0.001	0.668	0.559	0.801
	2023	-0.654	0.091	<0.001	0.519	0.415	0.651
	2024	-4.06	3.5	0.942	0.0172	-10.9	2.79
	Diabetes	0.002	0.105	0.986	1.002	0.816	1.23
Comorbidity	Cardiovascular disease	-0.865	0.169	<0.001	0.421	0.302	0.587
	Malignancies	-0.507	0.123	<0.001	0.602	0.474	0.766
	Other diseases	-0.047	0.073	0.524	0.954	0.826	1.102
Regression item	Constant	-2.230	0.134	0.000	0.108	-	-

B; The effect of each variable, SE; Standard error, OR; Odds ratio, CI; Confidence interval, Lower; The smallest value, Upper; The largest value, ref: Reference category, Constant; the value at which the regression line crosses the y-axis.

Discussion

In this study, the distribution of viral respiratory infections (COVID-19, influenza, and human RSV) was assessed. Out of a total number of 10,567 patients (55.2 years old) that was suspected of viral respiratory infections, 83.34% and 16.65% suffered from COVID-19 and influenza, respectively. Females (49.80%) were more than males (50.19%). The underlying disease was related to cardiovascular disease (17.47%). Human RSV was detected in five patients. In a similar study, Ramzali et al. reported that the prevalence of human RSV, COVID-19, and influenza infection among children hospitalized with acute lower respiratory infections was 27%, 16.5%, and 4.1%, respectively. Following COVID-19 in 2019, a significant decrease in human RSV activity was observed, coinciding with the implementation of public health and social measures. After the gradual lifting of public health measures and the change in season, a delayed outbreak of human RSV was found in a larger number of infected patients (7).

The results of our study indicated that the number of patients ≥ 50 years old (775 patients, 12.95%) with positive COVID-19 was higher than that of patients ≤ 50 years old (416 patients, 12.79%). Females (666 patients, 14.14%) were more likely to be positive for COVID-19 than males (555 patients, 11.87%). In the report by Khalagi et al., the prevalence of COVID-19 was estimated to be 14.2%, which was lower than the results of our study (83.34%). The prevalence among males, females, and patients over 60 years of age was 14.6%, 13.8%, and 19.4%, respectively, which were relatively close to our study, but overall, in our study, unlike the study of Khalagi et al., the distribution of COVID-19 among females (14.14%) was higher than that among males (11.87%) (14). In the study conducted by Kalantari et al., similar to our study (≥ 50 years old, 12.95%), the highest prevalence of COVID-19 was in patients with old age (80 years, 89%) (2). Older age, underlying medical conditions, especially hypertension, diabetes, obesity, and cardiovascular disease, play a significant role in people's susceptibility to COVID-19. Communities with higher risk factors for these conditions are also at higher risk for COVID-19. It is possible that in a research population, more women are affected by these risk factors, and naturally, the number of positive cases of COVID-19 in that population will be higher than that of men. The hormone testosterone also decreases in older men, so a decrease in the hormone could make older men more susceptible to COVID-19 (15, 16). Although Fabião et al. reported that men are more likely to develop severe COVID-19 disease and die from it than women (17), Bai et al. also showed that women (especially pregnant, postmenopausal, and elderly women) are more likely to develop long COVID-19 than men (15).

Ahmadi et al. reported that 349/1237 (28.2%) cases were diagnosed as influenza-positive. In our study, 244/1665 (16.65%) were positive for influenza, which was lower than the frequency in the study by Ahmadi et al. (28.2%). These rates indicate the existence of influenza peaks with different frequencies in different parts of the country (16). Overall, the distribution of influenza in our study (n=244 patients) was lower than in the study of Salehi et al. (n=165 patients), but the mean age of the patients in our study was older (55.20 years old) than the mean age (49 years old) of the patients in the study of Salehi et al. They also reported that most of the patients with influenza were males (55.20%). In our study, there were also more female patients (14.90%) than males (15.62%) who were detected with positive influenza, but there was no significant association between gender and positive influenza distribution (18). Also, the number of patients with influenza in our study was higher in patients ≥ 50 years old (158 patients, 15.41%) than in patients < 50 years old (85 patients, 15.42%). Older people may be more susceptible to viral infections, so in studies that include older patients, the probability of positive influenza is higher. The populations studied in different studies vary, meaning that some patients have underlying medical conditions or, as mentioned, have received the influenza vaccination. The prevalence of influenza also varies in different geographical regions. In our study, sampling was done through a census, so the number of patients included in the study may have been higher among females. All of these factors may have contributed to a disparity in prevalence between females and males for both COVID-19 and influenza (15, 17).

The presence of underlying diseases among patients in our study population (cardiovascular disease (17.47%), diabetes (10.93%), malignancies (7.90%) was observed by Salehi et al. The most common underlying diseases in the present study were related to heart disease (17.47%). In the study by Salehi et al., the mortality rate (11.55%) was also related to cancer and liver disease. These similar results show that people with underlying diseases are more susceptible to viral infections due to weak immune systems (18). In our study, which was conducted from 2021 to 2024 (after universal COVID-19 vaccination), 1,217 patients with COVID-19 were identified (According to complete files). The highest distribution of positive cases of COVID-19 (n=864, 12.91%) was found in 2022. Kazempour Dizaji et al. showed that a failure to comply with health measures and restrictions increased the frequency of COVID-19 by an average of 1.03 per thousand people per day, while attention to public health measures and continued social restrictions by the government reduced the frequency of the disease by an average of 13.2 per thousand people per day. Even after vaccination and the end of the major COVID-19 epidemic, failure to comply with health measures and the reduction of social restrictions because of the spread of COVID-19 and influenza in the community (19). Alimohamadi et al. reported that there were nine influenza epidemics from 2015 to 2019 (21). The highest distribution of COVID-19-related cases was observed in the fall (630 patients, 25.08%) and winter (337 patients, 10.20%). The incidence of influenza was also in the fall (170 patients, 18.53%) and winter (74 patients, 10.88%). Influenza epidemics occur annually in the fall and winter in temperate regions and affect a significant proportion of adults and children, but seasons have different effects on age groups and severity (4).

During the cold months of the year, damage to respiratory cells is greater, so viruses that infect respiratory cells can also facilitate the occurrence of other respiratory diseases. Therefore, although the high incidence of respiratory viruses in cold areas has been proven, epidemiological and statistical studies of respiratory viral infections in different seasons, a specific area, and different periods have a significant impact on how patients are managed and treated, and the preparedness of healthcare systems in times of viral epidemics or pandemics (20). In our study, five cases (one case in 2023 and four cases in 2024) of human RSV were detected (5 patients, three patients with underlying disease, ≥ 18 years old). The decrease in human RSV activity could be due to the implementation of public health and pharmaceutical interventions to reduce the spread of COVID-19. Hernández-Rivas et al. reported that the epidemiology of human RSV has changed during the COVID-19 pandemic (21). A study of three groups: children (March 2021), pilgrims (July 2022), and outpatients (November and December 2022), by Yavarian et al. showed that no human RSV was detected in any age group (22). Human RSV is one of the most common viral respiratory infections worldwide, with a global prevalence increasing in children under five years of age (23). Tavakoli et al. reported that of the 206 children who had symptoms of respiratory infection, 74 (35.92%) samples tested positive for human RSV (24). In another study, Modarres Gilani and Rahbarimanesh reported that human RSV was positive in 33/268 (12.3%) children. Similar to our results (four cases in the winter), human RSV infections peaked in early winter, as 85% were diagnosed from December to March (25). Although the number of cases is increasing among infants and young children, human RSV can infect any age group. There are significant differences in the symptoms and diagnosis of human RSV infections in adults and children. Human RSV infections in adults are underestimated and underdiagnosed. Due to comorbidities, milder symptoms, and a lack of routine testing for human RSV in adults, many infections go undiagnosed and unreported. Human RSV is known to cause bronchiolitis and pneumonia in children, especially infants, which leads to more frequent testing and higher reporting rates. Therefore, the reporting rate of RSV infections in adults is lower than in children (higher rates recorded in children) (26, 27). However, some people are at greater risk than others. Premature infants, young children, the elderly, and immunocompromised individuals are most likely to develop more severe RSV disease than healthy adults. In our study, patients with human RSV suffered from malignancy and cardiovascular disease (23-25).

Chuang et al. also reported that human RSV affects not only children but also the elderly and immunocompromised patients. After the emergence of COVID-19, a significant decrease in human RSV activity was observed, which coincided with the implementation of public health and social measures; however, the gradual lifting of health measures and the change of seasons were accompanied by the spread of this virus (28). The distribution of human RSV disease is higher in the cold season than in other seasons, and in our study, all five cases of human RSV patients were identified in the cold season (fall and winter) (1). Although the number of cases is increasing among infants and young children, human RSV can infect any age group. However, some people are at greater risk than others. Premature infants, young children, the elderly, and people with weakened immune systems are most likely to develop more severe human RSV than healthy adults (28).

A study by Dadashi et al. showed that the co-infection frequency of influenza and COVID-19 was 0.8%. The frequency of influenza virus co-infection among patients with COVID-19 in Asia was 4.5% and 0.4% in the United States (9). Khodamoradi et al. reported that four patients were infected with COVID-19 and influenza A in the early days of the COVID-19 disease (29). A case report by Baala et al. also showed co-infection of COVID-19 and H1N1 influenza in one patient (30). Susceptibility and intrinsic factors of the virus (virus species and sequence) and the host (immunity and comorbidities), external factors including the infectious load of the virus and the time elapsed between the primary and secondary viral infections, can cause simultaneous viral infections. For example, since COVID-19 preferentially infects alveolar type II (AT) 2 Pneumocyte AT2) cells, which are the main site of influenza virus replication, co-infection could lead to exacerbation of COVID-19 manifestations (5, 6).

On the other hand, COVID-19 replication is inhibited by influenza viruses and the human respiratory syncytial virus. This inhibition is dependent on a functional antiviral response, and the level of inhibition is proportional to the time of secondary viral infection. COVID-19 replication is inhibited by both the influenza A virus and the respiratory syncytial virus. Positive and negative interactions between respiratory viruses at the epidemiological level can be mediated by transient innate immune responses. Some viruses induce an interferon-mediated response that can block secondary viral infection. Therefore, it is expected that the incidence of COVID-19 will decrease and be seen sporadically during periods of influenza A and respiratory syncytial virus circulation (6, 8). In addition to vaccination status and healthcare, socioeconomic factors can also influence the prevalence of viral respiratory infections in communities. Lower socioeconomic status is often associated with higher rates of infection and mortality from viral respiratory infections. Vaccination rates are also correlated with socioeconomic status, with wealthier countries and communities often having higher and better quality vaccination coverage. Access to health services also plays an important role in the spread of infections; limited access and inability to pay for tests can hinder testing, treatment, and preventive measures such as vaccination (31). Both COVID-19 and influenza A/B viruses can lead to a wide range of COVID-19 manifestations. Therefore, clinical symptoms are not useful for accurate diagnosis and differentiation of these two viruses, so different molecular (in our study, real-time-PCR detection of viruses was used) and serological techniques are required for accurate diagnosis of COVID-19 and influenza co-infection (8).

The strength of this study is that it reports observed associations and patterns of variables, but cannot make firm claims about statistical significance or causality. This research used eligible cases during the study period for statistical analysis, so there was no formal sample size calculation. To address this statistical problem and the lack of a priori power analysis, a prospective power analysis should be performed to determine the minimum sample size required to detect a clinically meaningful effect with sufficient statistical power typically 80% or higher). This is necessary to ensure that negative findings are truly negative (i.e., an effect does not exist) and not false negatives due to insufficient sample size. There were also limitations in this study. Complete records of all patients were not available, so to evaluate the various variables, complete information related to each data set was statistically interpreted separately. On the other hand, molecular diagnostic kits for influenza and human RSV were not available for all years. Therefore, the tests prescribed to patients varied from year to year. For example, not all patients were prescribed the same tests for COVID-19, influenza, and CVD every year. So, it was difficult to compare all patients based on the three tests and diseases (COVID-19, influenza, human RSV), which is considered a critical methodological and analytical limitation. Therefore, for analysis, the percentage method and frequency distribution were performed based on each year and each virus, separately, and only eligible cases were considered. Also, many patients had received the COVID-19 and influenza vaccine, which affects the prevalence of different viruses and their interactions. Vaccination status was not assessed in this study, and the lack of recorded vaccination status can confound the interpretation of trends and disease prevalence by age and season in a study. Therefore, observed differences in disease prevalence across age groups, seasons, and years could be due to fundamental differences in vaccination coverage. Vaccination status acts as a confounder, meaning that it is associated with both exposures (age, years, and season) and outcome (disease), and can influence the true relationship. Therefore, it is suggested that recording vaccination status be considered in future studies. Subgroup analysis, sensitivity analysis, and regression adjustment should be used in the statistical section (32, 33). The prevalence of infection by human RSV in children is also very high, but this study only examined adults, and children (from a specialized children's hospital) were not included in the study. For the study of human RSV, our patients were ≥ 18 years old. SO, there was also missing data and a single-center design. According to the results, this epidemiological study showed the distribution of viral respiratory infections. So, results can help to understand disease patterns and intervene for disease prevention and control. Given that the prevalence of human RSV is higher in children, it is recommended that the prevalence and risk factors for contracting this virus in children be examined and compared. In conclusion, patients with positive COVID-19, influenza, and human RSV were reported after universal COVID-19 vaccination. The distribution of cardiovascular disease was higher than that of diabetes and malignancies. Most of the patients were females and ≥ 50 years old. Gender was associated in this descriptive analysis with the distribution of COVID-19. The distribution of positive COVID-19 and influenza cases was shown in the fall and during 2022 and 2023. There was a significant relationship between seasons and years. Human RSV infection in five patients was confirmed. Investigating the distribution of different viral infections in the hospital at different times has a significant impact on how to manage and treat hospitalized patients.

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