



The Prevalence and Clinical Features of Co-Infection with SARS-CoV-2 and Influenza Virus during the COVID-19 Pandemic in Semnan, Iran

Mahdieh Tarahomi¹, Mohammad Sadegh Shokri Koohikheyli¹, Pegah Pooya², Majid Eslami^{3,4}, Mahshid Vakili⁴, Fatemeh Habibian¹, Ramtin Naderian⁵, Mohsen Zolfaghari⁵, Hadi Ghaffari^{3,4*}

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Abstract

Background: COVID-19 and influenza are both contagious respiratory diseases. Influenza virus can increase the severity of COVID-19 infection in the cold months of the year through damage to respiratory ciliated cells, which may cause an increase in hospitalization, disease symptoms and mortality rate. Therefore, the purpose of this study was to ascertain the frequency of co-infection with the influenza virus and SARS-CoV-2, as well as the impact of co-infection on clinical outcomes in hospitalized patients suffering from respiratory problems within Semnan City, Iran.

Methods: In this cross-sectional descriptive study, we investigated 1267 hospitalized patients with respiratory problems between September 2021 and March 2022. Two nasopharyngeal and oropharyngeal throat swab samples were collected from each patient and tested for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A, and influenza B viruses using real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR). The collected data were analyzed with χ^2 test, ANOVA, paired Student's t-test, and Pearson's correlation coefficient test in different groups. Analyzes were done with SPSS 26.0 software.

Results: In total, 29.6% (n = 375) of patients had confirmed positive results for SARS-CoV-2, and their median age was 55.4 ± 24.63 years. It was found that 1.9% (n = 7) and 0.5% (n = 2) of COVID-19 patients had co-infections with influenza viruses A and B, respectively. In 2.4% of the cases, co-infection with COVID-19 and influenza was found. 8 out of 9 patients (88.8%) recovered, while one patient (11.1%) died. Co-infection did not significantly correlate with cancer ($P = 0.588$), diabetes ($P = 0.202$), hypertension ($P = 0.530$), or any other illness.

Also, Associations of death and co-infection with diabetes, cardiovascular disease, or CKD showed that a statistically significant correlation was present only between diabetes and death. Based on the ANOVA test to look at associations of death and co-infection with diabetes, cardiovascular disease, or CKD, it showed that there was no significant association of co-infection with diabetes ($P = 0.202$), hypertension ($P = 0.530$), cancer ($P = 0.588$), and other diseases.

Conclusion: Although a low proportion of COVID-19 patients have influenza co-infection, the importance of such co-infection, especially in high-risk individuals and the elderly, cannot be ignored. Given the prevalence of influenza co-infection, increased coverage of flu vaccination is encouraged to mitigate the transmission of the influenza virus during the ongoing COVID-19 pandemic and reduce the risk of severe outcomes and mortality.

Keywords: COVID-19, Influenza A and B, Co-infection

Conflicts of Interest: None declared

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Corresponding author: Dr Hadi Ghaffari, Hghaffari@semums.ac.ir

¹ Semnan University of Medical Sciences, Semnan, Iran

² Department of Molecular Virology, Semnan Health Reference Laboratory, Semnan University of Medical Sciences, Semnan, Iran

³ Cancer Research Center, Semnan University of Medical Sciences, Semnan, Iran

⁴ Department of Bacteriology and Virology, Semnan University of Medical Sciences, Semnan, Iran

⁵ Student Committee of Medical Education Development, Education Development Center, Semnan University of Medical Science, Iran

↑What is “already known” in this topic:

COVID-19 co-infections with other respiratory infections, especially influenza infection may complicate the diagnosis, treatment and prognosis of COVID-19. Influenza infection can facilitate COVID-19 through damage to respiratory ciliated cells.

→What this article adds:

Our study has shown that SARS-CoV-2 and influenza co-infection was not common in Semnan city because health care during the COVID-19 pandemic may have helped to reduce the transmission of seasonal influenza virus.

Introduction

Respiratory virus infections account for significant morbidity and mortality across the globe (1, 2). Two pandemic diseases caused by respiratory viruses in the last two decades, H1N1 influenza, and SARS-CoV-2 (3, 4). Both COVID-19 and influenza diseases present with similar clinical symptoms including cough, sore throat, headache, muscle aches, breathlessness, fever, acute respiratory distress syndrome, and death (5, 6). Bao et al. showed that co-infection with these viruses increases the duration of clinical symptoms of COVID-19 and increases lung damage (7). Moreover, another study has found that the influenza virus can cause aggravate SARS-CoV-2 infection. Increased viral load of SARS-CoV-2 and aggravated lung damage occur in mice infected with influenza virus. However, increased infectivity of SARS-CoV-2 has not been observed in infections with other respiratory viruses, possibly because the influenza virus is associated with increased ACE2 expression (8). The combined effects of SARS-CoV-2 and influenza virus infection may be due to the fact that both viruses predominantly affect alveolar type II cells. Therefore, SARS-CoV-2 and influenza co-infection may aggravate respiratory epithelial damage. In addition, other studies showed that patients with Co-infection of these viruses are more likely to need mechanical ventilation (9, 10). Therefore, SARS-CoV-2 and influenza virus co-infection increases patient risk and complicates patient condition. The aim of this research is to determine the frequency of co-infection between influenza and COVID-19 viruses and assess their correlation with patient demographics and clinical data.

Methods

Study Design, Participants, and Sample Collection

This study was conducted cross-sectionally. A total of 1267 nasopharyngeal and oropharyngeal throat samples were collected between December 2021 and March 2022 in Kausar and Amir-Al-Momenin hospitals affiliated with Semnan University of Medical Sciences with approval certificate from the medical ethics committee of the university.

RNA extraction, SARS-CoV-2, influenza A and influenza B PCR detection

Using the Roje kit, viral RNA was isolated from samples. Using the Viga SARS CoV-2 and influenza A/B molecular diagnostic kit, RNA was obtained and then submitted to Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR). All molecular tests were carried out in accordance with the manufacturer's instructions (ROJE Technologies, Iran). All the RT-PCR reactions were carried out in the QIAquant 5-Plex 96 Real-Time PCR System (Qiagen, Hilden, Germany).

Data Collection and Statistical Analysis

All data and laboratory parameters were collected. Descriptive statistics were calculated for all of the variables. We calculated the prevalence of COVID-19 and influenza by the entire study group. We used the χ^2 test to compare the rates of sex, Comorbidities, Dexamethasone, and

Remdesivir consumption, In-hospital deaths and use of mechanical ventilation in different age groups. The mean of age, first SpO₂ and second SpO₂ in each of the groups were analyzed by analysis of variance (ANOVA) and paired Student's t-test. Furthermore, the relationship between the use of dexamethasone and remdesivir and the survival rate of patients was investigated by Pearson's correlation coefficient test. All variables with p-values of ≤ 0.05 were considered statistically significant. The SPSS statistic software (version 26) was used to perform the analysis.

Results

Demographic and clinical characteristics

A total of 1267 individuals from Semnan, Iran who had symptoms similar to the flu were evaluated. Of them, 614 females (48.4%) and 653 males (51.5%) had mean ages of 65.38 ± 110.83 and 59.40 ± 63.41 , respectively (Figure 1). A positive rate of 29.6% (n = 375) for SARS-CoV-2 was seen in 375 patients, with a median age of 55.4 ± 24.63 years. 10.2% (n = 35) of the SARS-CoV-2-positive patients were under 20, 41.5% (n = 142) were between 21 and 60 years old, and 48.2% (n = 165) were over 61. Compared to men (47.5%, n = 178), females had a greater prevalence of SARS-CoV-2 (52.5%, n = 197).

Influenza and COVID-19 co-infection were detected in 2.4% (n = 9) of the cases. According to influenza subtypes, in 375 COVID-19 RT-PCR-positive patients, the types of influenza in the form of co-infection were influenza A (1.9%, n = 7) and influenza B (0.5%, n = 2) was determined. Demographic and clinical information concerning the SARS-CoV-2 virus and co-infection with influenza virus are reported in Table 1.

Regarding comorbidities, the prevalence of diabetes was 18.9% (n = 71), hypertension 26.7% (n = 100), cancer 3.5% (n = 13), coronary artery bypass graft (CABG) 1.9% (n = 7), hyperlipidemia (HLP) 12.6% (n = 47), ischemic heart disease (IHD) 12% (n = 45), cerebrovascular accident (CVA) 5.6% (n = 21) and asthma 3.7% (n = 14) among positive COVID-19 patients. There was no significant association of co-infection with diabetes ($P = 0.202$), hypertension ($P = 0.530$), cancer ($P = 0.588$), and other diseases.

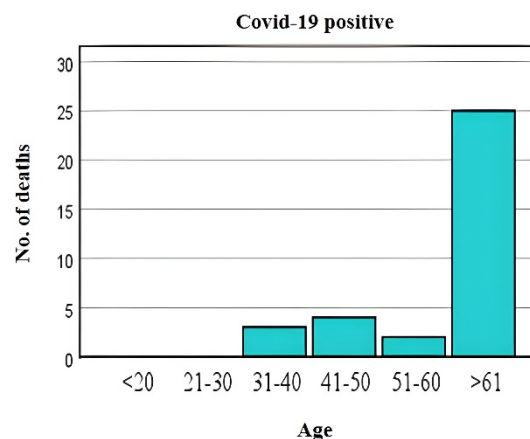


Figure 2. The mortality toll of Covid-19 positive patients by age

Table 1. Characteristics of the patients with COVID-19 and influenza co-infection

| Variable | Levels of variables | No. of patients (%) with only COVID-19 | No. of patients (%) with influenza and SARS-CoV-2 coinfection | P-value |
|-------------------------------|--|--|---|---------|
| No. of total (%) | | 366 (97.6%) | 9 (2.4%) | |
| Sex | | | | 0.218 |
| | Male | 173 (47.2%) | 5 (55.5%) | |
| | Female | 193 (52.7%) | 4 (44.4%) | |
| Age group | | | | |
| | <20 | 35 (9.2%) | 1 (11.11%) | |
| | 21-30 | 23 (6.1%) | 0 (0%) | |
| | 31-40 | 43 (11.3%) | 2 (22.22%) | |
| | 41-50 | 37 (9.7%) | 0 (0%) | |
| | 51-60 | 39 (10.3%) | 3 (33.33%) | |
| | >61 | 165 (43.4%) | 3 (33.33%) | |
| Influenza type | | | | |
| | A | - | 7 (77.7%) | |
| | B | - | 2 (22.2%) | |
| Comorbidities | | | | |
| | Cancer | 13 (3.5%) | 0 | 0.725 |
| | Diabetes | 68 (18.5%) | 3 (33.3%) | 0.500 |
| | Hypertension (HTN) | 97 (26.5%) | 3 (33.3%) | 0.775 |
| | Coronary artery bypass graft (CABG) | 6 (1.6%) | 1 (11.1%) | 0.061 |
| | Hyperkeratosis lenticularis perstans (HLP) | 46 (12.5%) | 1 (11.1%) | |
| | Ischemic heart disease (IHD) | 44 (12%) | 1 (11.1%) | 0.762 |
| | Cerebrovascular accident (CVA) | 21 (5.6%) | 0 | 0.590 |
| | Asthma | 14 (3.7%) | 0 | 0.706 |
| Blood oxygen saturation level | | | | |
| | The mean of the first SpO ₂ | 91.4 | 83.7 | 0.057 |
| | The mean of the second SpO ₂ | 95.2 | 94.2 | 0.592 |
| Medication | | | | |
| | Dexamethasone | 125 (34.1%) | 4 (44.4%) | 0.813 |
| | Remdesivir | 109 (29.7%) | 3 (33.3%) | 0.378 |
| In-hospital deaths | | 34 (9.2%) | 1 (11.1%) | 0.752 |

In addition, the mean of the first SpO₂ level in patients with positive COVID-19 and co-infected was 91.4 ± 9.58 and 83.7 ± 7.83 , respectively, and this difference was not significant.

Analysis of severity and treatment of patients with COVID-19 and coinfected with influenza

Among the positive COVID-19 patients, 35 people (9.3%) died, one of whom was coinfected with influenza and COVID-19. The mean age in dead patients was 68.46 ± 20.18 years, and in living patients was 54.09 ± 24.687 years ($P = 0.001$). Figure 2 shows that among the people who died due to COVID-19, most of them were 61 years old or older. In addition, among the people who died due to co-infection of COVID-19 and influenza, one person was older than 61 years.

An analysis of the relationships between death and co-infection with diabetes, cardiovascular disease, or chronic kidney disease (CKD) using the ANOVA test revealed (Table 1) that only the relationship between diabetes and death was statistically significant ($P = 0.020$).

Comparing the mean of first SpO₂ and second SpO₂ in people with only COVID-19 positive and people with co-infection showed that there is no significant difference in these two groups (respectively $P = 0.057$ and $P = 0.592$). However, the comparison between the COVID-19-positive patients in both survivor and deceased groups showed that there is a significant difference in the mean age, mean of first SpO₂, and second SpO₂ in these two groups (Table 2).

In addition, the relationship between first SpO₂ levels and co-infection with influenza viruses was investigated, and the findings showed that there is a negative correlation between co-infection with influenza in COVID-19-positive patients and first SpO₂ levels (Table 3).

The results of examining the relationship between the use of dexamethasone and remdesivir and the survival rate of patients (Pearson's correlation coefficient test) showed that there was no significant correlation between them. However, there was a negative correlation between remdesivir drug use and second SpO₂ levels in patients ($P = 0.011$, Pearson Correlation = -0.281) (Table 4).

Discussion

COVID-19 and influenza are both contagious respiratory diseases. The influenza virus can increase the severity of COVID-19 infection in the cold months of the year through damage to respiratory ciliated cells, which may cause an increase in hospitalization, disease symptoms and mortality rate (11, 12). Co-infection with COVID-19 and influenza can therefore represent a major risk to one's health. We aimed to study the epidemiology of COVID-19 and influenza co-infection in a cross-sectional study. COVID-19 and influenza co-infection were detected in 2.4% ($n = 9$) of the cases. Additionally, 1.9% and 0.5% of influenza A and B patients, respectively, were infected with the virus. Moreover, the relationship between first SpO₂ levels and co-infection with influenza viruses was investigated, and the findings showed that there is a negative correlation between co-

Table 2. Comparison of age average, first SpO₂, and second SpO₂ in dead and alive COVID-19-positive patients

| | Alive patients | Dead patients | P-value |
|---|-----------------|-----------------|---------|
| Age mean | 54.09 (±24.686) | 68.46 (±20.187) | 0.001 |
| The mean of the first SpO ₂ * | 92.15 (±8.981) | 84.23 (±11.664) | <0.001 |
| The mean of the second SpO ₂ * | 96.12 (±2.333) | 86.75 (±6.670) | 0.005 |

* First SpO₂ defines SpO₂ at the time of admission to the hospital and second SpO₂ is the patient SpO₂ at the time of discharge from the hospital.

Table 3. Mechanical ventilation used in people with positive COVID-19 and co-infection

| | Not Use | O ₂ therapy by mask 5_8 Lit/min | O ₂ therapy by reserve bag mask | O ₂ therapy by venturi mask | O ₂ therapy nasal cannula |
|------------------------|---------|--|--|--|--------------------------------------|
| COVID-19-positive | 330 | 16 | 13 | 5 | 2 |
| Influenza co-infection | 7 | 2 | 0 | 0 | 0 |

Table 4. Comparison of influenza and SARS-CoV-2 coinfection, Demographic data, and laboratory parameters

| | | Age | Sex | Cancer | Diabetes | Hypertension | First SpO ₂ | Second SpO ₂ | Survival status |
|-----------------------|-------------|--------|--------|--------|----------|--------------|------------------------|-------------------------|-----------------|
| Influenza coinfection | Pearson | -0.019 | -0.048 | 0.028 | 0.066 | -0.033 | -0.120* | -0.084 | -0.001 |
| | Correlation | | | | | | | | |
| | P-value | 0.720 | 0.354 | 0.588 | 0.202 | 0.530 | 0.038 | 0.453 | 0.981 |

The relationship between the use of dexamethasone and remdesivir and the survival rate of patients was investigated by Pearson's correlation coefficient test.

infection with influenza in COVID-19-positive patients and first SpO₂ levels. In Guan W et al. report from China, In 28 patients, 50.0% of patients were severe type and 14.3% died, which was higher than those reported in COVID-19 (13). Furthermore, a study from Northeastern Iran showed 22.3% of dead cases were co-infected with COVID-19 and influenza co-infection (14), indicating co-infection with COVID-19 and influenza co-infection may result in more severe conditions. These studies show that patients with COVID-19 and influenza co-infection developed more severe clinical conditions while having shorter hospital stays. In our opinion, the possible reason may be attributed to the timely use of effective antiviral drugs such as oseltamivir. In one study, 1.3% of COVID-19-positive patients also tested positive for influenza virus, which was lower than the results of our study (15). In the investigated studies, the rate of co-infection between COVID-19 and influenza has been observed to range from 0.24 to 44%. (6, 14, 16-20). This dispersion and difference can be due to the study population, the underlying conditions of the patients, and the method of confirming the infection as well as the time and place of the investigation in terms of the prevalence of Influenza. In this investigation, in patients with COVID-19, Hypertension (26.7%), diabetes mellitus (18.9%), cancer (3.5%), asthma (3.7%), and cardiovascular diseases were the most common underlying diseases, consistent with other recent reports (21-23). Previous studies reported 9% to 14% prevalence of diabetes in COVID-19 patients (21, 23-25). Here, we reported a higher prevalence rate of diabetes in these patients, which might be due to the larger proportion of geriatric COVID-19 patients in our study. In general, aged people are more susceptible to COVID-19 and more likely to be severe than people younger than 50 years; this may be due to more health issues and comorbidities in this population. Ding and colleagues (17) identified 5 COVID-19 and influenza co-infection patients out of 115 SARS-CoV-2 positive hospitalized patients. These 5 patients did not need ICU admission or IMV and all were discharged alive. On the other hand, Alosaimi and colleagues (26) identified 30 co-infected patients out of 48 hospitalized (14 ICU) SARS-CoV-2 positive patients and found

that influenza co-infection was associated with mortality.

In this study, the death rates were 9.3% for COVID-19-positive patients and 11.1% for COVID-19 and influenza co-infection patients. These findings demonstrated that there is no discernible link between the co-infection of COVID-19 and influenza and the patient death rate. According to research, COVID-19 individuals who had previously gotten an influenza vaccination had far decreased probabilities of both critical outcomes and death (27, 28). This might imply a potential competitive mechanism between COVID-19 and influenza like competitively bound to the receptors and thereby contributing to the reduction or block of SARSCoV-2 entry into lung cells. It is also possible that influenza vaccination could stimulate a short-term non-specific immune response that provides temporary protection against SARSCoV-2. Thirdly, a considerable reduction of cytokines among co-infection patients was detected in some studies, indicating that patients with co-infection might have a lower degree of hyper-inflammation and therefore lower risk for adverse outcomes (29).

In our study, there was no significant association of co-infection with diabetes ($P = 0.202$), hypertension ($P = 0.530$), cancer ($P = 0.588$), and other diseases. In addition, in this study, among the dead positive COVID-19 patients, 34.2% had Hypertension (HTN), 31.4% had Ischemic heart disease (IHD), 11.4% had diabetes, and 5.7% had cancer. Also, there was a patient with a co-infection of COVID-19 and Influenza who had IHD.

The study found that COVID-19 patients with diabetes were more likely to develop severe or critically ill subtypes, including more complications with Adult Respiratory Distress Syndrome (ARDS), and acute cardiac injury, resulting in receiving more antibiotic therapy and mechanical ventilation (30).

Furthermore, high blood pressure (hypertension) is one of the most common conditions in severe COVID-19 patients (31). In Ref. (32), systematic reviews show that hypertension is one of the major comorbidities of fatality in COVID-19 cases. Although, it should be noted that further studies of other comorbidities connected to hypertension are necessary.

Covid-19 problems are more common in cancer patients, according to published studies. It has been suggested by Liang et al. (33) that COVID-19 risk may be higher in cancer patients than in non-cancer patients. They also demonstrated worse COVID-19 outcomes for cancer patients. As a result of the COVID-19 infection, Zhang et al. (34) noted that cancer patients' circumstances are getting worse.

Also, regarding the relationship between heart diseases and death caused by COVID-19, ten observational studies have reported mortality data of acute influenza concurrent with cardiac abnormality upon presentation. Mortality in individuals hospitalized due to influenza or influenza-like illness ranged from 3.8 to 50% in these studies, except for one study by Chacko et al. (35) evaluating cardiac manifestations in patients with severe H1N1 virus during the 2009 pandemic, where a 92% overall mortality rate was observed. In contrast to acute Influenza, for the last 5 months, many (n = 16) observational studies have reported mortality data for patients with COVID-19 infection. The overall mortality in this patient cohort has ranged from 1.4 to 61.5%, which is higher than that observed in patients with acute Influenza.

The results of examining the relationship between the use of dexamethasone and remdesivir and the survival rate of patients showed that there was no significant correlation between them. However, there was a negative correlation between remdesivir drug use and second SpO₂ levels in patients. There was no significant relationship between the use of dexamethasone and the second level of SpO₂ in patients. The results of other studies have shown that among hospitalized patients with COVID-19 requiring supplemental oxygen at baseline, the use of remdesivir, compared to the best supportive care, is likely to improve the risk of mortality, recovery, and require oxygen assistance in patients receiving low-flow oxygen (LFO₂) and any additional oxygen (AnyO₂) (36).

Limitations

The following are some of the study's limitations: Firstly, the current study has a rather tiny sample size. Second, certain information was not recorded, which made it impossible for us to get precise and comprehensive clinical data on every patient. Furthermore, the absence of some data could have an impact on the study's findings. Third, we were unable to determine which patient had contracted which virus initially. In summary, our study's findings show that co-infection with SARS-CoV-2 and influenza was uncommon in Semnan City.

Conclusion

COVID-19 and influenza co-infection is predictable, especially during the cold season of the year. Because influenza viruses and COVID-19 share many similarities in terms of how they spread and clinical manifestations, we should investigate management strategies in the early diagnosis and treatment of these viral infections. We also encourage the rapid evaluation of patients presenting in respiratory distress to emergency departments for both SARS-

CoV-2 and influenza and, if co-infected, treatment with antiviral agents both for influenza A and B and for SARS-CoV-2. Future research is desperately needed to gather data on SARS-CoV-2 subtypes, influenza subtypes, and the sequence of infection in order to undertake stratified analysis and provide more specific information, given the ongoing co-circulation of these two viruses.

Authors' Contributions

H.GH and M.E designed and administrated the project. M.T and MS.SH wrote the manuscript. P.P, M.V and F.H collected the patient's data and samples. R.N and M.Z analyzed the data. All authors read and approved the final version of the manuscript.

Ethical Considerations

All procedures performed in this work followed the ethical standard of the committee of the Semnan University of Medical Sciences (Ethical Code: IR.SEMUMS.REC.1400.334).

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Conflict of Interests

The authors declare that they have no competing interests.

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