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Original Article

The Concurrent Identification of SARS-Cov-2 and Influenza A/B Viruses in Nasopharyngeal Swabs Using Multiplex Real-time PCR

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ABSTRACT

Introduction: Respiratory viral infections vary in their clinical presentation, treatment approaches and outcomes. Therefore, accurate, timely and cost-effective detection of key pathogens, particularly SARS-CoV-2 and influenza A/B, which are among the most common causes of respiratory infections, is crucial. This study aimed to identify the presence of COVID-19 and influenza A/B, as well as their co-occurrence, in hospitalized pediatric patients presenting with respiratory viral infections symptoms.

Materials & Methods: Nasopharyngeal specimens were collected from pediatric patients admitted to Mofid Children's Hospital in Tehran, Iran who exhibited symptoms of viral respiratory infections. Detection of SARS-CoV-2 and influenza A/B was achieved using multiplex real-time polymerase chain reaction (PCR) following total RNA extraction of samples. Data regarding symptoms and other pertinent information about the patients were collected via a questionnaire.

Results: A total of 2,353 hospitalized children in this study, ranging from under one year old to 18 years old. Of these 43% were female and 57% male. Fever was the most commonly reported symptom. The results of the multiplex real-time PCR were positive in 8% of cases, including 55% for COVID-19, 8.5% forinfluenza A, 26% for influenza B, and 10.5% for co-infections.

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Conclusion: The results of this study suggest a decline in seasonal influenza incidence compared to previous years, potentially due to the improved personal protection measures during to the COVID-19 pandemic. On the other hand, the presence of co-infection in this study is important and this co-infection should be considered in treatment and diagnostic systems in respiratory infection by physicians. Importantly, the presence of co-infections highlights the need for clinicians to consider dual pathogen involvement in diagnosis and treatment strategies for respiratory infections.

1. Introduction

The emergence of a novel strain of the human coronavirus, known as SARS-CoV-2, and the resulting COVID-19 pandemic have a profoundly impacted healthcare systems and communities worldwide [1, 2]. Given the transmission routes, clinical symptoms, and management strategies of COVID-19 and influenza, it is essential to establish targeted monitoring, testing, and therapeutic interventions for these respiratory diseases [3, 4]. Both viruses are primarily transmitted through respiratory droplets and close contact, making them highly contagious in community settings [4]. The World Health Organization (WHO) has emphasized the importance of maintaining robust influenza surveillance and management, even as global resources are heavily directed toward combating COVID-19 [3].

Influenza viruses remain among the most prevalent human pathogens because of their frequent seasonal occurrences and the persistent risk of a worldwide pandemic, with over 650,000 annual deaths attributed to influenza-related illnesses worldwide [5, 6]. Health officials within the government have implemented several measures to reduce the spread of COVID-19. These include educating the public on personal hygiene, enforcing limitations on social gatherings, and closing borders. These actions are intended not only to combat the spread of COVID-19 but also to potentially decrease the transmission of other contagious diseases. A recent study has demonstrated the effectiveness of facial masks in lowering the transmission of viral respiratory illnesses, underscoring the significance of these preventative measures in safeguarding public health [7]. However, during the COVID-19 pandemic, there was a surge in infections while influenza continued to spread. It seems that the actions taken by public health authorities to combat COVID-19 may have also contributed to reducing the transmission of the influenza in early 2020 [4, 8]. Furthermore, studying the medical and demographic attributes of influenza during the COVID-19 pandemic is vital. In addition, it is essential to explore the latest advancements in point-of-care molecular testing, which enable the simultaneous identification of both influenza and coronaviruses. Moreover, the development of innovative treatments for influenza remains crucial [9]. This study aimed to determine the frequency of COVID-19 and influenza semitonally in children presenting with pneumonia symptoms

2. Materials and Methods

2.1. Samples

The research was conducted from December 4, 2022, to August 23, 2023, in Iran. Nasopharyngeal swabs were collected from patients admitted to the Mofid Children's Hospital in Tehran, Iran, who were suspected of having COVID-19 and/or influenza from various hospital wards. The samples were transported to the Pediatric Infections Research Center (PIRC) using the standard viral transport medium (VTM) method. Patient demographic information was gathered through a data collection form after obtaining consent from the children's parents.

2.2. Total RNA extraction

Following sample preparation, total RNA extraction was performed using a commercial Viral Nucleic Acid (VNA) Extraction kit (SIMBIOLAB. Lot. No. 010502-2326-202N100), based on the silicon column method.

2.3. Real-time polymerase chain reaction (PCR)

Multiplex real-time PCR was used to identify COVID-19 and influenza A and B by detecting the *N* gene in coronavirus, and *M2* and *NS1* genes in influenza A and B, respectively. The Viga SARS-COV2 and influenza A/B multiplex real-time PCR kit (ROJE Technology. Lot No. 512623082310041) was employed. The one-step multiplex real-time PCR program consisted of: 50 ˚C for 20 minutes to cDNA synthesis followed by; 95 ˚C for one minute and 45 cycles of; 95 ˚C 10 seconds and 55 ˚C for 45 seconds. The process ended by 72 ˚C for 15 seconds.

2.4. Data analysis

Statistical analysis was performed using SPSS software, version 23. To determine the relationship between symptoms, gender and age with real- time PCR positive results for COVID-19 and/or influenza,the t-test was applied. A P≤0.05 was considered statistically significant.

3. Results

During the study period, a total of 2,353 hospitalized individuals who were included. The age of the patients ranged from under one year to 18 years old. Among all patients, 43% were female and 57% were male. The most common symptom observed was fever, which was associated with respiratory infections. A detailed frequency of symptoms is presented in Table 1.

Specimens were collected from various departments within the hospital, including the emergency room, infectious diseases unit, respiratory ward, pediatric intensive care unit (PICU), neonatal intensive care unit (NICU), and transplant ward. The emergency room and transplant ward accounted for the highest and lowest number of specimens, representing 27% and 0.1% of the total samples, respectively.

Real-time PCR were positive in 303 cases (12.8%), including 167(55%) COVID-19, 26(8.5%) influenza A, 78(26%) influenza B and 32(10.5%) for different co-infections. Among the co-infections; 28 cases (87.5%) involved COVID-19 and influenza B, 2 cases (6.25%) involved influenza A and B. All co-infections were observed in children under 11 years, with 66% occurring in children under 6 years old. The seasonal distribution of co-infections is illustrated in Figure 1. Symptoms such as fever and cough were more frequently observed in co-infection patients; however, the difference was not statistically significant (P>0.05).

4. Discussion

According to global clinical monitoring, the incidence of influenza and respiratory syncytial virus (RSV) decreased during the COVID-19 pandemic [10-12].

In a study conducted by Pourmomen et al. in 2023, researchers found that patients presenting with symptoms of viral respiratory tract infection, the rates of positive real-time PCR tests for COVID-19 and influenza A/B were 10.5% and 11%, respectively [13]. These results exceed those of the present study, which may be attributed to differences in the populations studied. Pourmomen et al. included participants across all age groups, [13] whereas the present study focused exclusively on children. Conversely, the prevalence of influenza B reported in their study [13] was lower than that of the present study. This variation could be due to seasonal differences in the timing of the studies. Notably, in the current study, conducted during spring and summer, the incidence of influenza B was unexpectedly higher than that of influenza A. Numerous studies have shown that the influenza A virus is generally more widespread than other strains of the influenza virus [13-15]. Influenza B typically accounts for approximately 25% of the annual influenza cases [13, 16]. Influenza B cause fewer and less severe epidemics, possibly because they mainly circulate among humans without a significant animal reservoir [17]. Importantly, the data in this study pertain to individuals vaccinated against COVID-19. Previous research on seasonal influenza have consistently shown a greater prevalence of influenza A compared to influenza

Table 1. Frequency of symptoms

Symptoms	No. (%)
Fever	20
Cough	12
Respiratory distress	11
Seizure	7
Diarrhea	5
Vomiting	3
Abdominal pain	2
Others such as; chest pain, imbalance and headache, decreased level of consciousness	40

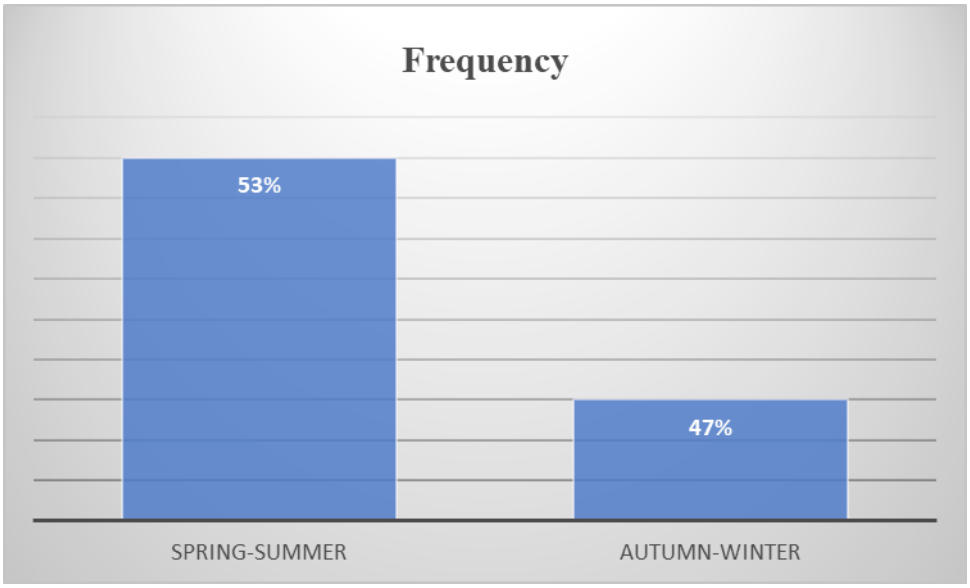

Figure 1. Frequency of co-infection in different seasons

B, especially during the pandemic and among individuals affected by COVID-19 or receiving the vaccines [13, 16, 17]. Interestingly, the current study observed a reversal of this trend, with influenza B being more prevalent than influenza A, contrary to earlier findings.

Further research is needed to understand the impact of the COVID-19 pandemic and associated factors on such trends. It appears that certain nonpharmaceutical interventions (NPIs) implemented to control COVID-19 have also been effective in reducing influenza epidemics. Data suggest a significant decline in influenza cases following the enforcement of public health strategies aimed at controlling COVID-19. On the other hand, the results of this study showed that the symptoms of COVID-19 and influenza are not significantly different, making it difficult to distinguish between the two diseases based solely on clinical symptoms. Additionally, viral co-infections were observed, particularly during spring and summer. Co-infection with COVID-19 and influenza A virus was reported in Iran, 2020 [18]. A systematic reviewand meta-analysis documented 51 cases of COVID-19 and influenza B co-infection in other countries [19]. Another study reported 0.5% co-infection rate of COVID-19 and influenza B in Iran in 2024 [20], whereas our study found a significantly higher rate of 9.2%. Since the highest frequency of influenza B virus was observed during spring and summer, and this virus also accounted for the highest share in co-infections, therefore, physicians and health policymakers should take into consideration the increased prevalence of influenza B virus following COVID-19 vaccination.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (Code: IR.SBMU.RICH.REC.1402.023). Informed consent was obtained from the parents of all participating children.

Data availability

The data presented in this study are available upon reasonable request from the corresponding author.

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Authors' contributions

Study design: Leila Azimi; Data collection: Hannan Khodaei, Masoud Alebouyeh, Nafiseh Abdollahi; Seyed Hossein Ardehali, and Mohammad Ehsan Yari; Writing: Leila Azimi and Hirad Rokni; Clinical consultation: Abdollah Karimi; Supervision: Fatemeh Fallah and Shahnaz Armin.

Conflict of interest

The authors declared no conflict of interest.

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