The concurrent identification of SARS-CoV-2 and influenza A/B viruses in nasopharyngeal swabs using multiplex Real-Time PCR

Running Title: Simultaneous detection of SARS-CoV-2 and influenza A/B virus

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Abstract

Respiratory viral infections can exhibit variability in their treatment approaches and outcomes. Therefore, accurate, timely and cost-effective detection of viruses such as SARS-CoV-2 and influenza A/B concurrently as a two important cause of the respiratory infections is crucial. This study aimed to identify the COVID-19 and influenza A/B and also, co-occurrence of them in hospitalized pediatric patients presenting with respiratory viral infections symptoms.

In this research, specimens were obtained from the nasopharyngeal of hospitalized patients in Mofid Children hospital, Tehran, Iran showing symptoms of viral respiratory infections. The detection of SARS-CoV-2 and influenza A/B viruses was achieved using multiplex Real-Time PCR after total RNA extraction of samples. Data regarding symptoms and other pertinent information about the patients were collected via a questionnaire.

During this research, a total of 2,353 patients who were hospitalized children participated. Their ages ranged from under one year old to 18 years old, with females accounting for 43% and males accounting for 57%. The most commonly reported symptom among these patients was fever. The results of the multiplex Real-Time PCR were positive in 8% of cases including; 55% COVID-19, 8.5% influenza A, 26% influenza B, and 10.5% co-infections.

The results of this study showed that the frequency of seasonal influenza has decreased compared to previous years, which could be due to the improvement of personal protection due 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.

Key words: COVID-19, influenza, co-infection, multiplex Real- Time PCR

1. Introduction

The emergence of a novel strain of the human coronavirus, known as SARS-CoV-2, and the ensuing COVID-19 pandemic, is having a profound impact on healthcare systems and communities globally (1, 2). With the shared characteristics in the transmission, symptoms, and management of COVID-19 and influenza, it is essential to prioritize the establishment of targeted monitoring, testing, and medical interventions for these respiratory diseases. (3, 4). Furthermore, the transmission of the influenza virus mirrors that of COVID-19 in that it is commonly spread through proximity and inhaling respiratory droplets suspended in the air. (4). The importance of maintaining high standards in the management of influenza cases has been emphasized by the World Health Organization (WHO), particularly as resources and attention are currently being diverted to address the COVID-19 pandemic. (3).

Conversely, influenza viruses are recognized as one of the prevailing human pathogens because of their frequent seasonal occurrences and the persistent risk of a worldwide pandemic. Annually, more than 650,000 people succumb to illnesses linked to seasonal influenza across

the globe. (5, 6). Health officials within the government have put in place several measures to reduce the spread of COVID-19. This includes educating the public on personal hygiene, enforcing limitations on social gatherings, and shutting down borders. These actions are not only intended to combat the spread of COVID-19 but also to potentially decrease the transmission of other contagious diseases. A recent study has shown 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 the influenza continued to spread. It seems that the actions implemented by public health authorities to combat COVID-19 may have also played a role in 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 delve into the latest advancements in point-of-care molecular testing, enabling the simultaneous identification of both influenza and coronaviruses. Moreover, the development of innovative treatments for influenza is crucial. (9). This study aimed to determine the frequency of COVID-19 and influenza semitonally in children 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 and suspected of having COVID-19 and/or influenza from various hospital wards. The samples were transported to the Pediatric Infections Research Center (PIRC) by using the standard method of viral transport medium (VTM). Patient demographic information

was collected through an information form after obtaining permission from the parents of the children.

2-2. Total RNA extraction

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

2-3. Real-Time PCR

Multiplex Real-Time PCR was used for COVID-19 and influenza A and B identification by detection the N gene in corona Virus, M2 and NS1 in influenza A and B, respectively by Viga SARS-COV2 and Influenza A/B multiplex Real-Time PCR kit (ROJE Technology. Lot No. 512623082310041). The one-step multiplex Real-Time PCR program was; 50^{oC} for 20 minutes to cDNA synthesis followed by; 95^{oC} for one minute and 45 cycles of; 95^{oC} 10 seconds and 55^{oC} for 45 seconds. The process ended by 72^{oC} for 15 seconds.

2-4. Data analysis

Statistical analysis was done by SPSS software version 23. To determine the relationship between symptoms, gender and age, Real- Time positive results for COVID-19 and/or influenza. The t-test was used. A p value ≤ 0.05 was

3. Results

In the duration of this study, a total of 2,353 individuals who were hospitalized were included in the study. The age of the patients ranged from under one year to 18 years old. Of all the patients, 43% were female and 57% were male. The most common symptom observed in patients was fever, which was linked to respiratory infections. The detailed frequency of symptoms can be found in Table 1.

Table1. frequency of symptoms

Symptoms	Frequency (%)
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

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 had the highest and lowest number of specimens, accounting for 27% and 0.1% of the total samples, respectively.

The results of the Real- Time PCR were positive in 303 (12.8%) cases that include; 167 (55%) COVID-19, 26 (8.5%) influenza A, 78 (26%) influenza B and 32 (10.5%) different co-infections. The co-infections were; 28 (87.5%) COVID-19 and influenza B, 2 (6.25%) COVID-19 and influenza A and 2 (6.25%) influenza A and B. All co-infections were observed in under 11 years old children and the 66% of them under 6 years old. Also, frequency of co-infections in different seasons shows in figure 1. Also, the symptoms like fever and cough were more observed in co-infection but it did not significant. (p value >0.05).

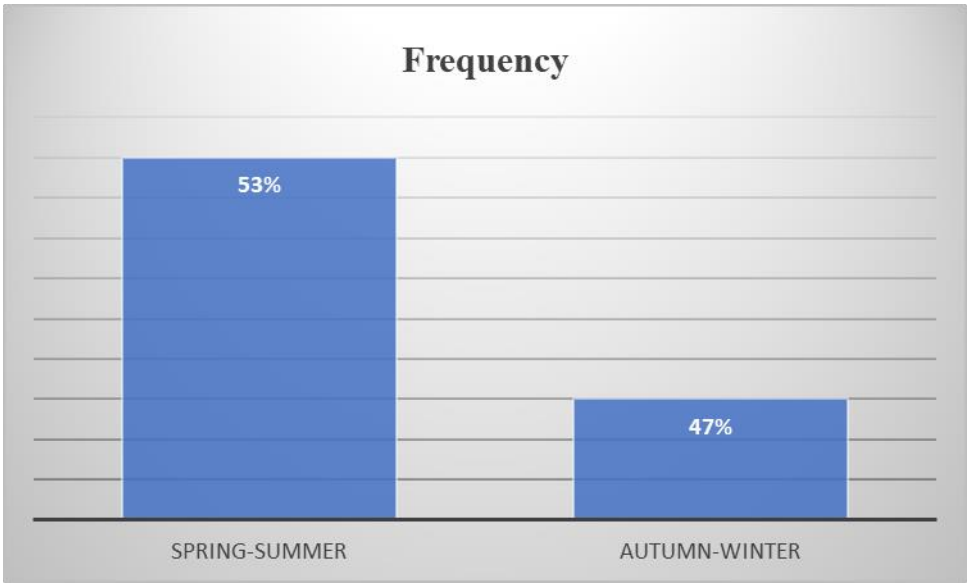

figure1. Frequency of co-infection in different seasons

4. Discussion

According to worldwide clinical monitoring, the happening of influenza and respiratory syncytial virus (RSV) decreased during the COVID-19 pandemic (10-12)**.**

In a study conducted by Pourmomen et al., and colleagues in 2023, researchers found that among patients showing 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). The results obtained exceed our own, which may be linked to the population under investigation. The research conducted by Pourmomen et al., included participants across all age groups (13) however, the present study focused solely on children. Conversely, the prevalence of influenza B reported in that study (13) is less than that of the present study. This variation could be attributed to the different seasons during which the studies were conducted.

It is noteworthy that in the current study, conducted during the spring and summer, the incidence of influenza B was surprisingly higher than that of influenza A.

Numerous research studies have shown that the influenza A virus is more widespread compared to other strains of the influenza virus (13-15) The influenza B virus contributes to approximately 25% of the annual influenza cases (13, 16). Influenza B viruses cause fewer and less severe epidemics, possibly because they mainly circulate among humans without a significant animal reservoir (17). Naturally, it is important to highlight that the data in this research pertains to individuals who have been vaccinated against COVID-19. Previous studies on seasonal influenza have shown a greater occurrence of influenza A compared to influenza B, especially in the context of the pandemic and individuals dealing with COVID-19 or receiving the vaccines (13, 16, 17). It is interesting to observe that following these events, there has been a shift in the prevalence of influenza type B being higher than type A in the current study, which is contrary to earlier findings. However, additional research is required 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 proven to be effective in reducing influenza epidemics as well. The data suggests a significant decrease in the number of 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, and no distinction can be made between the two diseases based on clinical symptoms. In addition, co-infection by viruses can exist according to the results of this study especially in spring and summer. Some Co-infection with COVID-19 and influenza A virus was reported in Iran, 2020 (18). In a systematic review/meta-analysis, COVID-19 and Influenza B co-infection were reported in 51 patients in other countries (19). A study reported 0.5% co-infection of COVID-19 and influenza B in Iran, 2024 (20) but this frequency increased in our study to 9.2%. Since

the highest frequency of influenza B virus was in spring and summer and this virus also had the highest share in co-infections, therefore, physicians and health policymakers should consider this increase of influenza B virus after COVID-19 vaccination.

5. Acknowledgment

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6. Authors' contributions

H.Kh, M.A and N.A; laboratory data collection.

S.H.A. data gathering.

M.E.Y. data extraction from record.

F.F. and Sh.A. supervised the study.

L.A. designed the study and prepare the manuscript.

A.K. clinical consult.

H.R. improve the manuscript.

7. Ethics approval and consent to participate

The ethical code of this study is IR.SBMU.RICH.REC.1402.023, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Also, the consent was approved by parents of the children.

8. Availability of data and materials

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

9. Competing interests

This study does not have any conflict of interest.

10. Funding

11. This study does not have any conflict of interest.**References**

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