



Screening of Influenza Virus A and B among COVID-19 Patients in a Tertiary Care Hospital, Southern India

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ABSTRACT

Background: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and influenza virus are quite distant viruses, but they share significant similarities such as mode of transmission and clinical manifestations. No specific clinical signs reliably distinguish early influenza sickness from the disease caused by SARS-CoV-2 (COVID-19); therefore, it will be critical in clinical practice to determine the viral etiology. The present study aimed to screen for influenza virus A and B among COVID-19 patients by quantitative reverse transcription polymerase chain reaction (RT-qPCR).

Materials & Methods: A total of 100 nasal swabs from COVID-19 patients were collected in viral transport medium (VTM) during June to July 2022. RNA extraction was done using QIAGEN RNA extraction kit, and then RT-qPCR was performed using HELINI swine flu (H1N1) kit.

Findings: The average age of the study participants was 31 years, and 13 patients were hospitalized due to the COVID infection. Hypertension, diabetes, and chronic lung, heart and kidney diseases were identified as comorbidities. It was found that none of the tested samples were positive for influenza A and B.

Conclusion: Although none of the patients were positive for influenza, the importance of co-infection could not be ignored. Screening of a large number of samples is needed during the seasonal period.

Keywords: Acute respiratory distress syndrome (ARDS), Co-infection, COVID-19, Influenza A and B, RT-qPCR.

CITATION LINKS

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Introduction

A number of viruses affect the human respiratory tract, leading to various manifestations ranging from mild to potentially fatal acute respiratory distress syndrome (ARDS). Prevention and management of this group of viruses is difficult due to their high transmission rates and ability to rapidly change to new forms that may cause epidemics and pandemics. Largely due to international travels, these viruses are able to quickly spread to any region of the globe. The pandemics of H1N1 influenza virus in 2009 and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in 2020 had a great impact on public health care, leading to increased morbidity and mortality. Direct, indirect, or close contact with an individual with SARS-CoV-2 infection (called as COVID-19) could lead to the transmission of the virus, which has the ability to cause pneumonia. Adults are more prone to this infection compared to children. Symptoms related to lower respiratory tract infection are commonly observed in infected individuals; an excessive inflammatory response results in an abnormal cytokine pattern that leads to tissue damage ending in ARDS. Multi-organ failure is observed in severe COVID-19 patients [1, 2]. Influenza virus similar to SARS-CoV-2 is mostly transmitted through direct contact and primarily via respiratory droplets. Common clinical symptoms of both disorders include headache, myalgia, dyspnea, rhinitis, fever, and cough. Although influenza virus infection is a self-limiting illness, some affected individuals develop severe complications [3]. To effectively differentiate early influenza illness from COVID-19 in clinical practice, there are no precise clinical symptoms that could be relied upon. This is more complicated because there are a few recorded cases of influenza with SARS-CoV-2 infection. The potential of COVID-19 coinfection with other respiratory infections impedes accurate diagnosis, care, and prognosis. These co-infections could po-

tentially worsen the disease and increase the fatality rate. Some viral infections that primarily affect respiratory ciliated cells may facilitate SARS-CoV-2 infection [4].

Correct and prompt diagnosis is essential for patients with respiratory diseases to receive the best care. Early diagnosis of influenza could minimize the need for unnecessary antibiotic treatment and give the option of antiviral medications. Circulation of SARS-CoV-2 during the pandemic may result in under reporting of other infections that might be etiological agents contributing to the disease severity. Influenza virus infections are frequently misdiagnosed as bacterial infections because of their non-specific clinical appearance and lack of rapid and definitive diagnosis [5].

During the pandemic, the World Health Organization (WHO) recommended influenza virus testing for patients who tested positive for SARS CoV-2; however, due to the increasing number of cases and high demand for medical services, only COVID-19 infection was tested using real-time polymerase chain reaction (RT-PCR). Understanding the coinfection of multiple respiratory viruses could help in different clinical aspects, including selecting the best therapeutic approach and infection control. In order to test for both influenza and COVID-19 concurrently, the WHO has designed a surveillance program called the Global Influenza Surveillance and Response system (GISRS), and the Indian Council of Medical Research (ICMR) has established a surveillance network at 22 viral research and diagnostic laboratories (VRDL) in India. However, the interaction between these viruses is not fully understood [6]. Although various drugs are under investigation for COVID-19, there are currently no drugs licensed despite the fact that treatments for influenza are available. Influenza infection in COVID-19 patients is reported sporadically worldwide, and India has few case reports of this coinfection. Patients with influenza and COVID-19 co-infection may experience identi-

cal symptoms. Identifying co-infections is important since some of them could be treated with antiviral drugs.

Objectives: Hence, the present study aimed to screen influenza (Inf) A and B virus among COVID-19 patients by RT-qPCR.

Materials and Methods

Standard precautions were followed during sample collection and processing. Institutional human ethical clearance was obtained (IHEC-I/1031/22). In this study, 100 nasal swabs from COVID-19 patients were collected in viral transport medium (VTM) during June to July 2022. Patients' characteristics (demographic, clinical variables and vaccination history) were collected in the information sheet, and consent forms were also obtained from the study participants.

RNA extraction was done according to QIAamp viral RNA kit procedure (Qiagen Inc, Hilden, Germany). Quantitative reverse transcription polymerase chain reaction (RT-qPCR) was performed to detect influenza A and B according to the kit procedure using Qiagen real-time PCR cycler. For the assay, the HELINI swine flu (H1N1) two-tube assay real-time PCR kit was used, which has Inf A, B, and H1N1/H3N2 universal primers and probes for specific identification.

The kit consists of a one-step RT-PCR master mix and RT taq enzyme in a 1.5 mL Eppendorf centrifuge tube, to which a mixture consisting of primer and probe mix was added in order to prepare a reaction mix. This is a two-tube assay, the master mix was prepared separately for H1N1 and Inf-B/H3N2. Each set had a negative control whereby 5 µL of nuclease-free water, non-template control (NTC), was added in one well. The extracted RNA samples obtained earlier from each sample were then pipetted in to their respective target wells. After adding RNA, the wells were immediately capped. Then 5 µL of positive control available in the kit was added immediately and capped.

Human RNase P gene was used for endogenous control.

PCR tubes were placed in the real time PCR system, and RT-qPCR was performed. Reverse transcription and amplifications were done under the following conditions: reverse transcriptase at -50 °C for 20 min and taq enzyme activation at - 95 °C for 15 min, followed by denaturation at 95 °C for 20 s, annealing at 56 °C for 20 s, and extension at 72 °C for 20 s in 42 cycles. The kit contains primers and probes specific for the following Influenza A and B strains.

- H1N1 [H1N1-2009pdm - California/2009, H1N1-2009pdm-NY/2009, globally reported human infected H1N1 strains in the year of 2010/11/12/13/14/15/16/17/18/19].
- H3N2- A/Fujian/411/2002(H3N2)
- Influenza B Victoria & Yamagata lineage strains

Interpretation of obtained data

The cycle threshold (Ct) value of the reaction curves for RNase P must be within the range of Ct: 23 +/- 9, indicating the presence of sufficient RNA from human sample and considered acceptable quality as per kit. A non-template control (NTC) reaction was considered valid if no amplification was observed for Influenza-A/H1N1/H3N2 and Influenza-B. For the assay to be valid, the positive control had to be amplified. To observe amplification, each test sample well was chosen individually. A sample was considered positive if the reaction growth curve crossed the cycle threshold when selecting specific fluorescent dyes in the PCR amplification. A sample was considered negative for influenza virus if there was no growth curve or reaction growth curve for Influenza-A/H1N1/H3N2 and Influenza-B after 40 cycles of PCR amplification.

Findings

In this study, 100 nasal swabs from COVID-19 patients were collected in VTM and screened for influenza A and B virus. The mean age of

the studied patients was 32 years (interquartile range: 16), and there were 62 male patients and 38 female patients. Of all patients, 13 cases were hospitalized for 2-4 days and discharged without any complications. Most patients had typical COVID-19 symptoms including fever, cough, myalgia, and breathlessness, regardless of whether they were hospitalized or not, which were also similar to influenza symptoms. Despite the presence of several comorbidities in one patient, diabetes, asthma, chronic obstructive pulmonary disease (COPD), renal disease, and heart disease were observed in certain patients in this study (Table 1). None of the tested samples were positive for Inf A and B virus in RT-qPCR test. The amplification plot was observed for positive controls while no amplification was observed for NTC and tested samples.

Table 1) Patient demographic details and clinical characteristics that includes the information related to 100 COVID-19 patients enrolled in this study

Patient Characteristics	All (n=100 patients)
Age	Median age:32
Sex	F/M: 38/62
Symptoms	
Fever	83
Cough	77
Myalgia	94
Headache	71
Comorbidities	
Hypertension	8
Diabetes	8
Asthma	4
Chronic kidney disease	1
Chronic heart disease	5
Chronic lung disease	17
History of vaccination	
Covishield	61
Covaxin	18
No vaccination	21
COVID-19 patients	100

Discussion

Co-infection usually increases the virus virulence, cell death, course of the disease, and the severity of symptoms. However, few studies have shown improved clinical outcomes. Conflicting findings on disease outcomes imply a complicated mechanism underlying how co-infection affects clinical outcomes. Patients with influenza and COVID-19 co-infection may experience identical symptoms. Recognizing co-infections is crucial since influenza could be treated with antiviral drugs. It is challenging to determine the effect of COVID-19 and influenza on intensive care unit (ICU) admission and death due to their comparable clinical presentations [7]. Given the widespread prevalence of influenza viruses, this study emphasized the importance of screening COVID-19 patients for co-infection with other viruses.

Implementation of COVID-19 prevention strategies has been linked to reduced influenza activity during 2020 flu season, including using mask, following hand hygiene, and social distancing. None of the tested samples in the current study were positive for influenza A and B. Additionally, numerous instances of COVID-19 and influenza co-infections have been reported worldwide [8-12]. Even though India experiences influenza throughout the year, it primarily strikes during the winter, also known as the flu season. The efficiency of the vaccine in this season, the percentage of the population receiving the vaccine, the kind of influenza strain, the features of other circulating viruses, and the length of the season all affect the illness burden. Sporadic case reports of influenza infection in India are available [13-16]. A sentinel surveillance study conducted by Aggarwal et al. (2022) in India during July 2021 to January 2022 reported 5 out of 13,467 (0.04%) samples as positive for influenza co-infection with COVID-19 [17].

The results in this study have some restrictions. First, despite the fact that the consis-

tency of results across numerous centers is compelling, an ecological analysis could not prove causality. Second, other variables that may contribute to reduced influenza transmission were not examined, such as the sharp decline in international travels or the rise in vaccination rates. Third, the viral interference may account for the absence of influenza during a pandemic. Fourth, it's possible that the drops were just the result of the influenza season ending naturally. However, the drop percentage modification suggest that more forces are at work. It is challenging to differentiate the potential impact of specific community mitigation strategies on the seasonal influenza outbreak. School-going children are a major contributor to the spread of influenza infection, it is unclear if school closures were helpful on their own. Since the result of each influenza test was reported separately, the impact of the interaction and activity of the viruses could not be assessed.

Hospitalization rates for patients with dual viral respiratory infections were higher than for patients with single viral respiratory infections, indicating a potential rise in morbidity due to coinfection. Both mono-infected and co-infected patients had comparable mortality rates, lengths of hospital stay, and needs for ICU-level treatment; however, those with co-infection were more prone to develop complications. The variation in influenza positivity rate may be caused by a variety of sources ^[18]. The sample size of this study needs to be further enlarged to make the results more convincing. The study samples were collected during June to July, and the COVID-19 transmission was at a low level during this period, seasonal flu starts later and peaks from September to January in India. As COVID-19 prevention measures are currently being progressively eased in India, an upsurge in influenza circulation is anticipated.

The noted decline in influenza virus circu-

lation might be due to mitigation measures followed during the COVID-19 pandemic. The spread of influenza virus has to be monitored when the community mitigation measures are eased. If these measures could reduce the activity of influenza virus, they should be used to control seasonal flu outbreaks and reduce transmission rates ^[19]. Planning prior to the season is important, and vaccination is important to prevent the infection.

In high-risk groups, influenza vaccination is important to reduce the risk of co-infection with COVID-19. Increased vaccine uptake may make it easier to manage respiratory outbreaks that occur during the peak influenza season, and in particular may address the lack of identification resources. A better understanding of the difference between COVID-19 and influenza in epidemiology is necessary to manage these illnesses. A mathematical model was developed to assess the dynamics of co-infection under various categories of influenza vaccine coverage, efficacy and coverage of COVID-19 booster, and testing capacity ^[20]. Initially, influenza testing was declined because of increased SAR-CoV-2 detection rates. In the event that influenza virus and SARS-CoV-2 co-circulate, circulating influenza strains used in vaccination may also assist in reducing influenza epidemics. In addition to taking the recommended daily preventive measures, doctors should promote influenza vaccine to all individuals aged 6 months when schools and workplaces reopen.

Rarely, COVID-19 and influenza co-infect. Some cases have been reported in screening trials, suggesting that coinfection is still underdiagnosed and underreported unless individuals are screened for both. Growing expertise in thoracic radiology helps narrow the clinical diagnosis, which may help identify viral etiologic agents. Influenza vaccination before the start of the flu season is recommended for larger population groups.

Conclusion

In conclusion, influenza was not detected in any of the tested COVID-19 patients. However, in the future, co-infection rates are likely to rise despite continuous immunization because it takes time to vaccinate a sufficient fraction of the population during the flu season; in addition, restrictions on COVID-19 are gradually being eased in many regions of India. High-risk patients should be screened for seasonal flu because there are particular treatment options for influenza, and influenza testing is generally accessible.

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Authors' contributions: Ms. Sushmitha Anand: conceptualization, methodology, investigation, data curation; Dr. Lavanya Mohanam: conceptualization, visualization, methodology, writing, validation; Dr. Priyadarshini Shanmugam: supervision.

Conflicts of interests: None.

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Consent to participate: This study included sampling of human participants, and the procedures performed in this study were in accordance with the guidelines. Consent forms were obtained from the study participants.

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