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Original Research Article

The impact of COVID-19 pandemic on seasonal influenza virus

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ABSTRACT

Background: The COVID-19 pandemic and subsequent implementation of non pharmaceutical interventions reduced transmission of Influenza viruses. The WHO has also noticed a drop-in influenza virus cases; however, it hasn't been precisely characterized. The prospect of concurrent influenza and COVID-19 outbreaks is a major concern for public health professionals. Therefore, in this study, we present our limited data on seasonal influenza activity during COVID -19 period (November 2021–October 2022).

Materials and Methods: A retrospective analysis was conducted in 100 positive and 100 negative COVID-19 cases to look for co-infection with Influenza viruses. Influenza screening was performed with multiplex real-time RT-PCR using standardized techniques for Influenza H1N1/H3N2 with Inf-B on archived respiratory samples.

Results: A total of 8% Influenza cases were found with coinfection in only 05 cases denoting a drop of influenza infection during this period. Influenza A(H1N1) was the most common influenza virus followed by Influenza B (lineage not determined).

Conclusion: The clinical outcomes of co-infections could not be anticipated and may worsen if novel COVID-19 variants continue to appear. The use of community mitigation measures for the COVID-19 pandemic, are likely to be effective in reducing the incidence of seasonal influenza.

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1. Introduction

The SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2) is still causing an unprecedented worldwide epidemic of COVID-19.¹ SARS-CoV-2 was 1st discovered in China in the 2019-20 influenza season in the northern hemisphere. As a result, there was an early worldwide drop-in influenza virus activity.² The decrease in influenza activity during COVID-19 pandemic varied by location. There was either no seasonal influenza activity seen in worldwide or only few cases were recorded. A wide

variety of signs, from minor cold-like symptoms to complex pneumonia, a severe inflammatory response, and death, could result from infection with the novel pandemic disease SARS-CoV2.³ Limited data on concurrent influenza virus infections have been reported in the form of a few case reports. The drop in the incidence of influenza and other respiratory viruses was mostly linked to NPIs (Non-Pharmaceutical Interventions) and IPC (Infection Prevention and Control) measures put in place to decrease the impact of SARS-CoV-2 and its transmission.⁴ The WHO has also noticed a drop-in influenza virus activity; however, it hasn't been precisely characterized or reported.

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The prospect of concurrent influenza and COVID-19 outbreaks is a major concern for public health professionals and doctors. Therefore, in this study, we present our limited data on seasonal influenza activity during COVID-19 period (November 2021- October 2022).

2. Materials and Methods

2.1. Study population

From November 2021 to October 2022, corresponding to the time of the COVID-19 epidemic wave, archived respiratory specimens from suspected cases of acute respiratory infection that tested positive for COVID-19 by standard real-time RT-PCR (“Reverse-Transcription Polymerase Chain Reaction”) assay were utilized. To maintain anonymity, the samples were deidentified and were serially numbered before testing. For comparison and control, negative samples for COVID-19 during this same period were also selected. After excluding patients with missing clinical data or less sample volume, 200 samples tested for COVID-19 between November 2021 to October 2022 were included in this study. A total of 100 samples negative for COVID-19 in patients with ARI (“Acute Respiratory Infection”) were used as the comparison controls.

2.2. Testing process and analysis

Influenza screening was performed with multiplex real-time RT-PCR using standardized techniques for Influenza H1N1/H3N2 with Inf-B on archived respiratory samples. RNA was extracted with MB615 HiPurA® Viral RNA Purification Kit and was measured by real-time RT-PCR [CFX96 Touch™, Bio-Rad] for detection of Influenza by using multiplex TRUPCR H1N1/H3N2 using Inf-B kit type 2.0. The term “threshold” refers to the key fluorescence signal values during the exponential development phase. Cut-off threshold (Ct) values describe the number of cycles needed for the fluorescence signal to reach the threshold. A value of Ct < 38 has been considered positive, and values of Ct >38 were considered negative. Lower values of Ct < 30 referred to greater viral load for SARS-COV-2, while for Influenza CT-values less than 32 were defined as positive, and >32 were defined as negative.

2.3. Clinical data collection

We studied written and electronic medical records for every specimen. The electronic medical records have been studied from specimen referral forms for 2019 n-CoV (SARS COV-2) from ICMR (“Indian Council of Medical Research”). Data gathered included co-morbid illnesses, demographic details, presenting signs and symptoms, ICU (Intensive Care Unit) admission, and stay length of hospital. Documentation of any adverse outcomes was taken from medical records.

The existence of pulmonary infiltrates on chest imaging was used to identify pneumonia. A hospital stay longer than 20 days was deemed to be a prolonged hospitalization. COVID-19 and Co-infection with H1N1 were expressed as positive nucleic acid detection in the same sample for both viruses at admission.

3. Results

A total of 100 SARS-CoV-2 negative and positive samples have been screened for the presence of the Influenza virus. 16 (8%) samples tested positive for Influenza RNA from 200 screened samples. Out of 100 COVID-19 positive samples, 05 samples were having co-infection with Influenza (5%). 02 samples had Influenza A (H1N1), 01 samples showed co-infection of Influenza A with Influenza B, and the other 2 have been co-infected with Influenza B (Lineage not determined). Out of 100 Covid 19 negative samples, 11 samples were flagged positive for Influenza infection (11%). Above influenza infections were distributed into the following lineages, 03 Influenza A(H1N1), 03 Influenza A(H3N2), 01 Influenza A(H1N1) pdm09, 02 Influenza B (Lineage not determined), and 02 coinfection of Influenza A(H3N2) along with Influenza B (Lineage not determined) respectively.

CT values of COVID-19 positive samples, coinfecting with Influenza were of lower range [Median CT value 25.5 (21-30)] while in case of mono-infection of SARS COV-2 CT values varied [Median CT Value 27 (18-36)]. This probably indicated high viral load in co-infected patients, though an association of CT values could not be established with the severity of disease in the patients.

Among the Influenza coinfecting and SARS COV-2 patients, all 05 patients were ≥ 40 (80%) years of age except one patient who was a 15-year-old female (20%). Out of these five patients, 03 patients were hospitalized and two were OPD patients. Two admitted patients were coinfecting with Influenza A and one patient was coinfecting with Influenza A(H1N1) and influenza B. All 3 were immunocompromised patients two of whom were elderly patients having both diabetes mellitus and hypertension, while one patient was suffering from hypertension only. The duration of hospital stay of all three patients was more than 2 weeks due to pneumonia with multilobar infiltrates, secondary bacterial infection, and acute kidney injury respectively. Influenza B was detected in the other two co-infected OPD patients. They were home isolated and had complaints of fever, cough, fatigue, myalgia, headache, sore throat, and diarrhoea but no complications. (Table 1)

In patients with Influenza alone, 07 patients were admitted to ICU while 04 patients were from OPD. 04 inpatients suffering from influenza H1N1 had ARDS with severe complications, morbidity was high and length of hospital stay was longer. Influenza A (H3N2) was detected in the other three admitted patients, all having an

Table 1: Clinical features of individuals with SARS-COV-2 and influenza virus infections

	Patient				
	1	2	3	4	5
Age	67 Years	57 years	51 Years	46 Years	15 years
Gender	Male	Female	Male	Male	Female
Hospital admission/Home isolation	Hospital admission (↑↑ duration)	Hospital admission (↑↑ duration)	Home isolation	Hospital admission (↑↑ duration)	Home Isolation
Co-morbidities	✓	✓	NA	✓	NA
Hypertension	✓	NA	✓	✓	NA
Diabetes mellitus	“Influenza A (H1N1)	Influenza A (H1N1) + Influenza B	Influenza B	Influenza A (H1N1)	Influenza B
Influenza Infection	1. multi-lobar infiltration 2. Secondary bacterial Infection	1. Pneumonia 2. Acute Kidney Injury	NA	1. multi-lobar infiltration 2. Secondary bacterial Infection	NA
Any other	✓	✓	✓	✓	NA
Sign and Symptoms					
Fever	✓	✓	✓	✓	NA
Cough	✓	✓	NA	✓	•
Breath Shortness	✓	✓	NA	✓	NA
Myalgia	✓	✓	✓	✓	•
Fatigue	✓	✓	✓	✓	NA
Sorethroat	NA	✓	NA	NA	•
Headache	NA	NA	✓	NA	•
Diarrhoea	NA	✓	✓	✓	•
Chest pain	NA	✓	NA	✓	NA
Expectoration	✓	✓	NA	✓	NA
Haemoptysis	NA	✓	NA	✓	NA
Abbreviations used: NA: not applicable; ↑↑ duration: prolonged duration of stay					

acute respiratory infection, less morbidity, and no severe complications. Out of 4 OPD patients, 2 had co-infection of influenza A (H3N2) and influenza B, other 2 had Influenza B only.

4. Discussion

According to the World Health Organisation (WHO), 769 million COVID-19 Positive cases and 6.9 million death were confirmed.⁵ Few reports have revealed that co-infection with other respiratory diseases among COVID-19-infected individuals may be linked to more severe clinical outcomes, longer duration of hospitalization, and increased morbidity and mortality (Swets et al., 2022).⁶ Due to the overlapping of the COVID-19 epidemic with influenza seasons, co-infections of influenza and COVID-19 were noted in numerous studies.^{7–10} Salient features of COVID-19 and Influenza are presented in the given table. (Table 2)

In our study, the incidence of influenza co-infection among people infected with SARS-CoV-2 has been 05% between November 2021 and October 2022, which was concordant with other studies which have shown the prevalence of co-infection of Influenza and Corona Virus from 0.2 to 45.7% [(Tang YC et al. (33%), 2022; Dadashi et al. (0.9%), 2021; Stowe et al. (5.92%), 2021; Wu et al. (45.7%), 2020].^{7–10} Low co-infection rates can be caused by low influenza activity, which historically has been low during influenza season owing to primarily non-pharmaceutical treatments adopted for COVID-19 control.¹¹ However, it is also possible that the incidence of influenza infection was underreported as a result of the pandemic's diagnostic emphasis on COVID-19.

Multiple variations of the SARS COV-2 virus were discovered during the early stages of the COVID-19 outbreak. SARS-Delta CoV-2's variant was initially discovered and predominated until a novel VOC (Variant Of Concern), Omicron developed in November 2021.¹² In the later phase of the pandemic, Omicron presented distinct phenotypes compared to earlier variants. A recent analysis revealed that the human influenza A virus has similarities with the Omicron variant.¹³ Omicron variant diseases are primarily caused by URT ("Upper Respiratory Tract") in humans than Delta variant. Omicron has shown a shift to human "Transmembrane Serine Protease 2" (TMPRSS2)-independent cell entry which favours URT infection.¹⁴ Our study duration was the influenza season of 2021-2022 when the Omicron variant was prevalent in India, and compared to the Delta variant it was related to fewer hospital admissions. Therefore, in our study, less incidence of SARS COV-2 co-infection with Influenza virus could be due to the then prevalent VOC, Omicron of SARS COV-2. Further evaluation is required to ascertain, whether the Omicron variant as well as other SARS-CoV-2 variants act as an "antagonist" against the influenza virus, resulting in a lower risk of co-infection.

The amount of literature discussing the potential connection between reported changes in SARS-CoV-2 and other prevalent respiratory virus interactions is limited. Between many common respiratory viruses, viral interference was well described. Viral interference can elucidate host immune response by various mechanisms. Homologous interference occurs when two viral infections of comparable kinds are subjected to a certain immune response. Heterologous interference relies on when the first virus induces a nonspecific innate immune response that prevents or decreases infection and replication of a 2nd virus. Superinfection exclusion is a third mechanism, in which the entry of the virion to an uninfected cell is favored (Bellocave P, 2009).¹⁵ This shields a primary virus from a competing virus (Piret J, 2022).¹⁶ Deleveaux S Et al (2023) in their study on animal and human models found that 24 hours after primary viral infection (SARS CoV-2), the growth rate of the secondary sequential viral pathogen (Influenza virus) decreased remarkably.¹⁷ The interferon pathway has been implicated for causing this interaction. A similar observation was seen in a few other studies (Bao L, 2021; Zhang AJ, 2021; Pinky L, 2022).^{18–20}

In our study, coinfection with influenza A(H1N1) was most common, which was concordant with a few other studies as well (Alosaimi et al. 2021; Bai L et al. 2021; Ding et al. 2020; Cheng et al. 2020).^{21–24} In this study co-infection of Influenza subtype H1N1 caused more moderate to severe disease in comparison with other types of Influenza viruses. A similar observation has been made earlier (Hay et al. 2001).²⁵ Since influenza A (H1N1) is recognized to cause a significant inflammatory cytokine/cytokine storm (chemokine response), the co-infection of H1N1 with COVID-19 may hasten and significantly contribute to the development of ARDS (Alosaimi et al.;2021).²¹

Influenza B was the second most common co-infection in our study. This is in line with a previous study that observed that the antigenic drift of the B virus is similar to that of the A virus (H3N2). These data suggest that influenza B re-infection among individuals will also occur more often (Caini et al.,2019).²⁶

Many risk factors such as CVD (Cardiovascular Disease), diabetes mellitus, older age, and a few others were related to the SARS-CoV-2's clinical severity. In our analysis, the incidence of diabetes was 60% and an important association was present in these cases. This is predicted since reduced innate and adaptive immunity, which may result in lower viral clearance is a risk factor for poor glycemic control. The age of our co-infected patients was >40 years as and similar age profile has been noted in other studies (Ding, Cheng, Nowak et al 2020).^{23,24,27}

Our study has some limitations. Our data and samples have been gathered from a single geographic area. Moreover, this study does not have any data on prevalence

Table 2: Summarizing salient features of the Influenza & COVID-19 Viruses

	Influenza	COVID-19
Virus Characteristics	Influenza viruses have segmented genomes and are composed of negative-sense single-strand RNA.	A coronavirus is a virus with a positive-sense, single-stranded RNA genome that is not segmented.
Virus shedding	1- 4 days (average 2 days)	Most instances appear 4 to 5 days after exposure, with the majority occurring within 14 days of contact.
Transmission	Respiratory droplets and contact	Antigen detection assays, (NAAT (Nucleic Acid Amplification Testing) most frequently with RT-PCR” test, Multiplex PCR
Diagnostic Tests	Antigen detection tests RT-PCR, multiplex PCR, as well as rapid molecular assays	Diarrhea, myalgias, nasal discharge, dyspnea, cough, fever, and taste or smell disorders. Conjunctivitis and dermatologic manifestations are also present.
Sign and Symptoms	Nasal discharge, sore throat, cough, malaise, myalgia, headache, and fever. Diarrhoea (not very common)	Consolidation and ground glass opacities
Radiological intervention	Reticulonodular opacities or Bilateral reticular with or without superimposed consolidation	Lymphopenia raised aminotransaminase levels, raised LDH (“Lactate Dehydrogenase”)levels, C-reactive protein, elevated ferritin, and erythrocyte sedimentation rate as well as deranged coagulations tests
Laboratory parameters	Leukocyte levels are normal or low during the beginning of the disease, although they may increase later on.	ARDS, Guillain–Barre syndrome, multisystem inflammatory syndrome, mucor-mycosis, thromboembolic complications, cardiogenic shock, arrhythmias, acute coronary syndrome, heart failure, and myocarditis
Complications	ARDS (“Acute Respiratory Distress Syndrome”), encephalopathy, transverse myelitis, toxic shock syndrome, Guillain–Barre syndrome, pericarditis and myocarditis, acute myocardial infarction, rhabdomyolysis, myositis	Less Prevalent
Secondary Bacterial Infection in ICU patients	More Prevalent	FDA-approved anti-viral drugs
Treatment	FDA-approved anti-viral drugs	Vaccines available (Efficacy is greater for avoiding death and severe infection than for preventing mild infections. Vaccine effectiveness declines over time but could be improved with a booster)
Vaccines	Vaccines available (Efficacy varies from season to season)	

or infection data on vaccines. The effect of vaccination would have serious implications for managing co-infections of SARS-CoV-2 with Influenza viruses. Multi-institutional studies including the prevalence and implications of vaccination, and the prevalence of other respiratory viruses would be needed to address further questions. Overall, patients with respiratory disease symptoms should continue to be encouraged to get tested for influenza & SARS-CoV-2 diseases.

5. Conclusion

Reduced circulation of influenza virus appears to be real and concurrent with the COVID-19 pandemic. The clinical outcomes of co-infections could not be anticipated and may worsen if novel COVID-19 variants continue to appear. Clinician should consider the resumption of circulation of above viruses, therefore clinical suspicion must increase for and testing for influenza and SARS-CoV-2 viruses in persons with influenza-like illness must be promoted, specifically in those with higher risks of complications. Prompt treatment for COVID-19 and/or influenza may assist in avoiding more severe illness and/or hospitalization. Improved non pharmaceutical interventions can play an important role on the transmission of respiratory viruses and can guide future prevention recommendations.

6. Source of Funding

None.

7. Conflict of Interest

None.


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