Section: Microbiology

Original Research Article

TRENDS OF INFLUENZA AND RESPIRATORY SYNCYTIAL VIRUS INFECTIONS DURING AND POST COVID-19 PERIOD

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ABSTRACT

Background: Influenza and Respiratory syncytial virus (RSV) infections were significantly decreased during COVID-19 pandemic, but a rise in the cases was noted during the post pandemic era. During COVID-19 pandemic, many countries gave less importance to annual Influenza and RSV vaccination; as a result many people became less immune to Influenza and RSV. The increase in Influenza and RSV cases in the second half of 2022 might be due to relaxation of international travel restrictions. **Materials and methods:** Record based cross sectional study was done. RT-PCR was done for COVID19, Influenza and RSV. **Results**: In our study, the overall COVID 19 positivity and Influenza cases were higher in 2022 compared to 2021. The test positivity of COVID 19 was higher (27.6%) during the months of January & February 2022 due to the third wave hit of Covid-19. Afterwards, a declining trend of Covid-19 was noted, compared to Influenza (17.4%). Also, the overall test positivity was significantly higher for COVID 19 (49.5%) in 2022 followed by a decrease in trend compared to RSV (2.6%). Higher test positivity was noted for Influenza B (54.9%) in comparison to Influenza A (45.1%). **Conclusion:** Infection control measures mainly focus on practical implications to reduce transmission, manage outbreaks and perform surveillance within a wide range of communicable diseases. Although Non Pharmaceutical interventions (NPI) reduce the incidence of other viral respiratory infections, we need to focus more on traditional respiratory viral infections. The policy makers, stake holders and clinicians should be more aware of the emerging viral infections so as to adopt appropriate preventive measures.

INTRODUCTION

Viral infections of the respiratory system range from self-limiting illnesses to Acute Respiratory Distress Syndrome (ARDS). They cause global health burden. Most frequently involved agents are Influenza virus, Respiratory Syncytial virus (RSV), Rhinovirus and SARS CoV2 viruses.

Human corona viruses are enveloped RNA viruses with four major subgroups alpha, beta, gamma, delta. Beta corona virus causes deadly diseases SARS (Severe Acute Respiratory Syndrome) and MERS (Middle East Respiratory Syndrome). SARS CoV2 (COVID-19) was declared as a public health emergency of international concern by the World Health Organization (WHO) on March 11, 2020.[1] Since then, infection control practices were implemented including social distancing, hand washing, use of face mask and other personal protective equipment and stay at home orders. These measures were primarily intended to prevent the spread of COVID-19, but they significantly reduced the incidence of other respiratory infections as well. Prior to the COVID-19 pandemic, Influenza and

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RSV were the most common causes of lower respiratory tract infection in the pediatric and adult population.

The Influenza virus is unique, which can cause seasonal epidemics as well as global pandemics. Influenza virus infects many vertebrates with subtypes A, B,C. Seasonal Influenza is an annual threat and it imparts high financial burden on the countries. According to CDC, around 131 subtypes of Influenza A strains have been identified based on hemagglutinin and neuraminidase transmembrane glycoproteins. Influenza B virus is divided into two lineages-Yamagata and Victoria.[2] It can cause acute febrile respiratory disease in an explosive manner. Paules and Subbarao et al in 2017 found that severe pneumonia associated with seasonal Influenza can contribute to 6-29% of mortality.[3]

RSV is an enveloped single stranded non segmented, negative sense RNA virus belonging to Paramyxoviridae, single antigenic serotype with two subgroups A and B, and carries 13 and 20 genotypes respectively.[4] It is a highly infectious pathogen that causes acute lower respiratory tract illness in all age groups and seasonal outbreaks, most commonly reported during cold climate.[5]

SARS CoV-2 was discovered by deep sequencing analysis from lower respiratory tract samples, which showed its binding to ACE-2 receptors in humans.[6] Patients presented with fever, malaise, myalgia, rhinorrhea, sore throat, non-productive cough, shortness of breath, diarrhea and other organ involvement. Both Influenza and RSV cause similar symptoms. During the pre COVID-19 era, annual incidence of respiratory virus illnesses mostly comprised of Influenza and RSV. According to WHO, SARS pandemic in 2002-2003 affected 8422 people in 32 countries with 916 deaths.[7]

In 2016, Centers for Disease Control and Prevention (CDC) conducted a study to evaluate the effectiveness of Non-Pharmaceutical Interventions (NPI) to mitigate the spread of seasonal and pandemic Influenza within communities which provided evidence that face masking, hand hygiene, cough etiquette, social distancing are effective in reducing the spread of Influenza, but also showed the limitations in implementing these measures on a large scale.[8] In the United States, during October 2020 to May 2021, incidence of Influenza reported was as low as <0.4% of respiratory specimens with positive test results for each week of the season.[9] Two groups of antiviral agents are available for treatment of Influenza virus-neuraminidase inhibitors block virus which release via cleaving the cellular receptor sialic acid residues and M2 inhibitors which interfere with viral un-coating.[10,11]

RSV causes mild cold like symptoms, most people recover in one to two weeks, but it can cause serious infections in infants and elderly individuals.[12] In the United States, incidence of RSV associated hospitalization reduced to 0.3 per lakh persons during October 2020 to April 2021, which is very less compared to previous years report of around 27.1.[9] A study conducted in France during 2020-2021 by Jojanneke van et al states that median age of incidence of RSV increased to 4.8 months when compared with previous year reports which was around 3 months in 2019-2020.[13]

Currently we are in the COVID-19 pandemic transition phase. It is essential to have accurate trends and epidemiologic information of respiratory viruses for developing, implementing, monitoring and evaluating health policies. Limited data is available regarding trends of Influenza and Respiratory Syncytial Virus infections during and post COVID-19 period, both in India and as well as in Kerala. Hence, it was decided to undertake the present study.

The objectives of the study were to know the Trends of Influenza and Respiratory Syncytial Virus infections during and post COVID-19 period from January 2021-December 2022and to know the Influenza virus subtypes in the above period.

MATERIALS AND METHODS

Protocol: The clinical sample collection, processing, and laboratory testing were based on WHO/ICMR guidelines.

Record based cross sectional study was done on samples received in Virus Research & Diagnostic Laboratory (VRDL) for suspected COVID-19, Influenza &RSV infections during the period January 2021-December 2022. Ethical clearance was obtained from Institutional Ethical Committee and proceeded with the study. Patients having combination of COVID-19 infection with Influenza or RSV and postmortem samples received for testing were excluded.

RT-PCR was done for COVID-19, Influenza and RSV which is the gold standard for detecting viral infections using nucleic acids.

Procedure:

RNA extraction: Nucleic acids were extracted from the Nasopharyngeal and Throat swab samples in Viral Transport Medium using the automated KingFisher Flex platform. For extraction using the KingFisher Flex platform, the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Fisher Scientific, cat. no. A42352) was used according to the manufacturer's directions. The KingFisher Total RNA extraction procedure uses magnetic-particle technology for total RNA purification. For RNA extraction briefly, 5 µL of proteinase K was placed in each well of the extraction plate (Deepwell 96 Plate), and 200 µl sample was added. Then, 275 µl of the extraction mixture containing binding buffer (265 µl) and the magnetic beads (10 µl) were added. The sample plate was loaded on the instrument along with two washing reagent cartridge, one elution plate and a tip comp plate. In an automated manner, the equipment agitated the plate for 5 minutes and performed a first wash with 500 µl of wash buffer, followed by 500 µl of 80% ethanol and

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a final wash with 250 µl of 80% ethanol. After drying the beads for 5 minutes at 72°C, the purified RNA was collected in 50 µl of elution buffer.

Multiplex RT-qPCR Analysis: The CFX96 real-time PCR detection system (Bio-Rad) was used for detection of positive samples by Taqman real time PCR technology using Applied Biosystems CoviPath COVID-19 RT-PCR Kit by Thermo Fisher Scientific, RealStar Influenza Screen & Type RT-PCR Kit 4.0 (Altona Diagnostics –Germany), RealStar RSV RT-PCR Kit 3.0 (Altona Diagnostics –Germany) for COVID 19, Influenza and RSV respectively. The Multiplex RT-qPCR Analysis were performed following manufacturer's directions of each diagnostic kit.

RESULTS

Comparison of COVID 19 between 2021 and 2022

The comparison of COVID 19 between 2021 and 2022 is shown in Figure 1. Here the p-values are less than the significance level 0.05. Hence the difference between COVID 19 cases in 2021 and 2022 is significant

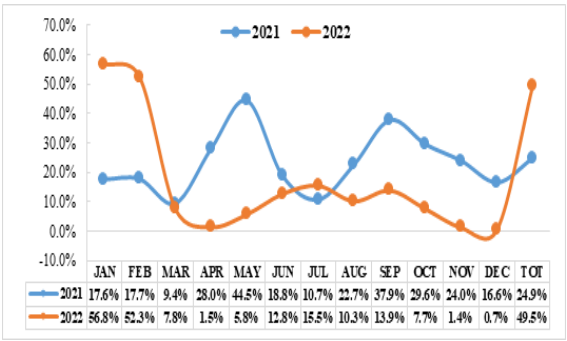

Figure 1: Comparison of COVID 19 between 2021 and 2022

Comparison of Influenza between 2021 and 2022

The Figure 2 shows comparison of Influenza cases between 2021 and 2022. The p-values are greater than the significance level 0.05 which indicates that the difference between Influenza cases in 2021 and 2022 is not significant.

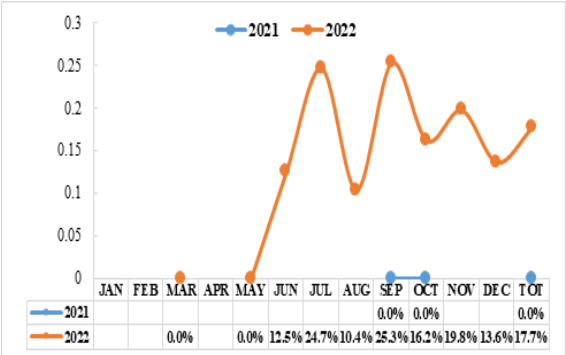

Figure 2: Comparison of Influenza between 2021 and 2022

Comparison of Covid 19 and Influenza in 2021

The comparison of Covid 19 and Influenza is depicted in Figure 3. Here, the difference in test positivity between COVID 19 and Influenza in 2021 is not significant as the p-values are greater than 0.05.

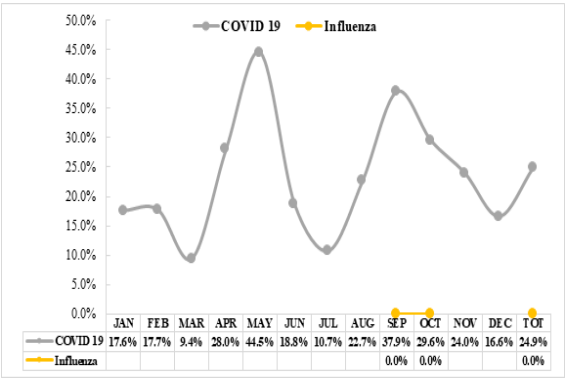

Figure 3: Comparison of COVID 19 and Influenza in 2021

Table 1 illustrates Comparison of COVID 19 and Influenza in 2022. Here the p-values suggest that the difference in test positivity in 2022 is significantly higher in COVID 19 compared to Influenza throughout the second half of 2022. Also, the overall test positivity in 2022 is significantly higher in COVID 19 (49.5%) compared to Influenza (17.7%).

Table 1: Comparison of COVID 19 and Influenza in 2022

Month	COVID 19		Influenza		p – value
	Tested	Positive	Tested	Positive	
January	30091	17100 (56.8%)	0	--	--
February	12365	6473 (52.3%)	0	--	--
March	2485	194 (7.8%)	10	0 (0.0%)	0.358
April	581	9 (1.5%)	0	--	--
May	462	27 (5.8%)	1	0 (0.0%)	0.804
June	493	63 (12.8%)	8	1 (12.5%)	0.980
July	566	88 (15.5%)	81	20 (24.7%)	0.038
August	426	44 (10.3%)	77	8 (10.4%)	0.979
September	510	71 (13.9%)	83	21 (25.3%)	0.008
October	300	23 (7.7%)	148	24 (16.2%)	0.006
November	296	4 (1.4%)	81	16 (19.8%)	0.000
December	141	1 (0.7%)	88	12 (13.6%)	0.000
Total	48716	24097 (49.5%)	577	102 (17.7%)	0.000

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Comparison of COVID 19 and Influenza:
[Figure 4] demonstrates the overall comparison of COVID 19 and Influenza in year 2021 and 2022.

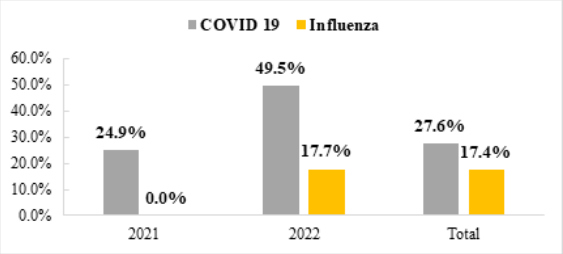

Figure 4: Comparison of COVID 19 and Influenza

While comparing COVID 19 and Influenza [Figure 4], the p-values suggest that there is no difference in test positivity between COVID 19 and Influenza in 2021 and a significantly high difference in test positivity between COVID 19 and Influenza in 2022. The overall (2021+2022) test positivity is also higher in COVID 19 (27.6%) compared to Influenza (17.4%).

Comparison of Influenza A and Influenza B
Influenza A and Influenza B are compared in [Table 2].

Table 2: Comparison of Influenza A and Influenza B

Type	Influenza		p - value
	Positive	Percent	
Influenza A	46	45.1%	0.162
Influenza B	56	54.9%	
Total	102	100.0%	

The difference in positivity rate between Influenza A and Influenza B is not significant as the p-value is greater than 0.05. Test positivity is higher in Influenza B (54.9%) as compared to Influenza A (45.1%).

Comparison of H1N1 and H3N2
The difference in positivity rate between H1N1 and H3N2 [Figure 5] is not significant because the p-value is 0.095. The figure shows that the test positivity is highest with H3N2 (58.7%) than H1N1 (41.3%).

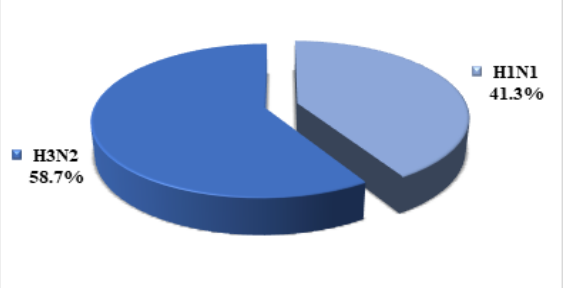

Figure 5: Comparison of H1N1 and H3N2

Comparison of COVID 19 and RSV in 2022
The comparison of COVID 19 and RSV in 2022 [Figure 6] suggests that the difference in test positivity in 2022 is significantly higher in COVID 19 compared to RSV from August through end of 2022. Significant p value was observed in February (0.010), August (0.086), September (0.002), October (0.000), November (0.000), December (0.001).It is also noted that the overall test positivity in 2022 is significantly higher in COVID 19 (49.5%) compared to RSV (29.6%).

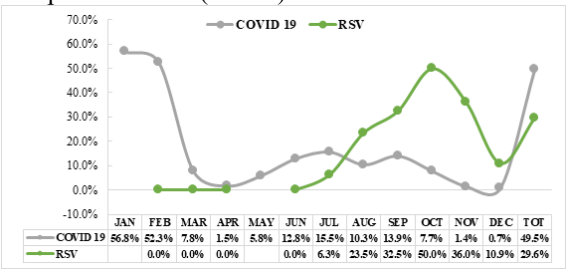

Figure-6: Comparison of COVID 19 and RSV in 2022

DISCUSSION

The present study compared the trends of the most relevant respiratory viruses Influenza and RSV during and post-COVID-19 period. We analyzed the results of total four lakh patients in all age groups by RTPCR which is gold standard for detecting viral infections using nucleic acid.[14]

In our study, the overall COVID 19 positivity is significantly higher in 2022 (49.5%) compared to 2021 (24.9%), mainly due to very high positivity rate during the third wave of COVID-19 (January & February 2022). The positivity rate is higher in 2021 compared to 2022 in all months except January & February. The rate was tremendously decreased after February 2022. Although there are no effective antiviral agents for COVID-19,appropriateNPIs and active vaccination might have reduced the incidence COVID-19 during the second half of 2022.[15]

The comparison of Influenza between 2021 and 2022 shows that, the difference is not significant. The Influenza cases are higher in 2022 (17.7%) compared to 2021 (0.0%); only 10 samples were received for Influenza testing in 2021. This might be probably because during the COVID-19 pandemic, the patients visiting the health care facilities with respiratory symptoms were prioritized for COVID-19 testing, and only samples negative for COVID-19 were being tested for Influenza. Besides, during the severe period of COVID-19, most of the patients did not want to visit hospitals for mild clinical issues; also globally the resources and man power were mostly reserved for COVID-19. So the total testing for Influenza was decreased during 2021.The Influenza test positivity is higher after June 2022, which coincides with the decrease in COVID-19 cases.

The COVID-19 pandemic through 2020 and 2021 had a large impact on Influenza circulation in World population. In the non-pandemic years, Influenza had resulted in a high burden on the healthcare system and the population.[16,17] The present study suggests that there is no difference in test positivity between COVID 19 and Influenza in 2021 and a

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significantly high difference between the test positivity in 2022. Also, the overall (2021+2022) test positivity is higher in COVID 19 (27.6%) compared to Influenza (17.4%), the probable explanation for these findings are the very high COVID 19 positivity rates during the third wave of COVID-19 in 2022. A study conducted in Korea by Hyunju Lee, Heeyoung Lee et al, investigating the impact of public health interventions on seasonal Influenza activity during the COVID-19 outbreak in Korea -2019/ 2020, showed Influenza rate of 49.8 ILIs (interstitial lung infection) /1000 visits compared to 71.9-86.2 ILIs/1000 visits in the previous season.[18] In another study by Cornelia Adlhoch, Piers Mook et al, Influenza transmission was decreased in the European region during 2020/21.[19]

The difference in positivity rate between Influenza A and Influenza B was not significant. Table 5 shows that the test positivity is higher in Influenza B (54.9%) as compared to Influenza A (45.1%). A study conducted in China in 2021/2022 says Influenza B increased during the COVID 19 period as compared to Influenza A, and it lead to substantial morbidity and mortality.[20] Influenza A has several reservoir hosts like, birds and pigs, but humans are the major natural hosts for Influenza B.[21,22] Another probable reason for Influenza B is the over usage of Oseltamivir during COVID-19 period, which might have led to emergence of drug resistant strain (mutations in Neuraminidase inhibitor) of Influenza B Yamagata lineage.[23,24] Also, the usage of trivalent vaccine in most of countries for Influenza which contain 2 strains of type A viruses and 1 strain of type B could have contributed to Influenza B cases.

The difference in positivity rate between H1N1 and H3N2 was not significant among the Influenza A positive samples. The sample positivity was higher with H3N2 (58.7%) compared to H1N1 (41.3%). A meta-analysis conducted during 2018 suggested that, H3N2 prevalence in National Influenza Centres and other national Influenza laboratories from 91 Countries was 92.5% during February 2017. The study also mentions that H1N1 & H3N2 were most common subtypes in Iran, Afghanistan & Egypt.[25]

In our study, comparison of COVID-19 and RSV showed that the difference in test positivity is significantly higher in COVID 19 compared to RSV from August through end of 2022. It was also noted that the overall test positivity in 2022 is significantly higher in COVID 19 (49.5%) compared to RSV (29.6%), mainly due to very high COVID 19 positivity rates in January and February. A study from Australia suggested that COVID-19 public health measures suppressed the 2020 southern hemisphere RSV season, and resurgence of RSV infections was observed after the relaxation of interventions.[26] Other areas of the world have also reported resurgences of cases.[27] A study conducted by Weiling Qiu, Chen Zheng et al in China to know the Epidemiological trend of RSV infection before and during COVID-19 pandemic showed that, out of 11,290 patients, RSV positive cases were 402, 288, 415 in 2019, 2020 and 2021 respectively with most patients under 1year old with a tendency to increase with age.[28]

CONCLUSION

During the COVID-19 pandemic, globally, the health care professionals and government gave more importance to containment of COVID-19, hence resources and man power were mostly limited to COVID 19. Testing and screening for other common respiratory viruses like Influenza and RSV were reduced. Until the third wave of COVID-19 pandemic till March 2022, the cases of Influenza and RSV were very less which may be due to effective non pharmaceutical interventions such as hand hygiene, face mask, quarantine, personal protective equipment and reduced testing for other respiratory viral infections during that period. Following the third wave of COVID-19, Influenza and RSV cases increased and also Influenza B is at a rise as compared to Influenza A. The reason for increased cases of RSV in post- COVID-19 period is mainly due to low vaccination coverage during COVID-19 period. So in the current era of post COVID-19, we should give more importance to seasonal respiratory infections like Influenza and RSV to prevent further pandemic attacks in the upcoming years. Other commonly encountered viruses should be screened along with the pandemic strain for a better understanding of the epidemiological load of individual pathogens.

Limitations

Being a record based cross-sectional study, clinical details as well as follow up of patients could not be done. Other human respiratory viruses such as Parainfluenza virus, Rhinovirus, Meta-pneumoviruses were not included in the study.

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