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ORIGINAL ARTICLE

Hospital Based Prevalence of Respiratory Viral Infections in Clinically Suspected Cases of SARI with or without SARS-CoV-2 Infection: A Study from a Tertiary Care Hospital of North India.

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Abstract

Background: In this COVID-19 era, it is worth to know the circulation of other respiratory viruses (ORVs), in SARI (severe acquired respiratory infections) patients with or without SARS-CoV-2 for clinic-epidemiological perspective. Therefore, present study was conducted to estimate the prevalence of respiratory viruses among the clinically suspected cases of SARI with or without SARS-CoV-2 infection in a tertiary care hospital setup. **Methods**: A total of 175 adult patients fulfilling the inclusion criteria were included. Nasopharyngeal/Oropharyngeal swabs were collected in VTM tubes and processed for viral nucleic acids extraction and multiplex-real-time-RT-qPCR as per standard protocols. **Results:** Out of 175 patients, 115(65.71%) were SARS-CoV-2 negative and 60(34.28%) were SARS-CoV-2 positive. Females were predominant with male: female ratio of 1:1.16. Majority of the patients (n=161;92%) were from 18-60yrs. Most of the patients had fever (n=105;60%) followed by cough (n=91;52%), sore throat (n=83;47.4%), malaise/body ache (n=81;46.3%), breathing difficulty (n=28;16%), loss of smell & chest pain (n=9;5.1%) and diarrhoea (n=5;2.9%). Overall, 12(10.4%) patients had infection of ORVs; Human Rhinovirus (n=8; 6.96%), Influenza B virus (n=2;1.7%), Human Parainfluenza Virus Type-1 & Type-3 in single patient each. Significant statistical differences were found in gender, clinical symptoms and diabetes mellitus ($P \leq 0.05$). **Conclusions**: This study highlighted the infection of ORVs in SARS-CoV-2 negative patients and suggests after ruling out SARS-CoV-2 infection, the laboratories should screen all the patients for ORVs.

Key-words: Respiratory viruses; SARS-CoV-2; COVID-19; Human Rhinovirus.

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Introduction

Severe acute respiratory infections (SARI) are one of the major causes of illness and death throughout the world, especially in developing countries. The World Health Organization (WHO) estimates that acute respiratory infections (ARI) cause approximately 4 million annual deaths, at a rate of more than 60 deaths/100,000 populations [1,2]. Viruses contribute to 30 –70% of ARI, where respiratory Syncytial virus (RSV), Influenza virus, Parainfluenza virus (PIV), human Bocavirus, human Metapneumovirus (hMPV), Human Rhinovirus (HRV), Adenovirus (ADV), Enterovirus and Coronaviruses account for the majority of these cases [2,3].

Risk factors associated for ARI include age, family history of frequent upper respiratory tract infections, asthma, allergies, heart disease or other lung problems, active and passive smoke, poor personal hygiene, and failure to comply with the vaccination program [4]. However, anyone whose immune system might be weakened by another disease is at risk for ARI [5,6].

Data collected from the Global Burden of Disease study 2016 showed that after pneumococcal pneumonia, RSV was the second leading cause of lower respiratory infection, morbidity & mortality globally [5] and is the leading cause of hospitalization [7]. In the absence of influenza epidemics, HRV is the most frequently detected viral pathogen causing SARI among influenza-negative samples and it account for ~30–50% of all respiratory illnesses annually [8,9)]. Even though, HRV is typically associated with the common cold, recent studies suggest that this virus may also be associated with more severe illness, including lower respiratory disease [9-11].

Since ARI symptoms such as cold, sore throat, and fever are common to different pathogens and opportunistic microorganisms, it is important to make use of a technique that allows the detection of various pathogens simultaneously [5]. Distressingly, this issue is especially in low and middle-income countries, where these diseases can emerge and spread rapidly and poses a serious threat to public health, socioeconomic growth and development, as well as highlights the need for advanced diagnostic services and strengthen disease surveillance and control [9].

Diagnostic services within developing countries like ours are typically limited for the detection of respiratory viruses. Although specific pathogens commonly cause characteristic clinical manifestations, the detection of viral pathogens by PCR, cell culture, or serologic tests is useful for patient treatments or epidemiology surveillance to identify and determine the cause of an outbreak [9,12,13]. The main objective of this study was to describe the circulation of respiratory viruses among the suspected/ confirmed cases of SARI with or without SARS-CoV-2 infection.

Materials and Methods

This Pilot study was conducted at a tertiary care centre of North India from January 2021-June 2021, included 175 adult (18 years) suspected/confirmed SARI cases with or without SARS-CoV-2 infection. Group-A-comprising of 115 adult suspected/confirmed SARI cases without SARS-CoV-2 infection and Group-B comprising of 60 adult suspected/confirmed SARI cases with SARS-CoV-2 infection. Demographic information along with detailed clinical history was obtained in predesigned-proforma.

Nasopharyngeal/Oropharyngeal swab samples were collected using sterile nylon flocked swab in Hi-Viral transport medium (HiMedia, Mumbai, India). The labelled VTM tubes with samples were transported to the laboratory on ice at earliest for further processing and samples were stored at - 80°C until further use. Sample collection, packaging and transportation were done according to standard WHO guidelines for SARS-CoV-2.

Viral Nucleic Acid Extraction

Viral nucleic acid extraction from the samples was carried out using "Viral RNA/DNA Extraction QIAmp Kit" (Qiagen, India Pvt. Ltd.) according to standard manufacturer's instructions. During viral nucleic acid extraction process, 2µl of internal control (per 55µl elution volume) was added directly to the sample and to the negative control after the lysis stage of the extraction process.

Real Time–RT-qPCR for SARS-CoV-2 Testing

The real time-RT-qPCR for SARS-CoV-2 testing was carried out using "ICMR NIV Multiplex Single Tube RT–qPCR Version-3" kit as per manufacturer's instructions. All the reactions were multiplexed and an amount of 7µl of the template RNA was used per reaction. The RT-qPCR data generated as cycle threshold (Ct) values, were interpreted as per ICMR SOP "Detection of 2019 novel Coronavirus (2019-nCoV) in suspected human cases by Multiplex Single Tube RT–qPCR: Version 3", as positive or negative. Samples were considered positive when Ct value was ≤ 35 and were considered negative with no Ct or Ct ≤35 [14].

Multiplex real time–qRT-PCR for other respiratory pathogens

Multiplex real time RT-qPCR for other respiratory pathogens was performed by using "FTD Respiratory pathogens-21 (RUO) kit" as per manufacturer's instructions (Siemen Healthneers/Fast Track Diagnosis, Luxembourg). The kit used contains five tubes of primer-probe mix to identify 21-different pathogens; Tube-1: Influenza-A-virus (IAV), Influenza-A-subtype-H1N1 (Pandemic H1N1) virus, Influenza-B-virus (IBV), HRV. Tube-2: human coronaviruses (HCoV) NL63, 229E, OC43 and HKU1. Tube-3: Human parainfluena viruses (HPIV) 2, 3 and 4 and equine arteritis virus (EAV) which serves as an internal control (IC). Tube-4: HPIV-1, human metapneumoviruses (HMPV) A and B, Mycoplasma pneumonia, human Bocavirus (HBoV). Tube-5: RSV-A and B, human adenovirus (HAdV), Enterovirus (EV), human parechovirus (HPeV).

A PCR master mix for one reaction was prepared as: 1.5µl primer-probe + 1µl Enzyme + 12.5µl Buffer. 15µl of PCR master mix was aliquoted in each well of a PCR plate. To the aliquoted PCR master mix 10µl of positive control or the extracted sample was added. Then PCR plate sealed, briefly centrifuged and loaded in the CFX-96-touch real time-PCR detection system (Bio-Rad Laboratories, India Pvt. Ltd). The multiplex real time-RT-qPCR program for "FTD Respiratory pathogens-21" kit was as follows: Hold for 15 minutes at 50°C; hold for 1 minute at 94°C; cycling (40 cycles) for 8 seconds at 94°C and 1 min at 60°C.

Interpretation of RT-qPCR results

The presence of specific sequences detected by an increase in fluorescence observed from the relevant dual-labelled probe. The RT-qPCR data generated as Ct values were interpreted as per manufacturer's instructions. Samples were considered positive when Ct value was ≤35 and were considered negative with no Ct or a Ct >35 (Figure 1) [5].

Statistical Analysis

Statistical analysis was performed by using SPSS software (version 21.0; SPSS S.L., Madrid, Spain). Categorical variables were analysed by using chi-square-test or Fisher's-exact-test as appropriate. For all statistical tests, $P \leq 0.05$ was considered to be statistical significance. All tests of statistical significance were two-tailed.

Ethical Approval

All procedures were performed in compliance with relevant laws and guidelines, and with ethical standards of the Declaration of Helsinki and of the Institutional Ethics Committee. Informed consent was taken from the patients/guardian.

Results

Out of 175 patients, 115(65.7%) were COVID-19 negative (Group-A) and 60(34.3%) patients were positive for COVID-19 (Group-B). Among these 175 patients, 94(53.7%) were females and 81(46.3%) were males with male: female sex ratio of 1:1.16 and it was found to be statistically significant. Mean age of the patients was 38.7 years (S.D=±15.5 years) ranged from 18–74 years and median age was 31 years.

The majority of patients were from 18-60 years, accounted for 161(92%) of the total patients; 73(41.7%) patients were 18-30 years of age followed by 53(30.3%) patients 41-60 years of age, and 35(20%) patients 31-40 years of age while 14(8.0%) patients of >60 years (Figure-1). Most common clinical symptoms were fever (n=105;60%) followed by cough (n=91;52%), sore throat (n=83;47.4%), malaise/body ache (n=81;46.3%), breathing difficulty (n=28;16%), loss of smell & chest pain (n=9;5.1%) and diarrhoea (n=5;2.9%). All the clinical symptoms presented in the Group-A & Group-B patients were found to be statistically significant (Table-1).

A total of 32(18.3%) patients had at least single co-morbidity and 143(81.7%) patients had no co-morbidity. Diabetes mellitus (DM) was the most common co-morbidity in 17(9.7%) patients was statistically significant (P=0.03) followed by hypertension (HTN) in 9(5.1%), cardio vascular disease (CAD) in 4(2.9%), chronic obstructive pulmonary disease (COPD) & chronic kidney disease (CKD) in single (0.57%) patient each. Except DM all other co-

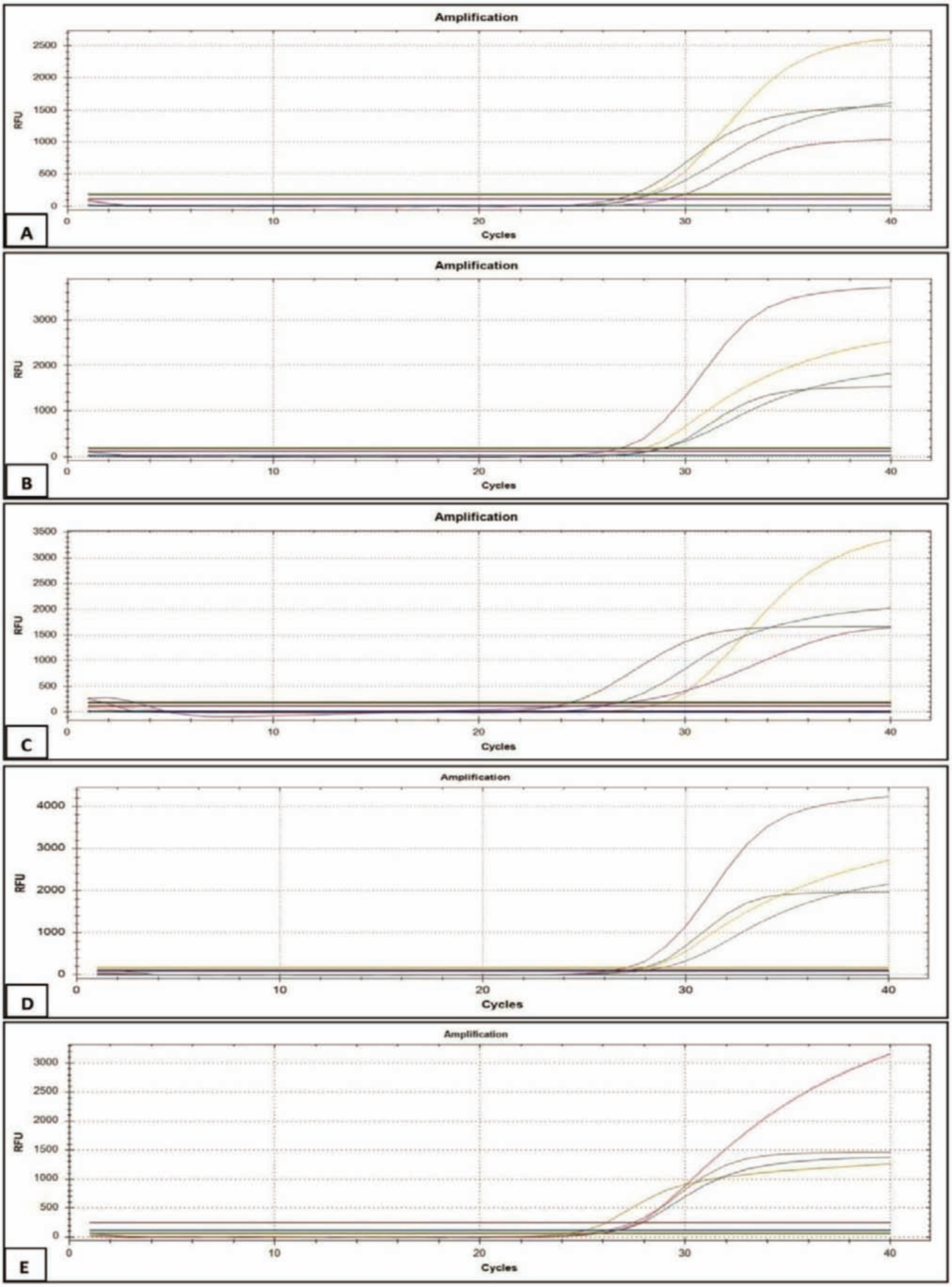

Figure 1: Amplification Curves: (A.) Positive Template Control of Tube-1; (B.) Positive Template Control of Tube 2; (C.) Positive Template Control of Tube 3; (D.) Positive Template Control of Tube 4; (E.) Positive Template Control of Tube-5.

Table 1: Clinical and epidemiological characteristics of adult suspected/confirmed SARI patients

Clinical and Epidemiological Characteristics		Total [n=175]	Group A (n=115)	Group B (n=60)	P value
	(mean ± S.D)	38.74 ± 15.51	37.11 ± 14.74	40.38±14.28	0.16
Age in years	18-60	161 (92.0%)	107 (61.14%)	54 (%)	0.56
	>60	14 (8.0%)	8 (6.95%)	6 (10%)	
Gender	Male	81 (46.29%)	54 (46.96%)	27 (45%)	**0.01**
	Female	94 (53.71%)	61 (53.04%)	33 (55%)	
Clinical symptoms	Fever	105 (60%)	56 (48.7%)	49 (81.67%)	**<0.001**
	Cough	91 (52%)	36 (31.3%)	55 (91.67%)	**<0.001**
	Sore Throat	83 (47.43%)	31 (27.0%)	52 (86.67%)	**<0.001**
	Malaise/Bodyache	81 (46.29%)	37 (32.2%)	44 (73.33%)	**<0.001**
	Loss of smell	9 (5.14%)	1 (0.9%)	8 (13.33%)	**<0.001**
	Difficulty in Breathing	28 (16%)	13 (11.3%)	15 (25%)	**0.03**
	Chest Pain	9 (5.14%)	3 (2.6%)	6 (10%)	**0.04**
	Diarrhoea	5 (2.86%)	1 (0.87%)	4 (6.67%)	**0.04**
Co-morbidities	Diabetes Mellitus	17 (9.71%)	11 (9.56%)	6 (10%)	**0.03**
	Hypertension	9 (5.14%)	6 (5.22%)	3 (5%)	0.62
	CAD	4 (2.86%)	3 (2.6%)	1 (1.67%)	0.57
	COPD	1 (0.57%)	1 (0.87%)	0 (0%)	NA
	CKD	1 (0.57%)	1 (0.87%)	0 (0%)	NA
COVID Vaccination	Complete	25 (14.86%)	16 (13.91%)	9 (15%)	
Status	Partial	22 (12.57%)	18 (15.65%)	4 (6.67%)	0.24
	No	128 (73.14%)	81 (70.43%)	47 (78.33%)	

Table 2: Clinical and Demographic details of patients having Respiratory Viruses

Clinical and Epidemiological Characteristics		N (%)
Age in years	mean ± S.D	31.4±13.2
	18-59	11 (91.67%)
	≥60	1 (8.33%)
Gender	Male	6 (50%)
	Female	6 (50%)
Clinical symptoms	Fever	5 (41.67%)
	Sore Throat	2 (16.67%)
	Malaise/Bodyache	4 (33.33%)
	Cough	4 (33.33%)
COVID Vaccination Status	Complete	3 (25%)
	Partial	2 (16.67%)
	No	7 (58.33%)
Past COVID History	Positive	2 (16.67%)
	Negative	10 (83.33%)
History of Alcohol & Smoking	Yes	2 (16.67%)
	No	10 (83.33%)

Table 3: Etiological agent distribution among suspected/confirmed SARI patients negative for SARS-CoV-2 infection

Etiological Agent		n=12
Influenza Virus A	pH1N1	0
	H3N2	0
Influenza Virus B	Yamagata/Victoria	02
Human Parainfluenza Virus	Type 1	01
	Type 2	0
	Type 3	01
	Type 4	0
Human Coronavirus	Type 229E	0
	Type OC43	0
	Type HKU1	0
	Type NL63	0
Respiratory Syncytial Virus	Type A	0
	Type B	0
Human Rhinovirus		08
Adenovirus		0
Human Metapneumovirus		0
Human Bocavirus		0
Mycoplasma Pneumonia		0
Single Infection		12
Multiple Infection		0

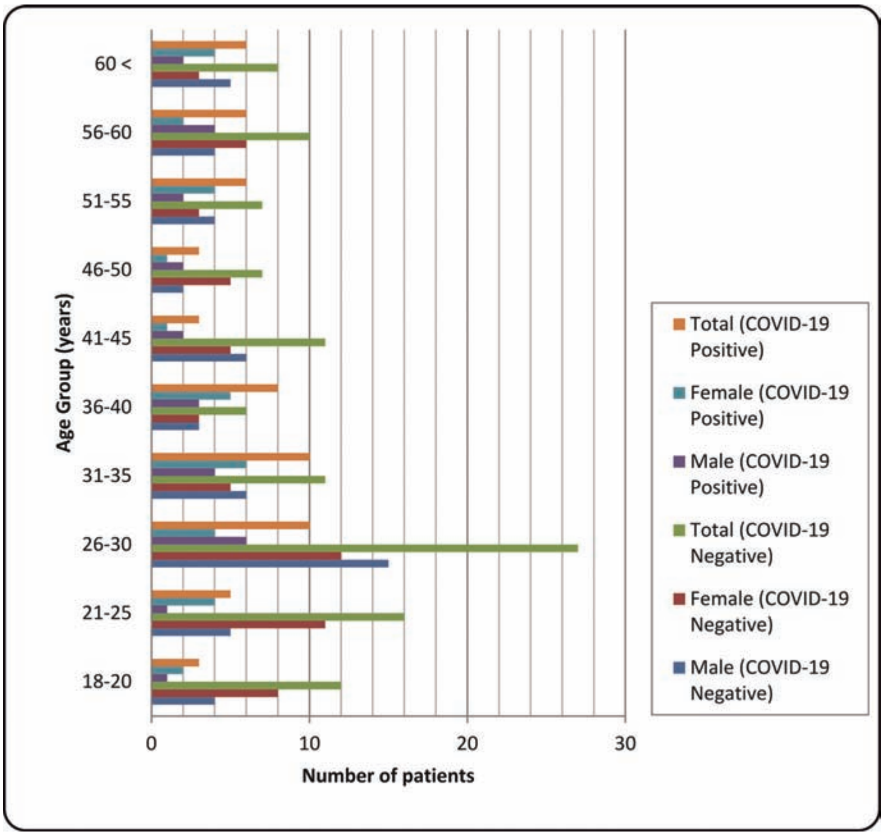

Figure. 2: Age and sex distribution of the patients

morbidities were statistically non-significant (Table-1).

All the samples of Group-A and Group-B patients were put up for multiplex-real-time-RT-qPCR to identify 21-different pathogens (Table-3). Of 175 patients, overall, 12(10.4%) patients had infection of ORVs, which were of Group-A patients. Other respiratory virus infections (n=12,10.4%) were more common in 18-59 years age group patients and equally distributed in male and female gender. Fever (n=5,41.7%) followed by cough (n=4,33.3%), malaise/body ache (n=4,33.3%) and sore throat (n=2,16.7%) was the most common clinical symptoms (Table-2).

The most prevalent pathogen identified was HRV in 8(6.96%) patients, followed by Influenza-B virus in 2(1.7%) patients, HPIV-1 and HPIV-3 in one (0.87%) patient each. No multiple infections were present, only single infection was found among 12 patients who had ORVs infection (Table-3).

Discussion

In this COVID-pandemic-era, as virus-virus interference is well known, trend of ORVs should always be kept under check including RSV, HRV, influenza (flu), human metapneumovirus (hMPV) (15). Due to non-specific clinical symptoms, self-limiting nature of disease and expensive cost of testing, ORV infections are under-diagnosed [16]. Therefore, there is a need of rapid etiological identification which supports the correct management and treatment of patients, allowing where necessary and prompt isolation. To this need, multiplex-molecular assays respond by identifying more than 80% of lower respiratory infections in hospitalized patients [17].

In this study, females were more with male: female ratio of 1:1.16 shows slightly female predominant study. Similarly, Hatem et al. [2] study reported that females (n=569;53%) were dominant than males (n=506;47%). In addition, various worldwide studies reported females to be dominant among SARI patients ranged from 51.9%-62.4% [2,18-20]. However, a study by Nguyen et al. [9] and Xu et al. [21] reported males to be predominant with 608 (62.4%) and 336 (57.4%), respectively.

In this study, mean age of the patients was 38.7 years, ranged from 18-74 years was almost similar to Elhakim et al. [19] study, in which an average age of 40.4 years was reported. Median age of our patients was 31 years is consistently similar to Nguyen et al. [9] study, in which they reported 36.9 years median age of the patients. However, Li et al. [18] study reported a higher median age of the patients (68 years) ranged from 16-99 years.

Majority of the patients in this study were from 18-60 years age-group, accounted for 161(92%) patients and 14(08%) patients were from >60 years of age-group. Our results were corroborating with study by Nguyen et al. [9] reported that the adult age-group (19–59 years) accounted for the highest percentage of patients (63%) and 140 (14.4%) patients were of $\geq$ 60 years. Another study by Elhakim et al. [19] reported 36% of the patients were of 18-64 years age-group.

In this study, most common clinical symptom was fever (n=105;60%) followed by cough (n=91;52%), sore throat (n=83;47.4%), malaise/body ache (n=81;46.3%), breathing difficulty (n=28;16%), loss of smell & chest pain (n=9;5.1%) and diarrhoea (n=5;2.9%) patients, were found to be statistically significant ($P \leq 0.05$). Similarly, a study by Elhakim et al. [19] reported that the most common clinical symptoms associated with SARI patients were fever (100%), cough (99.9%), sore throat (21.7%), vomiting (9.7%), lethargy (2.6%), dyspnoea (2.2%), pain (0.8%), haemoptysis (0.7%), and convulsions (0.2%). In most studies, fever was the most common clinical symptom followed by cough, difficulty in breathing and sore throat [18,19,21].

In this study, only 32(18.3%) patients had at least one co-morbidity whereas in a study by Li et al. [18], 70% patients had at least one co-morbidity. The main co-morbid disease presented in our patients was DM (9.7%) followed by HTN (5.1%), CAD (2.9%) and COPD & CKD (0.6%) while study by Xu et al. [21] also observed similar findings, the main underlying diseases were HTN (32.9%), CAD (31.9%), DM (25.9%) and COPD (10.4%). Further, Li et al. [18] in their study also observed HTN and CAD in 38.3% and 7.6% of the study population, respectively.

In this study, among 12 patients positive for ORVs, majority were suffered with fever, cough & body ache/malaise and sore throat. This was similar to a study conducted by Wang et al. [22] in which it was reported that patients with HRV presented with similar symptoms, and Chen et al. [6] study, involved individuals with HRV presenting influenza like illness similar to our study.

The worldwide distribution of viral-aetiology as a cause of SARI varies between 2%-78% [2]. In this study, we found a viral-aetiology of 10.4% (n=12), which is comparable to previous studies [2,21,23]. In this study, most prevalent pathogen identified was HRV in 8 (6.96%) patients, followed by Influenza-B virus in 2 (1.7%) patients, HPIV-1 and HPIV-3 in one (0.9%) patient each. The positive detection rate (PDR) of HRV in this study was 6.96%, which is almost similar to Grunberg et al. [24] study in which it was 7.8% whereas Xu et al. [21] study reported very low PDR of HRV (0.6%). Among these, HRV is a very common pathogen causing respiratory tract infection. In the COVID-era, symptoms of patients with HRV may mimic patients presenting with COVID like illness.

In this study, higher prevalence of HRV was observed in males, whereas in Xu et. al. [21] and Grunberg et. al. [24] study, higher prevalence of HRV was observed in females. Patients affected by ORVs were mostly of age group 18-59 years; hence, this study yet again corroborates this well-known observation made worldwide. This might be due to their active lifestyle atmosphere, where they are at a higher risk of getting infected with these ORVs, to which they have never been exposed previously [25].

Earlier, it was thought that HRV cause only upper-respiratory-tract-infections like common cold, sinusitis, otitis-media but now with advent of more recent diagnostic methods it is clear that they can also cause lower-respiratory-tract-infections like pneumonias, bronchiolitis and breathing difficulties [6]. Individuals with underlying chronic diseases like cystic-fibrosis, asthma, COPD, infants, elderly and immunocompromised hosts are more susceptible.

Coronavirus and HRV can be differentiated with the help of serological tests and RT-PCR testing. With the help of newer diagnostic methods, HRV is being identified as cause of ARI [26]. Nowadays, several single-use cartridge assays are available for diagnosis of multiple respiratory infections. These systems are completely automated and provide rapid results, but they are difficult to execute in a standard routine because they are very expensive [5]. Recently, in the pandemic-COVID-19, real-time-RT-PCR has become more important in routine diagnostic tests, and its application in future could yield more sensitive results.

In this study, reason of low prevalence of ORVs may be, first, small sample size and further studies with large number of sample size are needed. Second, patients of this study were with lower co-morbid diseases. Third, this study included adult patients however, various studies reported lower positivity of viral aetiologies in adult patients compared to children. Finally, this might be due to differences in geography, etiological agents, or diagnostic methods [2].

Limitations of this study, primarily this was a pilot study, conducted at a single tertiary care hospital, data may not represent the entire country, where climate and other factors may cause variations. Secondly, we did not obtain information regarding date of onset or hospital stay length. The use of real-time RT-PCR may have yielded false-negative results if samples were collected after viral shedding had ceased. However, sample collection, transportation and storage were done according to standard WHO guidelines for SARS-CoV-2 and hence, possibility of RNA degradation is negligible. Finally, there was paucity of data regarding bacterial infections or non-viral etiologies. This must be improved in future studies, as there is currently a limited understanding within the country of how the use or misuse of antibiotics affects etiology of respiratory infections [9].

Conclusions

This study noted infection of ORVs only in Group-A patients (SARS-CoV-2 negative). It is matter of concern that in this pandemic-COVID-era, worldwide laboratories were focusing on COVID-19 testing. It is important that after ruling out SARS-CoV-2 infection, we should screen all the individuals for ORVs. It should always be kept in mind that not all respiratory illnesses are always influenza (flu), other causes of illnesses should always be ruled out.

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Conflict of Interest:	All authors declare no COI
Ethics:	There is no ethical violation as it is based on voluntary anonymous interviews
Funding:	This study has been funded by the DHR, ICMR, Govt. of India, New Delhi
Guarantor:	Dr. Sanjib Gogoi will act as guarantor of this article on behalf of all co-authors.

References

1.) *Murray C, Lopez A, Mathers C and Stein C. The Global Burden of Disease 2000 Project: aims, methods, and data sources. Global programme on evidence for health policy. Geneva: World Health Organization; 2001. Available from; http://www.who.int/ healthinfo/ paper36.pdf.*

2.) *Hatem A, Mohamed S, Usama E, Ismael EAM, Rizk MS, El-Kholy A, et al. Clinical characteristics and outcomes of patients with severe acute respiratory infections (SARI): results from the Egyptian surveillance study 2010–2014. Multidiscip Respir Med. 2019; 14:11.*

3.) *Tregoning JS and Schwarze J. Respiratory viral infections in infants: Causes, clinical symptoms, virology, and immunology. Clin Microbiol Rev. 2010; 23:74–98.*

4.) *Benediktsdóttir B. Upper airway infections in preschool children—frequency and risk factors. Scand J Prim Health Care. 1993; 11(3):197–201.*

5.) *Concato C, Piccioni L, Ranno S, Antonelli F, Buonomini A, Coltella L, et al. Comparison of the AllplexTM respiratory panel assays and the automated fast track diagnostics Respiratory pathogens 21 assay for the diagnosis of pediatric respiratory viral infections. Archives of Virology. Published online: 30 March 2020. https://doi.org/10.1007/s00705-020-04593-8.*

6.) *Chen WJ, Arnold JC, Fairchok MP, Danaher PJ, McDonough EA, Blair PJ, et al. Epidemiologic, clinical, and virologic characteristics of human rhinovirus infection among otherwise healthy children and adults. J ClinVirol. 2015; 64:74–82.*

7.) *Gaunt ER, Harvala H, McIntyre C, Templeton KE and Simmonds P. Disease burden of the most commonly detected respiratory viruses in hospitalized patients calculated using the disability adjusted life year (DALY) model. J Clin Virol. 2011; 52(3):215–221.*

8.) Hong CY, Lin RTP, Tan ESL, Chong PN, Tan YSL, Lew YJ, et al. Acute respiratory symptoms in adults in general practice. Fam Pract. 2004; 21: 317–323.

9.) Le-Nguyen HK, Nguyen SV, Nguyen AP, Hoang PMV, Le TT, Co-Nguyen T, et al. Surveillance of Severe Acute Respiratory Infection (SARI) for Hospitalized Patients in Northern Vietnam, 2011–2014. Jpn J Infect Dis. 2017; 70, 522–527.

10.) Lee WM, Lemanske RF, Evans MD, Vang F, Pappas T, Gangnon R, et al. Human rhinovirus species and season of infection determine illness severity. Am J Respir Crit Care Med. 2012; 186:886-91.

11.) Do AHL, Van-Doorn HR, Nghiem MN, Bryant JE, Hoang THT, Do QH, et al. Viral etiologies of acute respiratory infections among hospitalized Vietnamese children in Ho Chi Minh City, 2004–2008. PLoS One. 2011; 6:e18176.

12.) Centre for Disease Control and Prevention. Real-time RT-PCR assays for non-influenza respiratory viruses. 2015:1-16.

13.) Dhakad MS, Gogoi S, Kumari A, Singh AK, Jais MB, Prakash A, et al. Comparative evaluation of cost effective extraction free molecular technique for detection of SARS-CoV-2 with reference to standard VTM based RT-qPCR method. Iran J Microbiol. 2021; 13(6):748-756.

14.) Watanabe Y, Kamioka Y and Seki M. Rhinovirus-Infected Patients in the COVID-19 Pandemic Period. Infect Drug Resist. 2021; 14:609–11.

15.) Palani N and Sistla S. Epidemiology and phylogenetic analysis of respiratory viruses from 2012 to 2015 – A sentinel surveillance report from union territory of Puducherry, India. Clin Epidemiol Glob Health. 2020; 8(4):1225–1235.

16.) Martínez-Roig A, Salvadó M, Caballero-Rabasco MA, Sánchez-Buenavida A, López-Segura N, Bonet-Alcaina M, et al. Viral coinfection in childhood respiratory tract infections. Arch Bronconeumol. 2015; 51(1):5–9.

17.) Li J, Song CL, Wang T, Ye YL, Du JR, Li SH, et al. Etiological and epidemiological characteristics of severe acute respiratory infection caused by multiple viruses and Mycoplasma pneumoniaein adult patients in Jinshan, Shanghai: A pilot hospital-based surveillance study. PLoS ONE. 2021; 16(3):e0248750.

18.) Elhakim MM, Kandil SK, Abd-Elaziz KM and Anwar WA. Epidemiology of severe acute respiratory infection (SARI) cases at a sentinel site in Egypt, 2013–15. J Public Health (Oxf). 2020; 42(3):525–533.

19.) Le YH, Nguyen KC, Coleman KK, Nguyen TT, Than ST, Phan HH, et al. Virus detections among patients with severe acute respiratory illness, northern Vietnam. PLoS ONE. 2020; 15(5): e0233117.

20.) Xu W, Guo L, Dong X, Li X, Zhou P, Ni Q, et al. Detection of viruses and Mycoplasma pneumoniaein hospitalized patients with severe acute respiratory infection in northern China, 2015–2016. Jpn J Infect Dis. 2018; 71(2):134-139.

21.) Wang K, Xi W, Yang D, Zheng Y, Zhang Y, Chen Y, et al. Rhinovirus is associated with severe adult community-acquired pneumonia in China. J Thorac Dis. 2017; 9(11):4502-4511.

22.) Huo X, Qin Y, Qi X, Zu R, Tang F, Li L, et al. Surveillance of 16 respiratory viruses in patients with influenza- like illness in Nanjing, China. J Med Virol. 2012; 84(12):1980-4.

23.) Grunberg M, Sno R and Adhin MR. Epidemiology of respiratory viruses in patients with severe acute respiratory infections and influenza-like illness in Suriname. Influenza Other Respi Viruses. 2021; 15(1):72–80.

24.) Falagas ME, Cholevas NV, Kapaskelis AM, Vouloumanou EK, Michalopoulos A and Rafailidis PI. Epidemiological aspects of 2009 H1N1 influenza: the accumulating experience from the Northern Hemisphere. Eur J Clin Microbiol Infect Dis. 2010; 29(11):1327-47.

25.) Jacobs SE, Lamson DM, St.-George K and Walsh TJ. Human Rhinoviruses. Clin Microbiol Rev. 2013; 26(1):135–62.