Correspondence

Important co-infections in the first wave of COVID-19 pandemic in India

The novel coronavirus, SARS-CoV-2 causative agent of COVID-19 disease, is transmitted mainly through respiratory droplets and close contact, aerosol mostly like other respiratory viruses. It implies the increased chances of occurrence of mixed respiratory tract infections or co-infections. It has been shown that the weakened immune system due to infection with one pathogen causes enhanced susceptibility to co-infection with other pathogens resulting in severe complications[1]. Respiratory infections especially caused by viruses predispose susceptible patients to co-infections which in turn cause increased disease severity resulting in high morbidity and mortality[2]. Co-infection of H1N1 especially with *Staphylococcus aureus* often results in significantly higher rates of morbidity and mortality among intensive care unit (ICU) patients as reported by Rice *et al*[3]. The occurrence of simultaneous infection caused by SARS-CoV-2 with other infectious agents causing respiratory disease has been reported recently from around the world[4-6]. Seasonal prevalence of respiratory viruses, common transmission means and weakened immunity of individuals contribute significantly towards the occurrence of co-infection. Active surveillance is important in controlling disease spread and the possibility of co-infection[7]. The present investigation was undertaken to study the co-infection of SARS-CoV-2 and other respiratory viruses in patients suspected to have SARS-CoV-2 infection.

This observational cross-sectional study was conducted in regional level Viral Diagnostic and Research Laboratory, ICMR-Regional Medical Research Centre (RMRC), Bhubaneswar, India, from March to December, 2020. Patients enrolled in the study were suspected COVID-19 patients showing respiratory symptoms belonging to all age groups. Asymptomatic COVID-19 suspected cases and those with known contact history with confirmed COVID-19 patients, not showing any symptoms of COVID-19,

were excluded from the study. The oropharyngeal and nasopharyngeal swab samples in viral transport medium (VTM), collected 2-14 days post-onset of illness were received from all districts of Odisha, government medical colleges and private hospitals. During the study, a total of 590 samples satisfying the inclusion criteria were tested for the presence of novel coronavirus causing COVID-19 as well as other viruses known to cause respiratory illness. Detailed clinical history along with travel history, if any, was collected from the patients in a predesigned questionnaire form.

Approval from the human Ethical Committee of the Institute was obtained, and written informed consent was obtained from all participants.

Swab samples in VTM were collected from each patient practicing strict aseptic and biosafety precaution and transferred to the laboratory under a cold chain with triple-layer packaging. The samples were subjected to RNA extraction using QIAamp Viral RNA mini kit (QIAgen, Germany, UK) following instructions given by the manufacturer. Storage of extracted RNA at −80°C was done till further processing. For molecular detection of novel coronavirus using real-time PCR, TaqPath™ COVID-19 Combo Kit (Applied Biosystems™, Thermo Fisher Scientific Inc., Massachusetts, USA) was used. This multiplex PCR assay consisted of three sets of primer/probe, *i.e.* N, ORF and S targets, specific to the SARS-CoV-2 genome and one set of primers/probes for MS2 bacteriophage, provided with the kit was used as control. A known positive control provided with the kit and a no template control was included in all reactions. Real-time PCR 7500 fast machine (Applied Biosystems™, Thermo Fisher Scientific Inc., Massachusetts, USA) was used for processing of the suspected samples. The samples were further investigated for the presence of other respiratory viruses *i.e.* influenza viruses (Inf A/B), human parainfluenza viruses (HPIV type – 1, 2, 3 and 4), human

metapneumovirus, rhinovirus, enterovirus, human respiratory syncytial virus (RSV A and B), human adenovirus, endemic coronaviruses (HCoVs – HKU1, NL63, 229E, OC43) using FTD respiratory pathogens 21 kit (Fast Track Diagnostics, Luxembourg, SARL, Germany) following manufacturer's instructions.

Data were collected on demographic characteristics, clinical features and laboratory parameters and analyzed using Stata version 14. (StataCorp LP, College Station, TX, USA). Characteristics of the study population are presented in the Table. Categorical data were presented as count with proportions, while continuous data were summarized as mean/median, as appropriate. Chi-square/Fisher's exact/Kruskal–Wallis test as appropriate was used to compare variables. Dunns multiple comparison test with Bonferroni correction was utilized to compare subgroups if Kruskal–Wallis test was significant.

Patients with the oldest median age belonged to the study population infected only with SARS-CoV-2 virus with median [interquartile range (IQR)] age 40 yr (27.5, 53) while youngest median age was observed in patients with non-SARS-CoV-2 virus infection with median (IQR) age 30 yr (18, 45). Significant differences in ages distribution were observed between SARS-CoV-2 mono-infection and non-SARS-CoV-2 mono-infection (*P*=0.009) and between patients infected with non-SARS-CoV-2 mono-infection when compared to non-infected study population (*P*=0.006). Of the total patients (n=590), 375 were male and 215 were female. There was no significant difference in the distribution of gender across all groups infection and co-infection. A total of 269 patients had a history of International or within-country travel, of whom 170 (63.2%) have travel history outside India. Maximum patients belonged to category 1 of ICMR criteria for testing[8]. A significant association (*P*<0.01) was seen between the history of comorbidity and different categories of SARS-CoV-2 and non-SARS-CoV-2 infection/co-infection. Among the four categories of SARS-CoV-2 and non-SARS-CoV-2 infection/co-infection, running nose was the most common finding while gastrointestinal symptom was the least common finding.

Of the 590 samples tested for novel coronavirus and other human respiratory viral pathogens, SARS-CoV-2 was detected in 129 (22%) samples and in 98 (16.6%) samples one or more respiratory viral agents other than SARS-CoV-2 were detected.

A total of 17 (2.9%) patients were co-infected with SARS-CoV-2 and one or more respiratory viral agents. Of the 129 samples found positive for SARS-CoV-2, six (4.6%) tested positive for one or more endemic HCoVs (229E, OC43, NL63 and HKU1). The presence of rhinovirus co-infection was found to be highest among COVID-19 positive patients. A total of 81 (13.7%) individuals presenting with respiratory illness were found to be negative for novel coronavirus but positive for one or more viruses causing respiratory illness. Among these patients, majority were found positive for Influenza A (H1) virus (n=31, 6.7%) followed by detection of endemic HCoVs (n=14, 3.03%).

Common modes of transmission, seasonal prevalence and host immune status contribute towards the occurrence of co-infection with respiratory viruses. The existence of co-infection increases morbidity and mortality in patients and therefore is significant during patient care, especially in immunocompromised patients. As most respiratory viruses, including influenza viruses and SARS-CoV-2, share the same season of occurrence and modes of transmission, the diagnostic algorithm should be inclusive of several respiratory viruses for an early identification of the aetiological agent followed by appropriate therapeutic intervention. Due to overlapping clinical manifestations, it is difficult for clinicians to differentiate between COVID-19 and non-COVID-19 infected patients. Simultaneous occurrence of COVID-19 disease with immunosuppressive diseases such as HIV, TB and other bacteria has been reported from India[9,10].

In this study, individuals presenting with respiratory illness were screened for the presence or absence of SARS-CoV-2 followed by screening for other respiratory viruses. Laboratory investigations showed that 22 per cent of patients tested positive for novel coronavirus and in 16.6 per cent of patients, respiratory viruses other than novel coronavirus were detected. Detection of other known human respiratory viruses in patients presenting with respiratory illness during the pandemic has been reported[11,12]. Of the total patients included in the study, 2.9 per cent were found to be co-infected with novel coronavirus as well as other human respiratory viruses confirming co-infection. The result was in concordance with the findings reported by Kim *et al*[13]. HCoVs that are endemic in nature usually cause mild to severe respiratory illness[14]. In immunocompromised patients, HCoVs can cause severe pneumonia with acute respiratory distress syndromes (ARDS). HCoVs are known to cause

Table. Characteristics of study population according to type of SARS-CoV-2 and non-SARS-CoV-2 infection and co-infection					
Characteristics	Categories of SARS-CoV-2 and non-SARS-CoV-2 co-infection (n=590)				P
SARS-CoV-2	Positive (n=129)		Negative (n=461)		
Non-SARS-CoV-2	Positive[a] (n=17)	Negative[b] (n=112)	Positive[c] (n=81)	Negative[d] (n=380)	
Age, median (IQR), yr	37 (18-5)	40 (27.5-53)	30 (18-45)	33 (25-55)	0.012[e]
Age categories, n (proportion)					
<20, n=69	5 (7.25)	16 (23.19)	22 (31.88)	26 (37.68)	-
20-39, n=276	4 (1.45)	39 (14.13)	33 (11.96)	200 (72.46)	
40-59, n=142	4 (2.82)	41 (28.87)	19 (13.38)	78 (54.93)	
≥60, n=103	4 (3.88)	16 (15.53)	7 (6.8)	76 (73.79)	
Sex, n (proportion)					
Male, n=375	9 (2.4)	72 (19.2)	56 (14.93)	238 (63.4)	0.556[f]
Female, n=215	8 (3.72)	40 (18.60)	25 (11.63)	142 (66.0)	
Travel history, n (proportion)					
International travel, n=170	0	12 (7.06)	22 (12.94)	136 (80)	-
Within country travel, n=99	2 (2.02)	3 (3.03)	26 (26.26)	68 (68.69)	
No travel history, n=321	15 (4.67)	97 (30.22)	33 (10.28)	176 (54.8)	
Category of patients[g], n (proportion)					
Category 1/2/3, n=106	0	7 (6.6)	16 (15.09)	83 (78.30)	
Category 4, n=69	1 (1.45)	3 (4.35)	11 (15.94)	54 (78.26)	
Category 5a/5b/6, n=21	0	0	1 (4.76)	20 (95.24)	
Others, n=196	14 (7.14)	92 (46.94)	3 (1.53)	87 (44.39)	
Site for sample collection*, n (proportion)					0.32[h]
URT, n=578	16 (2.77)	109 (18.86)	79 (13.67)	374 (64.7)	
LRT, n=10	1 (10)	1 (10)	2 (20)	6 (60)	
Comorbidities index, n (proportion)					
0 (no comorbidity), n=528	16 (3.03)	111 (21.02)	73 (13.83)	328 (62.1)	0.001[h]
At least one comorbidity, n=62	1 (1.61)	1 (1.61)	8 (12.90)	52 (83.87)	
Symptomatology of patients, median (IQR)					
Symptom index[x]	4 (3-5)				
Have 1 symptom, n=11, n (proportion)	0	0	0	11 (1.9)	
Have≥2 symptoms, n=579, n (proportion)	17 (2.9)	112 (19)	81 (13.7)	369 (62.5)	
Type of symptoms, n (proportion)	n=17	n=112	n=81	n=380	
Running nose	17 (100)[M]	112 (100)[M]	81 (100)[M]	380 (100)[M]	
Fever	12 (70.5)	85 (75.89)	57 (70.37)	222 (58.4)	
Cough	12 (70.5)	71 (63.39)	62 (76.54)	203 (53.4)	
Sore throat	13 (76.4)	79 (70.54)	35 (43.21)	116 (30.5)	
Body ache	2 (11.76)[m]	75 (66.96)	48 (59.26)	51 (13.42)	
Breathless ness	5 (29.41)	29 (25.89)	18 (22.22)	115 (30.2)	
Head ache	3 (17.65)	33 (29.46)	12 (14.8)[m]	73 (19.21)	
Chest pain	4 (23.53)	24 (21.43)	13 (16.05)	47 (12.37)	
Wheeze/crepitation's	3 (17.65)	10 (8.93)	15 (18.52)	44 (11.58)	

Contd...

Characteristics	Categories of SARS-CoV-2 and non- SARS-CoV-2 co-infection (n=590)				*P*
SARS-CoV-2	Positive (n=129)		Negative (n=461)		
Non-SARS-CoV-2	Positive[a]	Negative[b]	Positive[c]	Negative[d]	
	(n=17)	(n=112)	(n=81)	(n=380)	
GI symptoms	0	4 (3.57)[m]	12 (14.8)[m]	39 (10.26)[m]	

[*]Patient category as per infection detected. [a]Positive for SARS-CoV-2 and non-SARS-CoV-2 infection, [b]Positive for SARS-CoV-2 but negative for non-SARS-CoV-2 infection, [c]Positive for non-SARS-CoV-2 but negative for SARS-CoV-2 infection, [d]Not positive for either SARS-CoV-2 or non-SARS-CoV-2 infection, [e]Kruskal-Wallis rank test with total *P* value for all groups of co-infection, [f]Chi-square test with total *P* value for all groups of co-infection, [g]Categories 1/2/3 include symptomatic international traveller/ laboratory confirmed case/health worker, Category 4 Includes hospitalized SARI patients, Category 5a/5b/6=Asymptomatic contacts/ healthcare workers of confirmed cases/influenza-like illness and others, [h]Fisher's exact test with total *P* value for all groups of co-infection, [x]Total number of symptoms/per patient, [m]Least common symptom among the subgroup, [M]Most common symptom among the subgroup. IQR, interquartile range; GI, gastrointestinal; URT, upper respiratory tract; LRT, lower respiratory tract

co-infection with respiratory viruses such as influenza viruses, metapneumovirus, RSV, adenovirus and enterovirus[15]. In our study, co-infection was detected between SARS-CoV-2 and endemic HCoVs as reported by Chaung *et al*[16]. To avoid misdiagnosis, clinicians should be aware of the possibility of co-infections among SARS-CoV-2 and HCoVs. Both SARS-CoV-2 and influenza virus infections are presented with similar initial respiratory clinical manifestations and could progress towards ARDS and respiratory failure. Hence, early detection of simultaneous infection of novel coronavirus with other viruses is important in the clinical management of patients. Although in the present study detection of simultaneous infection of SARS-CoV-2 with influenza viruses was not found but the occurrence of such co-infections cannot be ruled out. The study had a few limitations. As the study was carried out in a virology laboratory, the existence of concurrent bacterial infections could not be studied. Another limitation of the study was that the outcome of patients with co-infection was not available. To conclude, the identification of other viruses should be considered while diagnosing and managing COVID-19 patients.

***Conflicts of Interest*:** None.

Subhra Subhadra[1], Jyotsnamayee Sabat[1],
Alka Turuk[3], Sonalika Rath[1],
Lal Mohan Ho[1], Madhab Charan Mandal[1],
Sailendra Panda[1], Demeyol Rhetso[1],
Sanghamitra Pati[2] & Jyotirmayee Turuk[2,*]

[1]Regional Virus Research & Diagnostic Laboratory, [2]ICMR – Regional Medical Research Centre, Bhubaneswar 751 023, Odisha & [3]Clinical Study, Trial and Projection Unit, Division of Epidemiology and Communicable Diseases, Indian Council of Medical Research, New Delhi 110 029, India
*For correspondence:
drjyotirmayeeturuk@gmail.com

Received February 23, 2021

References

1. Cauley LS, Vella AT. Why is coinfection with influenza virus and bacteria so difficult to control? *Discov Med* 2015; *19* : 33-40.

2. Metzger DW, Sun K. Immune dysfunction and bacterial coinfections following influenza. *J Immunol* 2013; *191* : 2047-52.

3. Rice TW, Rubinson L, Uyeki TM, Vaughn FL, John BB, Miller RR 3rd, *et al*. Critical illness from 2009 pandemic influenza A virus and bacterial coinfection in the United States. *Crit Care Med* 2012; *40* : 1487-98.

4. Ding Q, Lu P, Fan Y, Xia Y, Liu M. The clinical characteristics of pneumonia patients coinfected with 2019 novel coronavirus and influenza virus in Wuhan, China. *J Med Virol* 2020; *92* : 1549-55.

5. Jiang S, Liu P, Xiong G, Yang Z, Wang M, Li Y, *et al*. Coinfection of SARS-CoV-2 and multiple respiratory pathogens in children. *Clin Chem Lab Med* 2020; *58* : 1160-1.

6. Blasco ML, Buesa J, Colomina J, Forner MJ, Galindo MJ, Navarro J, *et al*. Co-detection of respiratory pathogens in patients hospitalized with coronavirus viral disease-2019 pneumonia. *J Med Virol* 2020; *92* : 1799-801.

7. Gregianini TS, Varella IRS, Fisch P, Martins LG, Veiga ABG. Dual and triple infections with influenza A and B viruses: A case-control study in Southern Brazil. *J Infect Dis* 2019; *220* : 961-8.

8.   Indian Council of Medical Research. India. *Strategy for COVID-19 testing in India (Version 5, dated 18/05/2020)*. Available from: *https://www.icmr.gov.in/pdf/covid/strategy/ Testing_Strategy_v5_18052020.pdf*, accessed on August 2, 2021.

9.   Marimuthu J, Kumar BS, Gandhi PA. HIV and SARS CoV-2 coinfection: A retrospective, record-based, case series from South India. *J Med Virol* 2021; *93* : 163-5.

10. Singh A, Gupta A, Das K. Severe acute respiratory syndrome coronavirus-2 and tuberculosis coinfection: Double trouble. *Indian J Med Spec* 2020; *11* : 164-8.

11. D'Abramo A, Lepore L, Palazzolo C, Barreca F, Liuzzi G, Lalle E, *et al.* Acute respiratory distress syndrome due to SARS-CoV-2 and Influenza A co-infection in an Italian patient: Mini-review of the literature. *Int J Infect Dis* 2020; *97* : 236-9.

12. Zhu X, Ge Y, Wu T, Zhao K, Chen Y, Wu B, *et al.* Co-infection with respiratory pathogens among COVID-2019 cases. *Virus Res* 2020; *285* : 198005.

13. Kim D, Quinn J, Pinsky B, Shah NH, Brown I. Rates of co-infection between SARS-CoV-2 and other respiratory pathogens. *JAMA* 2020; *323* : 2085-6.

14. Liu DX, Liang JQ, Fung TS. Human Coronavirus-229E, -OC43, -NL63, and -HKU1 (Coronaviridae). *Encyclopedia of Virology*. 2021; 428-40. doi:10.1016/B978-0-12-809633-8.21501-X

15. Killerby ME, Biggs HM, Haynes A, Dahl RM, Mustaquim D, Gerber SI, *et al.* Human coronavirus circulation in the United States 2014-2017. *J Clin Virol* 2018; *101* : 52-6.

16. Chaung J, Chan D, Pada S, Tambyah PA. Coinfection with COVID-19 and coronavirus HKU1-The critical need for repeat testing if clinically indicated. *J Med Virol* 2020; *92* : 1785-6.