Received: 5 January 2022 | Accepted: 26 February 2022

DOI: 10.1002/jmv.27685

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

No human respiratory syncytial virus but SARS-CoV-2 found in children under 5 years old referred to Children Medical Center in 2021, Tehran, Iran

Arash Letafati[1] | Fahimeh Sadat Aghamirmohammadali[1] | Abbas Rahimi-Foroushani[2] | Seyed Abbas Hasani[3] | Talat Mokhtari-Azad[1] | Jila Yavarian[1]

[1]Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[2]Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[3]Department of Pediatrics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Correspondence

Jila Yavarian and Talat Mokhtari-Azad, Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

Email: yavarian@tums.ac.ir and mokhtari@hotmail.com

Abstract

Acute respiratory infections (ARIs) are one of the leading causes of illness and death among community members worldwide. Viral infections are the most common agents estimated to be involved in these patients. This study aimed to investigate the prevalence of human respiratory syncytial virus (hRSV) and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) among children with ARIs. This study evaluated the presence of SARS-CoV-2 and hRSV in 168 throat and nasopharyngeal swab samples using real-time RT-PCR. All samples were collected from children under 5 years old with ARIs who attended Children's Medical Center, Tehran, Iran, and sent to the Iranian National Influenza Center with appropriate conditions in 2021. Chi-square and Fisher's exact tests were used for comparison of the data of the prevalence of hRSV and SARS-CoV-2 infections among children. Of 168 patients examined, 95 (57%) were male and 73 (43%) female. Out of them, 47 (28%) cases were younger than 1 year old and 121 cases (72%) were 1–5 years old. The most common clinical manifestations of patients were cough (78%), nausea (31%), diarrhea (27%), and fever (18%). Among 168 patients, no hRSV was detected, while the SARS-CoV-2 genome was identified in 16 (9.5%) patients. Among 16 positive cases of SARS-CoV-2, 8 (50%) were under 1 year old and 8 positive cases were 1–5 years old. This study was performed at cold months of the year but due to the coronavirus disease 2019 pandemic and adherence to health protocols, school closures, and virtual classes, no cases of hRSV infections were identified.

KEYWORDS

hRSV, prevalence, respiratory tract infection, SARS-CoV-2

1 | INTRODUCTION

Acute respiratory infections (ARIs) are associated with high morbidity and mortality among community members throughout the world. ARI is the most common reason for referral to a medical clinic, accounting for 70% of respiratory diseases in young children and newborns under 1 year of age.[1] The mortality rate for children under 5 years of age is high, especially for young children with pneumonia,[2] as well as for the elderly and immunosuppressed individuals.[3] These infections affect the upper and lower respiratory tract from the pharynx to the alveoli and can be associated with mild colds to severe pneumonia.[4]

Respiratory infections spread in the community through the inhalation of pathogen-containing aerosols and fomites. All microbiota, including bacteria, viruses, and protozoa may be presented in respiratory infections.[5] Among the pathogens, viruses play an important role in the development of respiratory infections, accounting for 30%–40% of ARIs.[6] Common viruses associated with ARIs are human respiratory syncytial virus (hRSV), human Bocavirus, influenza viruses (IFV) A and B, human metapneumovirus, and adenoviruses. Other viruses involved in ARIs include human parainfluenza virus, rhinovirus, and human coronaviruses.[7] Viral infection occurs alone or in combination with other viral/bacterial pathogens. Several studies have shown that viral co-infections may lead to the severity of respiratory diseases.[8] Therefore, identifying the most common viruses among respiratory infections can help to better manage and treat these patients.

hRSV is a single-stranded negative-sense RNA virus and belongs to *Pneumoviridae* family, orthopneumovirus genus and human orthopneumovirus species.[9]

hRSV is the leading cause of respiratory infections among infants and young children worldwide. The economic and public health burden of pediatric hRSV infection is considerable. hRSV infection can cause many symptoms, ranging from mild self-limiting upper respiratory tract infections to severe bronchiolitis, pneumonia, and hypoxemia.[10] Before the coronavirus pandemic, 24% of hospitalizations of children under 5 years of age with ARI were due to the hRSV, but during the pandemic, the rate of hospitalization because of hRSV was decreased.[11]

SARS-CoV-2 is a single-stranded positive-sense RNA virus and belongs to *coronaviridae* family and beta coronavirus genus.[12] After its appearance in China, in December 2019, SARS-CoV-2 spread rapidly around the world. The World Health Organization declared a pandemic on March 11, 2020. By December 5, 2021, more than 265 million infections had been detected worldwide and the novel coronavirus disease 2019 (COVID-19) had claimed more than 5.2 million lives.[13] Epidemiological data from the early stage of the pandemic suggested that children have much milder disease course than adults. Overall, children younger than 18 years are thought to account for only 1%–2% of detected COVID-19 cases worldwide.[14]

This study aimed to investigate the prevalence of hRSV and SARS-CoV-2 infections among children younger than 5 years old who were referred to Children's Medical Center in Tehran with ARIs.

2 | MATERIALS AND METHODS

2.1 | Study population

This was a cross-sectional study including 168 children with ARIs. They were referred to Children's Medical Center affiliated with Tehran University of Medical Sciences in the period between March and May 2021. Ethical approval for this study was obtained from TUMS Medical Ethics [IR.TUMS.REC.1399.306]. Written informed consent was obtained from the parents of all patients.

2.2 | Collection and preparation of the samples

Throat and nasopharyngeal swab samples were collected from each patient. Samples were refrigerated at 2–8°C and transported on ice to the Iranian National Influenza Center and stored at −70°C until use.

2.3 | Nucleic acid extraction and virus detection

Ribonucleic acid (RNA) extraction was performed by the High Pure Viral Nucleic Acid kit (Roche). For SARS-CoV-2 RNA detection, one-step Real-time RT-PCR was performed using Ampliqon mastermix based on the manufacturer's instructions. Primers and probes for Envelope (E) and RNA-dependent RNA polymerase (RdRp) genes were selected for amplification of SARS-CoV-2 RNA. RNA was added to a 25 µl PCR mixture containing 12.5 µl of Ampliqon mastermix, 1 µl (10 pmol) of each primer, and 0.5 µl (5 pmol) probe. The reaction consisted of 40 min at 38°C and 15 min at 95°C, followed by 45 cycles of amplification, including 15 s at 94°C and 30 s at 57°C for E gene detection and 30 s at 58°C for RdRp gene detection.

Detection of hRSV was performed using a one-step real-time RT-PCR. The nucleocapsid gene was selected for amplification of hRSV RNA. RNA was added to a 25 µl PCR mixture containing 12.5 µl of mastermix, 1 µl of each primer (10 pmol) and 0.5 µl probe (10 pmol). The reaction consisted of 40 min at 38°C and 15 min at 95°C, followed by 45 cycles of amplification, including 15 s at 94°C and 30 s at 54°C.

2.4 | Statistical analysis

The statistical analysis was performed using SPSS software, version 16. Chi-square and Fisher's exact tests were used for comparison of the data of the prevalence of hRSV and SARS-CoV-2 infections among children. The significance level was determined at $p < 0.05$.

3 | RESULTS

Of 168 patients examined, 95 (57%) were male and 73 (43%) female. We divided patients into two age groups: less than 1 year old and 1–5 years old which 47 (28%) cases were younger than 1 year old and 121 (72%) cases were 1–5 years old. The most common clinical manifestations of patients were cough (78%), nausea (31%), diarrhea (27%) and fever (18%), respectively. The SARS-CoV-2 genome was detected in 16 (9.5%) patients, while no hRSV genome was found in our study.

Among SARS-CoV-2 positive patients, 50% were under 1 year old and 50% were between 1 and 5 years old. Association between age groups and SARS-CoV-2 prevalence was significant in statistical analysis ($p < 0.048$) (Table 1). Also, among clinical manifestations, cough, nausea, and diarrhea were associated with SARS-CoV-2

TABLE 1 Comparison of clinical manifestations between SARS-CoV-2 positive and negative patients

Variable	Overall N = 168	Negative N = 152	Positive N = 16	p value
Age				0.048
Under 1 year old	47 (28%)	39 (26%)	8 (50%)	
1–5 years old	121 (72%)	103 (74%)	8 (50%)	
Sex				0.6
Female	73 (43%)	67 (44%)	6 (38%)	
Male	95 (57%)	85 (56%)	10 (62%)	
Fever				0.5
Positive	30 (18%)	26 (17%)	4 (25%)	
Negative	138 (82%)	126 (83%)	12 (75%)	
Cough				0.024
Positive	130 (77.4%)	114 (75%)	16 (100%)	
Negative	37 (22%)	37 (24.3%)	0	
Unknown	1 (0/6%)	1 (0.7%)	0	
Nausea				0.001
Positive	52 (31%)	41 (27%)	11 (69%)	
Negative	116 (69%)	111 (73%)	5 (31%)	
Diarrhea				0.002
Positive	45 (27%)	35 (23%)	10 (62%)	
Negative	123 (73%)	117 (77%)	6 (38%)	

Note: $p < 0.05$ statistically significant.
Abbreviation: ARI, acute respiratory infection.

TABLE 2 Comparison of clinical manifestations of patients with ARIs based on sex and different age groups

Variable	Overall N = 168	Female N = 73	Male N = 95	p value
Age				0.065
Under 1 year old	47 (28%)	19 (26%)	28 (29%)	
1–5 years old	121 (72%)	44 (74%)	67 (70%)	
Fever				>0.9
Positive	30 (18%)	13 (18%)	17 (18%)	
Negative	138 (82%)	60 (82%)	78 (82%)	
Cough				0.12
Positive	130 (77.4%)	61 (84%)	69 (72.6%)	
Negative	37 (22%)	12 (16%)	25 (26.3%)	
Unknown	1 (0.6%)	0	1 (1.1%)	
Nausea				0.026
Positive	52 (31%)	16 (22%)	36 (38%)	
Negative	116 (69%)	57 (78%)	59 (62%)	
Diarrhea				0.051
Positive	45 (27%)	14 (19%)	31 (33%)	
Negative	123 (73%)	59 (81%)	64 (67%)	
SARS-CoV-2				0.6
Positive	16 (9.5%)	6 (8.2%)	10 (11%)	
Negative	152 (90%)	67 (92%)	85 (89%)	

Note: $p < 0.05$ statistically significant.
Abbreviation: ARI, acute respiratory infection.

infection ($p < 0.05$). While fever and gender had no statistical correlation with SARS-COV-2 infection ($p > 0.05$). Furthermore, nausea was found mostly in males ($p < 0.05$) (Table 2).

4 | DISCUSSION

ARIs have a high incidence and are easy to spread in society. Viruses are the most common pathogens in the development of ARIs. The study of the prevalence of respiratory viruses is of great importance for the control and treatment of these infections.[6,7] Therefore, our study evaluated the prevalence of hRSV and SARS-CoV-2 in children with ARIs.

In studies before the COVID-19 pandemic, the prevalence of hRSV and IFV was higher in these patients. A meta-analysis study by Salimi et al.,[15] in 2016, reported that the prevalence of hRSV infection among ARIs from Iran was 18.7%. Controversy, Varela et al.[16] evaluated the prevalence of hRSV and IFV during the period of social distancing due to the COVID-19 pandemic in a heavily affected community. The results showed that SARS-CoV-2 was positive in 32.7% while IFV and hRSV were not detected. Similarly, Wu et al.,[17] also described that during the COVID-19 pandemic in China, influenza reports in early 2020 showed a downward trend, in contrast to the two peaks observed before the pandemic. The first SARS-CoV-2 infection in Iran was detected in a 68-year-old man in Qom, Iran on February 12, 2020.[18]

Similar to Valera's study, in our study hRSV was not detected among children with ARIs, while 9.5% of patients were positive for SARS-CoV-2 infections. In Liu et al.,[19] study, SARS-CoV-2 was detected in (6/330) 1.6% of hospitalized children (1–7 age range). In Oualha et al.,[20] study the prevalence of SARS-CoV-2 among 27 children (1–18 years old) admitted for confirmed or highly suspected SARS-CoV-2 infections was 89%. A meta-analysis study by Bhuiyan MU, et al., reported that among 1214 children younger than 5 years old with laboratory-confirmed COVID-19 infection 50% of young COVID-19 cases were infants (under 1 year old).[21]

There appears to be a significant reduction in the number of hospital admissions for acute viral bronchiolitis due to hRSV and IFV during the COVID-19 pandemic.[16]

Lindsay Kim[22] reported that the cumulative COVID19-related hospitalization rate for children under the age of 18 in March and July 2020 was 8 per 100 000 population with the highest among children under the age of two. In addition, 33.2% of inpatient children who had a complete overview of medical records were admitted to the intensive care unit.

A meta-analysis study by Badal et al.[23] reported that 13% of the pediatric COVID-19 cases were asymptomatic. Also headache (67%), fever (55%), and cough (45%) account for the most prevalent physical signs and symptoms seen in symptomatic patients. Elevated ferritin (26%), procalcitonin (25%), CRP (19%), leukopenia (12%), and lymphopenia (15%) were the most common laboratory findings. In addition, 33% of pediatric COVID-19 cases had normal computed tomography scans, while ground-glass opacities were observed in 36% of patients. 29% of the cases were nonsevere, whereas only 5% were severe. Mortality was observed in 0.3% of the overall cases. They reported that COVID-19 is prevalent across all pediatric age groups and presents with varying degrees of symptoms.

Eventually, our study reports valuable information on the spread of hRSV and SARS-CoV-2 in children with ARIs. Hygiene habits and social distancing measures seem to be related to the drastic reduction in hRSV transmission. Although it is not feasible to maintain such restrictions, similar measures can be taken to control outbreaks caused by respiratory viruses. Moreover, as the current prevention of SARS-CoV-2 only relies on vaccine injection and supportive interventions, it is necessary to continuously monitor its transmission dynamics.

ACKNOWLEDGMENTS

We thank the staff of the National Influenza Center, Virology Department, School of Public Health, Tehran University of Medical Sciences. This study was supported by the School of Public Health, Tehran University of Medical Sciences under Grant No.1400-1-99-52190.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

Talat Mokhtari-Azad and Jila Yavarian: conception and design of this study. **Arash Letafati**: wrote the manuscript and performed the laboratory tests. **Fahimeh Sadat Aghamirmohammadali:** helped in the laboratory tests. **Abbas Rahimi-Foroushani:** performed the statistical analysis. **Seyed Abbas Hasani:** medical diagnosis. Approval of the final version of the manuscript was done by all authors.

DATA AVAILABILITY STATEMENT

The dataset is available on request.

REFERENCES

1. Girard MP, Cherian T, Pervikov Y, Kieny MP. A review of vaccine research and development: human acute respiratory infections. *Vaccine*. 2005;23(50):5708-5724.
2. Caballero MT, Bianchi AM, Nuño A, et al. Mortality associated with acute respiratory infections among children at home. *J Infect Dis*. 2019;219(3):358-64.
3. Falsey AR, Walsh EE. Respiratory syncytial virus infection in elderly adults. *Drugs Aging*. 2005;22(7):577-587.
4. Fernandes-Matano L, Monroy-Munoz IE, Angeles-Martinez J, et al. Prevalence of non-influenza respiratory viruses in acute respiratory infection cases in Mexico. *PLoS One*. 2017;12(5):e0176298.
5. Yahia S, Kandeel AY, Hammad E, El-Gilany A-H. Human metapneumovirus (hMPV) in acute respiratory infection: a clinic-based study in Egypt. *Indian J Pediatr*. 2012;79(10):1323-1327.
6. Hatipoğlu N, Somer A, Badur S, et al. Viral etiology in hospitalized children with acute lower respiratory tract infection. *Turk J Pediatr*. 2011;53(5):508-516.
7. Bezerra PG, Britto MC, Correia JB, et al. Viral and atypical bacterial detection in acute respiratory infection in children under five years. *PLoS One*. 2011;6(4):e18928.
8. Cebey-Lopez M, Herberg J, Pardo-Seco J, et al. Does viral co-infection influence the severity of acute respiratory infection in children? *PLoS One*. 2016;11(4):e0152481.
9. Salimi V, Viegas M, Trento A, et al. Proposal for human respiratory syncytial virus nomenclature below the species level. *Emerging Infect Dis*. 2021;27(6):1-9.
10. Stockman LJ, Curns AT, Anderson LJ, Fischer-Langley G. Respiratory syncytial virus-associated hospitalizations among infants and young children in the United States, 1997–2006. *Pediatr Infect Dis J*. 2012;31(1):5-9.
11. Tahamtan A, Askari FS, Bont L, Salimi V. Disease severity in respiratory syncytial virus infection: Role of host genetic variation. *Rev Med Virol*. 2019;29(2):e2026.
12. Castagnoli R, Votto M, Licari A, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and adolescents: a systematic review. *JAMA pediatrics*. 2020;174(9):882-889.
13. Organization WH. COVID-19 weekly epidemiological update, edition 45, 22 June 2021. 2021.
14. Tönshoff B, Müller B, Elling R, et al. Prevalence of SARS-CoV-2 infection in children and their parents in Southwest Germany. *JAMA pediatrics*. 2021;175:586-593.
15. Salimi V, Tavakoli-Yaraki M, Yavarian J, Bont L, Mokhtari-Azad T. Prevalence of human respiratory syncytial virus circulating in Iran. *J Infect Public Health*. 2016;9(2):125-135.
16. Varela FH. Absence of detection of RSV and influenza during the COVID-19 pandemic in a Brazilian cohort: likely role of lower transmission in the community. *J Glob Health*. 2021;11:05007.
17. Wu D, Lu J, Liu Y, Zhang Z, Luo L. Positive effects of COVID-19 control measures on influenza prevention. *Int J Infect Dis*. 2020;95:345-346.
18. Yavarian J, Shafiei-Jandaghi N-Z, Sadeghi K, et al. First cases of SARS-CoV-2 in Iran, 2020: case series report. *Iran J Publ Health*. 2020;49(8):1564-1568.
19. Liu W, Zhang Q, Chen J, et al. Detection of Covid-19 in children in early January 2020 in Wuhan, China. *N Engl J Med*. 2020;382(14):1370-1371.
20. Oualha M, Bendavid M, Berteloot L, et al. Severe and fatal forms of COVID-19 in children. *Archives de Pédiatrie*. 2020;27(5):235-238.
21. Bhuiyan MU, Stiboy E, Hassan MZ, et al. Epidemiology of COVID-19 infection in young children under five years: a systematic review and meta-analysis. *Vaccine*. 2021;39(4):667-677.

22. Kim L, Whitaker M, O'Halloran A, et al. Hospitalization rates and characteristics of children aged< 18 years hospitalized with laboratory-confirmed COVID-19—COVID-NET, 14 States, March 1–July 25, 2020. *Morbidity and Mortality Weekly Report*. 2020; 69(32):1081-1088.
23. Badal S, Bajgain KT, Badal S, Thapa R, Bajgain BB, Santana MJ. Prevalence, clinical characteristics, and outcomes of pediatric COVID-19: a systematic review and meta-analysis. *J Clin Virol*. 2021;135:104715.

How to cite this article: Letafati A, Aghamirmohammadali FS, Rahimi-Foroushani A, Hasani SA, Mokhtari-Azad T, Yavarian J. No human respiratory syncytial virus but SARS-CoV-2 found in children under 5 years old referred to Children Medical Center in 2021, Tehran, Iran. *J Med Virol*. 2022;94: 3096-3100. doi:10.1002/jmv.27685