A delayed resurgence of respiratory syncytial virus (RSV) during the COVID-19 pandemic: An unpredictable outbreak in a small proportion of children in the Southwest of Iran, April 2022

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Abstract
The global outbreak of coronavirus disease 2019 (COVID-19), an emerging disease caused by severe acute respiratory syndrome virus-2 (SARS-CoV-2), and strict restrictions implemented to control the infection have impacted the circulation and transmission of common seasonal viruses worldwide and subsequently the rate of hospitalizations in children at young ages. Respiratory syncytial virus (RSV) surprisingly disappeared in 2020–2021 in many countries due to lockdown and precautions were taken because of the COVID-19 pandemic. Herein, we showed a notable change in the rate of hospitalization and reported an unpredictable outbreak of RSV in a small proportion of children admitted to a children's hospital in Dezful (a city in Southwest Iran) in the early spring of 2022. We performed a descriptive study of hospitalized young children (aged ≤ 5 years) with acute respiratory infections. Together with clinical information, 30 nasopharyngeal swabs were prospectively collected and 3 important respiratory viruses (RSV, influenza viruses, and SARS-CoV-2) were tested through the real-time polymerase chain reaction (real-time PCR) method. The age distribution of 30 hospital-admitted children was 1 month to 5 years old and males were the most included subjects 18/30 (60%) in this study. Although the viral genome of SARS-CoV-2 and influenza viruses was not detected, the presence of RSV was confirmed in 16/30 (53.33%) patients. Results showed that the majority of RSV-infected cases were males 10/16 (62.5%), within 12 months of life, and had changes in parameters of the complete blood count. Almost all patients with RSV infection had a cough as the most common clinical manifestation and had no history of past medical conditions as a risk factor. The presented study is the first investigation that documented an outbreak of RSV infection in young children reported since the onset of the COVID-19 outbreak in Iran. Our cases highlight the potential threats of important but neglected pathogens during the ongoing pandemic as described here for RSV, which would be challenging by easing the preimposed restrictions.

Keywords
COVID-19, influenza, Iran, outbreak, RSV, SARS-CoV-2
1 | INTRODUCTION

Respiratory syncytial virus (RSV) that belongs to the family Pneumoviridae, genus Orthopneumovirus is a common, seasonal (as we expect the peak of the virus in autumn and winter in temperate climates), and infectious virus in humans. This virus antigenically falls within RSV-A and RSV-B subtypes. The virus has been recognized as a major responsible pathogen to cause lower respiratory tract infections (LRTI), for instance, pneumonia (hospital admission rate: 5%–40%), tracheobronchitis (hospital admission rate: 10%–30%), and is the most medically important agent for acute bronchiolitis (hospital admission rate: 50%–90%) in children. An estimation of the global burden of virus-associated acute LRTI in 2019 linked RSV to 25.4–44.6 million infection episodes, 2.9–4.6 million hospitalizations, and 15 100–49 100 deaths in hospitalized children below the age of 2 years. These rates are high in low- and middle-income countries. In Iran, between 1996 and 2013, the frequency of RSV was estimated at 18.7%, and the virus reached the highest number in cold months between November and March at this time interval. More recently, in 2018–2019, two genotypes of RSV, ON1 (RSV-A) and BA9 (RSV-B), were found to be the most detected viral isolates in Iran.

In late 2019, the world was shocked by the emergence of coronavirus disease 2019 (COVID-19), a disease caused by a new human bat-originated coronavirus (severe acute respiratory syndrome virus-2, SARS-CoV-2). As a global pandemic declared in an official declaration on March 2020, the virus has infected millions of men and women worldwide (https://www.worldometers.info/coronavirus/). Inter-human viral transmission in a rapid dynamic led us to take massive actions to reduce the risk of viral distribution on a global scale. Not surprisingly, these measures have also impacted the active circulation of other common and, of course, important respiratory viruses in children, for instance, what has been seen for RSV and influenza viruses recently. The activity of such viruses remained low and the number of new cases followed a sharply downward trend during this interval time. Herein, we presented the first cases of delayed RSV-associated outbreaks in early spring 2022, in Iran.

2 | MATERIALS AND METHODS

In April 2022, the National Influenza Center (NIC) of Iran received a total of 30 nasopharyngeal swabs from hospitalized children (children hospital, Khuzestan province, Dezful) to investigate the etiological agent(s) responsible for an unexpected increase, a possible outbreak, in the number of hospitalizations among a cluster of children aged between 1 month and 5 years old who were 18 (60%) males and 12 (40%) females. Together with clinical information, 30 nasopharyngeal swabs were prospectively collected and sent to NIC in a viral transport medium on ice for molecular detection of RSV, SARS-CoV-2, and influenza viruses. The SARS-CoV-2 RNA Extraction Kit (Viragene, Tehran, Iran) was used for the extraction of viral ribonucleic acids (vRNA) in accordance with the manufacturer's instructions. The extracted RNAs were checked with a Nanodrop spectrophotometer (Thermo Fischer Scientific) for quality and were stored at –80°C for further analysis. All samples were analyzed by real-time reverse transcription-PCR assay using SuperScript™ III Platinum™ One-Step qRT-PCR Kit (Invitrogen) to confirm viral infection. To test RSV, the nucleoprotein (N) gene was amplified following an optimal thermal cycling condition.

3 | RESULTS

Between 9 and 26 April 2022, 30 inpatients children were admitted to the Dezful Children’s Hospital. Table 1 shows the details of demographic and clinical characteristics as well as the test results for RSV, influenza, and COVID-19 for the studied children. The patient's ages ranged from 1 month to 5 years with median and mean ages of 9 and 18 months, respectively. The time from the onset of symptoms to hospitalization is between 0 and 7 days. The average time is 1.4 days (onset of symptoms/hospitalization). Our analysis showed no detectable influenza viruses and SARS-CoV-2 in none of the tested samples; however, RSV was confirmed in 16/30 (53.33%) swabs. Of 16 RSV-infected children, 10 (62.5%) cases were male and 6 (37.5%) cases were female, which shows males are more affected than females by the recent outbreak. The ratio of male/female was 1.67. Cough was the most complained symptom by children with RSV infection. High fever (more than 38°C), respiratory-related problems, and restlessness were other common manifestations in these patients. Almost all RSV-infected children had no history of past medical conditions. In the current study, children aged <1 year old were the most affected age group by RSV infection.

4 | DISCUSSION

For over 2 years of struggling, Iran has been one of the most affected countries in the Middle East by the outbreak of COVID-19 with more than 7.2 million laboratory-confirmed cases, more than 141 000 deaths, and more than 7 million recoveries by July 14, 2022 (https://www.worldometers.info/coronavirus/country/iran/). As elsewhere and from the very beginning of the pandemic, policy measures (i.e., national lockdowns, stay-at-home orders, travel and daily movement restrictions to infected areas, the closure of educational centers throughout the academic year, etc.) were taken on a wide scale to reduce the transmission and the rapid spread of SARS-CoV-2 and subsequently to save the human lives. These all strategies unintentionally resulted in the absence of seasonal and frequently detected viral pathogens worldwide in both adults and children during the pandemic. Here, we showed a notable change in the rate of hospitalization and reported an unexpected outbreak of RSV in 16/30 (53.33%) symptomatic children with about 1–5-month delay from the expected time a short after the relaxation of preimposed strict confinements on schools and social activities in April 2022. Fourteen out of 30 (46.67%) samples
| Sex/age | Date of symptoms onset | Clinical manifestations | Date of hospitalization | Past medical conditions | Symptoms onset—Hospitalization (day) | Virus detection |
|---------|------------------------|-------------------------|-------------------------|-------------------------|--------------------------------------|-----------------|
| M/8 months | April 9, 2022 | Cough, high fever, diarrhea, muscle pain, and fatigue | April 9, 2022 | — | 0 | RSV Influenza COVID-19 |
| M/5 years | April 10, 2022 | Cough, high fever, rhinorrhea/sneezing, recurrent fever and cough | April 10, 2022 | Immunodeficiency and chronic lung disease | 0 | RSV Influenza COVID-19 |
| M/5 months | April 10, 2022 | Cough, shortness of breath | April 11, 2022 | — | 1 | RSV Influenza COVID-19 |
| F/3 months | April 13, 2022 | Cough, shortness of breath | April 13, 2022 | — | 0 | RSV Influenza COVID-19 |
| M/1.5 months | April 13, 2022 | Difficulties in breathing, cough, restlessness | April 15, 2022 | — | 0 | RSV Influenza COVID-19 |
| M/1.5 months | April 13, 2022 | Restlessness, cough, high fever | April 15, 2022 | — | 2 | RSV Influenza COVID-19 |
| M/6 months | April 14, 2022 | Diarrhea, cough, restlessness | April 15, 2022 | — | 1 | RSV Influenza COVID-19 |
| F/10 months | April 14, 2022 | Cough, high fever, chill, shortness of breath, conjunctivitis | April 15, 2022 | — | 1 | RSV Influenza COVID-19 |
| M/2.5 years | April 14, 2022 | Conjunctivitis, difficulties in breathing, high fever, cough, sore throat, nausea/vomiting, shortness of breath | April 15, 2022 | — | 1 | RSV Influenza COVID-19 |
| F/2 months | April 14, 2022 | Restlessness, cough | April 15, 2022 | — | 1 | RSV Influenza COVID-19 |
| F/2.5 years | April 14, 2022 | High fever, cough, restlessness, difficulties in breathing, shortness of breath, headache, anorexia, diarrhea | April 15, 2022 | — | 1 | RSV Influenza COVID-19 |
| F/4 years | April 14, 2022 | High fever, sore throat, chill | April 15, 2022 | — | 1 | RSV Influenza COVID-19 |
| F/8 months | April 9, 2022 | Cough, high fever, nausea/vomiting, anorexia | April 16, 2022 | — | 7 | RSV Influenza COVID-19 |
| M/1 month | April 11, 2022 | Restlessness, cough | April 16, 2022 | — | 5 | RSV Influenza COVID-19 |
| F/2 months | April 14, 2022 | Cough | April 16, 2022 | Chronic cardiovascular disease | 2 | RSV Influenza COVID-19 |
| M/11 months | April 15, 2022 | Cough, high fever, restlessness, anorexia | April 16, 2022 | — | 1 | RSV Influenza COVID-19 |
| F/8 months | April 15, 2022 | Cough, nausea/vomiting | April 16, 2022 | — | 1 | RSV Influenza COVID-19 |
| M/4 months | April 15, 2022 | Cough, nausea, nasal congestion | April 17, 2022 | — | 2 | RSV Influenza COVID-19 |
| M/3 months | April 15, 2022 | Restlessness, nausea, diarrhea | April 17, 2022 | — | 2 | RSV Influenza COVID-19 |
| M/5 years | April 24, 2022 | Cough | April 24, 2022 | — | 0 | RSV Influenza COVID-19 |
| M/10 months | April 24, 2022 | Cough, sore throat, diarrhea, nausea/vomiting | April 25, 2022 | — | 1 | RSV Influenza COVID-19 |
| M/1 year | April 24, 2022 | Cough, high fever, diarrhea, nausea/vomiting | April 25, 2022 | — | 1 | RSV Influenza COVID-19 |
were negative for any of the tests. Several reasons can justify this issue: lack of standardization for sample collection, the different incubation periods of the disease in patients, late sampling, and low viral RNA levels in the later stages of the disease (the time interval between sampling and onset of symptoms), insufficient viral specimens, delays or poor storage conditions before arrival in the laboratory, PCR inhibitors and other respiratory viruses circulating at the same time.

To our knowledge, this is the first report from outbreaks of RSV experienced in Iran since the official announcement of the COVID-19 outbreak on February 19, 2020.9 Compared to data with the years before COVID-19, the pandemic has had a marked impact on the number of children referred to local hospitals and emergency departments due to non-COVID-19 viral infections (i.e., metapneumoviruses, and parainfluenza in addition to influenza and RSV which are mentioned above).8,10 As discussed in the literature, the pandemic of the influenza virus in 2009 had a similar impact on RSV seasonality and morbidity as similarly described for the 2020–2022 COVID-19 pandemic.11

In the meantime, viruses that were active in their typical seasons have mysteriously and nearly or completely disappeared (contrary to what has been seen for instance, for rhinoviruses12), and attempts have failed to laboratory confirm such viral infections. In the case of RSV which typically causes autumn and winter epidemics,13 Iran, until very recently, has not been the only nation to report a low number of RSV infections and hospitalizations14 as the virus has followed a marked reduction in terms of distribution and activity in other parts of the world (i.e., Italy8 and Korea15 in 2020/2021) regardless of how the nations of the world have responded to the global outbreak. The absence of these viruses appears to be attributed to the restrictions applied against the pandemic. In this context, since the pandemic began, nonpharmaceutical interventions (NPIs) (i.e., hand and respiratory hygiene practices) were implemented to control the spread of SARS-CoV-2 and to mitigate the virus transmission. These public health measures have also prevented successfully (as reported for South Africa in 2020,16 New Zealand in 2020,17 Korea in 2020–2021,15 and France in 2020–202118) both influenza viruses and respiratory syncytial virus, for instance, to make comeback in the pandemic era. We are still not sure which NIP has had the best effect in curbing the expected community infections during this period since the global expansion of the pandemic. In one example, in Western Australia, in the 2020 winter season, due to the closure of borders, both influenza viruses and RSV were strikingly reduced by 99.4% and 98.0%, respectively.19 Here, we observed a delayed and unprecedented RSV reappearance in the off-season (as described for New South Wales and Western Australia in 2020,20 Victoria [Australia] in 2021,20 Madrid [Spain] in 2021,13 Argentina in 2021,21 Ashdod [Israel] in 2021,22 and Tokyo [Japan] in 202123) shortly after the schools and daycare centers fully reopened (the risk of RSV rebound for ~23-fold24) and many of internal restrictions began to gradually relax in early 2022. Lifting such restrictions, however, provides an opportunity for viruses to arise; it might not be the only reason explaining the outbreak we encounter,
as in children’s communities where facial masks were not mandated and educational centers were not closed, off-seasonal epidemics were not noted. In Finland, for instance, no notable increase in the levels of RSV was seen when the schools and daycares reopened in 2020 during the spring months. As a hypothesis, this shifted epidemic may also be explained by a phenomenon designed as “viral interference,” which has explained a delayed circulation of the Influenza A virus in France during the pandemic of 2009. As rhinoviruses continued to be detected in the era, the role of such viruses in modifying and shifting RSV seasonality remains to be unresolved. The competition trend presumed for what has been seen in China and Switzerland during the pandemic is an open question that can determine the possible role of other respiratory viruses in suppressing the activity of seasonal viruses such as influenza viruses and RSV. The lack of natural immunity due to not the circulation of RSV and not being exposed to the virus is another factor that may have resulted in virus resurgence in susceptible communities as cited by countries mentioned above and more severe infection in infancy.

The current outbreak of RSV has been described in young children aged less than 5 years old with a higher prevalence rate in males than females and infants aged below 12 months than in other age groups who were diagnosed with such viral infection. Although the impact of gender differences is controversial, in Iran, from 1996 to 2013, the male to female ratio was analyzed at 1.5:1 in studied RSV-tested positive subjects suggesting males are the most affected sex groups by RSV infection. A similar finding has also been evidenced by recent reports from other parts of the world. In a systematic review from the Middle East and North Africa (MENA) region, male infants within the first year of life were shown to be more susceptible to developing RSV infection. In agreement with the results of a descriptive study from northern Spain, respiratory problems are common clinical features of RSV infection in both in- and outpatients. Here, as we reported similar findings in our patients, a previous description found coughing as the most common symptom seen in 98% of the cases followed by fever in 75% and labored respiration in 73% of RSV-admitted outpatients. These symptoms were also common in hospitalized children as described: 69% for fever and 95% for labored respiration.

There were some limitations to this study. The first limitation was the relatively small sample size which might not represent the real risk and define the magnitude of the outbreak. The work has focused on inpatients; however, the same outbreak might also observe in nonhospitalized children who were clinically diagnosed with respiratory infections at the time of the outbreak. The second limitation was the lack of genotyping of positive samples, which allows us not to identify the genotype(s) responsible for the outbreak. The third limitation was not investigating other respiratory viruses other than the three viruses mentioned above and investigating the possibility of simultaneous infections in the samples taken from the study subjects.

5 | CONCLUSION

In conclusion, it is time for neonatologists and pediatricians to be aware of and shift their focus toward other respiratory viruses instead of SARS-CoV-2 alone since RSV is just a sample for other viruses with the same site of localization. Given that RSV is a threat and the main cause of healthcare visits in infants and young children in the absence of successful and licensed vaccines, early detection is critical to determine the cause of death from respiratory illnesses in children and to take appropriate control and preventive measures. The presented small case series indicated that almost consistent with other reports, RSV can potentially flare and re-emerged concomitant with restrictions eased. Further epidemiological studies, in particular, among young children and infants with respiratory symptoms are needed to determine the outbreak nationwide and for timely and principled control of the epidemic.

AUTHOR CONTRIBUTIONS

Vahid Salimi conceived and designed the study. Leila Mohebi and Maysam Mard-Soltani collected the clinical specimens and information. Vahid Salimi and Hassan Karami analyzed the data and wrote the manuscript. Talat Mokhtari Azad and Jila Yavarian revised the manuscript. Hassan Karami, Negar Mirsalehi, and Nima Hoveidi Ardestani performed the experiments. Approval of the final version of the manuscript was done by all authors.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Ethical approval for this study was obtained from TUMS Medical Ethics [IR.TUMS.SPH.REC.1397.134]. Written informed consent was obtained from the parents of all patients.

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