

Original Article

Molecular Investigation of Seven Respiratory Viruses in Patients with Acute Respiratory Tract Infections

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Introduction: Respiratory tract infection (RTI) is among the important factors that can threaten human health and is one of the most prevalent etiological agents of death, especially in young children. Viruses account for approximately 80% of various RTIs worldwide. Our study aimed to investigate the molecular prevalence of seven respiratory viruses in RTI. Our results can help develop appropriate public health strategies and treatment management of RTIs.

Materials and Methods: 120 respiratory samples were obtained from patients with flu-like manifestations from hospitals referred to the Iran University of Medical Sciences during 2020-2022. Nucleic acid extraction was performed, and then real-time polymerase chain reaction was performed to analyze the specimens to investigate seven respiratory viruses.

Results: Of the 120 respiratory specimens collected from patients with RTI, viruses were identified in 41 (34.16%) samples, encompassing 24 (40.7%) females and 17 (27.9%) males. SARS-CoV-2 (n = 22, 18.3%) and Parainfluenza virus (n=18, 15%) were the predominant detected viruses in the present study, followed by respiratory syncytial viruses (n=1, 0.8%). Other viruses like Metapneumovirus, Adenovirus, Bocavirus, and Rhinovirus were not found in our study. We could not detect any co-infection in our analysis.

Conclusion: SARS-CoV-2 and Parainfluenza-virus were the predominant viruses detected in our study, followed by respiratory syncytial viruses. Other viruses, such as Metapneumovirus, Adenovirus, Bocavirus, and Rhinovirus, have not been found in our study. We could not detect any co-infection in our consideration. This result can help develop appropriate public health strategies, targeting prevention and avoiding inappropriate treatment such as using unnecessary antibiotics.



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Introduction

Acute respiratory tract infection (ARI) is considered one of the major infections in children and adults. The significant role of ARIs in worldwide morbidity and mortality was established [1]. ARIs, according to the anatomical location of the infection, are categorized into lower and upper respiratory tract infections and can induce various clinical manifestations, such as common cold to pneumonia [2, 3]. These infections are responsible for about 2 million deaths in young children, inpatient and outpatient visits in different age groups, especially children between 0-5 years of age [4]. Various organisms, such as viruses and bacteria, can cause ARI. Studies indicate that viruses are responsible for approximately 80% of ARIs worldwide. Viruses that can induce ARIs include Influenza viruses (types A and B), respiratory syncytial viruses (RSV), parainfluenza viruses (PIVs), human metapneumovirus (hMPV), human rhinovirus (HRV), coronavirus, Adenovirus (ADV), and bocavirus [5]. The seasonality transmission of infection diverges from virus to virus, and infections can occur during the year [6]. The clinical manifestations and diseases caused by these viruses differ according to the virus type and the person's immune status. Some viruses can induce serious disorders in immune-compromised people, while others can only cause Respiratory tract infection (RTI) [7-9]. Some risk factors that increase the risk of respiratory infection encompass age, health conditions, obesity, asthma, and smoking. For these reasons, young children, the elderly, and

immune-compromised adults are more prone to being infected by respiratory viruses. Severe clinical manifestations and high mortality rates are major concerns about these individuals [10-12].

Discovering which respiratory pathogen causes ARI is critical for developing appropriate public health strategies, targeting prevention, and avoiding inappropriate treatment such as using unnecessary antibiotics [13]. Our study aims to investigate the molecular prevalence of seven respiratory viruses in 120 individuals with RTIs who visited the hospitals referred to the Iran University of Medical Sciences during 2020-2022. The viruses that we analyzed in our study include Rhinovirus, Bocavirus, ADV, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Parainfluenza virus, hMPV, and RSV.

SARS-CoV-2 belongs to the Coronaviridae family and structurally is an enveloped virus with positive-sense single-stranded RNA. This virus, infecting over 20 million people, causes over 700,000 deaths globally as of 2021 [14]. PIVs, HMPV, and RSV, belong to the Paramyxoviridae family, and structurally, they have enveloped viruses with negative-sense single-stranded RNA. Parainfluenza viruses can induce respiratory infections, especially in young children and immunocompromised individuals [15]. Metapneumovirus is one of the etiological organisms of respiratory diseases, usually causing mild illness. However, hMPV can be associated with severe disease characterized by severe cough, high fever, myalgia, bronchiolitis,

and pneumonia in certain groups, such as the elderly over 65 years old and immunocompromised individuals [16]. RSV is the main etiological agent of respiratory infection and hospitalization among infants. RSV can cause about 34 million lower respiratory tract infections (LRTI) and 3.4 million hospitalizations annually in infants and young persons [17]. Rhinovirus is a small, nonenveloped virus classified in the Picorna viridae family. Due to the importance of rhinoviruses in inducing mild respiratory diseases like the common cold, these viruses were neglected for years. However, the ability of HRV to induce LRTI and severe illness was established in the last decade [18]. ADV is one of the major viruses in RTIs in different age groups. Children under five years old, immunocompromised individuals and young men in military training courses are susceptible to adenovirus respiratory infections [19]. Human Bocavirus (HBoV) is one member of the Parvoviridae family. This virus was first detected in respiratory specimens in 2005. Despite the frequent detection of HBoV in RTI specimens, the exact role of this virus in causing ARI is unclear [20]. Molecular methods are the most commonly used among the various methods available for detecting respiratory viruses.

These methods offer precise detection in a short time and can facilitate timely treatment decisions, thereby aiding in the management of infections [21, 22]. For these reasons, TaqMan real-time polymerase chain reaction (PCR) was performed to accurately and rapidly diagnose the target viruses in our study. We hope our study's results will help enhance our knowledge of the

viral etiology of respiratory infections, reduce unnecessary antibiotics, develop appropriate public health strategies, and prevent future outbreaks.

Materials and Methods

Sample collection

This study was performed on 120 patients with RTI admitted to hospitals referred to the Iran University of Medical Sciences during 2020-2022. Patients had flu-like manifestations such as fever (temperature ≥ 38), cough or sore throat, chills, headache, difficulty in breathing, nasal discharge, nausea, or vomiting. Throat or nasopharyngeal specimens were obtained, transferred in viral transport media, and maintained at -70°C . Then, specimens were transferred on ice to the laboratory for virus isolation and identification. Samples were analyzed to investigate seven respiratory viruses, including Rhinovirus, Bocavirus, Adenovirus, SARS-CoV-2, Parainfluenza virus, Metapneumovirus, and RSV.

Nucleic acid extraction

The samples' nucleic acid (RNA/DNA) was extracted by a highly pure viral nucleic acid kit (Yekta Tajhiz Kit, Tehran, Iran) based on the manufacturer's guidance. Extracted genomes were maintained at -70°C until tests were performed.

Real-time PCR

Extracted genomes were analyzed for specific virus detection using TaqMan Real-time PCR. We designed seven primers to detect viruses, including Rhinovirus, Bocavirus, ADV, SARS-CoV-2, Parainfluenza virus, metapneumovirus, and RSV. The PCR master mixture contained

TaqMan one-step PCR 2X Master Mix (10 µl), forward primer (1 µl), reverse primer (1 µl), probe (0.5 µl), and double-distilled water (2.5 µl). We mixed 15 µl of the master mixture with a 5 µl sample, in a total volume of 20 µl. The mixtures were amplified by 40 cycles of 94 °C and 60 °C for 10 and 30 sec at the denaturation and annealing-extension steps, respectively (Table 1).

Statistical analysis

We analyzed the data using SPSS, version 26 for Windows. Data comparisons were done by using the Fisher exact test. A Pvalue < 0.05 was considered significant.

Results

One hundred twenty respiratory samples were obtained from children with RTI to screen seven respiratory viruses. Participants suffered from flu-like manifestations such as fever (temperature ≥ 38), cough or sore throat, chills, headache, difficulty breathing, nasal discharge, nausea, or vomiting. Of 120 participants, 61 (50.8%) were men and 59 (49.2%) were women. The mean age of all patients was 38.9 years (ranging from under 5 to upper 20 years), including 36.3 years in males and 41.7 years in females. Viruses were identified in 41 (34.16%) samples, including 24 (40.7%) females and 17

(27.9%) males. SARS-CoV-2 (n = 22, 18.3%) and Parainfluenza virus (n=18, 15%) were the most prevalent viruses in our findings, followed by RSV (n = 1, 0.8%). Our study did not find other detected viruses such as Metapneumovirus, Adenovirus, Bocavirus, and Rhinovirus. We could not detect any co-infection in our sample. A high proportion of respiratory viruses were detected in adults > 20 years old, including SARS-CoV-2 (n = 19, 15.7%), Parainfluenza (n = 17, 14.2%), and RSV (n = 1, 0.8%).

Other viruses were found in two age groups: adults between 11-15 years old and children < 5 years old. SARS-CoV-2 (n = 2, 1.6%) and Parainfluenza (n = 1, 0.8%) were found in adults between 11-15 years old, and (n = 2, 1.6%) were detected in children < 5 years old. Our study detected no respiratory viruses in children 5-10 years old (Table 3). SARS-CoV-2 was more prevalent in females than males (10 females/8 males) in our study. The mean age of the patients was 47.2 years (ranging from 1-83 years). Meanwhile, Parainfluenza virus was more prevalent in males than females (12 males/10 females), and the mean age of infected persons was 37.8 years (ranging from 19-74 years). RSV was detected in a 37-year-old man. Virus-specific general features of the patients are indicated in Table 4.

Table 1. The chart for Real Time PCR

No.	Cycle	Step	Temperature	Time
1	1	Reverse transcription	50 °C	15 min
2	1	Initial denaturation	94 °C	3 min
3	45	Denaturation	94 °C	10 sec
		Annealing-extension	60 °C	30 sec
4	1	Device cooling	25 °C	1 sec

Table 2. Demographic features of the studied participants

Characteristic	All cases	Female	Male
Mean Age	38.9	41.7	36.3
Gender (M/F)	61/59		
Virus Detection (+/-)	41/120	24/59	17/61

Table 3. Distribution of viral infections in different age groups

Age groups	Detected Viruses No. (%)				
	Human Metapneumovirus	Parainfluenza virus	Respiratory syncytial virus	Severe acute respiratory syndrome coronavirus 2	Human rhinovirus
<5	0 (0%)	0 (0%)	0 (0%)	1 (0.8%)	0 (0%)
5-10	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
11-20	0 (0%)	1 (0.8%)	0 (0%)	2 (1.6%)	0 (0%)
>20	0 (0%)	17 (14.2%)	1 (0.8%)	19 (15.7%)	0 (0%)

Table 4. Virus-specific general characteristics of the study population

Parameters	Detected Viruses No. (%)				
	Human Metapneumovirus	Parainfluenza virus	Respiratory syncytial virus	Severe acute respiratory syndrome coronavirus 2	Human rhinovirus
Age range (years)	-	19-74	37	1-83	-
Age mean	-	37.8	37	47.2	-
Gender (M/F)	-	8/10	1/0	12/10	-

Discussion

RTI is among the major factors that can threaten human health and is one of the most prevalent etiologies of death, especially in young children. [1]. The significant role of viruses as the main cause of ARTI was established [2]. Influenza viruses (type A and B), RSV, PIVs, hMPV, HRV, coronavirus, adenovirus, and bocavirus can cause human respiratory tract infections [5]. In the current study, we investigate five prevalent respiratory viruses in 120 respiratory samples of patients who visited hospitals referred to the Iran University of Medical Sciences during 2020-2022. Viral agents were detected in 41

(34.1%) patients. Among the investigated viruses, SARS-CoV-2 was the most common virus, while PIVs and RSV were the second and third viruses detected in our study, respectively.

Different studies demonstrated that RSV, ADV, CoV, MPV, and PIV are associated with LRTI. RSV is the most prevalent pathogen in lower respiratory infections, and ADV infection is one of the major reasons for severe chronic disease and mortality among young children [23, 24]. Meanwhile, MPV, PIV, and CoV are responsible for severe RTI and pediatric hospitalization [25-27].

Similar to our study, Tai et al. analyzed 2292 respiratory specimens from inpatient adults with RTIs in Taiwan. They indicated that rhinovirus/enterovirus were the most prevalent viruses in patients. Similar to our results, PIV was their study's second most prevalent RV [28]. Although our study identified that the most prevalent respiratory virus was SARS-CoV-2, we could not find rhinovirus infection in our samples. One of the major causes of this difference can be the time difference between the two studies and the conditions of the COVID-19 pandemic.

Mengelle et al. used a multiplex real-time PCR assay to diagnose 15 RVs in 996 samples of 914 young children with respiratory symptoms in the emergency unit of the Toulouse University Hospital during the 2010-2011 winter seasons. They found that rhinovirus (40%) and RSV (49.9%) were the most common RVs in upper and lower respiratory infections. They detected other viruses, including influenza viruses (14.4%), adenovirus (9.6%), coronaviruses (9.1%), metapneumovirus (5.3%), and parainfluenza (4.9%) in lower and upper respiratory specimens. Unlike our study, they detected 174 co-infections in their study. The most prevalent co-infections included RSV (associated with rhinovirus, adenovirus, and CoV) and rhinovirus associated with adenovirus [29]. The importance of co-infection in causing severe respiratory diseases is based on the type of infecting viruses. RSV and rhinovirus are the most prevalent lower and upper respiratory tract pathogens in young children. Co-infection of these two viruses or other respiratory viruses can cause severe RTI [29-31]. Co-infections,

including RSV and MPV or RSV [32] and Rhinovirus [33], can induce severe bronchiolitis, whereas co-infections without RSV do not [34]. Hasan et al. analyzed the role of respiratory tract infection in 7388 inpatient children in rural Thailand during 2005–2010. They found the prevalent viruses were RSV (20%), HRV (18.7%), bocavirus (12.8%), and influenza viruses (8%) [35].

Various studies have been performed in Iran to find the epidemiology of respiratory viruses. Pourakbari et al. used molecular methods to detect the prevalence of three respiratory viruses-RSV, influenza virus, and adenovirus-among 232 hospitalized children with ARI. The most prevalent virus was RSV (40 cases, 17.2%), followed by influenza (10 cases, 4%) and adenovirus (8 cases, 3.4%) [36]. Ramezannia Z et al. used the same approach for screening the prevalence of RSV and HPIV-3 in Iranian children with RTI between April 2019 and March 2020. They reported that the frequency of RSV and HPIV-3 in children under five years of age was 18% and 2%, respectively, and RSV is one of the major causes of ARI in young children [37]. In another study, Keyvani et al. investigated 40 respiratory samples obtained from Idiopathic Pulmonary Fibrosis patients referred to IUMS hospitals between April 2015 and December 2016. They showed RSV, parainfluenza, rhino, corona, and influenza viruses were detected in 2.5% (1/40), 7.5% (3/40), 10% (4/40), 2.5% (1/40), and 0% (0/40) of cases, respectively [38].

The low prevalence of the respiratory virus in our investigation compared with the other

studies can be due to various reasons, including the current COVID-19 pandemic, seasonality transmission of viruses, and type of sampling. Different studies emphasized seasonal variations of respiratory viruses. RSV and CoVs are more prevalent in winter and cold climates, while hMPV is more detected in spring and early summer in children [39-42]. Rhinovirus depends on the climate and the geographic area and has two peaks in spring and autumn [43]. Parainfluenza virus can appear throughout the year, but most are detected in summer and autumn [44, 45].

Conclusion

SARS-CoV-2 and Parainfluenza-virus were the predominant viruses detected in our study, followed by RSV. Our study has not found other viruses such as Metapneumovirus, Adenovirus, Bocavirus, and Rhinovirus. We could not detect any co-infection in our consideration. This result can help develop appropriate public health strategies, targeting prevention and avoiding inappropriate treatment such as using unnecessary antibiotics. The present study has some limitations that should be mentioned. Our sample size was small, so further study with a bigger sample size might provide better

information about the role of the mentioned virus among various age groups. Second, we only used a nasopharyngeal swab in our analysis. Using other types of respiratory samples, such as tracheal/bronchial secretion and bronchial lavage fluid, could be helpful in a more accurate diagnosis of respiratory viruses. Third, the current COVID-19 pandemic may affect the fear of respiratory patients coming to the hospitals during our study. Further studies with bigger sample sizes and the utilization of various types of respiratory samples are recommended.

Ethical Considerations

All ethical considerations were followed in compiling this work. Ethical approval for this study was obtained from the Ethics Committee of Iran University of Medical Sciences under the Ethic code (IR.IUMS.REC.1400.100).

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Conflict of Interest

All authors confirmed that they do not have any conflict of interest.

Acknowledgments

Not applicable.

Authors' Contributions

SHR.M designed the study. SJ. K performed all statistical analyses. A.T and Z.S wrote, reviewed, and edited the manuscript. All authors read and approved the final draft.

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