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# Prevalence of Respiratory Syncytial Virus, Adenovirus, and Rhinovirus in Patients With Flu-like Symptoms Admitted to Mazandaran Province Health Centers in North of Iran: A Cross-sectional Study

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## ABSTRACT

**Background:** The main viral causes of acute respiratory diseases (ARDs) include influenza viruses A and B, respiratory syncytial virus (RSV), adenovirus (ADV), rhinovirus, and parainfluenza viruses (PIV). This study aimed to investigate the prevalence of RSV, ADV, and rhinovirus in patients with flu-like symptoms admitted to Mazandaran Province health centers in northern Iran from December 21, 2018, to March 21, 2019.

**Materials and Methods:** The nasopharyngeal samples were collected from the patients with flu-like symptoms admitted to the health centers. ADV, RSV, influenza virus, and human rhinovirus (HRV) were detected by polymerase chain reaction (PCR) and reverse-transcription PCR. Then, the products were visualized by gel electrophoresis on 1.5% agarose under UV light.

**Results:** In the present study, 26 samples (26%) contained ADV, while 9% and 5% of the samples were positive for RSV and HRV, respectively. Also, co-infection with the influenza virus was identified in 12 patients, while 8(66.66%) patients had a co-infection of influenza virus and ADV. Also, 2(16.66%) co-infected patients had RSV and influenza virus. This rate of co-infection was found about HRV and influenza virus. There were no triple or more co-infections in this study. Moreover, one death was reported among the patients with co-infection of influenza virus and adenovirus, while other co-infected patients were cured.

**Conclusion:** The findings will help public health officials and physicians to prepare strategies to control respiratory virus infections. Further molecular monitoring of respiratory viruses should be performed to investigate their epidemiological and clinical features.

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## Introduction

Acute respiratory diseases (ARDs) account for about 75% of all severe illnesses in developed countries, of which 80% occur due to the respiratory system colonizing viruses [1]. Acute respiratory infections (ARIs) are the most important causes of hospitalization and mortality in children and adults. Based on statistics, 6.6 million under 5-year children die each year due to ARIs [2]. Influenza virus (type A and B), respiratory syncytial virus (RSV), adenovirus (ADV), rhinovirus, and parainfluenza virus (PIV) type 1-3 are the most common ARDs emerging viruses (35% to 87%) in children and adults [3]. So, the prevalence of these viruses is significant in patients with acute respiratory diseases.

Adenovirus was first detected from adenoid tissue cell culture in 1953 by Rowe and his colleagues [4]. The adenoviruses are non-enveloped and contain a linear double-stranded DNA (dsDNA) by a terminal protein attached at the end of their genome [5]. These viruses can spread through fecal-oral route, respiratory droplets, contaminated objects, and direct contact with infected persons [5]. The respiratory complications caused by ADVs include laryngitis, croup, bronchiolitis, and pneumonia [6]. Also, at least 5%–10% of pediatric respiratory diseases, 1%–7% of adult respiratory infections, and 5%-15% of severe diarrhea in children are caused by adenoviruses [7]. The infection rate in children with bone marrow transplantation is 22%, with a mortality rate of 60% in these patients [8].

Respiratory syncytial virus (RSV) was first discovered in chimpanzees in 1955 and subsequently confirmed as a human pathogen [9]. This single-stranded negative-sense RNA virus can be transmitted through nasopharyngeal mucosa and conjunctiva by respiratory droplets, followed by rapidly spreading in the respiratory system [10]. RSV is the most common cause of acute lower respiratory tract infections among infants (1-3 months) and children, with a high mortality rate in infants under one year. The primary clinical symptom of the RSV infection is rhinitis, followed by bronchiolitis, pneumonia, cough, wheezing, and respiratory distress [10]. The RSV accounts for 43%-74% of bronchiolitis and 19%-54% of pneumonia [11]. Approximately 95% of the infected children are 2 years old, and some may be infected twice [11]. On average, this virus caused almost 33 million cases of acute lower respiratory tract infections, 3 million cases of hospitalization, and over 199000 cases of mortality in children worldwide [12].

Rhinovirus was first discovered in 1956 by Winston Price. This single-stranded positive-sense RNA virus is the most common cause of adult colds (2-3 times/year in adults and 8-12 times/year in children) [13]. More than 150 types of rhinoviruses have been identified and classified into 3 distinct classes (HRV-A to -C)—HRV-C can cause serious diseases [14]. The rhinoviruses can transmit through respiratory droplets, contact with infected people, and fecal-oral route [15]. The common clinical symptoms, such as cough, shortness of breath, chest pain, and wheezing, are non-specific [15]. Inducing immunodeficiency, the rhinovirus triggers severe chronic obstructive pulmonary disease (COPD), asthma symptoms in children and adults, severe bronchiolitis in infants and children, and fatal pneumonia in adults [16].

In the past two decades, the serological and viral antigen detection techniques, such as hemagglutination inhibition test (HAI), enzyme of immunoassay (EIA), investigation of the cytopathic effect (CPE), direct fluorescent antibody (DFA) staining, and shell vial culture (SVC) were the common diagnostic methods in the clinical laboratories for the detection of respiratory viruses, while they are too time-consuming with low sensitivity [7].

Today, molecular diagnostic techniques such as PCR with relatively high speed, accuracy, sensitivity, and specificity are developed and used to detect respiratory viruses. Rapid detection of these pathogens can guide physicians to more accurate diagnosis, reduce the length of hospital stay and unnecessary costs, and control nosocomial infections. So, we aimed to investigate the prevalence of HRV, RSV, and ADV in hospitalized patients with flu-like symptoms in Mazandaran Province health centers.

## Materials and Methods

### Study design and data collection

This study was conducted in Mazandaran Province, North of Iran, from December 21, 2018, to March 21, 2019. The nasopharyngeal samples were collected from the patients with flu-like symptoms (fever, sore throat, cough, and auxiliary symptoms such as shivering, muscle pain, and lethargy) admitted to the health centers. The age of the patients was under 91 years. The inclusion criteria for this study were the presence of flu-like symptoms according to demographic and clinical data extracted from structural questionnaires. Data collected included age, sex, history of underlying disease, symptoms at the first visit, postoperative supportive symptoms, history of medication use, need for oxygen, rate,

length of hospital stay, complications, and secondary infections. The relevant physician completed these questionnaires.

### Sample collection

The clinical samples were collected by a Dacron sterile swab (Maxwell, China) from the oropharynx of the patients. The specimens were transported by a viral transport medium (Hanks) to the Influenza Laboratory affiliated with Mazandaran University of Medical Sciences under cold conditions and stored at -70° C for polymerase chain reaction (PCR) and reverse-transcription PCR (RT-PCR).

### RNAs/DNAs extraction

According to the manufacturer's instructions, the viral RNAs and DNAs were extracted from the oropharyngeal swab samples by PureLink™ Viral RNA and DNA kits (Invitrogen, USA). First, 25 µL proteinase K was added into a sterile microtube containing 200 µL clinical sample. Then, a 200 µL lysis buffer (containing 5.6 µg carrier RNA) was added, and the contents were vortexed for 15 s and incubated for 15 min at 56°C. Next, 250 µL of 96%-100% ethanol was added to the microtube, mixed for 15 s, and incubated for 5 min at room temperature. In the next step, 675 µL of this product was transferred into the viral spin column tube and centrifuged for 1 min at 12000 rpm (~6.800 g). The viral spin column filter was moved into a new sterile microtube, washed twice with 500 µL of wash buffer, centrifuged for 1 min at 12000 rpm, and then for another minute at high speed. Afterward, the viral spin column filter was moved into a new sterile 1.5 mL microtube, and 50 µL of distilled water or RNase-free water was added to the middle of the viral spin column filter and incubated at room temperature for 1 min and centrifuged for 1 min at high speed. Finally, the extracted RNAs/DNAs concentration was determined using a spectrophotometer (Thermo NanoDrop, USA) and stored at -70°C until further use. All samples were examined at the Influenza Laboratory affiliated with Mazandaran University of Medical Sciences, North Iran.

### Polymerase chain reaction (PCR)

The PCR reaction was done in a volume of 15 µL using the specific primers as follows: RSV-forward-5′-TCTTCATCACCATACTTTTCTGTTA-3′, RSV-reverse-5′-GCCAAAAAATTGTTTCCACAATA-3′ [17] targeting *L* gene, ADV-forward-5′-GCCSCARTGGKCWTACATGCACATC-3′, ADV- reverse -5′-CAGCACSCCICGRATGTC -3′ [18] detecting *vp6* gene, and HRV-forward-5′-CCCCTGAATGYGGCTAACCT-3′, HRV-reverse-5′-CGGACACCCAAAGTAGTYGGTC-3′ (designed in this study) targeting 5′ *NCR*. Each reaction contained 7.5 µL of 2x reaction mix (Ampliqon, Denmark), 0.5 µL of each forward and reverse primer, 5 µL of the extracted DNA, and 1.5 µL of RNase/DNase-free water. The amplification progressed in 40 cycles, including denaturation at 95°C for 40 s, annealing at 61°C for 35 s, extension at 72°C for 30 s, and a final extension at 72°C for 4 min by a Rotor-Gene Q Thermal Cycler (Qiagen, USA).

### Revers-transcription polymerase chain reaction (RT-PCR)

Viral RNAs were subjected to reverse transcription using a First Strand cDNA Synthesis Kit (high capacity cDNA reverse transcription kit, Applied Biosystems, Germany Cat No: 4368813) according to the manufacturer's instructions. The reverse transcription method was used to detect RNA viruses. The RT-PCR was performed in two steps: the RT step for synthesizing cDNAs and the PCR step for amplifying the cDNAs using the specific primers (Metabion, Germany). We utilized 10 µL of cDNA in PCR mixture comprising 10 mM of Tris-HCl (pH=8.5), 50 mM of KCl, 1.5 mM of $MgCl_2$, 200 mM of each dATP, dCTP, dGTP, and dTTP, along with 0.5 µM of each primer, and 1.25 U of Taq polymerase (AmpliTaq; Perkin-Elmer Cetus) in a final volume of 50 µL by a Rotor-Gene Q Thermal Cycler (QIAGEN, USA). The RT-PCRs were done in 40 cycles at 60°C and 55°C for RSV and HRV, respectively. Moreover, the human respiratory syncytial virus and rhinovirus, obtained from the Influenza Laboratory at Mazandaran University of Medical Sciences, were the positive controls in this test. At the same time, these strains were verified using FTD respiratory pathogens 21 plus kit (Siemens Healthiness, Luxembourg) by the real-time method. The PCR products were visualized by gel electrophoresis on 1.5% agarose (Wizbiosolutions, South Korea) comprising 3 µL of DNA green viewer in 0.5x TBE (Tris/Borate/EDTA) buffer under UV light.

## Results

### Characteristics of the study population

A total of 100 non-duplicated oropharyngeal swabs were collected from hospitalized patients (50 males and 50 females) with flu-like symptoms. The outpatients were excluded, while 29 cases were children (<19 years old), and 71 others were adults. The median age of the

**Table 1.** Demographic characteristics of the study patients

| Demographic Data | | Patients With Positive Results | | | | | |
|---|---|---|---|---|---|---|---|
| | | ADV | | RSV | | HRV | |
| | | No. (%) | P | No. (%) | P | No. (%) | P |
| Gender | Female (n=50) | 13(50) | 1.000 | 6(66.7) | 0.259 | 4(80) | 0.169 |
| | Male (n=50) | 13(50) | | 3(33.3) | | 1(20) | |
| Age (y) | ≤5 (n=20) | 6(23.1) | 0.818 | 6(66.7) | 0.040 | 3(60.0) | 0.655 |
| | 6-15 (n=6) | 1(3.8) | | 0(0.0) | | 0(0.0) | |
| | 16-25 (n=7) | 1(3.8) | | 0(0.0) | | 0(0.0) | |
| | 26-35 (n=11) | 3(11.5) | | 1(11.1) | | 0(0.0) | |
| | 36-45 (n=13) | 5(19.2) | | 0(0.0) | | 1(20.0) | |
| | 46-55 (n=4) | 0(0.0) | | 1(11.1) | | 0(0.0) | |
| | 56-65 (n=9) | 2(7.7) | | 0(0.0) | | 0(0.0) | |
| | 66-75 (n=6) | 1(3.8) | | 0(0.0) | | 0(0.0) | |
| | 76-85(n=15) | 5(19.2) | | 0(0.0) | | 1(20.0) | |
| | >85 (n=9) | 2(7.7) | | 1(11.1) | | 0(0.0) | |

Abbreviations: ADV: Adenovirus; RSV: Respiratory syncytial virus; HRV: Human rhinovirus.

**Table 2.** Frequency of symptoms observed in study patients and relationships between the symptoms and the presence of viruses

| Symptoms in 100 Patients | ADV (n=26) | | RSV (n=9) | | HRV (n=5) | |
|---|---|---|---|---|---|---|
| | No. (%) | P | No. (%) | P | No. (%) | P |
| Fever (n=84) | 24(92.3) | 0.179 | 9(100.0) | 0.170 | 5(100.0) | 0.137 |
| Sore throat (n=66) | 20(76.9) | 0.172 | 2(22.2) | 0.004 | 5(100.0) | 0.100 |
| Muscle pain and contusion (n=42) | 13(50.0) | 0.337 | 2(22.2) | 0.208 | 3(60.0) | 0.403 |
| Chills (n=35) | 9(34.6) | 0.962 | 3(33.3) | 0.912 | 2(40.0) | 0.810 |
| Anorexia (n=41) | 10(38.5) | 0.760 | 6(66.7) | 0.101 | 2(40.0) | 0.963 |
| Joints pain (n=36) | 14(53.8) | 0.028 | 2(22.2) | 0.367 | 2(40.0) | 0.848 |
| Nausea/Vomiting (n=30) | 7(26.9) | 0.691 | 1(11.1) | 0.195 | 3(60.0) | 0.133 |
| Rhinorrhea (n=16) | 6(23.8) | 0.253 | 3(33.3) | 0.137 | 4(80.0) | 0.802 |
| Cough (n=80) | 20(76.9) | 0.919 | 8(88.9) | 0.485 | 4(80.0) | 0.485 |
| Dyspnea (n=56) | 13(50.0) | 0.474 | 7(77.8) | 0.168 | 3(60.0) | 0.853 |
| Seizure (n=6) | 1(3.8) | 0.591 | 2(22.2) | 0.032 | 0(0.0) | 0.562 |
| Diarrhea (n=17) | (65.4) 17 | 0.000 | 0(0.0) | 0.155 | 0(0.0) | 0.299 |

Abbreviations: ADV: Adenovirus, RSV: Respiratory syncytial virus; HRV: Human rhinovirus.

**Table 3.** Prevalence of underlying diseases in study patients

| Viruses | No. (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Patients With Underlying Diseases | | | | | | |
| | Chronic Cardiac Disease (n=22) | Chronic Pulmonary Disease (n=21) | Chronic Renal Disease (n=4) | Chronic Nervous Disease (n=1) | Diabetes (n=29) | Pregnancy (n=5) | Cancer (n=1) |
| **ADV** | 9(26.9) | 5(19.2) | 0(0.0) | 0(0.0) | 5(19.2) | 1(3.8) | 0(0.0) |
| P | 0.481 | 0.797 | 0.226 | NS | 0.202 | 0.754 | NS |
| RSV | 0(0.0) | 1(11.1) | 0(0.0) | 0(0.0) | 1(11.1) | 1(11.1) | 0(0.0) |
| P | 0.095 | 0.445 | 0.521 | NS | 0.215 | 0.378 | NS |
| HRV | 1(20.0) | 2(40.0) | 1(20.0) | 0(0.0) | 2(40.0) | 0(0.0) | 0(0.0) |
| P | 0.912 | 0.285 | 0.061 | NS | 0.578 | 0.599 | NS |

Abbreviations: ADV: Adenovirus; RSV: Respiratory syncytial virus; HRV: Human rhinovirus; NS: Not statistically significant.

patients was 39 years. The demographic characteristics of the patients are presented in Table 1.

## Prevalence of the respiratory viruses

Out of 100 collected samples in the present study, 26 samples (26%) were reported ADV positive, while 9% and 5% were positive for RSV and HRV, respectively. Also, co-infection with the influenza virus was identified in 12 patients, while 8(66.66%) patients had a co-infection of influenza virus and ADV. Also, 2(16.66%) RSV and influenza virus co-infected patients and 2(16.66%) HRV co-infection with influenza virus were detected. There were no triple or more co-infections in this study. Moreover, one death was reported among the patients with co-infection of influenza virus and adenovirus, while other co-infected patients were cured.

## Clinical symptoms

The most common symptoms of adenovirus infections were fever and sore throat. Also, half of the patients had muscle pain and shortness of breath. All patients infected with RSV complained of a fever; shortness of breath and anorexia were other symptoms in these patients. Also, sore throat, runny nose, cough, and muscle pain were the most significant symptoms in patients with rhinovirus. Also, we noticed 3 cases of seizures that occurred in the patients. Table 2 presents the frequency of other symptoms.

**Table 4.** Prevalence of complications in study patients

| Viruses | No. (%) | | | |
|---|---|---|---|---|
| | Patients With Complications | | | |
| | Viral Pneumonia | Bacterial Pneumonia | Bronchiolitis | Respiratory Support |
| ADV | 12(46.2) | 12(46.2) | 2(7.7) | 8(30.8) |
| P | 0.001 | 0.001 | 0.872 | 0.615 |
| RSV | 3(33.3) | 4(44.4) | 1(11.1) | 2(22.2) |
| P | 0.440 | 0.132 | 0.612 | 0.735 |
| HRV | 1(20.0) | 1(20.0) | 0(0.0) | 0(0.0) |
| P | 0.870 | 0.830 | 0.529 | 0.163 |

Abbreviations: ADV: Adenovirus, RSV: Respiratory syncytial virus; HRV: Human rhinovirus.

### Underlying medical conditions

We investigated the patients with underlying diseases as the high-risk groups needing special care and respiratory support. Chronic heart failure and chronic pulmonary disease were more common than other underlying diseases in the present study, as shown in Table 3.

### Complications of the viral infections in the patients

Approximately half of the patients infected by adenovirus were complicated with viral and bacterial pneumonia after infection. However, these complications had a lower rate in the RSV-infected patients. Also, one case of viral pneumonia was detected in a patient with rhinovirus infection. Moreover, 10 patients received respiratory support, such as a nasal cannula, pressure support, mechanical ventilation, etc. The prevalence of the complications in the patients of the present study is described in Table 4.

## Discussion

Acute respiratory disease syndrome (ARDS) is a serious condition in humans. Influenza, parainfluenza, RSV, ADV, and HRV are the most significant respiratory viruses in patients with ARI [1]. The present study collected clinical samples from hospitalized patients with flu-like symptoms to detect the prevalence of common respiratory viruses (RSV, ADV, and HRV).

In this study, ADV, with 26%, and HRV, with 5%, were the most and least prevalent viruses. There was a significant relationship between the age group and the prevalence of RSV in our study. This virus was more common in children under 2 years old (66.7%), concordant with the study conducted by Radin et al. [19]. The RSV in children seems more prevalent than other significant respiratory viruses in this area. Another research conducted on Italian community-dwelling adults reported that the prevalence of RSV in this age range was 1.6%, indicating the significance of this virus in children [20]. Besides, we detected a significant relationship between the emergence of sore throat and anorexia and RSV, contrary to the results of Liu et al. in 2016 [21]. Considering that most of the positive patients in our study were children and the possibility of complaints about anorexia and sore throat is more in this group, maybe this is the reason for this relationship. However, we detected that 2 of the 9 patients with RSV had a seizure similar to the results of Liu et al., in which 18 patients with RSV had a seizure [21]. We found 2 cases of RSV and influenza co-infection in our study, in contrast to another research conducted by Duttweiler et al. [22]. However, it was concordant with the analysis by Bont et al. that RSV/influenza co-infection occurred in 11 patients when there were 599 single RSV infections [23]. The discrepancy in prevalence is probably due to the sampling season, sample type, study population, etc.

Adenoviruses are implicated in 4%–10% of lower respiratory tract infections (LRTI) cases in children [5]. According to the results of the epidemiological studies, respiratory diseases caused by adenoviruses are common in winter and spring, so the samples of this study were collected in winter and early spring. In our study, the prevalence of adenovirus was reported as 26%, which was higher than the results obtained in a study conducted by Lessa et al. may be due to the community chosen [24]. However, the prevalence of adenovirus in another study by Jin et al. in China was 6.33% [22]. On the other hand, similar to our results, some studies have shown that the incidence of adenovirus infection is inversely relevant to the age group of the patients [25, 26]. Also, similar to the present study, other medical reports showed that the highest and most common complications in patients with adenovirus Upper Respiratory Tract Infections (URTI) are viral and bacterial pneumonia [6] and bronchiolitis [25]. Besides, 8 cases (66.6) of adenovirus and influenza co-infection were detected in the present study, of which one died. Also, 26.9% of adenovirus-infected patients had an underlying cardiovascular disease, consistent with the study of Straliotto et al. [27].

Rhinoviruses are the cause of the common cold in children and adults [13]. In a study by Wildenbeest et al. in the Netherlands, the prevalence of rhinovirus was reported as 43.5%, which is inconsistent with our results. This difference may be due to the different samples (only from children with wheezing) [28]. Considering that the prevalence of rhinovirus in the winter is higher than other viral agents causing respiratory infections, perhaps the low prevalence of this virus in our study was related to the time of collecting the samples, which was in late winter and early spring. However, another study conducted in the Netherlands showed similar results [29]. Also, we detected that the highest incidence of rhinovirus was observed in under the 5-year age group, especially in infants. This finding was in agreement with other studies carried out by Linder et al. [30]. It seems that the prevalence of HRV is higher in children, emphasizing the significance of this virus in the pediatric healthcare problem of ARI. This finding was similar to other cross-sectional studies conducted in the USA, from which 29.6% of their positive respiratory viral infection were related to the rhino-

virus [31]. They detected that most positive participants were inpatients or older pediatric patients, suggesting the significant role of rhinovirus in severe ARI, especially in younger than 2 years, contrary to other viruses, such as RSV, which are more prominent in winter months [31]. However, runny nose, fever (low grade), sore throat, and cough were among the most significant symptoms observed in rhinovirus-infected patients, similar to another study conducted by Heikkinen et al. [32]. On the other hand, one case of rhinovirus and influenza co-infection was observed in our study who healed. The low rate of co-infections in our study may be because a previous infection with a respiratory virus can result in an intrinsic immunity up-regulation or a non-specific cross-reaction against other viruses that decrease shedding of a following infection [20].

## Conclusions

This study provided a better understanding of viral respiratory infections and demonstrated the potential for modeling and risk assessment. The findings will help public health officials and physicians understand the rate of respiratory virus infections and co-infections. Further molecular monitoring of respiratory virus infections should be performed to investigate their epidemiological and clinical features.

### Study limitations

This study was performed on hospitalized patients with respiratory symptoms, while outpatients and asymptomatic populations were not included. Also, the present study was conducted in only 3 hospitals and may not represent the total population.

## Ethical Considerations

### Compliance with ethical guidelines

In this study, informed consent was prepared by the patients or their close relatives before the sampling. This study followed the Declaration of Helsinki, and the collected information was kept confidential. Also, this research was approved by the Ethics Committee Mazandaran University of Medical Sciences (Code: IR.MAZUMS.REC.1397.3012).

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### Authors contribution's

Conceptalisation and study design: Mohammad Reza Haghshenas and Hamid Reza Goli; Acquisition of data: Zeynab Daneshyar and Mehdi Rabie Rudsari; Analysis and data interpretation: Zeynab Daneshyar, Mehdi Haghshenas, and Mehdi Rabie Rudsari; Drafting of the manuscript: Zeynab Daneshyar; Final approval: All authors.

### Conflict of interest

The authors declared no conflict of interest.

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