Laboratory Investigation of Human Rhinovirus Infection in Cheonan, Korea

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ABSTRACT

Annually, millions of children die from respiratory virus infections. Human rhinovirus (HRV) is a causative agent of severe respiratory infections in young, elderly, and asthmatic patients with weak immunity. In this study, 9,010 respiratory virus specimens were collected from January 2012 to December 2018 at Dankook University Hospital, Cheonan and examined by real-time reverse transcription polymerase chain reaction. Twelve respiratory viruses were detected. The mean detection rate was 21.3% (N=1,920/9,010), and the mean age of HRV-positive patients was 6.5 years (median age: 1.6 years, range: 0.0 ~ 96.0). The detection rate was the highest in July (32.4%) and the lowest in February (8.3%). When the detection rate was analyzed by age group, the detection rate was the second highest in patients aged 10 ~ 19 years. The co-infection rate of HRV was 35.3%, and the most common combination was with Adenovirus. Respiratory virus infections are known to occur in children and elderly people with weak immunity. However, in this study, the detection rate in this age group was more than 15%, except in January and February. These results suggested that steady-state studies on the infection patterns of HRV are required.

INTRODUCTION

Respiratory viruses are one of the most common infectious diseases in children, accounting for 30 ~ 50% of the total number of hospitalized patients worldwide, and are responsible for the deaths of millions of children each year [1, 2]. The major causative agents of respiratory virus infections are Adenovirus, Coronavirus, Human rhinovirus, Influenza A and B viruses, Metapneumovirus, Parainfluenza viruses (1 ~ 3), and Respiratory syncytial viruses A and B.

Human rhinovirus (HRV) is a species of the Picornaviridae family, Enterovirus genus. HRV is currently classified into three species, HRV-A, -B, and -C, and there are more than 100 serotypes of HRVs [3]. Recent studies have reported that HRV-A and -C are detected more frequently than HRV-B. Real-time reverse transcription polymerase chain reaction (RT-PCR) can be used to detect all three species of HRV (HRV-A, -B, and -C). HRV is the...
most common cause of respiratory infections, regardless of age. Headache, sore throat, and cough are the main symptoms, and 50% of cold cases in adults are caused by HRV \[4, 5\]. HRV has also been reported to cause severe respiratory infections in patients that are immunocompromised due to age, tumors, or asthma and can aggravate asthma, leading to chronic obstructive airway infection \[6\]. This virus is also associated with all types of upper and lower respiratory diseases, but is of particular concern when causing lower respiratory tract infections in children, infants, immunocompromised patients, and the elderly. Thus, because of the risk posed by these respiratory viruses, the Korea Center for Disease Control and Prevention (KCDC) has developed the Influenza and Respiratory Viruses Surveillance System (KINRESS), which includes surveillance of HRV \[7\].

In this study, we investigated the patterns of infection for HRV, including annual, monthly, and overlapping infections at a local university hospital for 7 years. Although common colds are not associated with major health concerns, they can result in enormous costs to society in the form of missed school and work and unnecessary medical care.

### MATERIALS AND METHODS

1. **Collection of samples**

   The patients in this study provided 9,010 respiratory specimens (nasopharyngeal aspirates, nasal swabs, and throat swabs), which were sent to the Department of Laboratory Medicine, Dankook University Hospital, Cheonan for real-time RT-PCR, from January 2012 to December 2018. The samples were immediately tested, or if they were not immediately available, they were refrigerated at 4°C and tested within 24 h.

2. **Extraction of RNA**

   The collected respiratory specimens were treated with a QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany) to extract the RNA.

3. **Synthesis of complementary DNA (cDNA)**

   cDNA was synthesized using a RevertAid First Strand cDNA Synthesis Kit (Fermentas, Ontario, Canada). The reverse transcription reaction was performed by mixing 50 ng extracted RNA with random hexamers (0.2 μg/μL) at 25°C for 5 min. RT buffer, 10 mM dNTP, RNase inhibitor (20 μg/μL), and reverse transcriptase (200 μg/μL) were added to the mixture and reacted in a final reaction volume of 20 μL at 42°C for 60 min.

4. **Real-time RT-PCR**

   Extracted nucleic acids were then amplified and probed for HRVs with the AdvanSure RV real-time RT-PCR (LG Life Science, Seoul, Korea) according to the manufacturer’s instructions. Five microliters of extracted cDNA was added to a tube containing 5 μL of primer probe mix and 10 μL of one-step RT-PCR premix. For the reverse transcription step, this mixture was incubated at 50°C for 10 min. Denaturation was performed at 95°C for 30 s, followed by 10 cycles of PCR (15 s at 95°C, 30 s at 53°C, and 30 s at 60°C). Thirty additional cycles of PCR were completed for the detection of fluorescence signals (15 s at 95°C, 30 s at 53°C, and 30 s at 60°C).

5. **Statistical analysis**

   HRV detected by real-time RT-PCR was analyzed by various criteria, including overlapping infection, sex, age, year, and month. Results with P values of less than 0.05 were significant.

6. **Ethics**

   This study was approved by the IRB Committee of Dankook University (No. 2019-04-006).

### RESULTS

Among the 9,010 respiratory specimens collected during the study period, 12 respiratory viruses were detected in 5,081 specimens, with a detection rate of 56.4%; 1,920 specimens were positive for HRV (detection
rate: 21.3%). The detection rate for males was 22.0% (N=1,155/5,242), and the detection rate for females was 20.3% (N=765/3,768). The average age of the patients was 19.8 years (median age: 2.7 years, range: 0.0 ~ 96.0 years). The mean age of patients with HRV was 6.4 years (median age: 1.6 years, range: 0.0 ~ 93.2 years) (Table 1).

The detection rate in 2015 was the highest (30.7%; N=358/1165), and the second highest detection rate was in 2016 (27.4%; N=365/1333). The detection rate in 2018 was the lowest (13.9%; N=119/1435) during the study period (Figure 1). The detection rate in July was the highest (32.4%; N=191/590), and the detection rate in February was the lowest (8.3%; Figure 2, Table 1). The detection rate for patients 2 ~ 3 years old was the highest (36.5%; N=212/581), and the detection rate for patients 40 ~ 49

Table 1. Detection rate and number of specimen of HRV aggregated by month

| Month   | Total specimen (No.) | Positive specimen (No.) | Detection rate (%) |
|---------|----------------------|-------------------------|-------------------|
| Spring  | 3 887                | 174                     | 19.6              |
|         | 4 892                | 220                     | 24.7              |
|         | 5 814                | 219                     | 26.9              |
| Summer  | 6 636                | 175                     | 27.5              |
|         | 7 590                | 191                     | 32.4              |
|         | 8 610                | 141                     | 23.1              |
| Autumn  | 9 543                | 169                     | 31.1              |
|         | 10 650               | 178                     | 27.4              |
|         | 11 829               | 188                     | 22.7              |
| Winter  | 12 1058              | 136                     | 12.9              |
|         | 1 681                | 61                      | 9.0               |
|         | 2 820                | 68                      | 8.3               |

Figure 1. Annual positivity rate for respiratory specimens containing HRV isolated from Cheonan, Korea from January 2012 to December 2018.

Figure 2. Detection rate of HRV aggregated by month in respiratory specimens isolated from Cheonan, Korea from January 2012 to December 2018.

Figure 3. Detection rate of HRV aggregated by age in respiratory specimens isolated from Cheonan, Korea from January 2012 to December 2018.
Table 2. Detection rate and number of specimen of HRV aggregated by age

| Age (year) | Total specimen (No.) | Positive specimen (No.) | Detection rate (%) |
|------------|----------------------|-------------------------|--------------------|
| <1         | 3005                 | 708                     | 23.6               |
| 1 ~ 2      | 1047                 | 372                     | 35.5               |
| 2 ~ 3      | 581                  | 212                     | 36.5               |
| 3 ~ 4      | 364                  | 120                     | 33.0               |
| 4 ~ 5      | 299                  | 83                      | 27.8               |
| 5 ~ 6      | 230                  | 68                      | 29.6               |
| 6 ~ 7      | 181                  | 57                      | 31.5               |
| 7 ~ 8      | 147                  | 42                      | 28.6               |
| 8 ~ 9      | 120                  | 26                      | 21.7               |
| 9 ~ 10     | 97                   | 22                      | 22.7               |
| 10 ~ 11    | 72                   | 14                      | 19.4               |
| 11 ~ 12    | 68                   | 19                      | 27.9               |
| 12 ~ 13    | 53                   | 9                       | 17.0               |
| 13 ~ 14    | 59                   | 10                      | 16.9               |
| 14 ~ 15    | 70                   | 12                      | 17.1               |
| 15 ~ 16    | 59                   | 15                      | 25.4               |
| 16 ~ 17    | 55                   | 8                       | 14.5               |
| 17 ~ 18    | 38                   | 7                       | 18.4               |
| 18 ~ 19    | 17                   | 3                       | 17.6               |
| 19 ~ 20    | 14                   | 2                       | 14.3               |
| 20 ~ 29    | 105                  | 9                       | 8.6                |
| 30 ~ 39    | 128                  | 6                       | 4.7                |
| 40 ~ 49    | 209                  | 3                       | 1.4                |
| 50 ~ 59    | 326                  | 13                      | 4.0                |
| 60 ~ 69    | 483                  | 20                      | 4.1                |
| 70 ~ 79    | 717                  | 37                      | 5.2                |
| >80        | 466                  | 23                      | 4.9                |
| Total      | 9010                 | 1920                     |                    |

Table 3. Number of double infections with other respiratory viruses

| Virus                   | Number of multiple infections |
|-------------------------|------------------------------|
| Adenovirus              | 241                          |
| Coronavirus 229E        | 9                            |
| Coronavirus OC43        | 21                           |
| Influenza virus-A       | 24                           |
| Influenza virus-B       | 11                           |
| Metapneumovirus         | 48                           |
| Parainfluenza virus-1   | 38                           |
| Parainfluenza virus-2   | 13                           |
| Parainfluenza virus-3   | 85                           |
| RSV-A                   | 78                           |
| RSV-B                   | 63                           |

In this study, we evaluated the detection rates of HRV in a cohort of 9,010 patients. Our results showed that HRV infections were most common in patients ages under 10. Our findings provided insights into the prevalence of HRV in Korean populations.

During the period evaluated in our study, we observed two peaks in 2012 and 2015. Another study conducted over a similar period reported similar results, albeit with higher rates in 2011 and 2015 [8]. In Cheonan, HRV appeared to be an epidemic for a period of 2~3 years. The prevalence period depends on the subtype of HRV [9]. For this reason, it seems to be an epidemic for a period of 2~3 years.

The detection rate in July was the highest, followed by that in September. Detection rates averaged more than 15% per year, except for those (9.0% and 8.3%) in January and February, respectively. In previous studies, the incidence of HRV has been reported to increase in autumn and late spring, with lower rates during the other periods [10, 11]. In contrast, high detection rates were reported in summer months (June and July), in Korea [10]. Moreover, the detection rates of HRV in June and July were higher than 20%. Seasonal differences in respiratory viruses have been well documented [12]; however, the reason for this seasonality is unknown, and few studies have examined this topic [2, 13]. As previously noted, we found that HRV was most active in Korea in the summer, which was unusual because another study reported that HRV is more
Table 4. Number of triple infections with other respiratory viruses

| Pathogen          | Specimen (No.) |
|-------------------|----------------|
| HRV Adenovirus    | 3              |
| Coronavirus 229E  | 7              |
| Influenzavirus-A  | 3              |
| Influenzavirus-B  | 2              |
| Metapneumo virus  | 6              |
| Parainfluenza virus-1 | 1     |
| Parainfluenza virus-2 | 2     |
| Parainfluenza virus-3 | 22    |
| RSV-A             | 10             |
| RSV-B             | 11             |
| Coronavirus OC43  | 1              |
| Influenzavirus-A  | 0              |
| Influenzavirus-B  | 0              |
| Metapneumo virus  | 3              |
| Parainfluenza virus-1 | 0     |
| Parainfluenza virus-2 | 0     |
| Parainfluenza virus-3 | 0     |
| RSV-A             | 0              |
| RSV-B             | 0              |
| Influenzavirus-A  | 1              |
| Influenzavirus-B  | 0              |
| Metapneumo virus  | 1              |
| Parainfluenza virus-1 | 0     |
| Parainfluenza virus-2 | 0     |
| Parainfluenza virus-3 | 3     |
| RSV-A             | 0              |
| RSV-B             | 2              |
| Influenzavirus-B  | 0              |
| Metapneumo virus  | 0              |
| Parainfluenza virus-1 | 0     |
| Parainfluenza virus-2 | 0     |
| Parainfluenza virus-3 | 0     |
| RSV-A             | 1              |
| RSV-B             | 1              |
| Metapneumo virus  | 0              |
| Parainfluenza virus-1 | 0     |
| Parainfluenza virus-2 | 0     |
| Parainfluenza virus-3 | 0     |
| RSV-A             | 0              |
| RSV-B             | 0              |
| Parainfluenza virus-1 | 1     |
| Parainfluenza virus-2 | 2     |
| RSV-A             | 1              |
| RSV-B             | 0              |
| Parainfluenza virus-3 | 2     |
| RSV-A             | 0              |
| RSV-B             | 0              |
| Total             | 88             |

likely to cause severe illness in winter and spring [12]. This may be because of unrelated factors: for example, yellow dust, which causes severe respiratory problems in the Asia-Pacific region, could cause an increase in the rate of HRV infection [14]. Although this has not yet been studied in Korea, similar results may be observed. Thus, further studies on the serotype, genotype, and seasonality of HRV are required to understand the causes of increased detection rates in the summer.

In Gwangju, Korea, researchers reported that detection rates decreased gradually with patient age. However, in this study, the detection rate was the lowest in the 40s and increased again in patients 50 years old and older. Previous studies have focused on pediatric patients; thus, there are not many studies on adults and the elderly. Respiratory virus infections are more common in elderly people with weakened immune systems, but can also affect young people with very strong immune systems, as demonstrated in this study. Therefore, further studies are required.

In our study, double infections with adenovirus were the most common. Other studies have reported higher rates of RSV co-infection [15]. RSV-A was identified in 78 cases, RSV-B was identified in 63 cases, and RSV was identified in 141 cases. Co-infection with adenovirus was also commonly observed. Further analyses of virus-host interactions and host immune responses are necessary to improve our understanding of co-infections with RSV.

Initial clinical symptoms are similar for most respiratory viruses, making it difficult to distinguish between causative pathogens based on clinical symptoms [16]. However, clinical severity varies according to the type of respiratory virus, subtype, and virus amount [17, 18]. Co-infection with multiple viruses also affects the severity of the disease and has been reported to result in more severe infections than single infection [19]. However, in a separate study, co-infection was found to yield less-severe symptoms than single infection [20]. The growth of HRV is not significantly affected by the presence of other respiratory viruses, although studies have shown that the presence of HRV reduces the replication of other viruses [21]. Thus, the roles of respiratory viruses in co-infections are still
unclear, and further studies are required.

The prevalence of HRV and the overall detection rate was highest in July during the Korean summer. In other countries, the HRV detection rates are not highest in July, highlighting a unique feature of HRV infection in Korea. Notably, the majority of positive samples (94.2%) were collected from patients under 20 years of age. Further analysis showed that high detection rates were observed in patients 2~3 years of age. Moreover, HRV and adenovirus were the most frequent multiple infections found in our samples, and this correlation should also be evaluated. The range of epidemics was diverse, resulting in localized epidemics or simultaneous epidemics worldwide. Therefore, it is important to diagnose the causative virus early, prevent the abuse of antibiotics, and provide proper treatment. The results of our study could be helpful for the development of preventive guidelines for the treatment of respiratory virus infections.

This study is a retrospective study, and we therefore could not evaluate relationships with clinical symptoms in patients. Additionally, we did not assess the distributions of serotypes and genotypes. In future studies, it is necessary to study the distribution of serotypes, genotypes, and nucleotide sequences.

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