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ORIGINAL ARTICLE

Laboratory Investigation of Human Rhinovirus Infection in Cheonan, Korea

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7년간 천안지역 대학병원에서의 라이노바이러스 감염 양상에 대한 연구

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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 was second highest in patients aged 10~19 years. Indeed, 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

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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

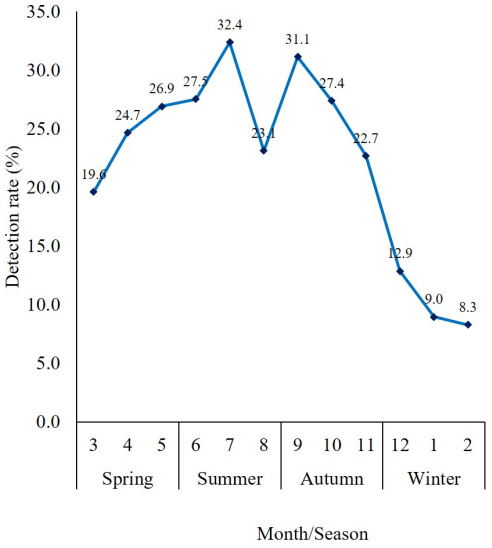

Figure 2. Detection rate of HRV aggregated by month in respiratory specimens isolated from Cheonan, Korea from January 2012 to December 2018.

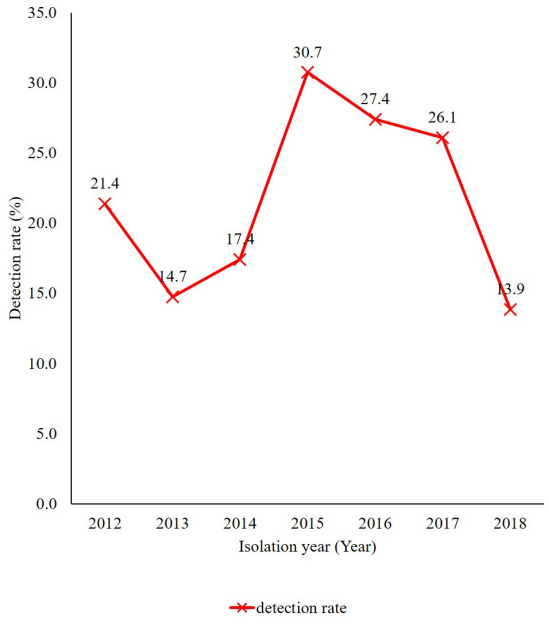

Figure 1. Annual positivity rate for respiratory specimens containing HRV isolated from Cheonan, Korea from January 2012 to December 2018.

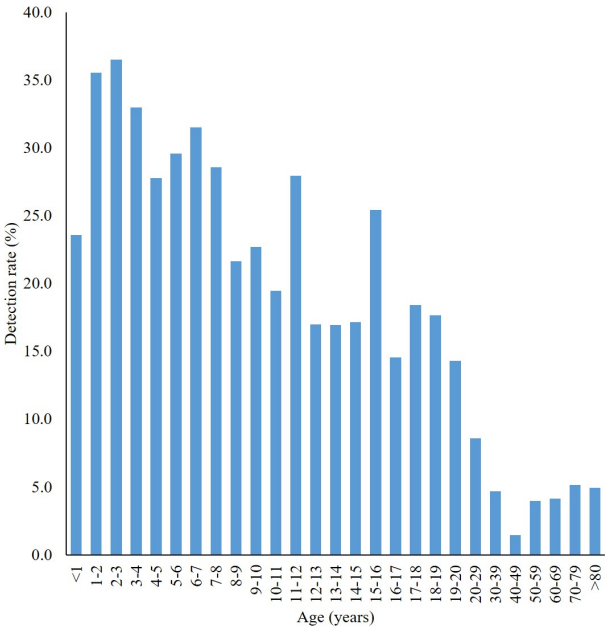

Figure 3. Detection rate of HRV aggregated by age in respiratory specimens isolated from Cheonan, Korea from January 2012 to December 2018.

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

years old was lowest (1.4%; N=3/209). The detection rate of patients less than 10 years old was the most highest (28.2%, N=1,710/6,071); Figure 3, Table 2).

In total, 677 specimens showed multiple infections of HRV plus other respiratory viruses. Among multiple infections, 585 specimens showed double infections, 88 specimens showed triple infections, and four specimens showed quadruple infections. Among quadruple infections, two cases showed infections with HRV, *Adenovirus*, *Coronavirus* OC43, and RSV; one case showed infections with HRV, *Adenovirus*, *Coronavirus* OC43, and *Parainfluenza virus*-3; and one case showed infections with HRV, *Adenovirus*, *Parainfluenza virus*-1, and *Parainfluenza virus*-3. Among multiple infections, HRV and *adenovirus* were the most frequent multiple infections found (Tables 3 and 4).

DISCUSSION

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	Coronavirus 229E	3
		Coronavirus OC43	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 229E	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
	Coronavirus OC43	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-A	Influenzavirus-B	0
		Metapneumo virus	0
		Parainfluenza virus-1	0
		Parainfluenza virus-2	0
		Parainfluenza virus-3	0
		RSV-A	1
		RSV-B	1
	Influenzavirus-B	Metapneumo virus	0
		Parainfluenza virus-1	0
		Parainfluenza virus-2	0
		Parainfluenza virus-3	0
		RSV-A	0
		RSV-B	0
	Metapneumo virus	Parainfluenza virus-1	1
		Parainfluenza virus-2	0
		Parainfluenza virus-3	2
		RSV-A	1
		RSV-B	0
	Parainfluenza virus-1	Parainfluenza virus-2	0
		Parainfluenza virus-3	0
		RSV-A	0
		RSV-B	0
	Parainfluenza virus-2	Parainfluenza virus-3	0
		RSV-A	1
		RSV-B	0
	Parainfluenza virus-3	RSV-A	0
		RSV-B	0
	RSV-A	RSV-B	3
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. Accordingly, it will be necessary to evaluate the cause of increased infections during the summer 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.

요 약

매년 호흡기 바이러스 감염으로 인해 수 백만명의 소아들이 사망한다. 호흡기 바이러스 감염의 원인 병원체 중 *Human rhinovirus*(HRV)는 코감기의 주요 원인 균으로 면역력이 약한 영, 유아, 노인 그리고 천식 환자에게 심각한 호흡기 감염의 원인으로 작용하는 병원체이다. 2012년 1월부터 2018년 12월까지 천안 단국대학교 병원 진단검사의학과에 호흡기 바이러스 검사가 의뢰된 호흡기 검체 9,010개의 검체를 real time reverse transcription PCR (real time RT-PCR) 방법으로 검사했다. 총 12종의 호흡기 바이러스를 real-time RT-PCR로 검출했다. 연구기간 중 평균 검출률은 21.3%이었고, HRV 양성 환자의 평균 연령은 6.5세였다. 7월의 검출률이 32.4%로 가장 높게 나타났고 2월이 8.3%로 가장 낮았다. 연령대별로 검출률을 분석해봤을 때 10세 미만의 검출률이 가장 높았다. HRV의 중복 감염률은 35.3%이고, 가장 흔한 조합은 *Adenovirus*와의 조합이었다. 호흡기 바이러스 감염증은 비슷한 임상 증상을 가지고 있어 빠른 진단이 이루어 져야 적절한 시기에 적절한 치료를 할 수 있다. 호흡기 바이러스 감염은 보통 면역력이 약한 어린아이와 노인에서 주로 발생하는 것으로 알려져 있다. 하지만 본 연구에서는 10세 미만에 이어 10대 환자들의 검출률이 높았다. 그리고 1,2월을 제외하고 15% 이상의 detection rate를 보였다. HRV의 감염 양상에 대한 꾸준한 연구가 필요할 것으로 사료된다.

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