

Human coronavirus infection in hospitalized children with community-acquired pneumonia

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Purpose : Human coronaviruses (hCovs) including hCoV-229E and hCoV-OC43 have been known as etiologic agents of the common colds and were regarded as clinically insignificant agents. However, recent identification of hCoV-NL63 and hCoV-HKU1 in children with lower respiratory tract infections has evoked the clinical concerns about their prevalence and the clinical significance of these hCovs in children. This study was performed to investigate the prevalence of hCovs in children with community-acquired pneumonia.

Methods : From March 2006 to January 2007, nasopharyngeal specimens collected from children hospitalized with pneumonia, were tested for the presence of common respiratory viruses (respiratory syncytial virus, influenza A, influenza B, parainfluenza viruses, and adenovirus) using multiplex reverse transcriptase polymerase chain reaction (RT-PCR). Human metapneumovirus (hMPV) infection was excluded by nested RT-PCR using primers for the F-gene. To detect the different strains of hCovs, nested RT-PCR assays specific for hCoV-NL63, hCoV-OC43, hCoV-229E, and hCoV-HKU1 were performed.

Results : Out of the 217 nasopharyngeal aspirate from children aged under 15 years, respiratory syncytial virus (RSV) was detected in 32 patients, hMPV in 18, human parainfluenza virus in 10, influenza virus A in 2, and adenovirus in 6. hCovs were detected by RT-PCR in 8 (3.7%) of the 217 patients, hCoV-229E in 1, hCoV-NL63 in 3, and hCoV-OC43 in 4 patients. hCoV-HKU1 was not detected in this study population.

Conclusion : Recently identified hCoV-NL63 and hCoV-HKU1 seemed to have a little clinical significance in Korean children with severe or hospitalized community-acquired pneumonia. (**Korean J Pediatr Infect Dis 2007;14:69-74**)

Key Words : Human coronaviruses, hCoV- HKU1, hCoV-229E, Pneumonia, Children

Introduction

Human coronaviruses (hCovs) such as hCoV-229E and hCoV-OC43 have been mainly regarded as etiologic agents of the common colds¹⁻³, although some reported that they can be a cause of lower respiratory tract infections in children and adults⁴⁻⁹.

Recent identification of hCoV-NL63 and hCoV-

HKU1, warrants the clarification of the clinical importance of hCovs in children with severe lower respiratory infections^{10,11}. hCoV-NL63 was found to be a cause of acute respiratory tract infection, especially in croup¹²⁻¹⁴. hCoV-HKU1 was recently identified in a patient with pneumonia in Hong Kong¹¹, and its prevalence was reported to be 0-3.1% in patients with acute respiratory tract infections¹⁵⁻¹⁸. However, the epidemiology of hCoV-HKU1 in Korean children is not known. The purpose of this study was to investigate the epidemiology of hCoV infections in children hospitalized for commu-

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nity-acquired pneumonia.

Material and Methods

1. Study population and Methods

Nasopharyngeal aspirates (NPAs) were prospectively collected from hospitalized children with community-acquired pneumonia at Sanggyepaik Hospital in Seoul during an 11-month period, between March 2006 and January 2007. The patients ranged in age from 1 month to 168 months (median age: 23.9 months), with children younger than 12 months of age comprising 27.6% (60/217) of the study population. Pneumonia was diagnosed when rales were documented on auscultation or evidence of pulmonary consolidation on radiograph. All NPAs were tested for common respiratory viruses (influenza A and B viruses, parainfluenza virus types 1, 2, and 3, hRSV, and adenovirus) using multiplex reverse transcriptase polymerase chain reaction (RT-PCR), as described previously¹⁹⁾. RT-PCR for hMPV using specific primers was performed, as described previously²⁰⁾.

2. RT-PCR for coronaviruses

Viral RNA was extracted from each sample using a QIAamp viral mini kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturers instructions. Reverse transcription was performed on 0.5 μg of each RNA in a final volume of 20 μL containing 5 μM random hexadexynucleotides, 1mM of each dNTP, 2 units of RNase inhibitor and 9 units of reverse transcriptase (Bioneer, Daejeon, Korea).

PCR for coronaviruses was performed using four different specific primers to amplify hCoV-HKU1, hCoV-NL63, hCoV-OC43, and hCoV-229E, respectively, as described previously^{11, 12, 19, 21)} (Table 1). To detect HCoV-NL63, previously described nested PCR assays for 1a and 1b gene were performed. Three different sets of primer for complete *pol* gene were used to detect hCoV-HKU1. Each primer mixture contained cDNA, PCR buffer, 200 uM concentration of each deoxynucleoside triphosphate, and 1 U Taq polymerase. PCR was done using the following reaction conditions: initial denaturation at 94°C for 5 minute; 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute; and

Table 1. Primers Used in PCR for Human Coronaviruses

Virus	Primer	Sequence	Ref.
hCoV NL-63	1b gene (F)	5-GTGATGCATATGCTAATTTG-3 (1 st round)	17)
	(R)	5-CTCTTGACAGGTATAATCCT-3	
	(F)	5-TTGGTAAACAAAAGATAACT-3 (2 nd round)	
	(R)	5-TCAATGCTATAAACAGTCAT-3	
	1a gene (F)	5-CTTTTGATAACGGTCACTATG-3 (1 st round)	
	(R)	5-CTCATTACATAAAAACATCAAACGG-3	
	(F)	5-GGTCACATATGTAGTTTATGATG-3 (2 nd round)	
	(R)	5-GGATTTTTTCATAACCACTTAC-3	
hCoV HUK-1	LPW1465 (F)	5-GTTC AAGTGTGCTGTTCA-3	11)
	LPW1822 (R)	5-CTATCATTATCACAATCCACAG-3	
	LPW1467 (F)	5-GGGTATGAAGTATCATCCTA-3	
	LPW1825 (R)	5-GATAATCCCAACCCATAAGAAC-3	
	LPW1826 (F)	5-CATCTTATAAAGGATGTTGAC-3	
	LPW1829 (R)	5-ACAAACAACCCATAAGAAC-3	
hCoV 229E	MD1 (F)	5-TGGGCCCATTTAAAAATGTGT-3	21)
	MD3 (R)	5-CCTGAACACCTGAAGCCAAT-3	
	MD-2i	5-CCGTATCAACACTCGTTATGTGG-3	
hCoV OC43	MF1 (F)	5-GGCTTATGTGGCCCTTACT-3	21)
	MF3 (R)	5-GGCAAATCTGCCAAGAATA-3	
	MF2i	5-CTCCAAAAACTTCCAGTTC-3	

final extension at 72°C for 10 minutes. The PCR products were separated by electrophoresis in 2% agarose gel, visualized with ethidium bromide and were sequenced with an ABI prism 3700 DNA analyzer (Applied Biosystem, Foster City, CA) to confirm the presence of hCoVs.

Results

A total of 217 children aged less than 15 years were enrolled (120 males; mean age, 23.9 months). The age distribution of the study population was: 27.6% (60/217) aged <12 months, 51.6% (112/217) aged 12–35 months, 13.4% (29/217) aged 36–59 months, and 7.4% (16/217) aged >60 months. HCoV infection was detected by RT-PCR in 3.7% (8/217) of patients; hCoV-229E in 1 patient, hCoV-NL63 in 3, and hCoV-OC43 in 4. HCoV-HKU1 was not detected in the study population. RSV was detected in 32 patients, hMPV in 18, human parainfluenza virus in 10, influenza virus A in 2, and adenovirus in 6 (Table 2).

Among 8 cases of hCoV infections, pure hCoV infection was found in 75% (6/8) of patients; hCoV-229E in 1 patient, hCoV-OC43 in 2, and hCoV-NL63 in 3. Co-infection with another respiratory virus was detected in 2 hCoV-OC43 positive patients. We

reviewed the medical records of the 8 patients with hCoV positive samples. Fever was noted in 6 (75%) of 8 patients and gastrointestinal symptoms in 1 (12.5%) patient. The seasonal distribution of hCoVs by month of the year is shown in Fig. 1.

Discussion

HCoVs are known to be associated with lower respiratory tract infection in children, but there is insufficient data regarding the clinical significance of hCoV infections in children⁴⁻⁶⁾. In the current study, a total of 8 (3.7%) children tested positive for one of the hCoVs, which suggests that hCoVs may have a minor role in children with community-acquired pneumonia. The lower prevalence of hCoVs in this study compared to those of previous studies^{3-5, 7, 22-24)} may be due to several factors; namely the lower pathogenicity of hCoVs, different study population, season of low prevalence and sensitivity of detection methods used for hCoV strains.

Co-detection of hCoV-OC43 and another respiratory virus was found in 50% (2/4), which is similar to those of previous studies^{5, 7, 23)}. Recently, Gerna et al.¹⁸⁾ reported that hCoVs have a great pathologic potential in infants and young children and suggested that co-infection by hCoVs and other respira-

Table 2. Prevalence of Different Respiratory Virus Infections during the Study Period

Patients	No. positive samples
No. tested	217
No. positive for respiratory viruses (%)	76 (35.0)
No. (%) for	
hRSV	32 (14.8)
hMPV	18 (8.3)
Influenza viruses	2 (0.9)
Parainfluenza viruses	10 (4.6)
Adenoviruses	6 (2.7)
hCoV-229E	1 (0.5)
hCoV-OC43	4 (1.5)
hCoV-NL63	3 (1.4)
hCoV-HKU1	0 (0)
Mixed detection	6 (2.8)
Total hCoV infections	8 (3.7)

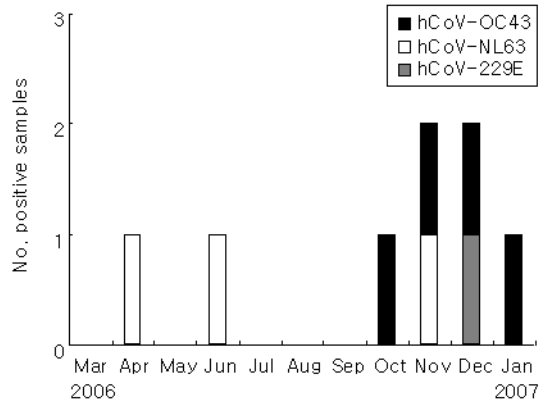


Fig. 1. Seasonal distribution of hCoV-229E, hCoV-NL63, and hCoV-OC43 in children with pneumonia from March 2006 to January 2007.

tory viruses may have a possible association with the severity of lower respiratory tract infections. However, it is still under debate whether the detection of upper respiratory tract viruses such as rhinovirus, hCoV-229E, and hCoV-OC43 in nasopharyngeal specimens from children with pneumonia were just incidental findings after a previous URI.

The prevalence of hCoV-NL63 worldwide is between 1.5%–9.3%^{12-14, 25, 26)}. In our previous report, hCoV-NL63 was found in 1.7% of children with acute respiratory tract disease and it is especially common in cases of croup²⁵⁾. Choi et al.²⁷⁾ reported that hCoV NL63 infection was detected in 1.6% of patients with lower respiratory infections and commonly found in croup, which is similar to those of our previous results²⁵⁾.

In a recent prospective study, hCoV-NL63 was found mostly in patients with mild disease and its clinical impact was no different from that of hCoV-229E and hCoV-OC43⁷⁾. Although, hCoV-NL63 has been suggested as an etiologic agent of severe pneumonia in an Australian study¹²⁾, the clinical role of this virus in our study was minor in children with pneumonia, which is similar to other recent studies^{8, 17)}.

In a previous study¹¹⁾, hCoV-HKU1 was shown to be responsible for 2.5% of community acquired pneumonia and the elevation of specific antibody for hCoV-HKU1 was confirmed in these patients. However, the clinical importance of hCoV-HKU1 infection in children with pneumonia is still difficult to define. In the present study, hCoV-HKU1 was not found in children with pneumonia, which is different from recent studies showing high prevalence of hCoV-HKU1 in patients with upper and lower respiratory tract infections^{16, 28)}. The difference in hCoV-HKU1 prevalence may be due to several factors such as size of the study population, methodological flaw, low sensitivity of primers used, geographical variance, disease severity, and cyclic pattern of hCoV-HKU1 infections. To eliminate the possibility of low prevalence due to the small study population, we tested for the presence of hCoV-HKU1 using RT-PCR

on 175 nasopharyngeal aspirates from hospitalized children with pneumonia collected during our previous study. HCoV-HKU1 was not detected in these additional samples (data not shown). The possibility of a technical problem is remote because we used positive control (kindly donated by Dr. Yuen KY, Honk Kong University) in each RT-PCR for hCoV-HKU1. HCoV-HKU1 infections have been suggested as an etiologic agent of severe lower respiratory tract infection in Hong Kong, USA, France, and Australia^{11, 16, 18, 29, 30)}. In a recent prospective study by Lau et al.¹⁵⁾, hCoV-HKU1 was detected in only 0.3% of patients with acute respiratory tract infection; with upper respiratory tract infection being the most common manifestation in young children. Our results indicate that hCoV-HKU1 may have a little clinical importance in Korean children with pneumonia. Further studies, involving a larger population with more varied clinical diseases over a longer period, are needed to elucidate the epidemiology of hCoV-HKU1 in Korean children.

In conclusion, recently identified hCoV-NL63 and hCoV-HKU1 seemed to have a little clinical significance in Korean children with severe or hospitalized community-acquired pneumonia. However, further studies are needed to clarify the epidemiological and clinical features of hCoVs including hCoV-HKU1 in children with lower respiratory tract infections.

국 문 요 약

입원한 폐렴 환아에서 코로나 바이러스 감염

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목 적 : 지금까지 코로나바이러스는 대부분 상기도 감염을 일으키며 임상적으로 큰 의미가 없는 것으로 여겨져 왔다. 하지만 최근에 발견된 코로나 바이러스인 hCoV-NL63와 hCoV-HKU1는 하부 호흡기 질환과의 연관성이 있는 것으로 보고되면서 임상적 의의에 대한

연구가 필요한 실정이다. 이에 저자들은 폐렴으로 입원한 소아 환자에서 최근에 발견된 hCoV-NL63과 hCoV-HKU1을 포함한 코로나바이러스 감염의 유행률을 알아보기 위하여 본 연구를 시행하였다.

방법 : 2006년 3월부터 2007년 1월까지 폐렴으로 상계백병원에 입원한 소아에게서 비인두 흡인물을 수집하였다. Multiplex RT-PCR 방법을 이용하여 RSV, influenza A, influenza B, parainfluenzavirus 및 adenovirus 감염을 진단하였으며, F 유전자 시발체를 이용한 nested RT-PCR에 의해 hMPV 감염을 진단하였다. 코로나바이러스 감염을 진단하기 위해 hCoV-NL63, hCoV-OC43, hCoV-229E 및 hCoV-HKU1에 각각 특이적 시발체를 이용하여 nested RT-PCR을 시행하였다.

결과 : 총 217명의 입원한 15세 이하의 폐렴 환아에게서 수집한 비인두 흡인물에서 multiplex PCR 방법에 의해 RSV 양성은 32명, hMPV는 18명, parainfluenza virus는 10명, influenza virus A는 2명, adenovirus는 6명에서 양성이었다. RT-PCR에 의해 hMPV 양성은 18명에서 확인되었다. 코로나바이러스는 RT-PCR 방법에 의해 8명 (3.7%)에서 양성이었으며, hCoV-229E 1명, hCoV-NL63 3명, 그리고 hCoV-OC43가 4명에서 검출되었다. 하지만 hCoV-HKU1은 연구 대상군에서 검출되지 않았다.

결론 : 아직 추가 연구가 필요하지만 최근에 발견된 hCoV-HKU1과 hCoV-NL63은 폐렴으로 입원한 국내 소아에서 임상적인 중요성이 적을 것으로 생각된다.

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