

Human Metapneumovirus Infection in Hospitalized Children with Acute Respiratory Disease in Korea

Human metapneumovirus (hMPV) is a recently isolated virus, mostly associated with acute lower respiratory infection in children, of which symptoms are similar to those of respiratory syncytial virus (RSV) infection. The aim of our study was to determine the frequency of hMPV in hospitalized children with acute respiratory tract disease in Korea. Nasal aspirates from hospitalized children with respiratory infections under 15 yr old between December 2003 and February 2005 were included in the study. Each sample was analyzed for RSV, adenovirus, influenza virus A and B, and parainfluenza virus by indirect fluorescent assay (IFA). F-gene sequences were used for PCR for the detection and sequencing of hMPV. In total 381 samples, negative samples in which any viral pathogen could not be identified by IFA were 231 cases. hMPV was detected using reverse transcriptase-PCR (RT-PCR) in 28 of 231 (12.1%) children who were not infected with another respiratory viruses. The hMPV-infected children were diagnosed as having pneumonia, bronchiolitis, bronchial asthma exacerbation, croup, and upper respiratory tract infection. Most of the RT-PCR positive samples for hMPV were collected in winter season. These results suggest that hMPV may be a responsible pathogen causing acute respiratory tract infection in Korean children.

Key Words : *Metapneumovirus; Child; Respiratory Tract Infections; Korea*

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INTRODUCTION

Human metapneumovirus (hMPV), which was identified recently in the Netherlands in respiratory samples from children with acute respiratory symptoms, is enveloped non-segmented RNA virus belonging to genus *Metapneumovirus*, family Paramyxoviridae (1, 2). hMPV has been emerged as an important etiologic agent of upper and lower respiratory tract infections in individuals of all age, especially in young children (3-8). The clinical manifestations associated with hMPV ranges from upper respiratory tract infection including cough, rhinorrhea, to lower respiratory tract diseases such as severe bronchiolitis and pneumonia, similar to those of human respiratory syncytial virus (RSV) (1, 9). Although the prevalence of hMPV infection by using reverse transcriptase PCR (RT-PCR) has been reported in several countries including U.S.A., Canada, Australia, England, Japan, and Hong Kong (3, 4, 8, 10-12), it is still unclear in Korean children. The purpose of our study was to determine the frequency of hMPV in hospitalized children with acute respiratory tract disease in Korea.

MATERIALS AND METHODS

Respiratory samples and study subjects

Between December 2003 and February 2005, we collected nasal aspirates from 381 children under 15 yr (median age=19 months; range 0-144 months) who was hospitalized with respiratory infections in the Department of Pediatrics, Sanggye-Paik Hospital, Inje University, Seoul, Korea. The specimens were immediately transferred to the laboratory and each sample analyzed for RSV, adenovirus, influenza virus A and B, and parainfluenza virus by immunofluorescent assay using fluorescein-labelled monoclonal antibodies specific for each virus (IMAGEN; Dako Diagnostics Ltd., Cambridgeshire, U.K.). The specimens were stored at -70°C until further testing. An informed consent was obtained at admission from patient's parents. The Ethics Committee of Inje University College of Medicine, approved the study.

RT-PCR

RNA was extracted using the QIAmp Viral RNA Mini Kit (Qiagen GmbH, Hilden, Germany). To synthesize cDNA,

0.2 μ g of RNA was incubated in a solution containing 5 μ M random hexamer (Bioneer, Daejeon, Korea), 1 mM of each dNTP, 2 units of RNase inhibitor, and 10 units of reverse transcriptase (Bioneer, Daejeon, Korea) in a final volume of 20 μ L at 42°C for 60 min. The cDNA (1 μ L) was subjected to PCR analysis to detect the hMPV F-gene. Published F-gene sequences of hMPV were used for PCR for the detection and sequencing of hMPV (13). The forward primer sequence was 5'-GCAACAATTGAA CTGATCTTCAGGAAAC-3' (AY304360-2; nucleotides 627 to 749) and the reverse primer sequence was 5'-GCAACATTGAACTGATCTTCAGGA-AAC-3' (AY304360-2; nucleotides 1350 to 1376). When the first PCR was negative, a nested PCR was performed using forward primer [5'-ACATGCCAACATCTGCAG-GA CAAATAAAAC-3' (AY304360-2; nucleotides 698 to 727)] and the reverse primer [5'-ACAT GCTGTTCACCTTCAACTTTGC-3' (AY304360-2; nucleotides 1285 to 1307)]. Amplification was performed in a 20 μ L reaction mixture containing the following: 0.2 μ M of each primer; 200 μ M dATP, dCTP, dTTP, and dGTP; 10 mM Tris-HCl (pH 9.0), 1.5 mM MgCl₂, 40 mM KCl; 1 unit of *Taq* polymerase (Bioneer, Daejeon, Korea); and 1 μ L of cDNA sample (or the first round PCR product). Amplification was performed in a DNA thermal cycler (iCycler Thermal Cycler, Bio-Rad Laboratories, Hercules, CA, U.S.A.). The amplification conditions were as follows: 95°C for 15 min followed by 35 cycles of amplification (95°C for 1 min, 55°C for 1 min, 72°C extension for 1 min) and a final extension at 72°C for 3 min. The amplified DNA fragments were size-separated on a 2% agarose gel with ethidium bromide, and visualized with UV light (Fig. 1). To examine the sensitivity and specificity of RT-PCR, ten of hMPV-positive RNA samples (0.1 μ g) and ten of RSV-positive samples were used. The amplified products were detected to 10 pg of RNA and no product was detected from RSV positive samples. cDNA from a clinical specimen was used as the positive control.

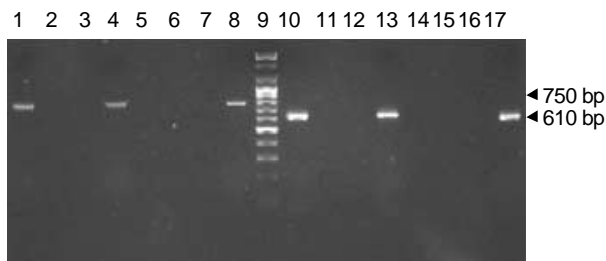


Fig. 1. Human metapneumovirus F gene amplification using nested polymerase chain reaction. Lane 9 shows a DNA ladder marker. Lanes 1 and 10 contain positive control (750 bp for first round PCR, 610 bp for second round PCR) Lanes 2 and 11 contain negative control. Lanes 3-8 and lanes 12-17 contain samples 1-6. Sample (lane 4 and 8) and (lane 13 and 17) show positive reactions and the other samples show negative reaction.

Phylogenetic tree analysis

The PCR products were purified using Accuprep PCR Purification kit (Bioneer, Daejeon, Korea) and subsequently sequenced directly on both strands using BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster, CA, U.S.A.) with ABI prism 377 analyzer (Applied Biosystems). Phylogenetic trees were constructed using MEGA version 3.0 (14). Sequences are available from GenBank under accession No. DQ092710-DQ092737.

RESULTS

Detection of hMPV by RT-PCR

In total 381 samples, negative samples in which any viral pathogen could not be identified by IFA were 231 cases (60.6%). Respiratory syncytial virus was detected in 129/381 children (33.8%). In addition, parainfluenza virus (n=9), influenza A (n=9), influenza B (n=2), and adenovirus (n=1) were identified (Fig. 1).

hMPV was detected using RT-PCR in 28 of 381 children (7.3%) and in 12.1% of children who were not infected with other common respiratory viruses. Of the 28 RT-PCR positive

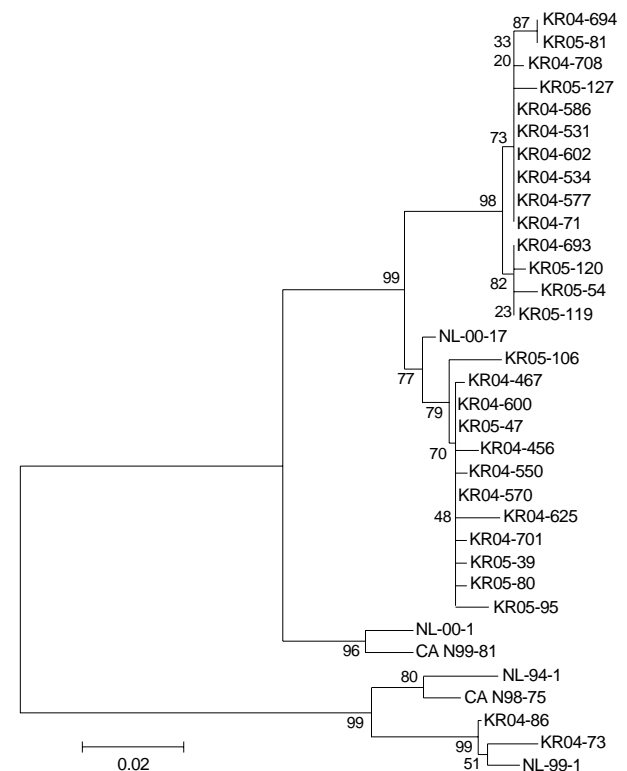


Fig. 2. Phylogenetic analysis of the F genes of hMPV isolated in Korean children, which was reconstructed using neighbour-joining method. Bootstrap proportions (500 replicates) are plotted at the branches of phylogram to show support values. GenBank accession numbers are DQ092710 to DQ092737.

samples for hMPV, 25 cases (89.2%) were collected between November 2004 and February 2005 (Table 1).

Clinical findings of hMPV positive patients

Clinical manifestations of the 28 children with hMPV infection are presented in Table 2. The median age was 15 months

Table 1. Virus detection in nasal swabs in hospitalized children with acute respiratory disease

	No. of tested samples (No. of positive)				
	Dec 2003- Feb 2004	Mar- May 2004	Jun- Aug 2004	Sep- Nov 2004	Dec 2004- Feb 2005
Common respiratory viruses					
RSV	27 (2)	20 (0)	48 (6)	191 (99)	95 (22)
Influenza A	27 (1)	20 (0)	48 (0)	191 (1)	95 (7)
Influenza B	27 (0)	20 (0)	48 (2)	191 (0)	95 (0)
Parainfluenza	27 (0)	20 (0)	48 (3)	191 (3)	95 (3)
Adenovirus	27 (1)	20 (0)	48 (0)	191 (0)	95 (0)
hMPV	5 (0)	18 (3)	33 (0)	120 (8)	54 (17)

*RSV, respiratory syncytial virus; hMPV, human metapneumovirus.

Table 2. Clinical manifestations of hMPV infection

Case No.	Sex	Age (months)	Symptoms	Diagnosis	Duration of hospitalization (days)	Underlying condition	Chest radiography findings
1	F	5	Cough, Rh, sputum	Bronchiolitis	4	None	Hyperaeration
2	F	26	Fever, cough, stridor	Croup	4	None	WNL
3	M	12	Fever, cough, sputum	Pneumonia	5	None	WNL
4	F	52	Cough, Rh, wheezing	Pneumonia	7	RAD	RLL
5	F	52	Cough, wheezing	B.A.	3	RAD	WNL
6	F	10	Fever, cough, sputum	Bronchiolitis	3	None	WNL
7	M	11	Cough	Bronchiolitis	10	None	WNL
8	M	8	Fever, Rh, sore throat	URI	5	None	WNL
9	M	15	Fever, Rh, cough, sputum	Bronchiolitis	7	None	LLL
10	F	24	Fever, Rh, cough	Bronchiolitis	6	None	Hyperaeration
11	F	14	Cough, Rh, wheezing	Pneumonia	10	None	WNL
12	M	32	Fever, Rh, cough, sputum	Bronchiolitis	6	None	RML
13	F	3	Fever, cough	B.A.	5	None	WNL
14	M	8	Cough, Rh, stridor	Croup	7	RAD	WNL
15	M	22	Fever, cough	Pneumonia	8	None	WNL
16	M	37	Fever, cough, sputum, wheezing, stridor, Rh	Croup	7	RAD	Interstitial
17	M	22	Fever, cough	Bronchiolitis	7	None	WNL
18	M	4	Cough	B.A.	18	RDS	WNL
19	M	79	Cough, wheezing	B.A.	4	RAD	WNL
20	M	6	Wheezing	Pneumonia	15	RAD	Interstitial
21	M	86	Fever, cough, sputum	Pneumonia	5	None	Interstitial
22	F	27	Fever, cough, sputum	ARDS, expired	2	None	BUL, BLL
23	F	22	Fever, cough	URI	8	None	WNL
24	M	26	Fever, cough, sputum	Pneumonia	6	None	Interstitial
25	F	9	Cough, diarrhea	Bronchiolitis	7	None	Hyperaeration
26	F	16	Fever, cough, sputum, Rh	Pneumonia	5	None	Interstitial
27	M	70	Fever, cough, sputum, Rh	Pneumonia	6	None	BLL
28	M	6	Cough, sputum	Bronchiolitis	5	None	Hyperaeration

URI, upper respiratory tract infection; B.A., bronchial asthma; RAD, reactive airway disease; ARDS, acute respiratory distress syndrome; RDS, respiratory distress syndrome; Rh, rhinorrhea; WNL, within normal limit; BUL, both upper lobe infiltration; BLL, both lower lobe infiltration; RML, right middle lobe infiltration; Interstitial, interstitial infiltration.

(range 7-86 months) in the 28 patients. Symptoms included cough (92.8%), fever (60.2%), sputum (42.8%), rhinorrhea (39.2%), dyspnea (17.8%), diarrhea (7.2%), and vomiting (7.2%). Expiratory wheezing and crackles were observed in 21.4% and 25% of the patients, respectively. Abnormal infiltrates of chest radiograph were noted in 10 (35.7%) of the 28 patients. The hMPV-infected children were diagnosed as having pneumonia (35.7%), bronchiolitis (28.5%), bronchial asthma exacerbation (14.3%), croup (10.7%), and upper respiratory tract infection (7.1%). Six of 28 patients had a history of reactive airway disease. A 27-month-old girl who was admitted with respiratory distress expired of cardiorespiratory failure 2 days after admission without known bacteremia. Only one patient of the 28 patients had underlying medical problem, chronic lung disease of prematurity.

Phylogenetic analyses of hMPV strains

Amplified products corresponding to part of the hMPV F gene (750 bp) were visualized on 1.5% gels. The PCR products from each positive specimen were sequenced and were

consistent with hMPV. The 28 hMPV strains detected in this study were classified into two distinct F lineages, 26 strains belonged to genogroup A (A2) and two strains to genogroup B (Fig. 2).

DISCUSSION

This study shows that hMPV is an important etiologic agent among Korean children hospitalized with acute respiratory tract infections. The prevalence of hMPV detected in nasopharyngeal samples from children with respiratory infections of unknown etiology has varied from 1.5% to 21% (1, 11, 15-18), but it has been unclear in Korea due to lack of published reports. The difference of prevalence in several studies may be explained by yearly variation in incidence, different group of patients and primers used in PCR assays for hMPV (15-18). Because we studied hospitalized children only, actual prevalence of hMPV infections may be higher. Some researcher suggested that hMPV cause lower respiratory tract infection in healthy children at a relatively high frequency (19).

It seems that hMPV shows a seasonal variation, with sporadic epidemics. In a study, hMPV was the most common virus isolates during the winter season 2002-2003 in children hospitalized for acute respiratory tract infection, but no specimen was found positive for hMPV in the following season (18). Maggi et al. (7) reported that the incidence of hMPV infection in infants varied from season to season over 3 yr period. The peak time of hMPV transmission in the Netherlands was in December, in Canada in April, and in Hong Kong in spring and early summer (1, 4, 12). Some reported that significant hMPV activity occurred in every month of the year with the peak incidence in the winter and spring seasons (19, 20). In our study, we detected 25 hMPV positive samples from November to February during the winter season of 2004-2005, with a peak in November. In the spring season of 2004, we also detected three hMPV positive samples. But, the exact prevalence of hMPV during the winter season of the 2003-2004 in this study is uncertain due to small number of available respiratory specimens.

The clinical features of children with hMPV positive samples observed in our study were similar to those of previous reports (8, 20). Exacerbation of asthma was observed in 14% (4/28) of hMPV only positive patients in this study. Like other viruses, such as hRSV and rhinoviruses, which have been suggested as important triggers of asthma exacerbation in children, an association between hMPV and asthma exacerbation has been implicated. In a Finnish study, the positive rate of hMPV was 32% (10/31) in hospitalized children with acute wheezing during the period of peak of hMPV infection (21). Although it may be possible that asthmatic bronchitis is triggered by hMPV, it is not certain to conclude definitely the association between and bronchial asthma (22). hMPV has

been suggested as a common and frequent etiologic agent of bronchiolitis in young children (8, 23, 24). Some reported that co-infection with RSV and hMPV may be a possible cause of increasing clinical severity in hospitalized children (25, 26), but others reported different results (7). The limitations of this study are relatively short study period, small number of samples in the first half of the 2004, which is not sufficient to know the exact epidemiologic characteristics. The data of combined infections of hMPV with other common respiratory viruses are also lacking.

RT-PCR is more useful method than cell culture, which has characteristics of slow growth and mild cytopathic effect of hMPV, and is now used commonly in epidemiologic studies in various populations (27, 28). The strains of hMPV can be divided into two major genetic lineages (1, 3). Variability of hMPV genes may affect the sensitivity of study due to limited available sequence information. We have performed the analysis of F-gene as van den Hoogen et al. described (13), which is known to have highly conserved sequence and allow the differentiation of all four sublineages in several studies (5, 8, 9, 13). Phylogenetic analysis of F gene sequences of our strains showed highly nucleotide identity with viruses belonging to genogroup A during the winter season of 2004-2005, all belonged to A2. Otherwise, three hMPV positive samples detected in spring of 2004 belonged to genogroup B2 in two, genogroup A1 in one. An Italian study reported striking variation in the overall circulation rates and co-circulation of multiple strains in the same area in different years (18).

This study suggests that hMPV may be an important etiologic agent of respiratory tract infection requiring hospitalization in children and two distinct groups of hMPV are co-circulating in Korea.

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