Short Communication

Human Metapneumovirus Infection in Febrile Children with Lower Respiratory Diseases in Primary Care Settings in Hiroshima, Japan

Michimaru Hara*, Shinichi Takao1, Shinji Fukuda1, Yukie Shimazu1 and Kazuo Miyazaki1

Hara Pediatric Clinic, Hiroshima 736-0035, and 1Department of Microbiology II, Hiroshima Prefectural Institute of Public Health and Environment, Hiroshima 734-0007, Japan

(Received May 19, 2008. Accepted October 1, 2008)

SUMMARY: Human metapneumovirus (hMPV) has been shown to be a leading cause of viral lower respiratory tract infections in children. Nevertheless, few reports regarding hMPV infections over consecutive years in children in primary care settings are available. We carried out virologic and clinical studies to determine the role of hMPV in febrile lower respiratory infections in children at a primary care clinic over 3 years and 5 months. Nasopharyngeal aspirates obtained from children with acute respiratory tract infections accompanied by high-grade fever (≥39°C) and productive cough were studied for hMPV by reverse transcription-polymerase chain reaction and for other respiratory viruses by viral cultures and immunoassays. Of 379 patients tested, 202 were positive for at least 1 virus, including 98 with hMPV, 69 with respiratory syncytial virus, 18 with adenovirus, 12 with enterovirus, 8 with parainfluenza virus, 3 with rhinovirus, 2 with influenza virus type C, and 1 with herpes simplex virus. The male:female ratio of hMPV-infected children was 0.96:1 with an overall mean age of 3.5 years (range, 2 months to 9 years). These infections occurred predominantly from February to July, and the hospitalization rate was 4%. Of 93 patients infected with hMPV alone, 52 (56%) showed evidence of a lower respiratory tract infection.

Respiratory syncytial virus (RSV), rhinovirus, parainfluenza virus, adenovirus, and influenza virus are common causes of lower respiratory tract infections in children. In 2001, researchers in the Netherlands isolated a new virus, human metapneumovirus (hMPV), from children with acute respiratory tract infections (1). Since then, investigators worldwide have reported that hMPV is a leading cause of lower respiratory tract infections in children (2-11). Because most studies have dealt with hospitalized patients with relatively severe lower respiratory tract infections (3,5-7,12-15), there have been few studies in primary care settings (2,16). We sought to determine the etiologic role of hMPV and to clarify the clinical features and epidemiology of hMPV infections in febrile children with lower respiratory tract infections at a primary care clinic.

Children with respiratory tract infections accompanied by cough and body temperature of 39°C or greater were studied for hMPV by reverse transcription-polymerase chain reaction (PCR) until tested. Viral RNA extracted from nasopharyngeal aspirates was subjected to nested RT-PCR using primers (MPVF1f: 5'-CTTTGGACTTAATGACAGATG-3', MPVF1r: 5'-GTCTTCTCTGTGTTAATCTTG-3') that amplify a 450-bp fragment in the region encoding hMPV fusion protein, as reported by Peret et al. (17), and primers (MPVF2f: 5'-CATGCGGACCTCTGACACGAC-3', MPVF2r: 5'-ATGTTGCAAYCTYTTTGATG-3'), designed by us, that amplify a 357-bp fragment targeting a further inner region of the above (18). The gene specific to hMPV was amplified. Nucleotide sequences were determined to confirm the identity of the virus detected by RT-PCR. As a result, the nucleotide sequences of the amplicons (GenBank accession nos. AB111371 to AB111374) had 95 to 97% homology with previously described F gene sequences in GenBank.

A 200-μL aliquot was inoculated into 2 wells each of MDCK, Vero, LLC-MK2, BGM, HEp-2, RD-18S, and FL cells seeded in 24-well culture plates using the microplate method (19) with modification. A hemagglutination test was performed with the supernatants of MDCK cell cultures. The isolates of influenza viruses were identified by the hemagglutinin-inhibition test. Identification of respiratory viruses other than influenza viruses was performed by the neutralization test.

Residual specimens were tested for rapid point-of-care detection of RSV using either a rapid immunoassay kit (Abbott TestPack RSV; Abbott Laboratories, North Chicago, Ill., USA) or the SAS RSV test (SA Scientific, San Antonio, Tex., USA).
USA).

We used the chi-square test to compare the prevalence rate of hMPV infections with that of infections due to other respiratory viruses. Analyses were performed using SPSS for Windows, version 15.0 J. \( P < 0.05 \) was considered to be significant.

Overall, 379 children were enrolled in our study. The mean age was 3.7 years, the median age was 3.1 years, and the age range was 3 months to 14.3 years. The male:female ratio was 1.1:1.0. Of 379 patients, 204 (54\%) underwent chest radiography.

Viral studies, including RT-PCR for hMPV, cell-culture, and rapid diagnosis tests for RSV, identified 98 patients with hMPV, 69 with RSV, 18 with adenovirus, 12 with enterovirus, 8 with parainfluenza virus, 3 with rhinovirus, 2 with influenza virus type C, 1 with herpes simplex virus, and 1 with poliovirus. The detection rate for hMPV was significantly higher than that for RSV \( (P = 0.011) \) and those for adenovirus, enterovirus, and parainfluenza virus \( (P < 0.001) \).

The 98 hMPV-infected children, 4 were coinfected with RSV and 1 with enterovirus. There were also 6 specimens containing dual viruses that did not include hMPV. As a result, 213 viruses were detected from 202 patients. In 98 hMPV-positive samples, the virus was detected in 93 specimens on the first PCR assay and in 5 on the second assay. Of 98 specimens found hMPV-positive by RT-PCR assays, 60 specimens were also positive for hMPV by viral culture methods using LLC-MK cells.

More patients between the ages of 1 and 4 years experienced febrile lower respiratory tract infections due to hMPV than patients 5 years and older (Fig. 1). The male:female ratio among children with hMPV infection was 0.96:1, with a mean age of 3.5 years, and an age range of 2 months to 9.7 years. Among these hMPV infections, 4\% occurred in infants, 86\% in children aged 1 to 6 years, and 8\% in children aged 7 to 9 years. Two children were infected with hMPV twice during the study, and they were counted as 4 patients.

The monthly distribution of infection among these 98 patients is shown in Fig. 2. Almost all infections occurred between February and July, whereas hMPV infection occurred in only 1 child from August to January.

Diagnoses of the 93 children with hMPV alone, of whom 53 underwent chest radiography, were respiratory tract infection with cough but without the clinical features of lower respiratory infection in 41 (44\%), wheezy bronchitis in 20 (22\%), bronchitis in 19 (20\%), pneumonia in 7 (8\%), and croup in 6 (6\%). Sixty-eight of these 93 patients (73\%) had rhinorrhea during their illness. There was no apparent difference in the severity of disease between the 5 coinfected patients and the 93 children infected with hMPV alone. Of the 98 total patients, 4 with a single infection were hospitalized.

There were 76 patients with infections due to only hMPV who were followed until the fever was resolved, and who showed no evidence of pneumonia. These 76 patients were assessed with respect to the duration of fever and the maximum body temperature during their illness. The mean duration of fever \( (\geq 38^\circ C) \) was 4.6 days (range, 2 to 8 days). Twenty-seven patients (36\%) had a maximum fever \( \geq 40^\circ C \) during their illness.

Of 43 patients undergoing blood tests, 25 had a single infection and did not have pneumonia according to chest radiographs. The mean leukocyte count of these 25 patients was \( 7,800 \pm 4,400/\mu L \) (mean \( \pm SD \), ranging from 2,400 to 22,800/\( \mu L \), and the mean CRP concentration was \( 2.4 \pm 2.0 \) mg/dL (mean \( \pm SD \), ranging from 0 to 6.7 mg/dL).

hMPV was present in 98 of 379 patients (26\%) and was the most frequent cause of lower respiratory tract infections in patients with high-grade fever \( (\geq 39^\circ C) \). Other reports on the study of hMPV in children noted a prevalence rate of 4 to 20\% (2,4,6-11,13). Because most of these studies dealt with hospitalized young children with lower respiratory infections, we cannot necessarily compare these findings with results of our study. Williams et al. reported on hMPV-infected pediatric outpatients at a primary clinic over a period of 25 years (2). In 248 patients with lower respiratory tract infection and with negative viral culture for other respiratory viruses, 20\% were observed to have hMPV infection using RT-PCR. Two additional studies on outpatients with lower respiratory tract disease noted an incidence of hMPV in 10 and 20\% of infections (4,11). These findings, along with our own, suggest that hMPV is a major cause of lower respiratory tract infections in outpatients.

In our study, over a period of more than 3 years, diseases due to hMPV occurred from February to July. Most reports from the northern hemisphere show that infections caused by hMPV predominantly occur from winter to spring (2,4,6-10,20). The seasonal occurrence from late winter to early summer in our study was somewhat different from that in other countries, but was generally consistent with that observed in other reports in Japan (11,21).

Further investigations are needed to clarify the role of hMPV in febrile outpatients with upper respiratory tract infections or afebrile children with lower respiratory tract infections.
REFERENCES