Original Article

Characteristics of Human Metapneumovirus Infection Prevailing in Hospital Wards Housing Patients with Severe Disabilities

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SUMMARY: Epidemics of infectious diseases often occur at long-term inpatient facilities for patients with severe motor and intellectual disabilities. However, the pathogens causing these infections remain unknown in approximately half of such epidemics. Two epidemics of respiratory tract infection occurred in 2 wards in the National Hospital Organization Ehime Hospital (prevalence 1, 34 infected out of 59 inpatients in the A ward in September 2011; prevalence 2, 8 infected out of 58 inpatients in the B ward in June 2012). Human metapneumovirus (HMPV) was detected from the nasal (and some pharyngeal) swabs from 17 patients. Based on phylogenetic analysis of viral genomes, the virus was grouped in subgroup A2 (prevalence 1) and B2 (prevalence 2). We considered that the viruses had spread through the 2 wards. The average duration of high fever in the 42 patients was 6.8 days, with the majority of fevers exceeding 38°C (79%) and being accompanied by a productive cough. Ten out of 17 patients (59%) in whom HMPV was detected had decreased lymphocyte and increased monocyte counts in the blood. Eleven cases (65%) had elevated-C reactive protein levels and fever protraction as well as images of bronchitis or pneumonia on chest radiographs approximately 1 week after onset. Anti-HMPV antibody in the blood was positive in 95% of patients (151 of 159 inpatients), indicating no relation between HMPV infection and antibody titer but revealing recurrent infections. In view of the fever protraction and frequent co-occurrence of bronchitis and pneumonia at long-term inpatient facilities for immunocompromised patients such as the ones in this study, the prevalence of HMPV must be carefully monitored, and preventive measures and early-stage treatments are required.

INTRODUCTION

In recent years, several different pathogenic viruses that cause respiratory tract infections have been discovered, one of which is human metapneumovirus (HMPV). This virus was discovered in 2001, and while analysis is underway, cases have been reported around the world (1–4). In Japan, following sporadic reported cases, there have also been reports of outbreaks among children and at facilities for the elderly (5–8). A domestic survey indicated that these cases constitute a constant proportion of respiratory tract infections (9). Because HMPV is highly infectious, the possibility of an outbreak at long-term inpatient facilities is a matter of concern.

In Japan, it is believed that there are more than 150 such facilities nationwide for patients with severe motor and intellectual disabilities. However, because people with disabilities at such facilities lead a life of long-term communal living, the frequent occurrence of outbreaks of infectious diseases (0.5–1 occurrence per ward per year) is a perennial issue. A survey of these facilities showed that there was a high frequency of outbreaks of influenza and norovirus infection and, otherwise, nearly 50% of outbreaks involved respiratory tract infections caused by an unknown pathogen (10).

Recently, we briefly reported that HMPV may be a causative pathogen (11). Our search for the causative pathogens of these respiratory tract infections is ongoing. We experienced two outbreaks of respiratory tract infections at Ehime Hospital that involved 34 patients in the A ward (59 inpatients) in 2011 and 8 patients in the B ward (58 inpatients) in 2012. HMPV genomes were detected in nasal and some pharyngeal swabs from 17 patients using RT-PCR, sequencing, and virus separation culture techniques. In conjunction with the respective clinical progressions, we report findings suggesting an HMPV outbreak. The results are similar to those described in our previous short report (11); therefore, the present study confirms the features of an HMPV outbreak among immunocompromised hosts in long-term inpatient facilities.

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MATERIALS AND METHODS

Patients: The average age of patients hospitalized at Ehime Hospital wards for severe motor and intellectual disabilities was 43.5 years (18–78 years), and the majority of patients were bedridden and had received full care for many years. There was a large number of cases with fever in the A ward from late August to early October 2011 (prevalence 1) and in the B ward in June 2012 (prevalence 2). Patients who had fever ≥37°C were subjected to follow-up observation. Their body temperatures were measured daily, and clinical symptoms including nasal discharge, productive cough, diarrhea, pharyngeal findings, and rales were examined. Blood and urine tests, bacterial cultures, and chest radiographs were conducted as necessary, and intravenous drips and/or medicines were administered as needed on the basis of symptoms and the test results. Rapid diagnostic kits for pharyngeal adenovirus, nasal respiratory syncytial (RS) virus, and group A streptococcal pharyngitis were also used to identify the pathogens of the infectious diseases.

Virus sample collection: Nasal and some pharyngeal swabs were collected using sterile swab collection sticks (MicroRheologics, Brescia, Italy) during periods of high fever (days 1–9 after onset, except for 1 patient on day 21) from 16 patients in prevalence 1 and 5 patients in prevalence 2. The swabs were stored at −80°C in 1 ml transport medium (Copan UTM-RT Medium; Copan Italia, Brescia, Italy). The frozen samples were transported on dry ice.

Virus detection by RT-PCR and virus separation cell culture: Samples from 21 patients were examined for HMPV and for RS virus, human rhinovirus, parainfluenza virus, enterovirus, and human bocavirus. RT-PCR for HMPV was performed in accordance with a previous report (6). In brief, RNA was extracted from the sample and cDNA was synthesized using a random primer. Part of the F gene was amplified in a primary PCR using a primer pair (hMPVf1: 5′-CTTGGGACCTTAATGACAGATG-3′ and hMPVf2: 5′-GTCTTCCTGTGCTAACTTTG-3′) according to the method of Peret et al. (11). In a secondary PCR, another primer pair (hMPVf2: 5′-CATGCCGACCCTTGCGGACC-3′ and hMPV2r: 5′-ATGTTGCCYAYT CYYTTGATTG-3′) was used to amplify a 357-bp fragment. The target gene was confirmed by 2% agarose gel electrophoresis and ethidium bromide or SYBR green-staining. In addition, we also searched for virus genes other than those for HMPV in accordance with previous reports. The results of all these tests were negative.

Virus separation cultures were run using Vero E6 cells. In brief, samples were centrifuged (3000 rpm for 10 min), added to an isolation medium (DMEM, BSA, and trypsin), and cultured for 2 generations at the rate of 1 generation in 3 weeks. A cytopathic effect was observed between day 15 of generation 1 and day 10 of generation 2.

Phylogenetic analysis of the viral genome: The 357-bp fragment of the F gene amplified by nested PCR was sequenced by direct sequencing. Homology with known base sequences registered in GenBank was examined for 317 bp of the fragment. Molecular Evolution Genetics Analysis (MEGA, version 4) was used for phylogenetic tree analysis using the neighbor-joining method (6).

Detection of anti-HMPV antibodies: The patient serum remaining after periodic health examinations was immediately stored at −80°C. The anti-HMPV antibody titer in the sera was measured by the fluorescent antibody technique using virus-infected cells. In summary, suspensions containing 3 × 10⁵ cells/ml of either HMPV-infected (strain isolated by the Shimane Prefectural Institute of Public Health and Environmental Science) or uninfected Vero E6 cells were added drop-wise into the respective wells on glass slides for fluorescent antibody techniques, dried, and acetone-fixed. The test samples were serially diluted from 20- to 640-fold in the wells, followed by reaction with the control sera at 37°C for 1 h. After rinsing, the cells were reacted with FITC-labeled goat anti-human IgG antibody (Invitrogen, Carlsbad, Calif., USA), rinsed, sealed, and then observed under a fluorescence microscope. The highest magnitude of dilution that exhibited positive staining represented the antibody titer (6).

Ethics statement: The research plan took ethical considerations into account and was discussed with and approved by the ethics committee of Ehime Hospital. The patients and/or guardians were given written explanations of the study protocol, and consent was obtained in writing.

RESULTS

Patient clinical course: In prevalence 1, a patient in the A ward had high fever on August 22, 2011, and subsequently, patients exhibiting similar symptoms were noted every 1–7 days, amounting to 34 patients in total. In prevalence 2, patients with low-grade fevers were first noted in the B ward on May 29, 2012, and the number increased every 1–4 days until there were 8 patients in total (Fig. 1). In each prevalence, the average duration of fever ≥37.5°C in all 42 patients was 6.8 days. Many cases had high fever ≥38°C (79%) and productive cough, with some patients also having nasal discharge, ear pain, and hoarseness. Rapid diagnostic kits showed negative results in all patients.

Detection of the HMPV viral genome: The HMPV genome was detected by RT-PCR in samples from 17 of 21 patients (prevalence 1, 13 out of 16 patients; prevalence 2, 4 out of 5 patients). The virus was separated in the separation cultures only from samples that were HMPV-positive by RT-PCR (Fig. 1). The 17 HMPV-positive samples were collected on days 1–9, and the 4 HMPV-negative samples were collected on days 2 and 7, except for 1 sample (day 21, from a patient with chronic pneumonia) (see Fig. 3). The duration during which the nasal swabs contained the viral genome was therefore not determined in this study.

Phylogenetic analysis of the HMPV genome: The results of phylogenetic tree analysis from the base sequence of a 317-bp part of the F gene of the virus classified the detected viruses as subgroup A2 (prevalence 1) and B2 (prevalence 2) (Fig. 2).

Clinical findings of HMPV-positive patients: Figure 3 illustrates the clinical findings of the 17 HMPV-positive patients. The average duration of fever ≥37.5°C was 10.1 days, and in all cases, high-grade fever ≥38°C was observed during the course of the illness. Coughing
and sputum were observed in a large number of cases (76%), with half exhibiting an increase in nasal discharge and congestion. Eleven cases (65%) progressed to bronchitis or pneumonia on days 3–7 after onset. Elevated C-reactive protein (CRP) levels and increased neutrophils were observed together with the onset of bronchitis or pneumonia. In general, the initial stages of onset were characterized by mildly elevated CRP levels, decreased lymphocytes, and an increased ratio of monocytes. Nine patients (53%) had an increased ratio of monocytes (≥8%) at onset of the infection, with the majority exhibiting an increase in monocyte counts when compared based on individual progressions to bronchitis or pneumonia. Bacterial identification was also performed for 11 patients (2 blood samples, 3 pharyngeal swabs, and 8 aspirated sputum samples), revealing that the blood samples were negative for bacteria and the other samples contained only indigenous bacteria such as Neisseria, Acinetobacter, and Pseudomonas aeruginosa). Pathogenic bacteria were therefore not detected in these patients.

Figure 4 depicts the patient (No. 2-4) who exhibited the typical progression of HMPV infection. The high fever ≥38°C persisted for 6 days despite the administration of antibiotics and then gradually dissipated. Despite pathogenic bacteria not being identified in the blood and sputum, wide-spectrum antibiotics were administered to prevent co-infection of bacteria. A reduction in lymphocytes and an increase in the ratio of monocytes were observed early in the illness, and on day 6, CRP levels were markedly elevated with a chest radiograph revealing the progression of pneumonia. Over the following 10 days, a favorable recovery was observed.

**Anti-HMPV antibody titer:** Anti-HMPV antibody testing of the serum remaining serum from periodic health examinations conducted 3 years previously (Table 1) revealed that 143 (95%) of the 151 cases for which a decision was possible were antibody-positive, while the remaining 8 cases were antibody-negative. The peak distribution of the antibody titer was 1:80, and only 1 case had a high titer of at least 1:640 (13). Examination of the patients showed that 30 of the 34 patients in prevalence 1 were antibody-positive, with the peak distribution of antibody titers being 1:80, similar to the overall results. Of the 8 patients in prevalence 2, a decision could be reached for 6 and the antibody titers were concentrated at 1:80. No significant difference was observed between the antibody titer distribution reported in hospitalized patients overall and that of patients in the present study (13). The antibody titers of the antibody-positive patients are shown in parentheses, with no deviation being observed.

The average age of the 159 patients was 41.1 years (range, 15–77 years) at the time of serum collection, while the average age was 16.7 years (range, 1–66 years) at the time of their hospitalization. The number of patients younger than 5 years and younger than 10 years at the time of hospitalization was 19 (12%) and 61 (38%), respectively. The ratio of the 42 patients in the present study was approximately the same (7%, 36%). There was no relationship between the age of hospitalization and antibody titer.

**DISCUSSION**

In Japan, there are at least 150 facilities that provide
Fig. 2. Phylogenetic tree based on the metapneumovirus genome F gene. E-numbers indicate viruses from patients in Ehime Hospital (prevalence 1, E-6–19; prevalence 2, E-V-1–3). Avian metapneumovirus type C is also shown as an outgroup.

Prevalence 1

<table>
<thead>
<tr>
<th>Patient Age</th>
<th>Sex</th>
<th>Body temperature</th>
<th>symptom</th>
<th>lower resp. infection</th>
<th>WBC(μL)</th>
<th>Mon(%)</th>
<th>CRP(μg/dL)</th>
<th>CRP(data)</th>
</tr>
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<tbody>
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<td>1-1 37 F</td>
<td>1-2 31 F</td>
<td>1-3 50 F</td>
<td>1-4 61 F</td>
<td>1-5 42 M</td>
<td>1-6 34 M</td>
<td>1-7 42 M</td>
<td>1-8 37 F</td>
<td>1-9 34 M</td>
</tr>
<tr>
<td>2-1 37 F</td>
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<td>2-3 47 F</td>
<td>2-4 37 F</td>
<td>2-5 37 M</td>
<td>2-6 37 M</td>
<td>2-7 27 F</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Clinical findings of 17 patients with a detectable metapneumovirus infection (prevalence 1, 13 patients; prevalence 2, 4 patients). * denotes the days of virus detection after onset. WBC, white blood cell; Mono, monocyte; CRP, C-reactive protein; nasal obs., nasal obstruction; nasal disc., nasal discharge.
Table 1. The number of patients whose sera had positive antibody titers

<table>
<thead>
<tr>
<th>Antibody titer</th>
<th>No. of inpatients</th>
<th>Patients with high fever</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Prevalence 1</td>
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<td>11 (6)</td>
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<td>1:320</td>
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</tr>
<tr>
<td>1:640 ≤</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Undetectable</td>
<td>8</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>159</td>
<td>31 (13)</td>
</tr>
</tbody>
</table>

The numbers were compared between all inpatients and patients who exhibited symptoms in this study. ( ) denotes the number of HMPV-positive patients.

Fig. 4. The clinical course of patient no. 2-4. Body temperature, examination data, and X ray photographs are shown. Seg, segmented nuclear cell; Lym, lymphocyte. Other abbreviations are in Fig. 3.

Table 1. The number of patients whose sera had positive antibody titers

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<tr>
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The large number of visitors who stay overnight with the patients or the patient’s participation in treatment and training activities outside the ward, there are many opportunities for infectious disease to be introduced into a ward. Outbreaks inside a ward are also commonplace following the entry of a highly infectious pathogen. In a previous survey, we showed that wards experienced outbreaks of infection 0.5–1 times per year and that common pathogens included influenza virus and norovirus. However, half of all the outbreaks were respiratory tract infections caused by an unknown pathogen (10). We briefly reported that HMPV subgroup B2 may be a causative pathogen (13).

HMPV was detected in the present study from numerous samples during two outbreaks of respiratory tract infection and belonged to subgroups A2 and B2. We therefore concluded that the pathogen somehow entered the wards and overcame the immune defenses of the patients as a result of its highly infectious nature, leading to an outbreak (12).

Increased rates of high fever and productive cough were observed in these outbreaks, although nasal discharge and congestion were comparatively low, a finding characteristic of patients with severe motor and intellectual disabilities. The early stages of the illness were characterized by a low to moderate increase in CRP levels, a reduction in peripheral blood lymphocytes, and an elevated ratio of monocytes. In general, the peripheral blood lymphocyte and monocyte ratios

care for an estimated number of 30,000 patients with severe motor and intellectual disabilities. Patients hospitalized at these facilities require long-term treatment and care. Many cases of long-term hospitalization span several decades after infancy and are subjected to a special environment in terms of infectious disease. These facilities contain multiple wards and employ a large number of staff members such as doctors, nurses, care workers, and nursery attendants. Because of either
promptly normalized together with mitigation of the symptoms. However, CRP levels tended to persist for some time. In cases that progressed to bronchitis or pneumonia, the high CRP levels and elevated peripheral blood neutrophils were observed with a delay of approximately 1 week.

Measurement of anti-HMPV antibodies revealed an antibody prevalence rate of 95%. In general, antibodies are thought to become positive by the age of 10 years (9). Antibody testing in the present study revealed that 95% of patients were antibody-positive even though they had been hospitalized for a long time, some since infancy. The proportion of the patients who were hospitalized before 5 years and 10 years of age was 12% and 38%, respectively. This suggested that most patients may have been infected with the virus before hospitalization and that the virus had caused outbreaks in the wards many times in the past. Infected patients in the present study exhibited no bias in antibody titer, and repeat infections appeared to occur irrespective of the presence of antibodies (13).

Although HMPV outbreaks have been previously reported in facilities for the elderly (7,8), the virus was also thought to have been involved in outbreaks at a constant rate in long-term inpatient facilities for patients with severe motor and intellectual disabilities. Such facilities house many patients with lowered immune defenses against infection, and respiratory tract infection is the leading cause of death. Our study, together with our previous short report (13), suggests that HMPV infection causes fever protraction and progression to bronchitis and pneumonia. Complications of bacterial infection and aspiration pneumonia also may occur. We therefore consider it necessary to establish preventative measures and early-detection strategies for HMPV outbreaks in facilities caring for patients with severe disabilities as well as facilities for the elderly.

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Conflict of interest None to declare.

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