Laboratory and Epidemiology Communications

Virus-Related Lower Respiratory Inflammation: Detection of Human Metapneumovirus from Severe Pneumonia Cases with A/H1N1pdm Virus

Kyoko Akiyoshi*, Takuya Tamura[1], Tsunekazu Haruta[1], and
Influenza Examination Team of Kobe Institute of Health

Kobe Institute of Health, Kobe 650-0046; and
[1]Kobe City Medical Center General Hospital, Kobe 650-0046, Japan

Communicated by Makoto Takeda

(Accepted December 13, 2010)

The first domestic infection in Japan was reported in Kobe on May 16, 2009. As the influenza A pandemic (H1N1) 2009 (A/H1N1pdm) virus spread, the number of patients hospitalized with pneumonia increased. Throat or nasal swabs were collected from 153 patients with suspected A/H1N1pdm virus infection and lower respiratory inflammation (LRI) detected as a result of pandemic influenza hospital admission surveillance and routine pathogen surveillance between June 2009 and April 2010. All samples were transferred to the Kobe Institute of Health for laboratory diagnosis.

*Corresponding author: Mailing address: Kobe Institute of Health, 4-6 Minatojima-nakamachi, Chuo-ku, Kobe 650-0046, Japan. Tel: +81-78-302-6252, Fax: +81-78-302-0894, E-mail: kyoko_akiyoshi@office.city.kobe.lg.jp

Specimens were analyzed by real-time RT-PCR to detect A/H1N1pdm virus (1), and by RT-PCR to detect human metapneumovirus (hMPV) (2) and respiratory syncytial virus (RSV) (3). Virus isolation was performed using RD-18S, HEp-2, FL, Vero-E6, and MDCK cells.

Viruses were detected in 106 of the 153 cases studied (69.3%) (Table 1). It can be seen from Fig. 1, which shows the seasonal distribution of viruses, that the highest number of A/H1N1pdm-positive samples from those cases with LRI were reported in the period October to January, with October having the most positive samples. The peak number of RSV infections overlapped with, but occurred later than, the peak for A/H1N1pdm infections, whereas hMPV infections peaked in warmer months.

The median ages of LRI cases infected with A/H1N1pdm virus, hMPV, and RSV were 8.4, 3.0, and

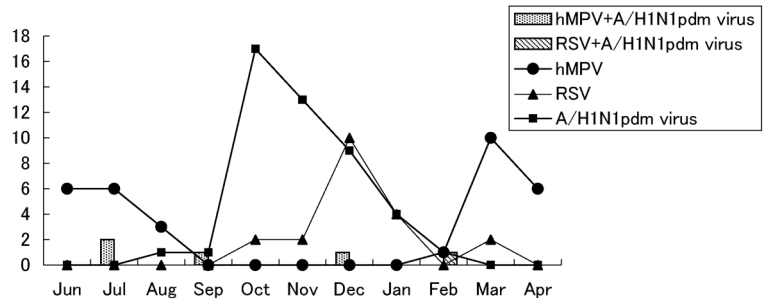

Fig. 1. Monthly incidence of viruses, except adenovirus, among 153 lower respiratory inflammation (LRI) cases studied from June 2009 to April 2010. hMPV, human metapneumovirus; A/H1N1pdm, influenza A pandemic (H1N1) 2009; RSV, respiratory syncytial virus.

Table 1. Viruses detected in the 153 LRI cases

Infected virus	No. of cases	
	Pneumonia	Bronchitis
A/H1N1pdm virus	23	23
hMPV	7	25
RSV	9	11
Adeno5	0	2
hMPV + A/H1N1pdm virus	4	0
RSV + A/H1N1pdm virus	0	1
Adeno1 + hMPV	0	1

2.1 years, respectively. Furthermore, the mean age of those LRI patients infected with A/H1N1pdm virus was significantly higher ($P < 0.01$) than those infected with RSV or hMPV.

A medical history of asthma together with pneumonia or bronchitis was reported in 47.8 and 8.7% of cases, respectively, for which only A/H1N1pdm virus was detected. Likewise, 14.3 and 0% of cases with hMPV infection only had a medical history of both asthma and pneumonia or bronchitis, respectively (0% in both cases for single RSV infection). Only one of four coinfected cases with A/H1N1pdm virus and hMPV had a medical history of asthma. Those cases infected with other viruses had no underlying disease. Our study therefore suggests that a previous history of asthma is a risk factor for progression to pneumonia when a patient is infected with A/H1N1pdm virus.

Two viruses, namely A/H1N1pdm virus and hMPV ($n = 4$), RSV and A/H1N1pdm virus ($n = 1$), and adenovirus serotype 1 and hMPV ($n = 1$), were detected simultaneously in six patients (5.7%). Furthermore, all four coinfected cases with hMPV and A/H1N1pdm had pneumonia. The other two cases had bronchitis. Likewise, four (14.8%) out of 27 A/H1N1pdm virus-infected cases with pneumonia were found to be coinfected with hMPV (Table 1).

Patients A and B: Twin boys aged 4 years and 7 months with no underlying disease. Both attended kindergarten. They both presented with fever on July 9, 2009 (day 1) and received treatment for upper respiratory inflammation on day 2. After administration of oseltamivir, their fever decreased on day 3. On day 5, boy A had exacerbation of coughing and both were hospitalized urgently. On admission, boy A had expiratory wheezing and a chest X-ray showed pneumonia. After treatment with intravenous fluids and bronchodilators, A's symptoms (cough and wheezing) disappeared. He was discharged on day 9.

On admission (day 5), a chest X-ray showed that boy B did not have pneumonia, although on the following day (day 6) B developed LRI with fever, cough, and wheezing. B was subsequently treated with a bronchodilator. This treatment reduced the fever by day 8, and B was also discharged on day 9.

Nasal swabs were collected from both boys on days 2 and 5 for laboratory diagnosis. The results are shown in Table 2. Cases A and B were laboratory confirmed as A/H1N1pdm virus coinfection with hMPV. B was infected with A/H1N1pdm virus first and then with hMPV. The cycle threshold (Ct) value of the real-time RT-PCR method for A/H1N1pdm virus for specimens obtained on day 5 was considerably higher than for those obtained on day 2. Furthermore, no A/H1N1pdm virus was isolated from the specimens obtained on day 5. These data therefore show that the loads of A/H1N1pdm virus on day 5 were lower than those on day 2, and that hMPV was associated with progression to pneumonia in patient A and secondary fever and deterioration of coughing in patient B.

An outbreak of upper respiratory inflammation with fever was reported in a kindergarten in Higashinada ward in early July 2009. Four other suspected cases of A/H1N1pdm infection were also examined. A/H1N1pdm virus was detected in one sample obtained from a 4-year-old (sw091549) on July 6, and hMPV was detected in the other three cases. All cases were negative for RSV (Table 2). The 250 nucleotide bases of the PCR products of hMPV's F-gene were sequenced and all products from hMPV-positive specimens were found to be identical. The data presented in Table 2, together with the sequencing results, strongly suggest that the hMPV outbreak was coincident with an A/H1N1pdm outbreak in this period and that patient A was coinfected with hMPV and A/H1N1pdm virus. It is thought that patient B was infected with hMPV by patient A.

The resulting sequences were compared with the hMPV F-gene sequences in the GenBank database of the National Center for Biotechnology Information and the nucleotide identity scores found to be highest (97%) for the 2008 isolates (GU048739) from Shanghai, China.

Patient C: A boy aged 13 years old with a medical history of asthma. He received treatment for coughing, headache, and sore throat with high fever (38.8°C) on

Table 2. Laboratory results for the kindergarten patients studied (including patients A and B)

Case	Age (y)	Date of sampling	Real-time RT-PCR	RT-PCR		Isolation of A/H1N1pdm virus	Oseltamivir susceptible
			A/H1N1pdm virus[1)]	hMPV	RSV		
sw091541	4	04/Jul/2009	−	+	−		
sw091549	4	06/Jul/2009	+	−	−	+	Yes
sw091555	5	08/Jul/2009	−	+	−		
sw091566	4	10/Jul/2009	−	+	−		
A (day 2)	4	10/Jul/2009	+ (28.3, 30.4)	+	−	+	Yes
(day 5)		13/Jul/2009	+ (36.4, 38.8)	+	−	−	Impossible[2)]
B (day 2)	4	10/Jul/2009	+ (27.5, 29.4)	−	−	+	Yes
(day 5)		13/Jul/2009	+ (31.9, 33.9)	+	−	−	Impossible

[1)]: Ct values are shown in parentheses (A/M, H1).
[2)]: Sequence-analysis was not possible due to the very low amount of PCR product obtained.

September 14 (day 1). Fast testing for influenza proved negative. He was hospitalized on day 2 as a result of breathlessness and worsening of his symptom. A chest X-ray and a chest computed tomography (CT) scan performed on admission showed pneumonia with atelectasis. However, fast testing for influenza again proved negative. As a result of his rapidly worsening respiratory status, patient C was transferred to a larger hospital. Upon admission to this hospital, C presented with dyspnea and high fever (39°C); fast testing for influenza was positive. C was placed on a ventilator with endotracheal intubation, and oseltamivir was administered. C's respiratory status had improved by day 5 and he was extubated. He was discharged on day 7. A/H1N1pdm virus and hMPV were detected from the specimen obtained on day 3 by real-time RT-PCR and RT-PCR, respectively; RSV was not detected.

Many cases of suspected A/H1N1pdm virus-associated severe pneumonia have been reported both inside and outside Japan (4–8). It is therefore important to determine whether a case of pneumonia is due to A/H1N1pdm virus only or to multiple pathogens. Herein we detected hMPV in four pneumonia cases with laboratory-confirmed A/H1N1pdm virus infection. Our study also suggests that coinfection with hMPV is a potential risk factor for clinical deterioration with A/H1N1pdm virus infection.

This article appeared in the Infectious Agents Surveillance Report (IASR), vol. 30, p. 298–299, 2009 in Japanese.

Conflict of interest None to declare.

REFERENCES

1. National Institute of Infectious Diseases (2009): Manual for the Diagnosis of H1N1 New Influenza Virus. 1st version. National Institute of Infectious Diseases, Tokyo, Japan (in Japanese).
2. National Institute of Infectious Diseases (2008): Manual for the Diagnosis of Human Metapneumovirus. National Institute of Infectious Diseases, Tokyo, Japan. Online at 〈http://www.nih.go.jp/niid/reference/hMPV-manual.pdf〉 (in Japanese).
3. Erdman, D.D., Weinberg, G.A., Edwards, K.M., et al. (2003): Genescan reverse transcription-PCR assay for detection of six common respiratory viruses in young children hospitalized with acute respiratory illness. J. Clin. Microbiol., 41, 4298–4303.
4. Gómez-Gómez, A., Magaña-Aquino, M., García-Sepúlveda, C.A., et al. (2010): Severe pneumonia associated with pandemic (H1N1) 2009 outbreak, San Luis Potosi, Mexico. Emerg. Infect. Dis., 16, 27–34.
5. Perez-Padilla, R., de la Rosa-Zamboni, D., Ponce de Leon, S., et al. (2009): Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. N. Eng. J. Med., 361, 680–689.
6. Chowell, G., Bertozzi, S.M., Colchero, M.A., et al. (2009): Severe respiratory disease concurrent with the circulation of H1N1 influenza. N. Eng. J. Med., 361, 674–679.
7. Jain, S., Kamimoto, L., Bramley, A.M., et al. (2009): Hospitalized patients with 2009 H1N1 influenza in the United States, April–June 2009. N. Eng. J. Med., 361, 1935–1939.
8. Kamiya, N., Moriyama, T., Nakajima, K., et al. (2009): Clinical case of pandemic (H1N1) 2009 influenza: a child with severe pneumonia that rapidly progressed to respiratory failure, August 2009—Tokyo. Infect. Agents Surveillance Rep., 30, 267–268 (in Japanese).