Sudden Death of a Patient with Pandemic Influenza (A/H1N1pdm) Virus Infection by Acute Respiratory Distress Syndrome

Akihiro Takiyama, Lei Wang, Mishie Tanino, Taichi Kimura, Naoki Kawagishi, Yasuyuki Kunieda, Harutaka Katano, Noriko Nakajima, Hideki Hasegawa, Tomoyuki Takagi, Hiroshi Nishihara, Tetsutarou Sata, and Shinya Tanaka

1Laboratory of Cancer Research, Department of Pathology and 2Department of Translational Pathology, Hokkaido University Graduate School of Medicine, Sapporo 060-8638; 3Department of Pathology and 4Influenza Virus Research Center, National Institute of Infectious Diseases, Tokyo 162-8640; and 5Wakkanai Municipal Hospital, Wakkanai 097-8555, Japan

SUMMARY: We describe an autopsy case of a patient with pandemic influenza (A/H1N1pdm) virus infection in Japan, who developed rapidly progressive viral pneumonia exhibiting diffuse alveolar damage. A 41-year-old female visited our hospital with a fever of 38.7°C. She was a public health nurse with no underlying disease and had had contact with a group of elementary school students who had been infected with the influenza (A/H1N1pdm) virus 1 week earlier. She was prescribed oseltamivir and returned to the hotel where she was staying alone. The next day, she was found dead in her hotel room. At autopsy, both lungs were voluminous and microscopic examination revealed acute-stage, severe diffuse alveolar damage with remarkable mononuclear cell infiltration and hyaline membrane formation in the lungs. CD8-positive T lymphocytes were dominantly observed. Immunohistochemically, influenza A viral protein was confirmed in the damaged type II pneumocytes and also in the infiltrated macrophages. Real-time RT-PCR analysis of both pre- and post-mortem pharyngeal swabs confirmed a novel influenza (A/H1N1pdm) virus infection. This is the second autopsy case of influenza (A/H1N1pdm) virus infection in Japan, and the findings indicated that the patient died due to an exceptionally rapid progression of viral pneumonia. This case indicates that patients with influenza (A/H1N1pdm) virus infection should be carefully monitored for acute respiratory distress syndrome.

In March 2009, a novel swine-derived influenza (A/H1N1pdm) virus was identified in California and Mexico (1,2). On June 11, 2009, the World Health Organization declared that infections caused by the new strain had reached pandemic proportion. On December 6, 2009, it was reported that 9,596 deaths had been associated with the pandemic worldwide (3), and on December 28, 2009, it was reported that 133 deaths had been linked to the virus in Japan (4). Here, we describe the second autopsy case of a patient with influenza (A/H1N1pdm) virus infection in Japan, which was characterized by rapidly progressive viral pneumonia exhibiting diffuse alveolar damage.

A 41-year-old female visited our hospital with a fever of 38.7°C. She was a public health nurse with no underlying disease and had had contact with a group of elementary school students who had been infected with the influenza (A/H1N1pdm) virus twice, once 5 days and once 8 days before presentation at our hospital. She had experienced a mild cough several days ago, but no other symptoms were observed. Oseltamivir was prescribed and she went back to her hotel alone. The next day, she was found dead in her hotel room. At autopsy, both lungs were voluminous (left, 560 g; right, 550 g; Figs. 1a and b), but there was little pleural effusion. Microscopically, prominent mononuclear cell infiltration with fibrous exudates was observed throughout the entire lung by HE staining (Figs. 1c, d, e, and f). Hyaline membrane formation was found in a part of the lung, suggesting diffuse alveolar damage (Fig. 1e). Hyperplasia of the alveolar pneumocytes was not evident. Necrotizing bronchitis and bronchiolitis were predominantly observed (Fig. 1g). Degenerative changes in the epithelial cells of the bronchial and bronchiolar mucosa and the bronchial glands were seen (Fig. 1g). Thrombosis of the capillaries and arteries was not evident (Fig. 1h). Immunohistochemically, the infiltrating lymphocytes were mostly positive for CD4 and CD8, and the CD4/CD8 ratio was lower than 1.0 (Figs. 1i and j).

To analyze the distribution of the influenza (A/H1N1pdm) virus antigen, 10% formalin-fixed paraffin-embedded tissue sections were immunostained by an avidin-biotin complex immunoperoxidase method (LSAB2 kit/HRP/DAB; Dako Cytomation, Copenhagen, Denmark) using a mouse monoclonal antibody against influenza A nucleoprotein (InfA-NP) (5). Positivity for InfA-NP was observed in the damaged cells, which were morphologically consistent with epithelial cells and macrophages (Fig. 2).

To characterize the virus-infected cells, a confocal laser scanning microscope was used to visualize double immunofluorescence staining for InfA-NP and for the cell type-specific marker proteins EMA (epithelial cells) and CD68 (macrophages) as previously described (6). Alexa Fluor 568-conjugated anti-rabbit IgG (Molecular Probes, Eugene, Oreg., USA) and Alexa Fluor 488-conjugated anti-mouse IgG (Molecular Probes) were used as secondary antibodies for InfA-NP and...
EMA/CD68, respectively. Double staining of epithelial membrane antigens and InfA-NP showed influenza A virus infection of the cytoplasm and/or nucleus of the damaged type II pneumocytes (Fig. 3a). In addition, combination staining with InfA-NP and CD68 revealed influenza virus infection in macrophages (Fig. 3b). Most of the tracheal epithelia were desquamated, only those of upper portion remained, and influenza (A/H1N1pdm) virus antigen was not detected in these areas. The liver showed fatty changes, mainly centrilobular. No pathognomonic findings were observed in other organs, including the brain (1,300 g) and the heart (430 g).

Real-time RT-PCR examination of both pre- and post-mortem pharyngeal swabs, performed by the Hokkaido Public Health Institute, confirmed the novel influenza (A/H1N1pdm) virus infection. Further analysis demonstrated the relatively lower copy number of infected virus in the lung tissue (data not shown).

Influenza (A/H1N1pdm) virus infection can cause acute respiratory distress syndrome (1,7,8). In the present case, a public health nurse was infected with influenza (A/H1N1pdm) virus and died of severe respiratory distress syndrome due to severe diffuse alveolar damage. She had no underlying disease except for obesity, which has been reported to confer an increased risk of death from influenza (A/H1N1pdm) virus (2). Alveolar involvement is unusual but such cases seem to have been common during the 1918–1919 pandemic (9). In the current case, immunohistochemical study clearly confirmed the influenza (A/H1N1pdm) virus infection of damaged type II pneumocytes. In consistent with our finding, Mauad et al. described 21 autopsy cases with confirmed novel influenza

Fig. 1. Autopsy findings of the lung. (a and b) Gross appearance of bilateral lung and the sectioned surface. (c-f) Microscopic findings. Prominent mononuclear cell infiltration with fibrinous exudates was observed throughout the entire lung. Partially, hyaline membrane formation was prominent (e). (g) Necrotizing bronchitis and bronchiolitis were observed. (h) Elastica-Masson stain. Thrombosis of the capillaries and arteries was not evident. (i and j) Immunostaining for CD4 (i) and CD8 (j).
(A/H1N1pdm) virus infection, and reported that diffuse alveolar damage was present in 20 individuals (10).

One of the significant features of this case was the remarkably rapid disease progression, with the patient dying less than 24 h after presentation. Viral overload leads to altered innate immune responses such as sustained TLR-3 activation, enhanced inflammation with high numbers of CD8-positive T lymphocytes, and local production of IFN-γ, all of which have been suggested to contribute to virally induced lung injury (11). In the present case, the cause of the diffuse alveolar damage was unknown, but an increase of CD8-positive T cells may have altered the immune response leading to lung injury. Therefore, when caring for the influenza (A/H1N1pdm) virus-infected patients, it is important to carefully exclude the possibility of severe acute distress syndrome, which can cause sudden death.

ACKNOWLEDGMENTS

We thank Kazuko Shimizu and Eiko Aoyanagi for the excellent pathologi- cal technique.

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

REFERENCES


