DOI:10.1111/irv.12065
www.influenzajournal.com

Original Article

Factors promoting the prolonged shedding of the pandemic (H1N1) 2009 influenza virus in patients treated with oseltamivir for 5 days

Seung M. Ryoo, Won Y. Kim, Chang H. Sohn, Dong W. Seo, Bum J. Oh, Jae H. Lee, Yoon S. Lee, Kyoung S. Lim

Department of Emergency Medicine, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea.
Correspondence: Won Y. Kim, Department of Emergency Medicine, University of Ulsan College of Medicine, Asan Medical Center, 86 Asanbyeongwon-gil, Songpa-gu, Seoul 138-736, Korea.
E-mail: wonpia@yahoo.co.kr

Accepted 29 November 2012. Published Online 26 December 2012.

Background The duration of viral shedding is an important determinant of infectivity and transmissibility and provides vital information for effective infection prevention and control. However, few studies have evaluated viral shedding in patients admitted to hospital with 2009 H1N1 influenza and treated with oseltamivir.

Objective To determine the incidence of prolonged 2009 H1N1 influenza viral shedding in patients treated for 5 days with oseltamivir and to identify factors that promote prolonged viral shedding.

Methods This was a prospective, observational cohort study of 173 patients infected with 2009 H1N1 influenza (confirmed by RT-PCR) who were admitted to isolation rooms in the emergency department of our hospital between August 25, 2009 and December 31, 2009. All of the patients were treated according to institutional protocols and received routine follow-up RT-PCR testing after 5 days of oseltamivir therapy. Prolonged viral shedding was defined as a positive follow-up RT-PCR result.

Result Of the 173 patients in our cohort, 88 (50·8%) showed persistent viral shedding after oseltamivir treatment. Viral shedding was significantly prolonged if antiviral therapy was started ≥ 2 days after symptom onset (OR 2·74, 95% CI 1·29–5·82), if there were major comorbidities (OR 3·07, 95% CI 1·29–7·32), and/or if respiratory symptoms were still present on the day 5 of antiviral treatment (OR 4·13, 95% CI 2·10–8·11).

Conclusions The presence of major comorbidities, a delay in initiating antiviral treatment, and continuing respiratory symptoms after 5 days of antiviral treatment are associated with prolonged shedding of the 2009 H1N1 influenza virus.

Keywords H1N1 subtype, influenza A virus, RT-PCR, viral shedding.

Please cite this paper as: Ryoo *et al.* (2013) Factors promoting the prolonged shedding of the pandemic (H1N1) 2009 influenza virus in patients treated with oseltamivir for 5 days. Influenza and Other Respiratory Viruses 7(5), 833–837.

Introduction

Influenza is a highly infectious, acute viral respiratory illness that causes worldwide outbreaks of variable extent and severity each year.[1] Recently, the pandemic 2009 influenza A (H1N1) virus spread rapidly, resulting in millions of laboratory-confirmed cases and over 16 713 deaths in over 200 countries.[2] Since then, numerous epidemiological and clinical findings on this influenza strain have been reported. However, few reports have evaluated viral shedding in patients admitted for 2009 H1N1 influenza and treated with oseltamivir.

The duration of viral shedding is likely to be an important determinant of infectivity and transmissibility and should be known when developing measures to prevent and control viral diseases. In the case of seasonal influenza viruses, the mean duration of viral shedding has been reported to be 4·8 days,[2] although this virus has also been detected for up to 5–7 days in patients admitted to hospital.[3] With regard to the 2009 H1N1 influenza strain, the World Health Organization (WHO) has recommended that patients receive 5 days of treatment with antiviral agents.[4] However, animal models show that the 2009 H1N1 virus replicates more efficiently than seasonal influenza viruses in the respiratory tract.[5–7] In addition, several studies have shown that the 2009 H1N1 virus can still be present for 8 days or more.[8–12] Hence, some patients require additional antiviral treatments and need to be isolated to prevent infection. However, despite these observations, there are still no guidelines or recommendations stipulating that patients should be assessed by RT-PCR testing when antiviral treatment courses are completed to determine whether viral shedding is still occurring.

The aim of this study was to determine the incidence of prolonged viral shedding of 2009 H1N1 influenza in patients

treated for 5 days with oseltamivir. Factors that promoted prolonged viral shedding were also identified.

Methods and materials

Study design and patients

This was a prospective, observational cohort study of patients with RT-PCR-confirmed 2009 H1N1 influenza who were admitted between August 25, 2009 and December 31, 2009 to the Asan Medical Center, the largest healthcare center (2800 beds) in Korea, and who were treated with oseltamivir according to WHO guidelines.[2] The patients were followed up according to institutional protocols. Hence, all patients were admitted to isolation rooms in the emergency department and treated for 5 days with oseltamivir. We repeated nasopharyngeal swab sampling at the conclusion of treatments, and additional swabs were collected at 7 days and beyond only if the follow-up PCR result was positive. According to hospital policy, only patients with negative RT-PCR results could be transferred from the isolation rooms to the general ward or discharged. This study protocol was approved by the institutional ethics committee, and informed consent was obtained from all patients.

Data collection

Nasopharyngeal samples were obtained from all patients with suspected influenza infections using flocked swabs (Flexible Minitip 503CS01 Flocked Swabs; Diagnostic Hybrids, Copan, Italy). A pH1N1 infection was suspected if the patient had an influenza-like illness, namely a temperature of $\geq 37{\cdot}5°C$ and at least one of the following symptoms: sore throat, cough, rhinorrhea, or nasal congestion.[13] RT-PCR was performed within 12 hours of sampling (without freezing) using Real-Time Ready Inf A/H1N1 detection kits (Roche Diagnostics, Rotkreuz, Switzerland) and multiwell plate-based real-time PCR platforms (LightCycler® 480 Real-Time PCR System; Roche Diagnostics) as previously described.[14] All patients admitted to isolation rooms underwent repeated RT-PCR testing after 5 days of oseltamivir therapy. Clinical data were recorded prospectively and included demographic characteristics, medical comorbidities, influenza vaccination status, time of symptom onset, influenza-related complications, antibiotic use, steroid and immunosuppressant treatment, presence of respiratory symptoms when the RT-PCR test was repeated, and clinical outcomes. Respiratory symptoms were defined as cough, sore throat, rhinorrhea, and nasal obstruction.[4] The medical comorbidities that were recorded included hypertension, diabetes mellitus, malignancies, chronic lung disease, chronic renal diseases, and organ transplantation. In our current analysis, prolonged viral shedding was defined as a positive follow-up RT-PCR test result after 5 days of oseltamivir therapy. The patients were divided into RT-PCR-positive and RT-PCR-negative groups on the basis of these follow-up results. The two groups were then compared statistically.

Statistical analysis

The data are presented as the mean ± standard deviation or median and interquartile range (IRQ) for continuous variables and as absolute or relative frequencies for categorical variables. Unpaired Student's *t*-tests or Mann–Whitney *U*-tests were used to compare continuous variables, whereas chi-square tests or Fisher's exact tests were used to compare categorical variables. Predisposing factors for prolonged viral shedding were assessed by univariate analysis, and variables that were statistically significant ($P < 0{\cdot}20$) in the univariate analysis were included in the multivariate analysis by applying a multiple logistic regression based on the enter method. Possible interactions and colinearity were tested. To adjust for confounding factors and to assess possible effect modifications, separate multiple logistic regressions were performed. The results of the multivariate logistic regression analyses were summarized by estimating the odds ratios (OR) and the respective 95% confidence intervals (CI). A P-value $< 0{\cdot}05$ was considered statistically significant. All statistical analyses were performed using SPSS for Windows, version 12·0 (SPSS Inc., Chicago, IL, USA).

Results

During this study period, 20 913 nasopharyngeal swab samples were obtained and tested by RT-PCR. Of these, 8016 (38·3%) samples were found to be positive for 2009 H1N1 influenza. Patients with 2009 H1N1 were hospitalized if they developed potentially serious medical conditions or if the exacerbation of their underlying chronic illnesses or their severe symptoms was considered to be unmanageable at home. In total, 173 patients were admitted to isolation rooms and RT-PCR tests were repeated after 5 days of oseltamivir treatment according to our institutional protocols.

The baseline characteristics of our patient subjects, including their demographic variables, comorbidities, presenting symptoms, and initial laboratory parameters, were assessed. The mean age was 43·4 ± 20·6 years and 102 patients were male (58·9%). During the study period, 94 (54·3%) patients exhibited abnormal findings upon chest radiography that were consistent with pneumonia and nine patients (5·2%) died during this period. The median interval from symptom onset to hospital visitation was 2·0 (range, 1·0–4·0) days, while the median duration between symptom onset and oseltamivir initiation was 2·0 (range, 1·0–4·0) days. Of the 173 patients who underwent repeated RT-PCR on day 5, 88 cases had positive results (50·8%).

The data for the RT-PCR-positive and RT-PCR-negative groups were compared (Table 1). The presence of any major

Table 1. Clinical characteristics of 173 patients with laboratory-confirmed 2009 H1N1 influenza who were treated for 5 days with oseltamivir and then underwent a follow-up RT-PCR test

	RT-PCR-negative (*n* = 85)	RT-PCR-positive (*n* = 88)	*P*
Demographic characteristics			
Age (y)	43·49 ± 18·81	45·13 ± 23·57	0·61
Male, *n* (%)	49 (57·6)	53 (60·2)	0·73
Vaccination*, *n* (%)	17 (20·0)	23 (26·1)	0·67
Comorbidities, *n* (%)			
Hypertension	14 (16·5)	20 (22·7)	0·30
Diabetes mellitus	11 (12·9)	13 (14·8)	0·73
Obstructive lung disease	11 (12·9)	17 (19·3)	0·26
Other underlying lung disease	22 (25·9)	28 (31·8)	0·39
Malignancy	17 (20·0)	26 (29·5)	0·15
Any major comorbidities	47 (55·3)	71 (80·7)	<0·01
Use of immunosuppressant	7 (8·2)	8 (9·1)	0·84
History of steroid use	14 (16·5)	25 (28·4)	0·06
Present symptoms, *n* (%)			
Fever	72 (85·7)	72 (81·8)	0·49
Cough	75 (89·3)	80 (90·9)	0·72
Sore throat	34 (40·5)	23 (26·1)	0·05
Rhinorrhea	28 (33·3)	33 (37·5)	0·57
Gastrointestinal symptoms	16 (19·0)	12 (13·6)	0·34
Treatment modality, *n* (%)			
Antibiotics treatment	61 (71·8)	72 (81·8)	0·12
Steroid treatment	23 (27·1)	41 (46·6)	<0·01
High-dose oseltamivir (150 mg)	43 (50·6)	42 (47·7)	0·71
Other antiviral agent combination**	6 (7·1)	14 (15·9)	0·07
Oseltamivir therapy commenced two or more days after symptom onset	42 (49·8)	58 (65·9)	<0·01
Laboratory tests			
White blood cell count (10^9/l)	9·06 ± 4·47	8·88 ± 5·88	0·83
Absolute neutrophil count	7327·0 ± 4393·8	7079·4 ± 5451·1	0·76
Lymphocyte (%)	14·4 ± 10·0	15·9 ± 13·6	0·51
Lymphocyte count (10^9/l)	1030·9 ± 554·5	999·3 ± 800·0	0·78
Hemoglobin (g/dl)	12·9 ± 3·7	12·1 ± 3·0	0·18
Platelet count (10^9/l)	179·9 ± 87·4	176·5 ± 92·8	0·82
Aspartate transaminase (IU/l)	324·4 ± 212·1	67·5 ± 76·3	0·50
Alanine transaminase (IU/l)	175·3 ± 103·2	37·2 ± 36·6	0·40
C-reactive protein (mg/dl), (*N* = 146)	8·2 ± 8·5	8·27 ± 9·4	0·97
Procalcitonin (ng/ml), (*N* = 78)	3·4 ± 7·7	5·6 ± 13·2	0·39

Values are expressed as mean ± SD, median and interquartile range (IRQ), or *n* (%).
*Seasonal influenza vaccination.
**Amantadine 100 mg po bid and ribavirin 300 mg po tid.

comorbidities or steroid treatments delayed oseltamivir therapy (two or more days after symptom onset). The continuation of respiratory symptoms 5 days after the start of treatment was significantly associated with prolonged viral shedding. Multivariate analysis revealed that prolonged viral shedding was significantly associated with delayed antiviral therapy (OR 2·74, 95% CI 1·29–5·82), major comorbidities (OR 3·07, 95% CI 1·29–7·32), and continuing respiratory symptoms after 5 days of treatment (OR 4·13, 95% CI 2·10–8·11) (Table 2).

Discussion

The duration of viral shedding provides important information regarding infectivity and transmissibility. However, only a few studies to date have described the duration of 2009 H1N1 shedding. Na *et al.*[11] reported that the median duration of viral shedding of 2009 H1N1 influenza, assessed by RT-PCR, was 9 days. Moreover, a further report from France has indicated a median shedding duration of 11 days for this virus.[12] Hence, these studies show that the shedding

Table 2. Multivariate analysis of factors associated with prolonged viral shedding in patients with laboratory-confirmed 2009 H1N1 influenza who were treated for 5 days with oseltamivir

	Multivariate analysis Odds ratio	95% CI	*P*
Oseltamivir therapy commenced two or more days after symptom onset	2·74	1·29–5·82	<0·01
Any major comorbidities	3·07	1·29–7·32	<0·01
Respiratory symptoms continuing on the 5th day of treatment	4·13	2·10–8·11	<0·01
Steroid treatment	1·87	0·56–6·25	0·31

period of 2009 H1N1 influenza is longer than that of seasonal influenza strains. Therefore, although the WHO recommends that influenza should be treated by 5 days of antiviral therapy, viral shedding may persist beyond this period and additional treatment and isolation should be considered for some infections. In our present study, the shedding of 2009 H1N1 virus was assessed in 173 patients: after 5 days of oseltamivir treatment, 88 patients (50·8%) still had a positive RT-PCR result. These results agree with those of recent studies that found that patients admitted to hospital with the pandemic influenza 2009 H1N1 virus had a longer duration of viral shedding than adults who were hospitalized with a seasonal influenza virus infection.[12–14]

In seasonal influenza, prolonged viral shedding can occur in children,[15] immunocompromised people,[16] and elderly hospitalized patients with comorbidities.[17] With regard to 2009 H1N1 influenza, Ling *et al.*[18] have reported that younger immunocompetent adults show prolonged shedding, while Cao *et al.*[13] have suggested that a delay from the onset of symptoms to oseltamivir therapy of >48 hours is associated with persistent RT-PCR positivity (>5 days). In our present study, the presence of major comorbidities was also found to be significantly associated with a persistent and positive RT-PCR result 5 days after oseltamivir therapy. The major comorbidities consisted of chronic systemic medical illnesses, which included cancer, chronic lung disease, renal diseases, heart disease, liver diseases, and stroke.

Our present findings show that prolonged shedding is also associated with the presence of respiratory symptoms after 5 days of antiviral therapy and the commencement of antiviral therapy two or more days after symptom onset. These results agree with those of previous studies.[13,18] A recent Italian study[19] has reported that the duration of viral shedding is significantly reduced when therapy is initiated within 2 days of symptom onset.

The Current Centers for Disease Control and Prevention Guidelines recommend that persons with influenza should not return to work or school until 24 hours after their fever has resolved without the use of antipyretics.[20] These guidelines are based on studies showing that shedding can occur after fever resolution and that many people infected with influenza will not have a fever.[20] Our present study also shows that prolonged shedding is associated with the continuing presence of respiratory symptoms (defined as cough, sore throat, rhinorrhea, and nasal obstruction) after 5 days of antiviral therapy. This is consistent with the results of a previous study in which the 2009 H1N1 influenza virus was found to be frequently detectable by RT-PCR for up to 10 days after fever resolution.[21] Hence, it should be considered that if respiratory symptoms remain after 5 days of antiviral treatment, additional antiviral treatment and isolation may be needed, especially if the patient commenced antiviral therapy more than 2 days after the onset of symptoms. However, further research is needed to clarify the effects of additional antiviral treatment and isolation.

Antiviral therapy has been proven to reduce the viral shedding period of seasonal influenza.[22–24] Oseltamivir (a neuraminidase inhibitor) is a first-line influenza drug that has been shown to effectively reduce both the severity of symptoms and the duration of infection.[23] In our present study, 75 and 150 mg of oseltamivir were administered twice daily for simple and complicated influenza infections, respectively. These two doses of oseltamivir do not differ significantly in terms of reducing viral shedding and titers in naturally acquired influenza infections.[22–24] In the present study, we found there is no significant difference in viral shedding between high dose of oseltamivir and standard dose ($P = 0·71$).

The severity of the (H1N1) 2009 influenza pandemic correlates with the extent of lymphopenia.[25] In particular, Li *et al.* showed that the viral load correlates inversely with the absolute lymphocyte counts in untreated patients.[26,27] However, in our present study, a significant correlation between the viral shedding period and lymphocyte count was not observed. In addition, viral shedding did not correlate with other laboratory data, including procalcitonin levels, leukocytosis, absolute neutrophil count, C-reactive protein levels, or liver enzyme levels.

This study has several noteworthy limitations. First, as this study included patients who were treated with oseltamivir, the data do not reflect the natural viral shedding period but the shedding period in patients treated with oseltamivir for 5 days. Second, samples that are positive for viral RNA by RT-PCR may not contain live virus. Third, the samples were not analyzed at a quantitative level. Nevertheless, our preliminary data reveal associations between prolonged viral shedding in patients with 2009 H1N1 influenza who were treated with oseltamivir for 5 days and also various clinical

characteristics of these cases. These observations may be pertinent for future epidemiological control in emergency department and in the clinical management of influenza.

Conclusions

Prolonged shedding of the 2009 H1N1 influenza virus strain was observed in 51% of patients who had been treated for 5 days with oseltamivir. Prolonged shedding was significantly associated with major comorbidities, delayed antiviral treatment, and continuing respiratory symptoms after antiviral treatment. Therefore, when patients exhibit these risk factors, they may require additional management beyond 5 days. Further research is needed to clarify the effects of additional antiviral treatment and isolation for patients with persistently positive RT-PCR results for influenza.

References

1 Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL. Harrison's principles of internal medicine, 6th edn. NewYork: McGraw-Hill, 2005.
2 World Health Organization. Global Alert and Response (GAR); Pandemic (H1N1) 2009 – Update. World Health Organization, 2009. Available at http://www.who.int/csr/don/2010_03_12/en/index.html (Accessed 12 March 2010).
3 Lee N, Chan PK, Hui DS *et al.* Viral loads and duration of viral shedding in adult patients hospitalized with influenza. J Infect Dis 2009; 200:492–500.
4 World Health Organization. Clinical Management of Human Infection with Pandemic (H1N1) 2009: Revised Guidance. World Health Organization, 2009. Available at http://www.who.int/csr/resources/publications/swineflu/clinical_management/en/index.html (Accessed 12 March 2010).
5 Itoh Y, Shinya K, Kiso M *et al.* In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. Nature 2009; 460:1021–1025.
6 Maines TR, Jayaraman A, Belser JA *et al.* Transmission and pathogenesis of swine-origin 2009 A(H1N1) influenza viruses in ferrets and mice. Science 2009; 325:484–487.
7 Munster VJ, de Wit E, van den Brand JM *et al.* Pathogenesis and transmission of swine-origin 2009 A(H1N1) influenza virus in ferrets. Science 2009; 325:481–483.
8 Witkop CT, Duffy MR, Macias EA *et al.* Novel Influenza A (H1N1) outbreak at the U.S. Air Force Academy: epidemiology and viral shedding duration. Am J Prev Med 2010; 38:121–126.
9 Eggertson L. Pandemic (H1N1) 2009 lives in some people for at least eight days after symptoms develop. CMAJ 2009; 181:E203.
10 To KK, Chan KH, Li IW *et al.* Viral load in patients infected with pandemic H1N1 2009 influenza A virus. J Med Virol 2010; 82:1–7.
11 Na S, Chong YP, Kim MN *et al.* Duration of viral shedding in patients admitted to hospital with pandemic influenza A/H1N1 2009 infection. J Med Virol 2011; 83:5–9.
12 Malato L, Llavador V, Marmier E *et al.* Pandemic influenza A(H1N1) 2009: molecular characterisation and duration of viral shedding in intensive care patients in Bordeaux, south-west France, May 2009 to January 2010. Euro Surveill 2011; 16:[pii: 19776].
13 Cao B, Li XW, Mao Y *et al.* Clinical features of the initial cases of 2009 pandemic influenza A (H1N1) virus infection in China. N Engl J Med 2009; 361:2507–2517.
14 Ong AK, Chen MI, Lin L *et al.* Improving the clinical diagnosis of influenza–a comparative analysis of new influenza A (H1N1) cases. PLoS ONE 2009; 4:e8453.
15 Nicholson KG, Wood JM, Zambon M. Influenza. Lancet 2003; 362:1733–1745.
16 Cox NJ, Subbarao K. Influenza. Lancet 1999; 354:1277–1282.
17 Leekha S, Zitterkopf NL, Espy MJ, Smith TF, Thompson RL, Sampathkumar P. Duration of influenza A virus shedding in hospitalized patients and implications for infection control. Infect Control Hosp Epidemiol 2007; 28:1071–1076.
18 Ling LM, Chow AL, Lye DC *et al.* Effects of early oseltamivir therapy on viral shedding in 2009 pandemic influenza A (H1N1) virus infection. Clin Infect Dis 2010; 50:963–969.
19 Meschi S, Selleri M, Lalle E *et al.* Duration of viral shedding in hospitalized patients infected with pandemic H1N1. BMC Infect Dis 2011; 11:140.
20 CDC Recommendations for the Amount of Time Persons with Influenza-Like Illness Should be Away from Others. Atlanta, GA: Centers for Disease Control and Prevention, 2009 [updated October 23, 2009]. Available at http://www.cdc.gov/H1N1flu/guidance/exclusion.htm (Accessed 26 April 2010).
21 Sato M, Hosoya M, Kato K, Suzuki H. Viral shedding in children with influenza virus infections treated with neuraminidase inhibitors. Pediatr Infect Dis J 2005; 24:931–932.
22 Treanor JJ, Hayden FG, Vrooman PS *et al.* Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized controlled trial. US Oral Neuraminidase Study Group. JAMA 2000; 283:1016–1024.
23 Aoki FY, Boivin G. Influenza virus shedding: excretion patterns and effects of antiviral treatment. J Clin Virol 2009; 44:255–261.
24 Nicholson KG, Aoki FY, Osterhaus AD *et al.* Efficacy and safety of oseltamivir in treatment of acute influenza: a randomised controlled trial. Neuraminidase Inhibitor Flu Treatment Investigator Group. Lancet 2000; 355:1845–1850.
25 Perez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S *et al.* Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. N Engl J Med 2009; 361:680–689.
26 Li IW, Hung IF, To KK *et al.* The natural viral load profile of patients with pandemic 2009 influenza A(H1N1) and the effect of oseltamivir treatment. Chest 2010; 137:759–768.
27 Choi WJ, Kim WY, Kim SH *et al.* Clinical characteristics of pneumonia in hospitalized patients with novel influenza A (H1N1) in Korea. Scand J Infect Dis 2010; 42:311–314.