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Original Article

Viral Shedding of 2009 Pandemic H1N1 and Evaluation of Quarantine Recommendations

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SUMMARY: Public health authorities recommend that isolation precautions for influenza should be continued for 7 days after illness onset or until 24 h after the resolution of symptoms, whichever event lasts longer. However, little data are available regarding the duration of isolation for patients with 2009 pandemic H1N1 (pH1N1). We recruited patients with confirmed pH1N1 virus infection at a 2,000-bed tertiary care center. Influenza viral loads from oropharyngeal swab specimens were serially determined by reverse transcriptase quantitative polymerase chain reaction every other day, and the risk factors for prolonged viral shedding were investigated. To evaluate the current recommendations for isolation precautions, we measured the intervals between symptom onset and the last viral RNA detection, and that between the last viral RNA detection and the point at which the patient was symptom-free for 24 h. From November 2009 to January 2010, 26 patients were enrolled, and viral RNA was detected in more than half of the eligible patients (10 of 19, 52.6%) for $\geq$7 days after symptom onset. While evaluating the policy for lifting quarantine, we found that viral RNA was detected in 4 of 15 patients (26.7%) beyond the recommended duration of isolation. In conclusion, viral RNA was detected in a substantial proportion of hospitalized patients even when they fulfilled the recommended conditions for lifting quarantine, and we believe that more prudence is required in this aspect.

INTRODUCTION

Since April 2009, pandemic H1N1 has disseminated globally with sustained human-to-human transmission (1). However, the introduction of effective vaccination and seasonal change decreased influenza activity in the Republic of Korea as well as other countries such as the United States. In Korea, the number of outpatient visits for influenza-like illness (ILI) per 1,000 patients had been 45.0 in the 45th week of 2009, and this number dropped to 6.0 in the 3rd week of 2010 (2). Similarly, in the United States, ILI outpatient visits had been 77.2 in the 42nd week of 2009, and this number decreased to 19.0 in the 3rd week of 2010 (3). However, a major cause of concern is that 2009 pandemic H1N1 (pH1N1) might spread worldwide in the next few years since most of the global population aged below 60 has very limited immunity against pH1N1 virus (4,5). The case fatality of pH1N1 is thought to be comparable with that of seasonal influenza, and even viral kinetics did not reveal any significant differences (6–10). Major health authorities recommend that isolation precautions for patients with influenza symptoms should be continued for 7 days after illness onset or until 24 h after the resolution of fever and respiratory symptoms, whichever event lasts longer, while a patient is in a healthcare facility (11). However, only few studies have evaluated these recommendations. In this study, we prospectively observed the viral kinetics of pH1N1 and evaluated the validity of the current quarantine policy.

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METHODS

Patients and specimen collection: This study was performed in Seoul, the Republic of Korea, from November 2009 to January 2010 at a single, 2,000-bed tertiary care center. Oropharyngeal swabs from the patients suspected to have pH1N1 infection were collected as clinical specimens of respiratory secretion. If the reverse transcriptase quantitative polymerase chain reaction (RQ-PCR) for pH1N1 was positive, a serial RQ-PCR test was performed every other day until the test revealed negative result, and another test was performed on the next day for confirming the negative result. All participants or legal guardians provided written informed consent. This study was approved by the institutional review board of the tertiary care center.

RQ-PCR: Total nucleic acid was extracted from each oropharyngeal swab specimen in a virus transport medium using the NucliSens easyMAG instrument 3 (bioMérieux, Durham, N.C., USA) according to the manufacturer's instruction.

RQ-PCR experiments were performed using the Real-Time ready Influenza A/H1N1 Detection Set® (Roche Diagnostics GmbH, Mannheim, Germany). Each 20-μL PCR reaction mixture contained 5 μL of RNA extract, 3

μl of premixed probe/primer, 4 μl of 5× reaction buffer, 0.4 μl of 50× enzyme mix, and 7.6 μl of water. Thermal cycling was done on the LightCycler 480 instrument (Roche Diagnostics) under the following conditions: reverse transcription for 8 min at 55°C, initial denaturation for 30 s at 90°C, and 45 cycles of 10 s at 95°C, 20 s at 60°C, and 10 s at 72°C.

For quantitative comparison, we used the positive control material included in the RealTime ready Influenza A/H1N1 Detection Set®, which contained plasmids with known copy numbers (2×10^6 copies/mL) of the amplification target. A series of 5 consecutive 10-fold dilution equivalents from 2×10^6 to 2×10^1 copies/mL were prepared and run to generate calibration curves correlating with the RQ-PCR results of the cycle threshold (Ct) value. The lower detection limit of the assay was 1,000 copies/mL of nucleic acid, and the viral titer of a specimen with an undetectable RQ-PCR result was considered as 500 copies/mL.

Data analysis: We collected data for demographics, underlying diseases, presenting symptoms, presence of pneumonia at initial presentation, time interval between symptom onset and antiviral treatment, total leukocyte counts, absolute lymphocyte counts, blood urea nitrogen, serum creatinine, and viral load of pH1N1. The presenting symptoms, i.e., nasal obstruction, sore throat, cough, myalgia, fatigue, headache, and fever, were scored on a 4-point scale. The sum of scores was regarded as the severity of presenting symptoms as mentioned in a prior study (12).

The logarithmic value of the initial viral load was determined by comparing the time intervals between symptom onset and initial test. In addition, the relationships between logarithmic value of initial viral load and patient's demographics, underlying diseases, presenting symptoms, and the presence of pneumonia were investigated. To assess the changes in viral load after antiviral therapy, the mean logarithmic value of the viral load was compared as the time interval between the initiation of antiviral therapy and subsequent viral load measurements.

To identify the risk factors for prolonged viral RNA detection in pH1N1, patients with available serial viral load data beyond 7 days after symptom onset were selected, and the relationships between prolonged viral RNA detection and patients demographics, underlying diseases, severity of presenting symptoms, timing of antiviral initiation after symptom onset ($\leq$48 h versus $>$48 h), and the presence of pneumonia were investigated.

To evaluate the current recommendations for isolation precautions of pH1N1 virus, we compared the data for the last positive day of viral RNA detection and the day when the patient fulfilled the criteria for lifting quarantine, either at least 7 days after illness onset or 24 h after the resolution of fever and respiratory symptoms, whichever event lasted longer. Demographics, underlying diseases, presenting symptoms, timing of antiviral therapy, presence of pneumonia, and initial log viral load were evaluated for their relationship with viral RNA detection beyond the recommended isolation period.

For statistical analysis, we compared the means for continuous variables by using independent Student's *t* tests and the proportions for categorical variables by using Fisher's exact test or Pearson's chi-square test. Significance was set as $P < 0.05$ using 2-sided comparisons, and all statistical analyses were performed using SAS version 9.1.3 (SAS Institute, Inc., Cary, N.C., USA).

RESULTS

Twenty-six patients were recruited during the study period, and the mean follow-up duration was 6.81 ± 3.90 days. The mean time interval between the symptom onset and the initial test for pH1N1 RQ-PCR was 1.84 ± 1.82 days.

The mean log value of initial viral load was 5.52 ± 1.78 copies/mL. The initial viral load was not related to underlying comorbidities, presence of pneumonia, and severity of initial presenting symptoms (data not shown). When the initial viral load was compared with the log values of the time interval between symptom onset and initial test, the mean log value was lower in the patients whose initial tests were performed more than 2 days after symptom onset, and the difference showed with borderline significance (Fig. 1, closed square). All the patients of our study population were prescribed neuraminidase inhibitor oseltamivir, and the mean interval between the pH1N1 test and initiation of antiviral therapy was 0.35 ± 0.56 days. The viral loads tended to decrease at least 3 days after the initiation of antiviral therapy (Fig. 1, open square).

To identify the risk factors related to prolonged viral RNA detection after symptom onset, 19 patients, whose serial viral load data were known for more than 7 days after symptom onset, were included for the analysis (Table 1). Among these patients, 10 (52.6%) were found to be shedding viral RNA from oropharynx for at least 7 days after symptom onset. However, no significant risk factor for prolonged viral RNA detection was identified, while an initial presentation with pneumonia was more frequent among the patients who were

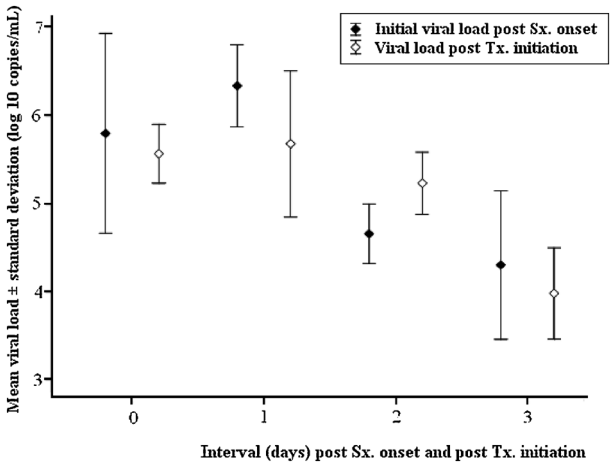

Fig. 1. Trends of initial viral load after symptom onset and changes in viral load after treatment initiation. The initial viral load decreased 2 days after symptom onset (closed square; $P = 0.060$) and the post treatment viral load decreased at least 3 days after antiviral treatment initiation (open square; $P = 0.059$) with borderline significance. Sx, symptom; Tx, treatment.

Table 1. Comparison of patients categorized by duration of viral RNA detection after symptom onset and viral RNA detection beyond lifting quarantine conditions

	Duration of viral RNA detection post symptom onset			Viral RNA detection beyond lifting quarantine conditions		
	≤6 days (n = 9, 47.4%)	≥7 days (n = 10, 52.6%)	P	No (n = 11, 73.4%)	Yes (n = 4, 26.7%)	P
Demographics						
Age	53.0±18.5	55.9±17.5	0.734	53.1±17.6	59.5±12.3	0.453
Sex (male:female)	3:6	6:4	0.370	4:7	4:0	0.077
Comorbidity						
CHF/IHD	1 (11.1)	2 (20.0)	1.000	1 (9.1)	1 (25.0)	0.476
Prior CVA	4 (44.4)	0	0.033	3 (27.3)	0	0.516
Malignancy	1 (11.1)	2 (20.0)	1.000	3 (27.3)	0	0.516
Chronic liver disease	0	0		0	0	
Renal disease	2 (22.2)	1 (10.0)	0.582	2 (18.2)	1 (25.0)	1.000
Immunosuppressive status	1 (11.1)	3 (30.0)	0.582	2 (18.2)	1 (25.0)	1.000
Chronic pulmonary disease	0	1 (10.0)	1.000	0	1 (25.0)	0.267
Diabetes mellitus	3 (33.3)	6 (60.0)	0.370	5 (45.5)	2 (50.0)	1.000
Presence of comorbidity	6 (66.7)	8 (80.0)	0.628	9 (81.8)	3 (75.0)	1.000
Presence of pneumonia	4 (44.4)	9 (90.0)	0.057	5 (45.5)	4 (100)	0.103
Sum of initial symptom scores	9.00±4.92	7.67±5.34	0.589	7.50±5.54	9.75±2.22	0.299
Initial laboratory parameters						
Total leukocyte count (cells/μL)	8,408±3,774	8,633±5,832	0.927	9,630±5,287	9,530±2,598	0.963
Lymphocyte count (cells/μL)	801±346	985±296	0.242	802±344	1,205±272	0.053
Lymphocyte increase on D7 (cells/μL)	857±1,488	−51.1±431	0.136	797±1,411	30±332	0.163
Platelet count (cells/μL)	248,200±202,125	101,334±69,097	0.270	248,800±99,922	199,500±41,315	0.367
Blood urea nitrogen (mg/dL)	15.7±12.6	21.4±12.4	0.357	19.8±14.5	20.2±13.5	0.980
Creatinine (mg/dL)	1.7±2.5	2.5±3.8	0.585	1.80±2.22	3.84±1.80	0.310
Log_{10} viral load (copies/mL)	4.95±1.83	5.65±1.76	0.414	5.12±2.02	4.61±0.80	0.499
Antiviral Tx within 48 h of Sx onset	4 (44.4)	5 (50.0)	1.000	5 (45.5)	1 (25.0)	0.604

Variables are given as no. (%) unless otherwise specified.
Continuous variable are presented as mean±standard deviation.
CHF, congestive heart failure; IHD, ischemic heart disease; CVA, cerebrovascular accident; Tx, treatment; Sx, symptom.

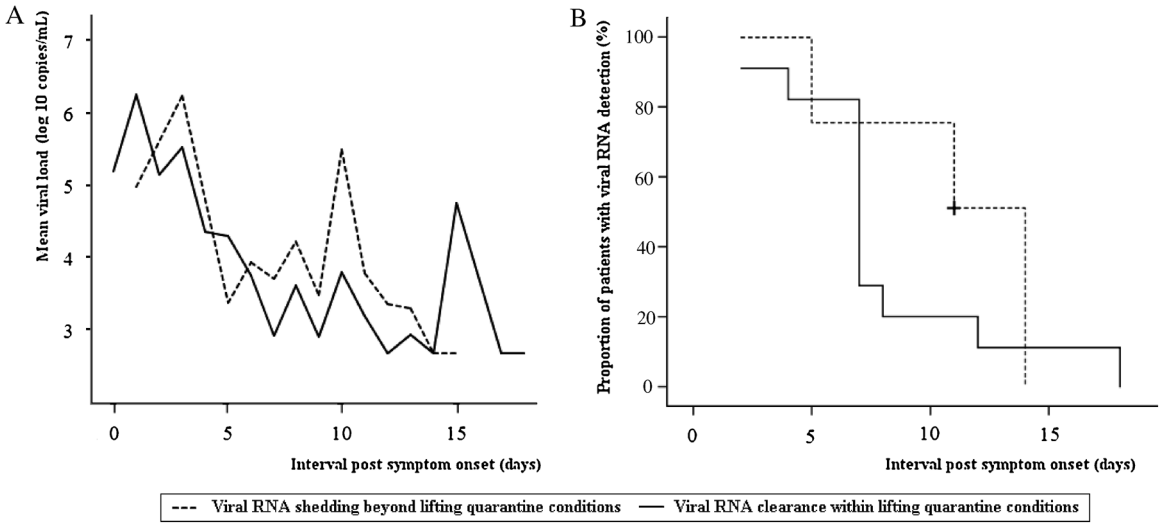

Fig. 2. Mean viral loads after symptom onset (A) and proportions of patients with viral RNA detection (B) regarding the recommended conditions for lifting quarantine. The median durations for viral RNA shedding were not different between the two groups (P = 0.382, log rank test).

shedding viral RNA for more than 7 days, with the difference showing borderline significance (90.0%, 9/10 versus 44.4%, 4/9; P = 0.057). A history of prior cerebrovascular accident (CVA) was less frequent among patients with prolonged viral RNA detection (0%, 0/10 versus 44.4%, 4/9; P = 0.033).

In the evaluation of the policy for lifting quarantine, 11 of 26 patients were excluded from the analysis. Six patients discontinued their follow-up while they were shedding viral RNA within 7 days after symptom onset,

Table 2. Evaluation of currently recommended conditions for lifting quarantine

Case no.	Age/sex	Underlying comorbidity	Pneumonia	Initial $\log_{10}$ viral load	Sx onset day (yr/mo/day)	Days post Sx onset				Viral RNA detection beyond lifting quarantine conditions
						Tx initiation	Last pos. RQ-PCR	First neg. RQ-PCR	24 h after Sx resolve	
1	41/M	DM	No	4.14	2009/11/26	4	5	7	6	No
5	33/M	None	No	7.11	2009/12/7	1	5	7	8	No
6	44/M	Asthma. Immune suppression	Yes	3.95	2009/12/3	6	12	14	9	Yes
8	74/M	DM, CHF	Yes	5.54	2009/12/7	3	11	NA	6	Yes
9	66/F	None	Yes	4.44	2009/12/7	5	5	7	8	No
11	70/F	DM, Malignancy	No	7.74	2009/12/12	1	10	12	13	No
12	90/F	DM, Malignancy, CKD, Old CVA	No	2.27	2009/12/16	0	0	2	2	No
13	49/F	Old CVA	Yes	6.18	2009/12/15	1	5	7	5	No
15	59/M	DM, CKD	Yes	7.74	2009/12/20	1	8	NA	4	Yes
16	57/F	Old CVA	No	2.27	2009/12/21	2	5	7	4	No
20	52/M	Immune suppression, Malignancy	Yes	4.35	2009/12/26	3	16	18	21	No
21	28/F	CKD	No	5.70	2010/01/08	2	1	4	4	No
22	61/M	None	Yes	3.93	2010/01/18	8	11	13	10	Yes
23	44/M	DM	Yes	7.93	2010/01/28	1	6	8	5	No
24	54/F	DM	Yes	1.96	2010/01/26	3	5	7	10	No

Sx, symptom; Tx, treatment; pos, positive; RQ-PCR, reverse transcriptase quantitative polymerase chain reaction; neg, negative; DM, diabetes mellitus; CHF, congestive heart failure; CKD, chronic kidney disease; CVA, cerebrovascular accident; NA, not available.

and 4 patients were not eligible for evaluation because of concomitant infections from organisms such as *Mycobacterium tuberculosis* or proven bacterial pneumonia. Another patient underwent liver transplantation a month before pH1N1 diagnosis and had related comorbidities, and therefore, the onset of symptoms was not measurable in this patient and he was excluded from the analysis. Among the 15 eligible patients, 4 (26.7%) were found to be shedding viral RNA when they satisfied the usual recommended conditions for lifting quarantine (Table 2, Fig. 2A). The median duration of viral RNA shedding in the group that showed prolonged viral RNA detection after lifting the quarantine conditions was slightly longer than that in the group that did not show viral RNA after the quarantine conditions (11.0 days versus 7.8 days), although the difference was not statistically significant ($P = 0.382$, log rank test, Fig. 2B). There was no identifiable significant risk factor with respect to the risk factors related for viral RNA shedding after fulfilling the conditions for lifting quarantine. However, an initial presentation of pneumonia was more frequent among the patients who were shedding viral RNA after fulfilling the conditions for lifting quarantine (100%, 4/4 versus 45.5%, 5/11), although the difference was not statistically significant ($P = 0.103$).

DISCUSSION

The duration of influenza virus shedding has been an issue of concern, especially in hospitalized patients, because it is related to infection control measures such as isolation and the use of surgical masks. Although resistance to antiviral agents is a matter of concern (13,14), antiviral stockpiling still remains an important strategy for the epidemiologic control of a future pandemic, even with the availability of a safe and effective pH1N1 vaccine (8). The natural kinetics of viral shedding of influenza A and the effect of antiviral therapy have been well-described through a randomized controlled study of experimental human infection (15). In this randomized study, the median time to cessation of viral shedding reduced from 107 h in the control group to 58 h in the oseltamivir treatment group. However, in one study, the proportions of patients with viral RNA detection were similar for up to 7 days regardless of oseltamivir treatment, although antiviral therapy reduced the duration and severity of acute influenza-related symptoms (17); these results were obtained in previously healthy young individuals who were naturally infected with the influenza virus. In our study, it was not until after 3 days of antiviral treatment that the viral load began to decrease. Although it is different from the studies performed in a healthy study population, which revealed a rapid decrease of viral load within 2 days after treatment (9,15,18), several studies revealed similar viral kinetics of delayed decrease or clearance despite antiviral therapy among pH1N1 patients with severe pneumonia or oxygen desaturation ($<90\%$) (6,20,21).

In the assessment of the initial viral load, the mean value was relatively lower when the first RQ-PCR test was performed more than 2 days after the symptom onset, and this is in accordance with prior studies that revealed that the viral titer peaked about 24–48 h after

symptom onset (15,19,22). In addition to the initial viral load, the mean lymphocyte count also tended to be higher in the group showing prolonged viral RNA detection. This is in contrast to the results of previous studies, since lymphocytopenia is a known risk factor for hypoxemia (6), prolonged infection (23), or respiratory failure (24). However, the increase in lymphocyte count during the 7 days after pH1N1 diagnosis was impaired in the groups showing prolonged viral RNA shedding in our study.

We used RQ-PCR not only to quantify the relative amount of viral RNA shedding but also to document the presence of viral material as an indirect index of infectivity. Viral culture is a superior method for the assessing the infectivity of a virus, and influenza virus culture study was positive only in about half of the patients showing positive results in the RQ-PCR assay (16). However, RQ-PCR has been accepted as the standard method for pH1N1 infection diagnosis and we think that the presence of a viral RNA can be used as the indirect index of virus infectivity. Major public health authorities recommend that isolation precautions for hospitalized patients with influenza symptoms should be continued for 7 days after the illness onset or for 24 h after the resolution of the fever and respiratory symptoms, whichever event is longer (11). This may be inferred from previous studies, which showed that influenza virus shedding generally diminishes over the course of 7 days (22), and viral clearance correlates with symptom resolution (16,25,26). However, most of these studies have focused on the viral dynamics of the initial phase and early response to antiviral therapy rather than the conditions for lifting quarantine, especially among hospitalized patients. Lee et al. (12) observed that hospitalized patients might show a high viral load as well as prolonged viral shedding, and they suggested a more stringent infection control strategy. However, there has been no report validating the policy of lifting quarantine for influenza infection with regard to both the duration of viral shedding after symptom onset and the condition of the patient's improvement. Viral shedding from asymptomatic patients after recovery from pandemic influenza is not a rare phenomenon (27). A recent study by Witkop et al. reported that 24% of the patients were shedding viable pH1N1 virus 7 days after symptom onset and 19% of those who were asymptomatic for more than 24 h were still found to shed viable virus (7). This is concordant with our study results, although in their study, different conditions were applied for patients 7 days after symptom onset and for those who had been symptom free for more than 24 h. Moreover, their study population comprised young healthy cadet trainees. In our study, 4 of 15 patients (26.7%) were found to be shedding the pH1N1 viral RNA when they satisfied the usually recommended conditions for lifting quarantine. Although we could not identify significant risk factors related to prolonged viral RNA detection after lifting the quarantine conditions, the frequency of accompanied pneumonia at initial presentation was more common in the patient group that showed prolonged viral RNA detection; however, the difference was not statistically significant.

Our work has several limitations. First, as mentioned before, we did not use a virus culture method, which is more relevant to determine the infectivity of virus. In addition, the small number of patients in our study population might have resulted in insufficient study data, and this may be related to a failure to identify significant risk factors for prolonged viral RNA detection after lifting of quarantine conditions.

In summary, a substantial proportion of pH1N1 infected hospitalized patients were shedding detectable viral RNA when they fulfilled the conditions for lifting quarantine. Further investigation regarding this result is crucial since the nosocomial spread of influenza is a critical issue in the management of influenza pandemic.

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Conflict of interest None to declare.

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