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CHEST

Original Research

INFLUENZA A(H1N1) INFECTION

The Natural Viral Load Profile of Patients With Pandemic 2009 Influenza A(H1N1) and the Effect of Oseltamivir Treatment

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Background: **The natural history of viral shedding from the upper respiratory tract of the new pandemic 2009 influenza A(H1N1) and the effect of oseltamivir treatment were uncertain.**
Methods: **A retrospective cohort study involving 145 consecutive patients with specimens positive by reverse transcriptase-polymerase chain reaction for the matrix and new H1 genes was conducted.**
Results: **The nontreated and oseltamivir-treated patients were comparable in their viral load at presentation, demography, and the presenting symptoms. No correlation was observed between viral load with age and number of symptoms. Viral load of nasopharyngeal aspirate (NPA) was significantly lower in treated than in nontreated patients at day 5 after symptom onset. When oseltamivir was initiated ≤ 2 days after symptom onset, a greater rate of viral load reduction in NPA of treated patients than that of nontreated patients was observed (−0.638 [95% CI, −0.809 to −0.466] vs −0.409 [95% CI, −0.663 to −0.185] $\log_{10}$ copies/mL/d post-symptom onset), and the viral load was undetectable at day 6 after oseltamivir initiation, which was 1 day earlier than that of those whose treatment was initiated > 2 days of symptom onset. The viral load was inversely correlated with concomitant absolute lymphocyte count in nontreated patients (Pearson correlation coefficient [*r*] = −0.687, *P* = .001) and treated patients (Pearson *r* = −0.365, *P* < .001). Resolution of fever was 1.4 days later in nontreated than treated patients (*P* = .012)**
Conclusions: **The natural viral load profile was described. Oral oseltamivir suppresses viral load more effectively when given early in mild cases of pandemic 2009 influenza A(H1N1) infections.**
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Abbreviation: H274Y = histidine 274-to-tyrosine mutation; Hb = hemoglobin; lym = absolute lymphocyte; NPA = nasopharyngeal aspirate; NPS = nasopharyngeal swab; NTS = naso-throat swab; pandemic A(H1N1) = pandemic 2009 influenza A(H1N1); plt = platelet; RT-PCR = reverse transcriptase-polymerase chain reaction

The novel 2009 influenza A(H1N1) [A(H1N1)] virus has disseminated globally from Mexico and the United States since April 2009.[1] Because of the sustained human-to-human transmission at all continents, the World Health Organization raised the pandemic alert level from phase 5 to phase 6 on June 11, 2009.[2] Because most of the global population aged < 60 years has undetectable neutralizing antibody,[3,4] the disease is expected to spread throughout the next few years with a more severe second wave in winter 2009/2010. The initial animal studies suggested that pandemic A(H1N1) also replicates in the lower respiratory tract and elicits more inflammatory damage than seasonal influenza A/H1N1 viruses.[5-7] Although the case-fatality rate outside Mexico was comparable to that of seasonal influenza, it was much higher in Mexico, and most occurred in those aged < 60 years.[3,8] Because an effective pandemic A(H1N1) vaccine was not available during the first 6 months of this pandemic, oral oseltamivir was recommended for chemoprophylaxis and treatment as containment and mitigation measures.[9,10] Although cases of oseltamivir-resistant isolates in relation to the neuraminidase histidine 274-to-tyrosine mutation (H274Y) were reported, most of the viral isolates are still susceptible to oseltamivir on *in vitro* testing.[11,12] Oseltamivir treatment had been shown to be safe and reduce disease duration by up to 1.5 days and incidence of secondary

complications, such as pneumonia or otitis media, when initiated within 36 h of symptom onset in seasonal influenza A.[13,14] It can be easily administered orally and is the only safe drug in patients with asthma.[15] The evidence of its effectiveness for the treatment of pandemic A(H1N1) remains uncertain. The current recommendations were based on the assumption that pandemic A(H1N1) will have similar biologic characteristics as seasonal influenza A/H1N1 viruses.[15] We compared the viral load profile of the first group of pandemic A(H1N1)-infected patients with or without oseltamivir treatment in Hong Kong.

Materials and Methods

During the containment phase of the pandemic A(H1N1) outbreak in Hong Kong from April 26, 2009, to June 18, 2009, all patients with laboratory-confirmed pandemic A(H1N1) infection, under the local Prevention and Control of Disease Ordinance, were compulsorily isolated.[16,17] Only those isolated in the three hospitals of Hong Kong were included in this study.[17] The study was approved by our institutional review board. In addition to drawing blood for routine hematologic and biochemical tests, a chest radiograph was taken if clinically indicated. If nasopharyngeal specimens tested positive by reverse transcriptase-polymerase chain reaction (RT-PCR) for the influenza A matrix gene and the pandemic A(H1N1) hemagglutinin gene, but negative for H3 and seasonal H1, 5 days of oseltamivir was recommended at a dosage adjusted for age and renal function.[18] Those who refused oseltamivir treatment were regarded as cases for our study of natural viral load profile from upper respiratory specimens, whereas those treated were controls. Demographic, clinical, laboratory, and radiographic data were retrospectively retrieved from the computerized Clinical Management System for entry on a standard form for analysis as previously described.[19] The number of classic initial clinical symptoms as predictors of influenza infections were used to correlate with viral load in respiratory specimens.[20,21] These included the presence of fever (temperature $\geq$ 37.8°C), cough, sore throat, nasal symptoms, myalgia, and headache.[20,21]

Specimen Collection

Respiratory specimens of patients were taken by nasopharyngeal aspirate (NPA), nasopharyngeal swab (NPS), or naso-throat swab (NTS) and sent in viral transport medium at presentation and days 1, 3, 5, and 6 after hospitalization.

RT-PCR

Total nucleic acid extraction using NucliSens easyMAG instrument (bioMerieux; Durham, NC) and RT-PCR for influenza A virus matrix, the H1 of pandemic A(H1N1), and seasonal H1 and H3 genes was performed as previously described.[22-25] Positive controls with a swine H1 virus (A/SW/HK/294/09), H1N1 (A/California/04/2009), and human seasonal H1N1 and H3N2 viruses were included. For quantitative assay, RT-PCR targeting matrix gene was performed with forward primer [5′-CTTCTAACCGAGGTCGAAACG-3′] and reverse primer [5′-GGCATTTTGGACAAAKCGTCTA-3′].[26] The cDNA was amplified in a Lightcycler instrument with a FastStart DNA Master SYBR Green I Mix reagent kit, and a reference standard was prepared using pCRII-TOPO vector (Invitrogen; San Diego, CA).

Statistical Analysis

Nontreated pandemic A(H1N1)-infected patients and those treated with oseltamivir were compared regarding their demographics, underlying comorbidities, initial presenting symptoms, laboratory parameters, and viral load of NPA at different days post symptom onset. Viral load of NPA of nontreated and those on oseltamivir initiated $\leq$ 2 and $>$ 2 days post symptom onset were compared. Viral load of nontreated and treated patients at each interval post symptom onset were compared. χ^2 Test or Fisher exact test were used for categorical variables and independent *t* test for continuous variables between two groups. One-way analysis of variance was used to compare viral loads of NPA, NPS, and NTS at each interval post symptom onset within nontreated and treated patients. Linear regression was used to determine the rate of viral load reduction. Pearson correlation was used to test the correlations between the age, number of presenting symptoms, concomitant total WBC count, absolute lymphocyte (lym) count, hemoglobin (Hb) level, and platelet (plt) count with viral load, respectively. SPSS 17.0 for Windows (SPSS Inc.; Chicago, IL) was used for statistical computation. A two-tailed *P* value $<$ .05 was considered significant.

Results

One hundred forty-five patients diagnosed with pandemic A(H1N1) infection from the period since the beginning of the epidemic in Hong Kong were included. Sixty-one (42%) were male patients. Seventy-six patients (52.4%) were aged $<$ 18 years but none were aged $<$ 1 year. Twenty-seven patients (18.6%) who refused treatment were enrolled as cases and 118 (81.4%) patients who received oseltamivir were controls. Refusals were related to fear of possible side effects of oseltamivir despite counseling by attending clinicians. None had diabetes mellitus or chronic obstructive airway disease. Only two (1.4%) patients had mild pneumonic changes over right middle and lower lobes on chest radiograph, respectively. None had evidence of rhabdomyolysis, myocarditis, or encephalitis. The nontreated and treated patients were comparable in demographics, comorbidity, presenting symptoms, and initial laboratory parameters (Table 1). Oseltamivir was initiated on the same day (16/118, 13.6%), 1 day (40/118, 33.9%), 2 days (27/118, 22.9%), 3 days

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Table 1—*Demographics, Initial Presenting Symptoms, and Laboratory Parameters of Treated and Nontreated Pandemic A(H1N1)-Infected Patients*

Characteristics	Nontreated Patients (n = 27)	Treated Patients (n = 118)	*P* Value
Age, y	17.6 ± 10.2	21.3 ± 11.8	.108
Sex, male:female	10:17	51:67	.557[a]
Non-Chinese ethnicity	7 (25.9)	21 (17.8)	.334[a]
Comorbidity			
Hypertension	0 (0)	1 (0.8)	1.000[b]
Ischemic heart disease	1 (3.7)	0 (0)	.186[b]
Asthma	1 (3.7)	2 (1.7)	.464[b]
Smoking	0 (0)	6 (5.1)	.594[b]
Contact history	17 (62.9)	50 (42.4)	.053[a]
Use of steroid or immunosuppressants	1 (3.7)	1 (0.8)	.339[b]
Presenting symptoms			
Fever	25 (92.6)	105 (89.0)	.738[b]
Sore throat	16 (59.3)	82 (69.5)	.305[a]
Cough	16 (59.3)	75 (63.6)	.677[a]
Running nose	17 (63.0)	77 (65.3)	.822[a]
Myalgia	4 (14.8)	25 (21.2)	.597[a]
Headache	1 (3.7)	20 (16.9)	.126[b]
Other symptoms			
Shortness of breath	0 (0)	6 (5.1)	.593[b]
Nausea or vomiting	0 (0)	4 (3.4)	1.000[b]
Diarrhea	0 (0)	6 (5.1)	.594[b]
Ear pain	0 (0)	1 (0.8)	1.000[b]
Initial laboratory parameters			
WBC count, 10^9/L	5.33 ± 1.93 n = 14	5.80 ± 1.8 n = 99	.407
Neutrophil count, 10^9/L	3.02 ± 1.82 n = 14	3.74 ± 1.89 n = 97	. 191
Lymphocyte count, 10^9/L	1.61 ± 0.76 n = 14	1.30 ± 0.66 n = 97	.166
Hb, g/dL	13.8 ± 1.42 n = 14	13.8 ± 1.3 n = 99	. 984
Plt count, 10^9/L	185 ± 32 n = 14	204 ± 49 n = 99	.076
Sodium, mmol/L	139 ± 3 n = 10	139 ± 2 n = 81	.936
Potassium, mmol/L	4.1 ± 0.5 n = 10	3.9 ± 0.4 n = 81	.147
Urea, mmol/L	3.32 ± 1.12 n = 10	3.80 ± 1.3 n = 81	. 195
Creatinine, μmol/L	68 ± 19 n = 10	74 ± 21 n = 81	. 409
Albumin, g/L	39 ± 13 n = 10	42 ± 3.2 n = 78	.459
Globulin, g/L	33 ± 4 n = 10	31 ± 4 n = 78	.183
Total bilirubin, μmol/L	10 ± 6 n = 10	9 ± 4 n = 78	.638
Alkaline phosphatase, International Unit/L	119 ± 64 n = 10	76 ± 43 n = 78	.069
Alanine transaminase, International Unit/L	16 ± 8 n = 10	22 ± 16 n = 78	.061
Aspartate transaminase, International Unit/L	27 ± 3 n = 7	37 ± 46 n = 26	.295
Creatinine kinase, International Unit/L	149 ± 105 n = 4	128 ± 114 n = 41	.721

Values given are No. (%) or mean ± SD unless otherwise noted. A *P* value < .05 was considered statistically significant. Hb = hemglobulin; pandemic A(H1N1) = pandemic 2009 influenza A(H1N1); plt = platelet.

[a]χ^2 test used for statistical analysis.

[b]Fisher exact test used for statistical analysis.

(15/118, 12.7%), and ≥ 4 days (20/118, 16.9%) post symptom onset. The mean (range) interval between symptom onset and oseltamivir initiation was 2.1 (0-8) days. Six patients had nonspecific complaints of nausea, vomiting, or loose stool on the initial 2 days of oseltamivir therapy but it was difficult to differentiate from clinical symptoms of pandemic A(H1N1) infection. None reported major side effects from oseltamivir therapy.

The viral loads of NPA, NPS, or NTS at different intervals post symptom onset in nontreated patients were comparable (Fig 1*A*, Table 2), whereas significant differences were observed at days 2 to 3 to days 8 to 9 post symptom onset in treated patients (Fig 1*B*, Table 3). In treated patients, viral loads of NPA were higher than those of NPS and NTS, and significant differences were observed at day 3 to 5 post oseltamivir initiation but not at 1 day before to 1 day after oseltamivir initiation (Fig 2, Table 4). Similar findings were observed in days 3 and 4 post oseltamivir initiation in those who received oseltamivir ≤ 2 days post symptom onset, and day 2 post oseltamivir initiation in those who received oseltamivir > 2 days post symptom onset. When only NPA specimens are analyzed, viral load of NPA was significantly lower in treated than nontreated patients at day 5 post symptom onset irrespective of the relation between oseltamivir initiation and the day post symptom onset (Fig 3*A*, Table 5).

When oseltamivir was initiated ≤ 2 days post symptom onset, a greater rate of viral load reduction in NPA of treated patients (−0.638 [95% CI, −0.809 to −0.466] vs −0.409 [95% CI, −0.663 to −0.185) $\log_{10}$ copies/mL/d post symptom onset) than that of nontreated patients was observed. Similar rate of viral load reduction in NPA was observed in those who received oseltamivir ≤ 2 and > 2 days of symptom onset (−0.711 [95% CI, −1.057 to −0.366] vs −0.695 [95% CI, −0.892 to −0.549] $\log_{10}$ copies/mL/d post oseltamivir initiation). At day 6 post oseltamivir initiation, > 90% of these patients had undetectable viral load level in respiratory specimens (Fig 3*B*) and their viral load level of NPA was undetectable, which was 1 day earlier than those received oseltamivir > 2 days post symptom onset. Moreover, the viral load of NPA in those who received oseltamivir ≤ 2 days post symptom onset was consistently lower than that of nontreated patients, and significant differences were observed at days 4 to 5 and days 6 to 7 post symptom onset (Fig 4, Table 6).

Among the 385 viral load tests performed, 19 out of 73 samples from 13 nontreated patients and 133 out of 312 samples from 96 treated controls had concomitant peripheral blood taken for hematologic test. The viral load level was inversely correlated with concomitant lym count (Pearson $r = -0.365$, $P < .001$), Hb level (Pearson $r = -0.234$, $P = .008$), and plt count (Pearson $r = -0.207$, $P = .019$) in treated patients.

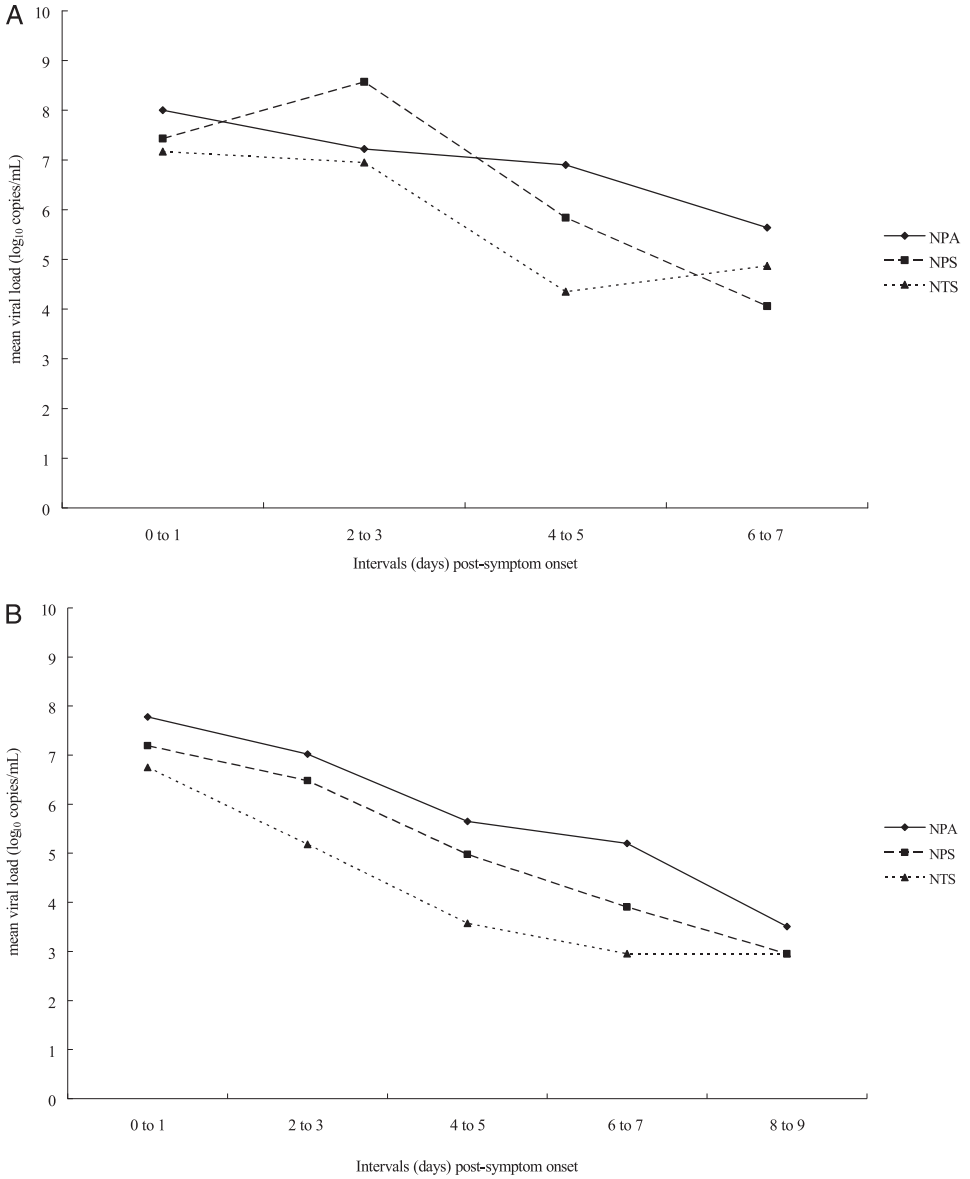

FIGURE 1. (*A*) The mean ± SD viral load (log_{10} copies/mL) profile in different respiratory specimens of pandemic A(H1N1)-infected patients not treated with oseltamivir at different intervals (days) post symptom onset. The detection limit of the quantitative RT-PCR was 2.95 log_{10} copies/mL. (*B*) The mean ± SD viral load (log_{10} copies/mL) profile in different respiratory specimens of pandemic A(H1N1)-infected patients treated with oseltamivir at different intervals (days) post symptom onset. The detection limit of the quantitative RT-PCR was 2.95 log_{10} copies/mL. NPA = nasopharyngeal aspirate; NPS = nasopharyngeal swab; NTS = naso-throat swab; pandemic A(H1N1) = pandemic 2009 influenza A(H1N1); RT-PCR = reverse transcriptase-polymerase chain reaction.

Similar findings were obtained with lym count (Pearson $r = -0.687$, $P = .001$) and WBC counts (Pearson $r = -0.489$, $P = .034$), but not for Hb level and plt count of nontreated patients. No significant correlation was observed between the viral load level with patient's age and the number of classic presenting symptoms. Resolution of fever (temperature ≤ 37.2°C) was 1.4 days earlier in treated than in nontreated patients (1.4 vs 2.8 days, $P = .012$). None required ventilatory or critical care and no death was reported.

DISCUSSION

Antiviral therapy for influenza is hampered by the early peaking of the viral load, which leaves a narrow window of opportunity for antiviral treatment.[14,24] In both seasonal and pandemic A(H1N1) influenza A infection, the viral loads peaked at around 1 to 2 days post symptom onset in natural infections of healthy adults with or without oseltamivir treatment.[14,24] As no data has yet been reported on the treatment effectiveness of oseltamivir on pandemic A(H1N1) infections, it is reassuring to observe that treated patients

Table 2—*Viral Load in Different Respiratory Specimens of Pandemic A(H1N1)-Infected Patients Not Treated With Oseltamivir at Different Intervals Post-Symptom Onset*

Respiratory Specimens	Interval Post-Symptom Onset, d			
	0-1	2-3	4-5	6-7
NPA	8.00 ± 1.30 (7)	7.22 ± 0.94 (10)	6.90 ± 1.80 (6)	5.64 ± 1.27 (8)
NPS	7.43 ± 0.80 (4)	8.57 ± 0.43 (2)	5.84 ± 1.81 (6)	4.06 ± 0.95 (5)
fNPS	(1)	(1)	(2)	(4)
sNPS	(3)	(1)	(4)	(1)
NTS	7.18 ± 4.77 (2)	5.74 ± 1.52 (3)	4.35 ± 1.59 (3)	4.87 ± 0.78 (3)
P value	.814	.183	.164	.079

Values given are mean ± SD viral load in $\log_{10}$ copies/mL (No. of specimens). The detection limit of the quantitative RT-PCR was 2.95 $\log_{10}$ copies/mL. fNPS = flocked nasopharyngeal swab; NPA = nasopharyngeal aspirate; NPS = nasopharyngeal swab; NTS = naso-throat swab; sNPS = standard nasopharyngeal swab; RT-PCR = reverse transcriptase-polymerase chain reaction.

had a greater rate of viral suppression in respiratory specimens throughout the disease course, with significantly lower viral load at day 5 post symptom onset. Moreover, when oseltamivir was initiated ≤ 2 days of symptom onset, viral load was not detectable at day 6 post oseltamivir, which was 1 day after treatment completion, and was 1 day earlier than that of those initiated > 2 days of symptom onset. The effect of oseltamivir as early treatment in suppressing pandemic A(H1N1) was similar to that of seasonal influenza, although its effectiveness for pandemic A(H1N1) chemoprophylaxis was not yet established.[14] Fever subsided 1.4 days earlier in treated patients, which is consistent with previous findings that oseltamivir shortens fever duration and reduces the quantity and duration of viral shedding in adults with seasonal influenza.[14,27,28]

Although oseltamivir was shown to hasten recovery and reduce viral load in this study, its long-term effectiveness for pandemic A(H1N1) remains uncertain. Primary resistance to the neuraminidase inhibitors among wild strains of human influenza viruses A(H1N1), A(H3N2), and B has been uncommon until the recent few years.[14] Oseltamivir resistance, which is largely associated with H274Y mutations in neuraminidase gene of influenza A(H1N1) and H5N1 viruses,[29-31] developed in 0.33% to 5.5% of patients following oseltamivir treatment.[14,29-32] In 2007, H274Y mutants of influenza A(H1N1) Brisbane-like variant, which were first detected in Norway,[33] have spread globally and become the dominant seasonal H1N1 virus. Initial bioinformatics analysis and phenotypic antiviral susceptibility tests showed that the H1N1 is susceptible to oseltamivir and zanamivir but resistant to amantadine or rimantadine because of a serine 31-to-asparagine mutation.[34] With the dramatic increase in oseltamivir use for treating pandemic A(H1N1) infection, resistance may continue to increase worldwide, including Hong Kong.[35,36] Even with the availability of a safe and effective pandemic A(H1N1) vaccine, antiviral stockpiling remains an important armamentarium for the epidemiologic control of future pandemic.

The early control of viral load in patients not treated with oseltamivir may be explained by the actions of innate immunity followed by early anamnestic adaptive immune response, possibly cytotoxic T lymphocyte response against cross-reacting epitopes of internal proteins or nonneutralizing surface proteins common to all influenza A viruses.[37] Failure to mount this early immune response because of innate humoral or cellular immunodeficiencies, concomitant use of immunosuppressive drugs, or transient immunoparesis due to severe concomitant coinfection may predispose some patients to develop severe clinical progression.[38] They are often those who presented late with hospitalization at a median of 7 days with respiratory failure and death at a median of 10 days post symptom onset.[8]

Viral load reflects the dynamic interaction between viral replication and clearance by body defense mechanisms. Monitoring viral load throughout the disease course has been used as an objective means of checking the clinical progress or response to antiviral therapy. We showed the inverse correlation between the absolute lymphocyte count and concomitant viral load level in

Table 3—*Viral Load in Different Respiratory Specimens of Pandemic A(H1N1)-Infected Patients Treated With Oseltamivir at Different Intervals Post-Symptom Onset*

Respiratory Specimens	Interval Post-Symptom Onset, d				
	0-1	2-3	4-5	6-7	8-9
NPA	7.78 ± 1.66 (45)	7.02 ± 1.88 (27)	5.65 ± 1.84 (21)	5.20 ± 1.84 (15)	3.51 ± 1.19 (9)
NPS	7.19 ± 1.95 (11)	6.48 ± 2.83 (19)	4.98 ± 1.73 (20)	3.90 ± 1.26 (19)	2.95 ± 0 (4)
fNPS	(0)	(8)	(12)	(14)	(0)
sNPS	(11)	(11)	(8)	(5)	(4)
NTS	6.75 ± 1.21 (9)	5.18 ± 1.78 (26)	3.57 ± 1.21 (32)	2.95 ± 0.70 (17)	2.95 ± 0 (12)
P Value	.317	.009	< .001	.001	.031

Values given are mean ± SD viral load in $\log_{10}$ copies/mL (No. of specimens). The detection limit of the quantitative RT-PCR was 2.95 $\log_{10}$ copies/mL. See Table 2 for expansion of abbreviations.

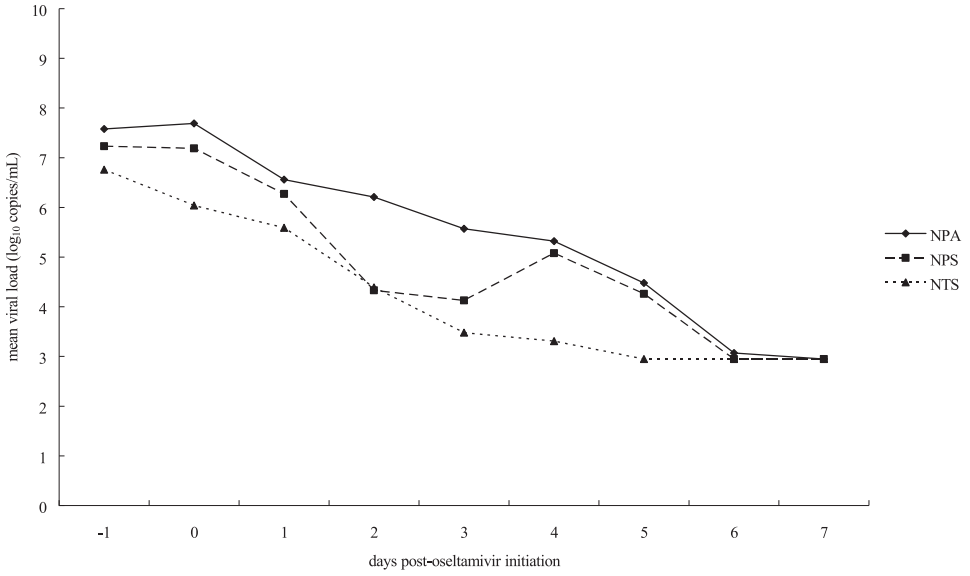

FIGURE 2. The mean ± SD viral load (log_{10} copies/mL) profile in different respiratory specimens of pandemic A(H1N1)-infected patients at different days post oseltamivir initiation. The detection limit of the quantitative RT-PCR was 2.95 log_{10} copies/mL. See Figure 1 legend for expansion of abbreviations.

nontreated and treated patients irrespective of the days post symptom onset at the time when the specimens were sampled, as in the case of SARS coronavirus and influenza A(H5N1) virus infections.[12,31,39-41] Lymphopenia was noted in pandemic A(H1N1)-infected patients of greater severity, especially those who were pregnant or morbidly obese.[8,42,43] Previous study in immunocompromised patients showed that lymphocyte recovery was associated with viral clearance, independent of oseltamivir.[44]

There was no difference in viral load with different sampling methods in nontreated patients, whereas the differences seen with NPA, NPS, and NTS in treated patients could be attributed to increased statistical power with the higher number of patient specimens in this group. As the dominant site of viral replication in these mild cases is the nasopharynx rather than the lower respiratory tract, the suction by nasopharyngeal aspirate is more likely to get a good sample of infected cells than swabbing the throat, anterior nares, or even the nasopharynx.[45] As the viral load is decreased by oseltamivir, the difference would be accentuated in the suboptimal sampling methods by NPS and NTS. But these factors are unlikely to affect the overall conclusion of this study because the results were similar when only nasopharyngeal aspirates were analyzed.

Similar to previous studies, oseltamivir is associated with few side effects. The most frequent side effects are gastrointestinal symptoms, which occurred in up to 40% of school children who received oseltamivir prophylaxis for the influenza A(H1N1) virus.[46] Previous reports suggesting serious neuropsychiatric manifestations after oseltamivir were not demonstrated in later studies and only 18% of school children taking oseltamivir prophylaxis had reported mild symptoms.[46-48]

Table 4—*Viral Load in Different Respiratory Specimens of Pandemic A(H1N1)-Infected Patients at Different Days Post-Oseltamivir Initiation*

Respiratory Specimens	No. of d Post-Oseltamivir Initiation								
	−1	0	1	2	3	4	5	6	7
NPA	7.58 ± 1.14 (18)	7.69 ± 1.62 (40)	6.56 ± 2.55 (13)	6.21 ± 2.21 (9)	5.57 ± 1.61 (10)	5.32 ± 1.54 (7)	4.48 ± 0.96 (6)	3.07 ± 0.72 (7)	2.95 ± 0.41 (5)
NPS	7.23 (1)	7.19 ± 2.40 (14)	6.27 ± 2.43 (13)	4.33 ± 1.50 (3)	4.13 ± 1.70 (17)	5.08 ± 1.65 (13)	4.26 ± 1.49 (7)	2.95 ± 0 (2)	2.95 ± 0.78 (6)
fNPS	(0)	(1)	(6)	(3)	(12)	(7)	(5)	(2)	(2)
sNPA	(1)	(13)	(7)	(0)	(5)	(6)	(2)	(0)	(4)
NTS	6.76 ± 0.37 (2)	6.04 ± 1.36 (6)	5.59 ± 2.12 (17)	4.39 ± 1.77 (19)	3.48 ± 0.92 (17)	3.31 ± 1.06 (10)	2.95 ± 0.37 (12)	2.95 ± 0 (9)	2.95 ± 0 (4)
P value	.606	.123	.604	.066	.003	.014	.002	.201	.535

Values given are mean ± SD viral load in log_{10} copies/mL (No. of specimens). The detection limit of the quantitative RT-PCR was 2.95 log_{10} copies/mL. See Table 2 for expansion of abbreviations.

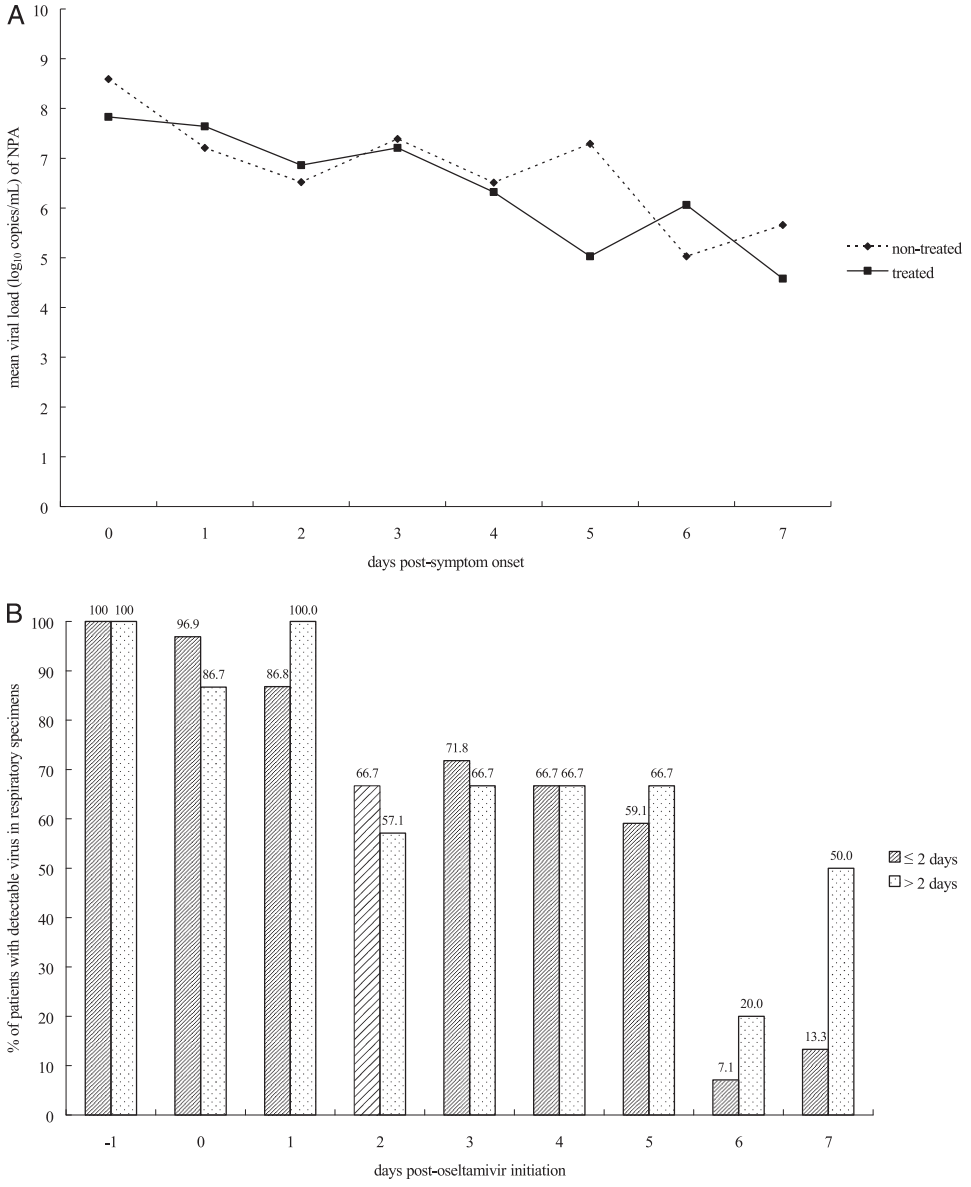

FIGURE 3. (*A*) The mean ± SD viral load (log_{10} copies/mL) profile in NPA at different days post symptom onset in pandemic A(H1N1)-infected patients not treated and treated with oseltamivir. The detection limit of the quantitative RT-PCR was 2.95 log_{10} copies/mL. (*B*) The percentage of pandemic A(H1N1)-infected patients with detectable virus in respiratory specimens at different days post oseltamivir initiation in those with oseltamivir initiated ≤2 and >2 days post symptom onset. See Figure 1 legend for expansion of abbreviations.

Table 5—*Viral Load in NPA at Different Days Post-Symptom Onset in Pandemic A(H1N1)-Infected Patients Not Treated and Treated With Oseltamivir*

	No. of d Post-Symptom Onset							
Patients	0	1	2	3	4	5	6	7
Nontreated	8.59 ± 0.71 (4)	7.21 ± 1.64 (3)	6.52 ± 0.20 (2)	7.39 ± 0.97 (8)	6.51 ± 2.29 (3)	7.29 ± 1.55 (3)	5.03 ± 1.76 (5)	5.66 ± 1.41 (4)
Treated	7.83 ± 1.27 (7)	7.64 ± 1.91 (38)	6.86 ± 2.03 (15)	7.21 ± 1.75 (12)	6.32 ± 2.01 (10)	5.03 ± 1.50 (11)	6.06 ± 1.89 (8)	4.58 ± 2.52 (11)
P value	.304	.709	.822	.799	.887	.040	.349	.440

Values given are mean ± SD viral load in log_{10} copies/mL (no. of specimens). The detection limit of the quantitative RT-PCR was 2.95 log_{10} copies/mL. See Table 2 for expansion of abbreviations.

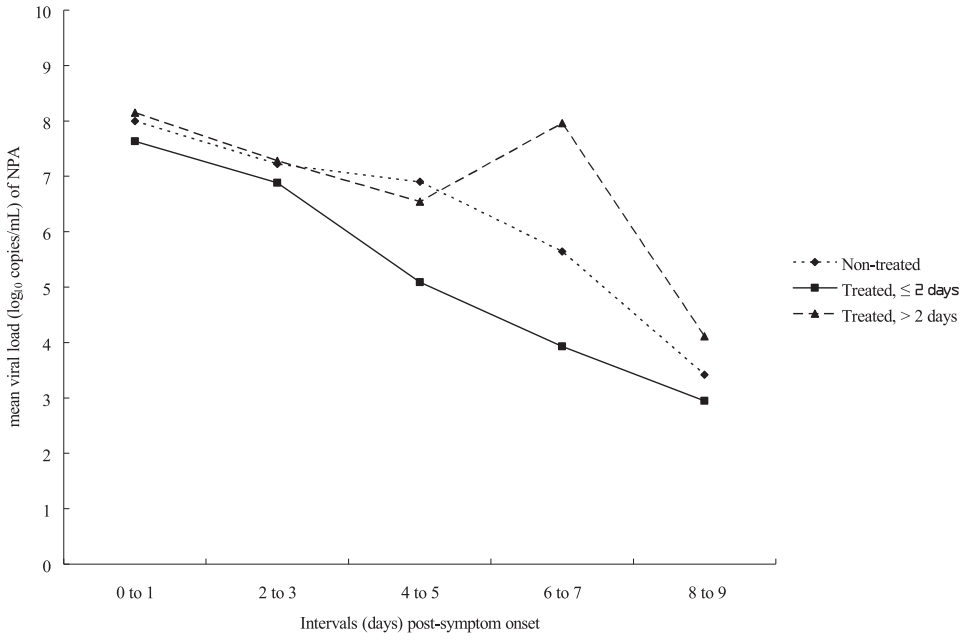

FIGURE 4. The mean ± SD viral load (log_{10} copies/mL) profile of NPA at different intervals (days) post symptom onset in pandemic A(H1N1)-infected patients not treated and treated with oseltamivir ≤ 2 and > 2 days of symptom onset. The detection limit of the quantitative RT-PCR was 2.95 log10 copies/mL. See Figure 1 legend for expansion of abbreviations.

A randomized control treatment trial is not possible at the beginning of the epidemic in our locality because of the uncertainties of its disease severity and the international recommendations on oseltamivir treatment.[9] Moreover, patients presented to us on different days post symptom onset and some have refused further nasopharyngeal sampling once their symptoms improved. Despite these limitations, the nontreated cases are still comparable to the treated patients in demographics, symptoms, and laboratory findings. A previous viral load study of pandemic A(H1N1) infection focused on oseltamivir-treated patients;[23] this is the first study, to our knowledge, of natural viral load profile of these patients without such treatment. Despite the apparent efficacy of oseltamivir in mild cases, its efficacy in stopping further disease progression of late cases remains uncertain.

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Dr Hung: contributed to collecting the clinical data and samples, and interpreting the data.
Dr To: contributed to performing the laboratory tests, interpreting the data, and performing statistical analysis.
Dr K.-H. Chan: contributed to performing the laboratory tests, interpreting the data, and performing statistical analysis.
Dr Wong: contributed to performing the laboratory tests, interpreting the data, and performing statistical analysis.

Table 6—*Viral Load of NPA at Different Intervals Post Symptom Onset in Pandemic A(H1N1)-Infected Patients Not Treated and Treated With Oseltamivir ≤ 2 and > 2 d of Symptom Onset*

Oseltamivir Treatment Status	Intervals Post Symptom Onset, d			
	0-1	2-3	4-5	6-7
Nontreated	8.00 ± 1.30 (7)	7.22 ± 0.94 (10)	6.90 ± 1.80 (6)	5.64 ± 1.27 (8)
Treated				
Time of initiation, days of symptom onset				
≤ 2	7.63 ± 1.86 (42)	6.88 ± 1.90 (18)	5.09 ± 1.41 (13)	3.93 ± 1.43 (13)
P value[a]	.621	.607	.029	.012
> 2	8.15 ± 1.11 (3)	7.28 ± 1.93 (9)	6.54 ± 2.17 (8)	7.96 ± 1.19 (2)
P value[b]	.864	.920	.749	.005
P value[c]	.638	.609	.078	< .001

Values given are mean ± SD viral load in log_{10} copies/mL (No. of specimens). The detection limit of the quantitative RT-PCR was 2.95 log_{10} copies/mL.
[a]Comparison between nontreated and treated patients with oseltamivir initiated ≤ 2 d of symptom onset.
[b]Comparison between nontreated and treated patients with oseltamivir initiated > 2 d of symptom onset.
[c]Comparison between treated patients with treatment initiated ≤ 2 and > 2 d of symptom onset.

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