Effects of Early Oseltamivir Therapy on Viral Shedding in 2009 Pandemic Influenza A (H1N1) Virus Infection

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Background. Pandemic influenza (H1N1) 2009 is susceptible to oseltamivir. There are few reports on its clinical and virologic response to oseltamivir.

Methods. During the pandemic containment response in Singapore, all patients with positive polymerase chain reaction (PCR) results for pandemic influenza (H1N1) 2009 were hospitalized, given oseltamivir for 5 days, and discharged when daily PCR results for combined nasal and throat swab samples became negative. Six patients had concurrent positive viral culture and PCR results.

Results. The median age of the first 70 consecutive patients was 26 years (interquartile range, 21–38 years); 60% were men, and 29% had comorbidity. The mean time (±SD) from illness onset to hospital admission was 3 ± 2 days. Influenza-like illness was noted in 63% of patients. Fever occurred in 91%, cough in 88%, sore throat in 66%, and rhinorrhea in 53% of patients. The mean duration (±SD) of viral shedding from illness onset was 6 ± 2 days. Viral shedding persisted beyond 7 days in 37% of patients. Clinical features and viral shedding were similar between those with and without comorbidity, except the former had more cough and lower oxygen saturation. Patients receiving oseltamivir on days 1 to 3 of illness had significantly shorter viral shedding duration, compared with those treated from day 4 onwards (P < .05). The mean durations (±SD) of positive PCR and viral culture results were 5 ± 0.8 and 4 ± 1.8 days, respectively, for 6 patients with concurrent positive viral culture and PCR results.

Conclusions. Prolonged viral shedding was noted in young immunocompetent adults with mild pandemic influenza (H1N1) 2009 despite receipt of oseltamivir. When prescribed during the first 3 days of illness, oseltamivir shortened the duration of viral shedding.

A novel influenza A H1N1 strain, pandemic (H1N1) 2009, emerged in late March 2009 [1] and spread rapidly to every continent with >399,232 cases and 4735 deaths reported to the World Health Organization as of 12 October 2009 [2]. It has become the predominant influenza strain in both the Northern and Southern hemispheres [3, 4]. Severe pandemic (H1N1) 2009 manifesting predominantly as cases of pneumonia requiring admission to intensive care occurred more frequently in young [5, 6], obese [7, 8], and pregnant persons [9], with neurodevelopmental disorders predominating among the affected young patients [5, 6]. Despite numerous epidemiological and clinical reports on pandemic (H1N1) 2009, few evaluated the efficacy of oseltamivir for treatment of pandemic (H1N1) 2009. The only case report documented cessation of viral shedding within 2 days after receipt of oseltamivir [10].

This study aims to describe the clinical and virological response to oseltamivir therapy in patients infected with confirmed pandemic (H1N1) 2009 and to compare the clinical illness and outcome of treated patients with pandemic (H1N1) 2009 with and without medical comorbidity.

METHODS

Study design. This is a prospective observational study of patients with laboratory-confirmed pandemic (H1N1) 2009 infection admitted to Communicable
Disease Center 2 at Tan Tock Seng Hospital (TTSH), Singapore, from 27 April through 24 June 2009. Communicable Disease Center is the national center for outbreak management including pandemic influenza; it is affiliated with TTSH, a 1200-bed teaching hospital. The study was approved by Domain Specific Review Board, National Healthcare Group, Singapore.

From 27 April through 24 June 2009, Singapore was in the containment phase of its pandemic response, the objective being to isolate infected case patients to delay community spread. Widespread public education led to heightened awareness and prompt referral of travelers and their contacts with acute febrile respiratory illness to TTSH for pandemic influenza screening. Patients came from primary care clinics, border entry, or via self-referral. All patients confirmed to have pandemic (H1N1) 2009 were admitted. Contact tracing and home quarantine for asymptomatic contacts was undertaken. All hospitalized patients underwent baseline full blood count, renal and liver function tests, C-reactive protein measurement, chest radiography, and daily combined nasal and throat swab specimen collection for polymerase chain reaction (PCR) for pandemic (H1N1) 2009. Oseltamivir (75 mg) was administered twice daily for 5 days on hospital admission; patients were discharged only when daily combined nasal and throat swab specimens were negative on PCR for pandemic (H1N1) 2009.

Clinical data collected prospectively included demographic data; medical comorbidities; travel history; contact with pandemic (H1N1) 2009 patients or patients with acute respiratory illness; date of illness onset, symptoms, and signs; timing of oseltamivir therapy; resolution of clinical illness; and duration of viral shedding. Medical comorbidities documented included diabetes mellitus, heart disease, chronic lung disease, renal failure, liver disease, human immunodeficiency virus infection, cancer, and receipt of immunosuppressive therapy including corticosteroids.

**Virologic investigations.** Combined nasal and throat samples obtained with flocked swabs were transported in universal transport medium (Copan) to the Department of Laboratory Medicine at TTSH for real-time PCR (in-house Taqman probe-based method) [11]. Two milliliters of universal transport medium with detectable pandemic (H1N1) 2009 was sent to National Public Health Laboratory, Singapore, for virus isolation. This was performed using Madin-Darby canine kidney cells (shell vial method) [12]. Isolated virus was identified by influenza A & B DFA kit and LIGHT DIAGNOSTICS (Millipore).

<table>
<thead>
<tr>
<th>Feature</th>
<th>Without comorbidity (n = 50)</th>
<th>With comorbidity (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years ± SD</td>
<td>28 ± 10</td>
<td>30 ± 12</td>
</tr>
<tr>
<td>Male sex</td>
<td>27 (54)</td>
<td>15 (75)</td>
</tr>
<tr>
<td>Duration of illness from onset to admission, mean days ± SD</td>
<td>3 ± 2</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Duration of viral shedding from illness onset, mean days ± SD</td>
<td>6 ± 2</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Duration of viral shedding from admission, mean days ± SD</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Length of stay, mean days ± SD</td>
<td>6 ± 2</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>Duration of fever, mean days ± SD</td>
<td>1.3 ± 0.6</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td>Duration of respiratory symptoms, mean days ± SD</td>
<td>3.9 ± 1.8</td>
<td>4.1 ± 2.0</td>
</tr>
<tr>
<td>Fever</td>
<td>45 (90)</td>
<td>19 (95)</td>
</tr>
<tr>
<td>Cough</td>
<td>40 (80)</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Rhinorrhea</td>
<td>27 (54)</td>
<td>10 (50)</td>
</tr>
<tr>
<td>Sore throat</td>
<td>31 (62)</td>
<td>15 (75)</td>
</tr>
<tr>
<td>Headache</td>
<td>11 (22)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>12 (24)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>Temperature, mean °C ± SD</td>
<td>38.0 ± 0.7</td>
<td>38.0 ± 0.6</td>
</tr>
<tr>
<td>Systolic blood pressure, mean mmHg ± SD</td>
<td>111 ± 13</td>
<td>112 ± 11</td>
</tr>
<tr>
<td>Respiration rate, mean breaths/min ± SD</td>
<td>20 ± 2</td>
<td>20 ± 1.0</td>
</tr>
<tr>
<td>Pulse rate, mean beats/min ± SD</td>
<td>104 ± 15</td>
<td>105 ± 17</td>
</tr>
<tr>
<td>Oxygen saturation on room air, mean % ± SD</td>
<td>97.4 ± 1.1</td>
<td>96.7 ± 1.1</td>
</tr>
<tr>
<td>Leukocyte count, mean cells/μL ± SD × 10^3</td>
<td>6.7 ± 2.3</td>
<td>7.1 ± 2.0</td>
</tr>
<tr>
<td>C-reactive protein, mean mg/dL ± SD</td>
<td>15 ± 13</td>
<td>18 ± 14</td>
</tr>
<tr>
<td>Pneumonia on chest radiography</td>
<td>1 (2)</td>
<td>3 (15)</td>
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**NOTE.** Data are no (%) of patients, unless otherwise indicated. SD, standard deviation.

a. *P < .05.*

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[13], and further subtyping of influenza A was performed by real-time PCR with use of an in-house method.

Detection of antiviral resistance in pandemic (H1N1) 2009 required sequencing of the neuraminidase segments. RNA extraction from viral cultures, via a high fidelity Taq DNA polymerase, formed the template for quantitative real-time PCR [14]. Three to four overlapping PCR fragments covering the coding region of the neuraminidase segment were sequenced. Following the neuraminidase sequence assembly, mutation mapping was performed. These investigations were conducted in a biosafety level 2 laboratory with use of biosafety level 3 precautions.

**Statistical analysis.** For most variables, descriptive statistics, such as mean ± standard deviation (SD; for data with normal distribution), median with interquartile range (IQR; for data with skewed distribution), and proportion (%), were calculated. The student’s t, Mann-Whitney U, and χ² tests were used for comparisons when appropriate, Spearman’s Rank correlation was used to assess the relationship between duration of clinical symptoms and viral shedding, and Kruskal-Wallis test was used for duration of viral shedding among groups. Statistical analyses were performed using SPSS software, version 16.0 (SPSS). In all analyses, a P value <.05 was considered to indicate statistical significance. All probabilities were 2-tailed.
Table 2. Viral Shedding at Days 5 and 7 from Start of Oseltamivir Treatment and Day of Illness at Initiation of Treatment

<table>
<thead>
<tr>
<th>Day of illness at initiation of oseltamivir treatment, no (%) of patients</th>
<th>Treatment day</th>
<th>≤2</th>
<th>&gt;2</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td></td>
<td>(n = 36)</td>
<td>(n = 34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>28 (78)</td>
<td>30 (88)</td>
<td>.34</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>8 (22)</td>
<td>18 (53)</td>
<td>.01</td>
<td></td>
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</tbody>
</table>

RESULTS

Patient description. During the study period, 70 consecutive patients were admitted to Communicable Disease Center 2 for isolation and treatment among 3490 patients who were screened. Forty-two (60%) were men, and 35 (50%) were Singaporeans. The remainder were foreigners residing in Singapore, in transit through Singapore, or visiting Singapore as tourists. Median age was 26 years (IQR, 21–38 years). Medical comorbidity was documented in 20 patients (29%), with 15 of 20 patients having chronic pulmonary disease or asthma.

Travelers comprised 91% of patients and presented at a median of 8 days (IQR, 7–12 days) from the start of travel and a median of 1 day (IQR, 0–2 days) from the end of travel. Exposure to people with acute respiratory illness was noted in 20 (29%), whereas 11 (16%) were close contacts of patients with pandemic (H1N1) 2009 infection. Patients presented at a mean of 3 ± 2 days after illness onset.

Clinical illness and effect of oseltamivir. Symptoms reported included fever in 64 (91%), cough in 62 (88%), sore throat in 46 (66%), rhinorrhea in 37 (53%), headache in 18 (26%), and myalgia in 19 patients (27%). Influenza-like illness, according to the Centers for Disease Control and Prevention (temperature ≥37.8°C and cough or sore throat) and World Health Organization (temperature ≥38°C, and cough or sore throat), was noted in 44 (63%) and 34 (49%) patients, respectively. Of 6 patients with no reported fever, 2 had temperature >37.5°C at the time of presentation.

After hospital admission, the mean duration (±SD) of fever (>37.5°C) was 1.3 ± 0.6 days, duration of constitutional symptoms was 1.3 ± 0.7 days, and duration of respiratory symptoms (either cough, rhinorrhea, or sore throat) was 3.9 ± 1.8 days. The mean duration (±SD) of hospital stay was 6 ± 2 days. All patients had an uneventful recovery; 4 (6%) developed pneumonia, but white cell count and C-reactive protein level were only mildly elevated. Leukocytosis >11 × 10³ cells/µL and thrombocytopenia <140 × 10³ platelets/µL occurred in 3 (4%) patients each; elevated alanine and aspartate transaminase levels >60 units/L occurred in 5 (7%) and 2 (3%) patients, respectively. The mean C-reactive protein level (±SD) was 16 ± 13 mg/dL.

Patients with comorbidities (n = 20) and those without (n = 50) were not significantly different in regard to age, sex, time to presentation, duration of fever, respiratory symptoms, viral shedding and hospital stay, symptoms and signs, inflammatory markers, and proportion with pneumonia (Table 1). Significantly more patients with comorbidities reported cough (100% vs 80%, P < .05) and had lower mean oxygen saturation (±SD) (96.7% ± 1.1% vs 97.4% ± 1.1%; P < .05), compared with those without comorbidities.

All patients received 75 mg of oseltamivir twice daily for 5 days on hospital admission. Of 64 patients who reported fever at presentation, fever persisted in 10 (29%) of 35 treated within the first 2 days of illness versus 8 (28%) of 29 treated after the first 2 days of illness (P = .99) when assessed on the second day of treatment; all patients defervesced by day 4. Of 68 patients who reported respiratory symptoms at presentation, the symptoms persisted in 15 (43%) of 35 treated within the first 2 days versus 11 (33%) of 33 treated after the first 2 days of illness (P = .46) when assessed on the fifth day of treatment (end of therapy).

Duration of viral shedding and effect of oseltamivir. The 70 patients had 351 combined nasal and throat swabs specimens obtained for influenza PCR. The mean duration (±SD) of viral shedding detected in combined nasal and throat swabs was 6 ± 2 days after illness onset and 4 ± 2 days after hospital admission. Of note, 26 patients (37%) had detectable viral shedding on day 7 of illness and 6 (9%) on day 10 of illness, despite oseltamivir therapy. Duration of respiratory symptoms (Spearman’s correlation ρ, 0.37; P = .002) (Figure 1A) was moderately correlated with the duration of viral shedding, in contrast to duration of fever, which did not correlate with viral shedding (Figure 1B).

Six patients had concomitant viral culture and PCR performed on combined nasal and throat swab specimens. Mean duration (±SD) of positive results for PCR was 5 ± 0.8 days and for viral culture was 4 ± 1.8 days. For all 6 patients, the H274Y mutation conferring resistance to oseltamivir was not detected.

The effect of oseltamivir started within (early treatment,
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n = 36) and after (delayed treatment, n = 34) the first 2 days of illness on the proportion of patients who had detectable viral shedding at day 5 (end of therapy) and day 7 from the start of oseltamivir was determined (Table 2). At day 5 from start of oseltamivir, similar proportion of patients had detectable viral shedding (78% vs 88%; P = .34) in the early versus delayed treatment groups. However, at day 7 from start of oseltamivir, significantly fewer patients had detectable viral shedding in the early versus delayed treatment groups (22% vs 53%; P = .01).

Compared with oseltamivir therapy on day 1 of illness, oseltamivir therapy on days 2 and 3 of illness was associated with similar duration of viral shedding. However, oseltamivir therapy on day 4 of illness and after was associated with more prolonged viral shedding (P < .01) (Table 3). Figure 2 shows statistically significant difference in duration of viral shedding, compared with day of illness on which a patient was treated with oseltamivir.

DISCUSSION

In contrast to randomized, controlled trials showing earlier alleviation of symptoms for oseltamivir and zanamivir given in the first 2 days of illness in acute upper respiratory infection from influenza [15], we did not observe earlier resolution of fever or respiratory symptoms in our patients who received oseltamivir within the first 2 days of illness. The mean duration of viral shedding in our patients was 6 days, and 37% and 9% had detectable viral shedding on days 7 and 10 of illness, respectively, despite early treatment with oseltamivir. Our findings call into question the clinical benefit of oseltamivir therapy for acute uncomplicated pandemic (H1N1) 2009 infection. However, our study was small and nonrandomized.

In contrast, available data on seasonal influenza showed benefits of oseltamivir therapy. Treanor et al [16] reported that viral shedding of seasonal influenza ceased within 3 days of oseltamivir treatment. Boivin et al [17] documented that following 2 days of oseltamivir treatment, PCR results were positive for 20.7% of patients with treatment commenced within 1 day, compared with 42.3% of patients with treatment commenced between 1 and 2 days after illness onset. In comparison, the mean duration of viral shedding was 4.8 days, although 20%–30% of patients with untreated seasonal influenza had persistent viral shedding until days 8–10 of illness [18].

Prolonged viral shedding of seasonal influenza can occur in children [19], the immunocompromised [20], and in those infected with human H5N1 [21]. Two recent studies reported prolonged viral shedding in seasonal influenza occurring in elderly hospitalized patients with comorbidities, a population which contrasts with our young patients with median age of 26 years and comorbidities in 29%. Leekha et al [22] studied 50 hospitalized patients with influenza A with a median age of 76 years, comorbidities in 96%, and receipt of antiviral treatment in 56%. Influenza A was detected by PCR in 54% and by culture in 29% of patients at ≥7 days of illness. Lee et al [14] reported on 147 hospitalized patients with a mean age of 72 years, comorbidities in 64%, and receipt of antiviral treatment in 75%. Influenza was detected by PCR in 33% and by culture in 2% at ≥7 days of illness. Notably, the apparent higher yield by PCR, compared with culture, in these 2 studies may result from detection of nonviable virus or the method’s superior sensitivity.

Interestingly, our small cohort comprising mainly young immunocompetent adults showed comparable mean viral shedding as detected by PCR (5 days) and culture (4 days). A recent Canadian study also observed that pandemic (H1N1) 2009 was detectable by PCR in 43% and culture in 30% of 44 patients on day 8 of illness [23]. An outbreak investigation of pandemic (H1N1) 2009 at a United States Air Force Academy reported that 24% of nasal wash specimens obtained at 7 days from illness onset were positive by viral culture [24]. In contrast, preliminary results from Vietnam demonstrated that most patients with pandemic (H1N1) 2009 ceased viral shedding within 5 days of receipt of oseltamivir. With 292 hospitalized patients (mean age, 26 years) receiving oseltamivir, virus detection by PCR occurred in 86%, 38%, and 14% on day 1, 3, and 5 of treatment, respectively [25]. The reasons for the differences in virological outcome between these studies are unclear.

Notably, our study revealed that oseltamivir therapy in the first 3 days of illness shortened duration of viral shedding for patients with pandemic (H1N1) 2009, compared with treatment after day 3. Furthermore, commencement of oseltamivir in the first 2 days of illness reduced persistent viral shedding at day 7 from the start of therapy. Three randomized, controlled trials (2 with zanamivir and 1 with oseltamivir) on seasonal influenza showed reduction in viral load with antiviral therapy.
[26–28], but 2 of the studies noted that duration of viral shedding was not statistically different between the patients who were treated and those who received placebo [26, 27]. More recently, Lee et al [14] found that antiviral treatment in the first 4 days of illness shortened viral shedding in elderly patients with comorbidities, but Leekha et al [22] did not find that antiviral therapy influenced prolonged viral shedding in a similar population. Nevertheless, neuraminidase inhibitors improved survival in severe pandemic (H1N1) 2009 [5, 7].

Our study detailed daily viral shedding in pandemic (H1N1) 2009 correlating with the timing of oseltamivir therapy. However, it is small, includes mainly uncomplicated pandemic (H1N1) 2009 infections, and lacks untreated controls. Qualitative rather than quantitative PCR was utilized. Importantly concurrent viral cultures were performed on a small subset of patients, limiting the extrapolation of our findings to the duration of transmissibility of pandemic (H1N1) 2009.

Prolonged shedding of potentially viable influenza virus in our cohort highlights the possible need for infection control measures (eg, use of surgical masks [29] and hand hygiene with soap and water [30]) to reduce spread. Respiratory symptoms appeared to correlate with detectable viral shedding by PCR, which may guide the decision to cease infection control measures. Patients who are young [19] or immunocompromised [20] may have a longer duration of viral shedding.

In conclusion, we demonstrated prolonged viral shedding in immunocompetent adults with mild pandemic (H1N1) 2009 infection treated with oseltamivir, and early treatment in the first 3 days of illness shortened duration of viral shedding. Further research on viral shedding in untreated patients with mild illness, treated patients with severe illness and surveillance for the emergence of oseltamivir resistance should be considered.

Acknowledgments

Potential conflicts of interest. Y.S.L. has received remuneration from Sanofi as advisor to a dengue vaccine trial. All other authors: no conflicts.

References


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