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Clinical Observation

Duration of viral shedding of Influenza A (H1N1) virus infection treated with oseltamivir and/or Traditional Chinese Medicine in China: A retrospective analysis

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Abstract

OBJECTIVE: H1N1 was a new and potentially serious infectious disease, in human, the severity of influenza can vary from mild to severe, thus to find an effective and safety way to control the influenza pandemic is of crucial importance. This retrospective study describes the duration of viral shedding in H1N1 patients that were hospitalized and treated in China.

METHODS: Clinical data were collected from May to July, 2009 in China for 963 patients with influenza A (H1N1) virus infection. Patients were treated based on the guidelines issued by the Chinese Ministry of Health. The primary outcome was duration of viral shedding and statistical comparisons were performed.

RESULTS: In the patients with body temperature greater than 38.0°C, there were no differences in virus shedding duration among the patients taking oseltamivir within two days, patients undergoing Traditional Chinese Medicine (TCM) therapy or those receiving no drug therapy. In patients with body temperature ≥38.1° C, TCM therapy reduced the viral shedding duration (*P<0.05*, vs. oseltamivir therapy). Furthermore, taking oseltamivir two days after onset of symptoms might prolong the virus shedding duration (*P<0.05*, vs. taking oseltamivir less than 2 days of onset).

CONCLUSION: TCM therapy is effective for reducing the length of virus shedding in patients with body temperature ≥38.0°C. Oseltamivir used for reducing virus shedding duration should be taken within two days of onset.

Key words: H1N1; Treatment of oseltamivir; Treatment of TCM

INTRODUCTION

The Chinese government has taken important steps for the prevention and treatment of 2009 pandemic influ-

enza A (H1N1, abbreviated as H1N1 pdm in this paper) virus infection. Two teams of experts from biomedicine and Traditional Chinese Medicine (TCM) were organized to generate guidelines for the prevention and treatment of H1N1 virus infection[1]. While the clinical characteristics of the 2009 H1N1 pdm virus infection in China have been previously reported[2], the effect of recommended treatment guidelines has yet to be ascertained by a retrospective study on collected cases for guiding future treatment strategies.

Since H1N1 was a new and potentially serious infectious disease, a network of hospitals that specialize in infectious diseases was formed to quarantine and treat patients infected with H1N1 virus. Beijing Ditan Hospital, one of the hospitals in this network, collected clinical data from H1N1 infected patients, as well as from patients with H1N1 at other hospitals in the network. There is little knowledge about how to treat such a new and potential serious infectious disease; thus, a retrospective analysis of clinical data would be helpful for future treatment strategies and exploring new therapies for the disease. The current retrospective study investigated the duration of viral shedding in 963 cases of H1N1 virus infection that were treated with different therapies from May to July, 2009 to determine the period of maximum transmission of the virus.This study was approved by the appropriate ethics committees and was performed in accordance with the ethical standards laid down in the Declaration of Helsinki. All persons signed their informed consent prior to their inclusion in the study.

METHODS

Patients

This retrospective study was conducted using data collected for 963 hospitalized patients who were recruited in eight hospitals, including Beijing Ditan Hospital affiliated with Capital Medical University, Beijing Youan Hospital affiliated with Capital Medical University, The Eighth Guangzhou Hospital, Chengdu Contagious Disease Hospital, The Second Hospital affiliated with Guangzhou University of Chinese Medicine, People's Hospital in Linyi of Shandong Province, People's Hospital in Liao City of Shandong Province, and Xinjiang Uygur Autonomous Region Contagious Disease Hospital. H1N1 infection was confirmed by real-time reverse transcription polymerase chain reaction (RT-PCR) assay performed at the Laboratory of Institute of Infectious Disease in Ditan Hospital operated under the auspices of the Chinese Center for Disease Control and Prevention. Patient characteristics are listed in Table 1. Symptoms and signs for each patient were recorded daily. Details of all treatments were recorded. Daily pharyngeal or nasopharyngeal swabs were analyzed for the presence H1N1 virus using real-time RT-PCR testing. All clinical data were sent to and stored at Beijing Ditan Hospital from May to July, 2009 as a secured file.

Table 1 Patient characteristics

Characteristic	Oseltamivir in 2 days	Oseltamivir after 2 days	Oseltamivir in 2 days plus TCM	Oseltamivir after 2 days plus TCM	TCM	No therapy
Number of cases	172	96	256	134	208	97
Male sex-no.(%)	97(56.4)	50(52.08)	150(58.59)	67(50)	113(54.33)	44(45.36)
Age-yr: Mean±sd	23.08±14.56	21.43±11.87	22.96±13.36	22.54±13.75	20.25±11.79	18.46±10.11
Age group-no.(%)						
<5 yr	4(2.33)	4(4.17)	6(2.34)	3(2.24)	1(0.48)	2(2.06)
5-14 yr	52(30.23)	19(19.79)	63(24.61)	41(30.60)	76(36.54)	36(37/11)
15-30 yr	77(44.77)	63(65.63)	137(53.52)	56(41.79)	100(48.08)	47(48.45)
31-50 yr	29(16.86)	6(6.25)	35(13.67)	31(23.13)	25(12.02)	11(11.34)
51-65 yr	8(4.65)	4(4.17)	14(5.47)	2(1.49)	5(2.40)	1(1.03)
>65 yr	2(0.16)	0	1(0.39)	1(0.75)	1(0.48)	0
Body temperature-no (%)*						
<37.3 °C	65(38.69)	56(58.95)	65(26.32)	57(44.19)	121(58.17)	69(71.13)
37.3-38.0 °C	45(26.79)	21(22.11)	87(35.22)	41(31.78)	50(24.04)	20(20.62)
38.1-39.0 °C	40(23.81)	15(15.79)	79(31.98)	25(19.38)	27(12.98)	6(6.19)

>39.0°C	18(10.71)	3(3.16)	16(6.48)	6(4.65)	10(4.81)	2(2.06)
Duration of fever	1.94±1.04	1.56±0.68	1.76±0.85	1.58±0.64	1.82±1.09	1.39±0.5

Notes: Oseltamivir used within two days (oseltamivir within two days), oseltamivir used two days after onset (oseltamivir after two days), TCM therapy (TCM), oseltamivir used in two days with TCM therapy (oseltamivir within two days plus TCM), oseltamivir used after two days with TCM therapy (oseltamivir after two days plus TCM), no drug therapy (No therapy). *P<0.001 among the cases treated with different therapies.

Real-time RT-PCR

The 2009 H1N1 pdm virus was detected using a real-time RT-PCR assay in accordance with the protocol from the United States Centers for Disease Control and Prevention (CDC), as recommended by the World Health Organization (WHO) [3]. The PCR products were sequenced using the BigDye Terminator, version 3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA) in accordance with the manufacturer's instructions.

Treatments

Two guidelines were used by physicians to direct treatment: 1) the National Diagnostic and therapeutic Guidelines for H1N1 Influenza A (published on May 9, 2009) [4] were adapted from guidelines provided by the CDC; and 2) guidelines on prevention and treatment with TCM issued by the State Administration of Traditional Chinese Medicine[5]. Physicians used these guidelines and clinical experience to make treatment decisions. Generally, for patients with elevated fever (body temperature between 38.1-38.9° C), only oseltamivir or TCM therapy was used. For the patients with higher fever (body temperature ≥39.0° C), combined therapy (oseltamivir and TCM) was used. Some patients with no or mild fever were not treated with drug therapy. For the recommended dosage, 75 mg oseltamivir was administered orally, twice a day. For TCM treatment, 20 mL Shuang Huang Lian liquor (Sanjing Pharmaceutical, Harbin, China) that contains Flos lonicerae, Radix scutellariae, Fructus forsythiae, was administered orally three times a day, four Lian Hua Qing Wen capsules (Hebei Yiling Pharmaceutical, Hebei, China; Fructus forsythiae, Flos lonicerae, honey-fried herba ephedrae, fried Semen armeniacae amarum, Gypsum fibrosum, Radix isatidis, cyrtomii rhizoma, Herba Houttuyniae, Herba Pogostemonis, Radix et Rhizoma Rhei, integripetal rhodiola herb, Mentholum, Radix Glycytthizae) were administered orally four times a day, and four Shu Feng Jie Du capsules (Anhui Jiren Pharmaceutical, Anhui Province, China; Rhizoma Polygoni Cuspidati, Fructus forsythiae, Dahurian patrinia herb, Herba Verbanae, Radix Glycytthizae, Radix Isatidis, Radix bupleuri, Rhizoma Phragmitis) was administered orally, three times daily. All TCM products were manufactured by pharmaceutical companies that were GMP(Good Manufacturing Practice) certified, officially marketed in China for at least three years and approved by the Chinese State Food and Drug Administration. A total of 598 cases were administered TCM products. Among these cases, 416 were administered two treatments at same time, and 182 were only administered one. Additionally, 390 cases were administered a TCM product plus oseltamivir at same time. According to TCM with similar pharmacological activity (even with different composition of herbs), patients that were administered a TCM product were treated as cases treated with TCM therapy. No further stratification based on different TCM products was conducted in this study since physicians also believed that the TCM products have similar activity.

Evaluation of efficacy

Since controlling viral spread was an important goal of H1N1 treatment, the duration of viral shedding was used to evaluate treatment efficacy. The duration of viral shedding was defined as the time period from the day of symptom onset to day with negative results by RT-PCR from two consecutive pharyngeal or nasopharyngeal swabs.

Data analysis

Continuous variables were summarized as means±standard deviation (SD). For categorical variables, the percentages of patients in each category were calculated. Clinical characteristics were compared among groups of patients with different therapies with the use of an analysis of variation (ANOVA) test, chi-square test, or Fisher's exact test, as appropriate. The differences in the duration of viral shedding among patients treated with different therapies were analyzed using ANOVA test and least significant difference (LSD) methods. A previous study showed that treatment with oseltamivir for less than two days might reduce the duration of viral shedding[2]. In this study, the intervals with less than or greater than two days between symptom onset and initiation of oseltamivir therapy were used as a stratification factor. Since body temperature was used to determine treatment (TCM therapy and/or oseltamivir), body temperature was used as a stratification factor for the analyses.

Symptoms and signs might be also used as the parameters to choose appropriate therapeutic approaches. Multiple logistic-regression analysis was used to identify independent predictors of viral shedding duration from all symptoms and signs among all patients. The independent predictors were also used as stratification factors in the analysis. All analyses were performed using SAS (Version 9.1.3; SAS Institute, Cary, NC). A P-value of <0.05 was considered statistically significant.

RESULTS

Patient characteristics

Table 1 shows that body temperature was the only difference in the baseline characteristics of patients treated with different therapies. Thus, body temperature was used as a stratification factor in subsequent analyses. Table 2 shows that there were some differences in the frequency of symptoms and signs in the cases treated with different therapies. Cases treated with oseltamivir + TCM within two days of onset and those treated with oseltamivir + TCM after two days of onset showed a higher frequency of sore throat. Cases treated with oseltamivir within two days and those treated with no drug therapy showed lower frequency of myalgia (arthralgia). Cases treated with no drug therapy and oseltamivir in two days showed a lower frequency of nasal congestion. Cases treated with no drug therapy showed lower frequency of tensile swelling. These symptoms and signs were also used as stratification factors in subsequent analyses.

Table 2 Frequency of symptoms and signs in cases treated with different therapies

Symptom and Sign	Oseltamivir in 2 days	Oseltamivir after 2 days	Oseltamivir in 2 days plus TCM	Oseltamivir after 2 days plus TCM	TCM	No therapy	*P* value
Cough	104/172(60.47)	73/96(76.04)	193/256(75.39)	100/134(74.63)	152/208(73.08)	63/97(64.95)	0.0071
Sore throat*	49/172(28.49)	42/96(43.75)	117/256(45.7)	60/134(44.78)	80/208(38.46)	28/97(28.87)	0.0014
Sputum production	41/172(23.84)	25/96(26.04)	67/256(26.17)	43/134(32.09)	54/208(25.96)	20/97(20.62)	0.4841
Rhinorrhea	28/172(16.28)	30/96(31.25)	43/256(16.8)	30/134(22.39)	55/208(26.44)	21/97(21.65)	0.0121
Headache	31/172(18.02)	20/96(20.83)	44/256(17.19)	15/134(11.19)	31/208(14.9)	11/97(11.34)	0.2558
Nasal congestion#	17/172(9.88)	20/96(20.83)	37/256(14.45)	19/134(14.18)	31/208(14.9)	6/97(6.19)	0.0441
Myalgia,arthralgia@	10/172(5.81)	7/96(7.29)	38/256(14.84)	11/134(8.21)	16/208(7.69)	4/97(4.12)	0.0053
Chill	10/172(5.81)	8/96(8.33)	24/256(9.38)	7/134(5.22)	16/208(7.69)	4/97(4.12)	0.4480
Conjunctival congestion	0/172(0)	2/96(2.08)	4/256(1.56)	4/134(2.99)	0/208(0)	2/97(2.06)	0.0953
Diarrhea	2/172(1.16)	1/96(1.04)	6/256(2.34)	5/134(3.73)	4/208(1.92)	1/97(1.03)	0.5806
Nausea,vomiting	5/172(2.91)	0/96(0)	6/256(2.34)	5/134(3.73)	10/208(4.81)	0/97(0)	0.0885
Chest pain	0/172(0)	2/96(2.08)	0/256(0)	2/134(1.49)	1/208(0.48)	0/97(0)	0.0826
Swelling of tonsils$	61/172(35.47)	25/96(26.04)	102/256(39.84)	51/134(38.06)	73/208(35.1)	22/97(22.68)	0.0226
Enlargement of lymph nodes	0/172(0)	1/96(1.04)	0/256(0)	1/134(0.75)	0/208(0)	1/97(1.03)	0.2997
Stomachache	0/172(0)	0/96(0)	4/256(1.56)	1/134(0.75)	3/208(1.44)	0/97(0)	0.3389
Dyspnea	2/172(1.16)	0/96(0)	1/256(0.39)	0/134(0)	4/208(1.92)	1/97(1.03)	0.3243

Notes: Data are presented as positive no./total no. (%). Oseltamivir used within two days (oseltamivir in two days), oseltamivir used two days after onset (oseltamivir after two days), TCM therapy (TCM), oseltamivir used in two days with TCM therapy (oseltamivir within two days plus TCM), oseltamivir used after two days with TCM therapy (oseltamivir after two days plus TCM), no drug therapy (No therapy). **P*=0.0014. Cases treated with oseltamivir in two days plus TCM, and oseltamivir after two days plus TCM showed higher frequency of sore throat. *@ P*=0.0053. Cases treated with oseltamivir in two days and no drug therapy showed lower frequency of myalgia (arthralgia). *#P*=0.0441. Cases treated with no drug therapy and oseltamivir in two days showed lower frequency of nasal congestion. *$P*= 0.0226. Cases treated with no drug therapy showed lower frequency of swelling of tonsils.

Duration of viral shedding

Body temperature was determined to be a key clinical sign for physicians to choose therapy, and Table 1 shows a difference in body temperature among patients treated with different therapies. Thus, body temperature at onset of therapy was one of the most important stratification factors for this retrospective analysis on viral shedding duration.

Figure 1 shows that no difference in the duration of viral shedding was observed in cases with normal body temperature treated with oseltamivir within two days and no drug therapy. However, the use of oseltamivir after two days from symptom onset (including oseltamivir and TCM) was shown to be associated with prolonged virus shedding duration. Similarly, Figure 1 also shows that there was no difference in the duration of viral shedding in cases with body temperature ≥37.3°C treated with oseltamivir within two days, TCM therapy and no drug therapy, and the use of oseltamivir after two days from onset (including oseltamivir and

TCM) was associated with prolonged virus shedding duration. These data suggest that patients with normal body temperature or ≥37.3°C should be treated with either oseltamivir within two days of symptom onset or just rest with no drug therapy, and administration of oseltamivir after two days from onset was not an effective treatment.

Table 1 shows that the body temperature in most cases treated with no drug therapy were normal or <38.0°C. Thus, the stratification based on body temperature < 38.0° C was used for subsequent analyses. Similar to the data presented in Figure 1, the use of oseltamivir after two days from onset does not reduce the duration of viral shedding.

Figure 2 shows that the duration of viral shedding in the cases with body temperature ≥38.1°C treated with TCM was shorter than those treated with oseltamivir within two days (P<0.05). These findings suggest that patients with body temperature ≥38.1°C could be effectively treated with TCM to reduce the duration of viral shedding.

Oseltamivir application

Figure 3 shows that the duration of viral shedding in cases treated with oseltamivir within two days was shorter than those treated with oseltamivir after two days in cases with normal body temperature or body temperature ≥37.3°C. These results further support the finding that taking oseltamivir within two days from disease onset is more effective at reducing viral shedding duration in patients either with a normal body temperature or body temperature ≥37.3°C.

Alleviation of symptoms and signs

Alleviation of symptoms and signs is also an important parameter used to evaluate the effect of administered therapies. After analyzing the duration of symptoms and signs, analyses revealed that there was no difference in reducing the duration of the symptoms and signs among cases treated with different therapies if the patients were stratified by body temperature (data not shown).

Rare adverse events were observed. Two cases treated with oseltamivir and TCM developed abnormal liver function tests. Two cases treated with oseltamivir developed an allergic reaction. One case treated with TCM developed nausea and vomiting. Two cases treated with TCM and one case treated with oseltamivir developed a rash.

Effect of symptoms and signs on the duration of virus shedding

Symptoms and signs might be taken as indications for the application of different therapies. Table 2 shows that there were differences in the frequency of some symptoms and signs in cases treated with different therapies, that included sore throat, myalgia (arthralgia), nasal congestion, and tonsillitis. In order to analyze whether the symptoms and signs influenced the duration of viral shedding caused by different therapies, multiple logistic-regression analysis was used to identify independent predictors of viral shedding duration among all patients by analyzing the predicted roles of age, sex, and all symptoms and signs (Table 2) for duration of viral shedding (5 days as risk point). Table 3 shows that patients aged <14 years old, sputum production, nasal congestion, or sore throat were found to be predictors for longer (>5 days) duration of viral shedding. Myalgia (arthralgia) and tonsillitis was not predictive of the duration of viral shedding. Thus, age, sputum production, sore throat, and nasal congestion were used as stratification factors in subsequent analyses of the duration of viral shedding in the patients treated with different therapies. No difference were observed for these four predictors among patients treated with different therapies (data not shown) and the predictors were not related to differences in viral shedding duration among the patients treated with different therapies.

DISCUSSION

Here we report a retrospective analysis of 963 patients hospitalized with the 2009 H1N1pdm virus infection between May and July of 2009 in China. Our major findings include that the use of oseltamivir within two days of onset of symptoms or TCM therapy in patients with normal body temperature does not significantly reduce the duration of viral shedding. Compared to those no treated with drug therapy, TCM therapy was shown to reduce virus shedding duration in patients with body temperature ≥38.1°C, and taking oseltamivir after two days of onset of symptoms might prolong virus shedding duration, even when compared to no drug therapy. These findings are similar to a previous study on the timing of oseltamivir use [2,6-8].

TCM with its natural products has been shown to have good therapeutic effect for the treatment of seasonal influenza [9,10]. At the beginning of the 2009 H1N1 pdm virus infection epidemic in China, a team of TCM doctors evaluated infected patients and developed expert consensus guidelines for the use of TCM therapy for treating H1N1 infection, and were issued by the State Administration of Traditional Chinese Medicine[1]. In the guidelines, Shuang Huang Lian Liquor, Lian Hua Qing Wen capsule, and Shu Feng Jie Du capsule were selected as the recommended herbal products for treatment of influenza. In cases treated with TCM therapy (including combination with oseltamivir therapy), more than 92% patients were given a single herbal product, either Shuang Huang Lian Liquor, Lian Hua Qing Wen capsule, or Shu Feng Jie Du capsule therapy, and only 8% patients were given a combination of two of the three herbal medicines. The current study

showed that TCM therapy could be a better choice for reducing the duration of viral shedding for patients with elevated body temperature (>38.0°C). These data suggest that TCM can be an effective therapy for reducing the transmission of the 2009 H1N1 pdm virus infection in China. Moreover, increasing resistance to oseltamivir among influenza A (H1N1) strains could limit the efficacy of treatment with oseltamivir[11-13], and in such a case, TCM therapies may be an alternative therapy for reducing the spread of influenza.

In clinical practice, physicians believe that if patients demonstrate an elevated body temperature and/or more symptoms and signs of infection, they should be treated with a combination of oseltamivir and TCM[1]. However, our data do not demonstrate the advantage for the use of combination therapy for the reduction of the viral shedding duration. These findings suggest that there needs to be more data to support the use of integrative medicine therapy (both TCM and oseltamivir) in the pandemic influenza A patients.

Table 3 Independent predictors of viral shedding duration obtained by multiple logistic-regression analysis

Variable	Odds Ratio (95% CI)	*P* value
Age: <14 yrs vs > or equal to 14 yrs	1.54 (1.07-2.20)	0.01962
Sputum production: without vs with	0.64 (0.44-0.92)	0.016084
Sore throat: without vs with	0.63 (0.45-0.88)	0.006291
Nasal congestion: without vs with	0.53 (0.31-0.91)	0.020932

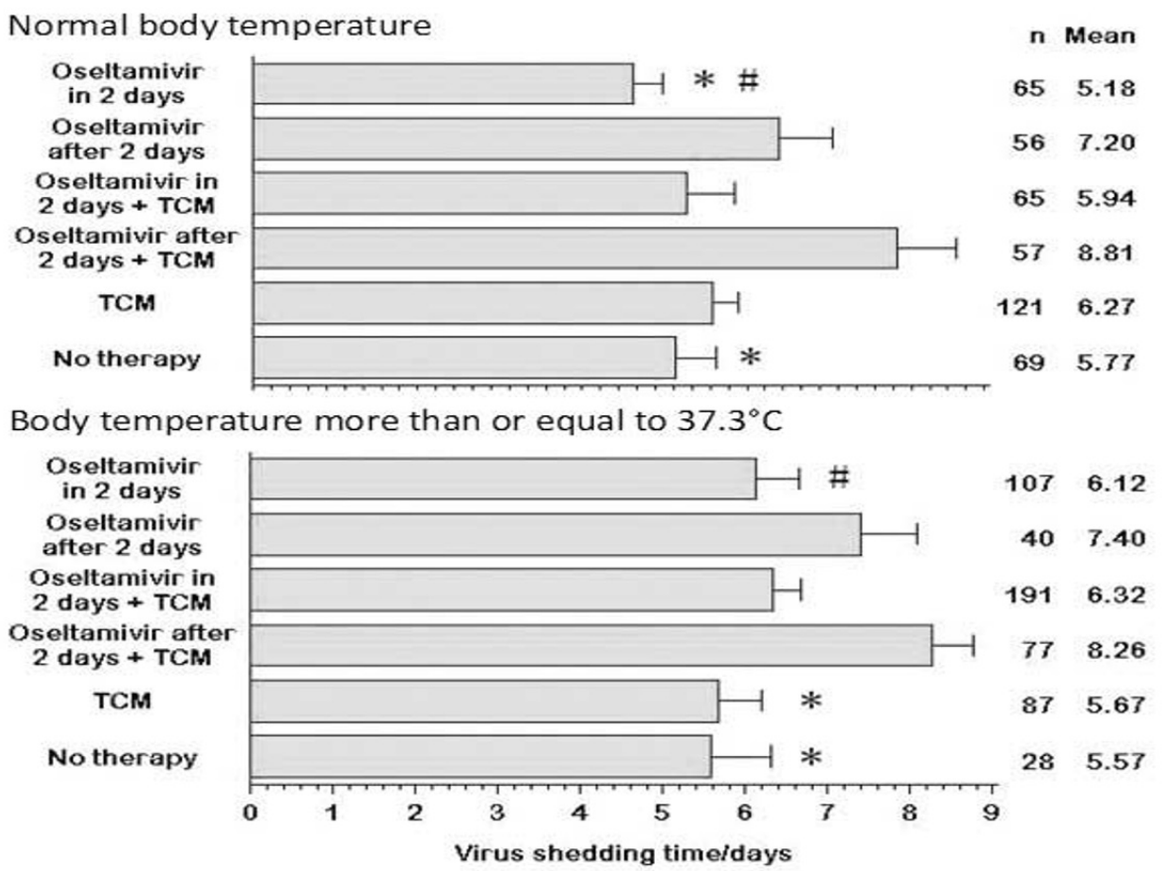

Figure 1 Duration of viral shedding in cases with normal body temperature (upper panel), and duration of viral shedding in the cases with body temperature≥37.3 °C (lower panel). Data are shown as mean±SD. Six therapies are listed: oseltamivir used within two days (Oseltamivir within two days), oseltamivir used two days after onset (Oseltamivir after two days), TCM therapy, oseltamivir used in two days with TCM therapy (Oseltamivir within two days plus TCM), oseltamivir used after two days with TCM therapy (Oseltamivir after two days plus TCM), no drug therapy (No therapy). Case numbers and means are listed on the right. In the upper panel, * P<0.05 (Oseltamivir within two days, No therapy vs Oseltamivir after two days); $^{\#}$$P$<0.05 (Oseltamivir in two days vs. Oseltamivir after two days + TCM). In the lower panel, *P<0.05 (TCM therapy vs. oseltamivir after two days and oseltamivir after two days plus TCM); $^{\#}$$P$<0.05 (Oseltamivir within two days vs oseltamivir after 2 days and oseltamivir after two days plus TCM).

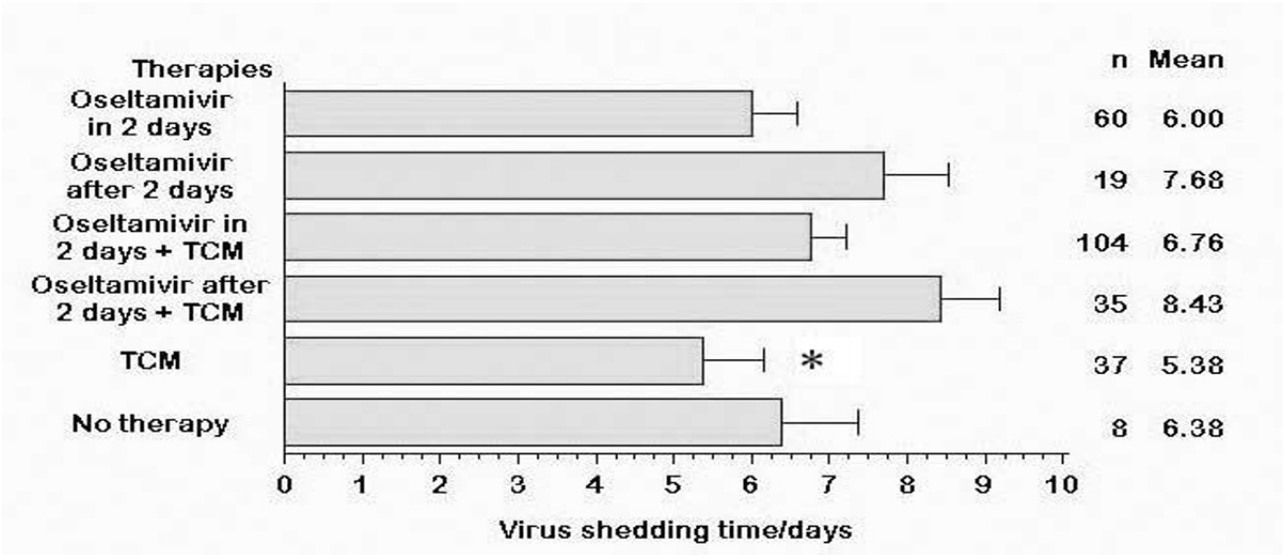

Figure 2 Duration of viral shedding in cases with body temperature ≥38.1°C. Data are shown as mean±SD. Six therapies are listed: oseltamivir used in two days (Oseltamivir within two days), oseltamivir used two days after onset (Oseltamivir after two days), TCM therapy, oseltamivir used in two days with TCM therapy (Oseltamivir within two days plus TCM), oseltamivir used after two days with TCM therapy (Oseltamivir after two days plus TCM), no drug therapy (No therapy). The case numbers and means are listed on the right. * *P*<0.05 (TCM therapy vs. Oseltamivir in two days, Oseltamivir within two days plus TCM, Oseltamivir after two days, Oseltamivir after two days plus TCM).

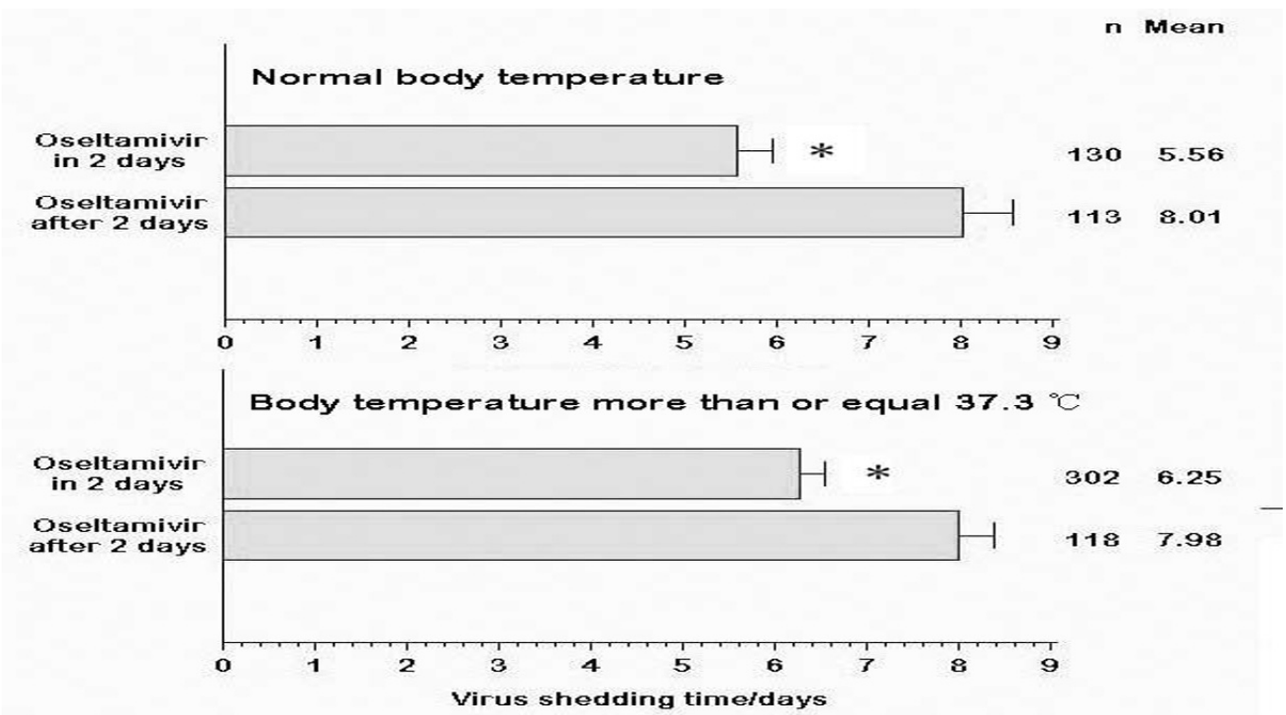

Figure 3 Duration of viral shedding in cases treated with oseltamivir. Data are shown as mean±SD. All patients treated with oseltamivir (including oseltamivir with TCM) were included. The therapies included oseltamivir used in two days (Oseltamivir within two days), oseltamivir used after two days (Oseltamivir after two days) listed on the left. Case numbers and means are listed on the right. **P*<0.05 (Oseltamivir within two days vs. Oseltamivir after two days).

It has been assumed that some symptoms and signs, which might reflect the severity of the disease, could influence the duration of viral shedding[1]. We found some symptoms and signs (Table 3) were associated with the duration of viral shedding in multiple logistic-regression analysis. However, using these predictors as stratification factors in subsequent further analyses, we did not find that symptoms and signs had an effect on viral shedding duration, which indicated that the correlation between these symptoms and signs and duration of viral shedding couldn't be confirmed in this study.

The major limitation of this study was that all data were collected retrospectively, thus a randomized-controlled clinical study must be conducted to confirm these findings. However, for this emerging infectious

disease, our present findings may be helpful for improving the design of future clinical trials, particularly with regard to stratification of patients, and oseltamivir and TCM therapy application. Another limitation of this study was that there are currently three TCM products used for TCM therapy, and it was difficult to define further sub-groups for different TCM products since at least nine different combinations of clinical data existed. Thus, focusing on one or two fixed TCM formulae in future clinical trials would better evaluate the effect of TCM therapy for the patients despite similar activities.

In conclusion, TCM or oseltamivir therapy or combinations thereof, do not reduce the duration of viral shedding in the patients with normal body temperature, and TCM therapy can be effective in patients with a body temperature >38.0° C. Furthermore, the use of oseltamivir two days after onset of clinical signs can prolong the viral shedding duration.

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