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Original Article

Association of early annual peak influenza activity with El Niño southern oscillation in Japan

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Background Seasonality characterizing influenza epidemics suggests susceptibility to climate variation. El Niño southern oscillation (ENSO), which involves two extreme events, El Niño and La Niña, is well-known for its large effects on inter-annual climate variability. The influence of ENSO on several diseases has been described.

Objectives In this study, we attempt to analyze the possible influence of ENSO on the timing of the annual influenza activity peak using influenza-like illness report data in Japan during 1983–2007.

Materials Influenza surveillance data for 25 influenza epidemics, available under the National Epidemiological Surveillance of the Infectious Diseases, was used in this study. ENSO data were obtained from the Japan Meteorological Agency.

Results Influenza-like illness peak week varied largely during the study period, ranging between 4th and 11th weeks (middle of winter to early spring). The average of peak week during ENSO cycles ($n = 11$, average = 4·5 ± 0·9) was significantly earlier than in non-ENSO years ($n = 14$, average = 7·6 ± 2·9; $P = 0·01$), but there was no significant difference in the peak timing between hot (El Niño) and cold (La Niña) phases. Earlier peaks of influenza activity were observed in 16, out of 25, epidemics. These coincided with 10 (90·9%) out of 11 ENSO and 6 (85·7%) out of seven large-scale epidemics.

Conclusion Influenza activity peak occurred earlier in years associated with ENSO and/or large scale epidemics.

Keywords Annual peak, El Niño southern oscillation, influenza, influenza-like illness, Japan.

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Introduction

Annual influenza epidemics affect 10–20% of the population resulting in substantial mortality and morbidity worldwide. The incidence of influenza in temperate areas of the northern and southern Hemispheres is characterized by seasonal cycles with marked peaks in winter,[1] hence, suggesting a role for climate. However, the year round pattern of epidemics observed in tropical countries[2] implies non-uniformity in the factors governing epidemics. In addition, to environmental factors, such as temperature and humidity, fluctuations in host immune response throughout the year, seasonal changes in host behaviors, and overcrowding have also been implicated to play a role in seasonal variation.[3] Annual epidemics are attributed to the continuous evolution of influenza viruses resulting from point mutations in its antigenic determinants.[3] Despite the availability of extensive time series and developed surveillance systems, there is still a lack in the understanding of mechanisms underlying seasonality.

The influence of interannual climate changes on the incidences and trends of a range of water- and food-borne diseases caused by microbiological agents has been reported.[4–6] El Niño southern oscillation (ENSO), which is associated with two extreme events, El Niño (warm) and La Niña (cold), is the most well studied climate phenomenon known to have the largest effect on periodic climate variability.[7,8] Thus, ENSO can serve as a marker to study the effect of climate variability on disease patterns.

Despite the strong seasonality, the timing of winter influenza epidemics changes from year to year. Influenza-like illness in Japan is mainly caused by influenza viruses and thus its incidence is highly representative of influenza activity.[9,10] In this study, we utilized influenza-like illness data from 25 epidemics to determine possible effect of ENSO on the timing of peak influenza activity.

Materials and methods

Influenza surveillance and data collection and analysis

Influenza-like illness report data were used as a marker of influenza activity.[9,10] Under the National Epidemiological Surveillance of Infectious Disease in Japan, clinically diagnosed influenza-like illness cases, defined as sudden fever ≥38°C, respiratory symptoms, and myalgia, are electronically reported on a weekly basis to the Infectious Disease Surveillance Center (IDSC) in the National Institute of Infectious Diseases, Tokyo. Reporting sentinels include pediatric and internal medicine clinics. The number of sentinels is decided on the basis of the size of population of the health center area where they serve. Ten percent of flu sentinels are appointed as laboratory diagnosis sentinels.

Influenza-like illness report data from 1983 through 2007 was obtained from the IDSC's webpage (http://idsc.nih.go.jp/index.html). The peak week for each influenza season was then defined as the week during which the most number of cases are reported. Large-scale epidemics were defined as those for which the peak was ≥38 cases per sentinel per week, which represents 70% of the largest peak observed during the study period, in 1994/1995. Data on circulating types and subtypes of influenza were also available from the IDSC.

Climate data

In Japan, ENSO cycles are identified using a 5 months moving average of the sea surface temperature anomalies. According to the definition of the Japan Meteorological Agency (JMA) El Niño events are associated with positive sea surface temperature anomalies (≥0·5°C), while La Niña events are associated with negative anomalies (≤−0·5°C). ENSO's data was obtained from the JMA's webpage (http://www.jma.go.jp/jma/index.html).

Statistical analyses

Statistical analyses were performed using Fisher's exact probability test (two-tailed) and Scheffe's multiple comparison method. Statistical significance was considered at $P < 0.05$.

Results

We analyzed approximately 14·7 million influenza like illness cases in Japan, which consist of weekly time series of disease incidence from 1983 through 2007, including 25 influenza seasons. Annual influenza seasons began between November and December, peaked between January and March, and returned to the baseline between April and June for the study period (Figure 1). The peak influenza-like illness activity varied between 4th to 10th weeks (late January and early March) during 1983–1994, between 4th and 5th weeks during 1995–2000, and ranged up to the 11th week (the middle of March) during 2001–2007.

Influenza A(H3N2) was dominantly circulating during the majority of seasons, followed by A(H1N1) and B. Large-scale epidemics were observed in seven seasons, namely the 1985/1986, 1989/1990, 1992/1993, 1994/1995 (largest), 1997/1998, 2002/2003 and 2004/2005 seasons. Major antigenic drift of A(H3N2) occurred in five of these epidemics (Figure 1). The average peak week for the study period was 6·2 (Figure 2). Early peak was observed in six of the large-scale epidemics (Figure 2).

Regarding ENSO, 10 episodes covering 11 influenza epidemics were identified during the study's period. The peak

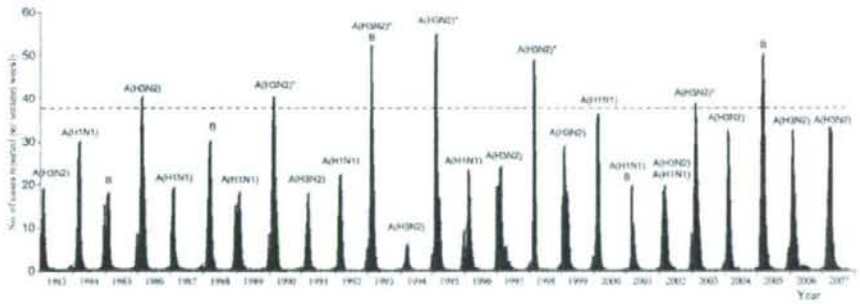

Figure 1. Weekly reported influenza-like illness cases per sentinel, 1983–2007. Data from Japan's Infectious Disease Surveillance Center. Dominant influenza types or subtypes circulating during each season are denoted on the top of the epidemic peak. Asterisks indicate a major antigenic drift in the A(H3N2). The dashed horizontal line indicates 38 cases per sentinel per week (representing 70% of the largest epidemic in 1994/1995). Epidemics with peak greater than 38 cases per sentinel per week were defined as large-scale epidemics.

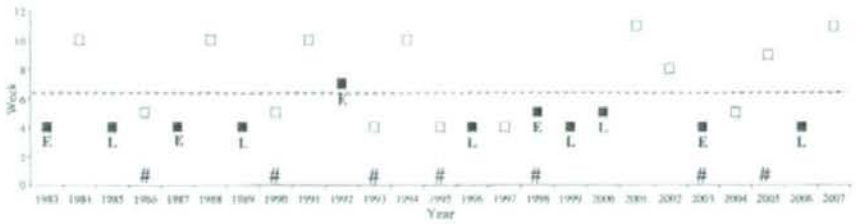

Figure 2. Yearly time series for peak week, defined as that during which the greatest number of influenza-like illness cases was reported, of influenza activity in Japan from 1983 to 2007 (data from Infectious Disease Surveillance Center). The dashed horizontal line, passing at 6·2, indicates the average peak week. # mark denotes a large-scale epidemic. Black boxes denote years during which El Niño southern oscillation (ENSO) episodes happened (E and L indicates El Niño and La Niña, respectively), while empty boxes resemble years with normal weather (non-ENSO years). ENSO data were obtained from Japan Meteorological Agency.

week of influenza epidemics was earlier than the average in 10 out of 11 ENSO years (90·9%) compared to 6/14 (42·9%) for non-ENSO years ($P = 0·03$ by Fisher's exact probability test, two-tailed). The average peak week for ENSO years, $4·5 \pm 0·9$ ($n = 11$), was significantly earlier than that for non-ENSO years (average = $7·6 \pm 2·9$, $n = 14$; $P = 0·01$ by Scheffe's multiple comparison method). No significant difference was found between the average peak weeks during El Niño ($n = 5$) and La Niña ($n = 6$) cycles, average = $4·8 \pm 1·3$ and $4·2 \pm 0·4$, respectively ($P = 0·85$ by Scheffe's method; Figure 2).

Discussion

Seasonality in disease incidence can often infer an association with weather factors and climate variability. We present here an evidence for a role of interannual climate variability on the temporal dynamics of influenza infections in Japan. The evidence is based on long-term time-series analyses of the relationships between peak influenza activity and ENSO as a major climate index.

El Niño southern oscillation arises from fluctuations in sea surface temperature of the tropical Eastern Pacific Ocean. It is well known for its wide-ranging and prominent consequences on weather around the world. It contributes to the likelihood of extreme weather events, such as strong winds, heavy rainfalls, and droughts.[8] The association between ENSO and cholera patterns in Bangladesh[11] and malaria epidemics in parts of South Asia and South America[12–14] were documented. Our data demonstrated that peak influenza-like illness activity occurred earlier in 16 out of 25 epidemics spanning 1983–2007. Early peaks were observed in 10 (90·9%) out of 11 ENSO and six (85·7%) out of seven large-scale epidemics (two of these occurred in ENSO cycles). Thus, we conclude that early peak influenza activity occurs in association with ENSO year or/and large-scale influenza epidemics.

Our observations demonstrated a strong association between the tendency to earlier peak activity and ENSO in Japan. The human response to weather fluctuations involved by ENSO, as inter-annual climate variability, may be different from their adaptation to usually experienced weather conditions. Changes in immunity, indoor crowding, and behavioral changes could set better conditions for virus transmission and consequently earlier peak influenza activity observed during ENSO cycles. Future studies on what factors of ENSO correlate mostly to influenza activity could provide better insight on such association.

Moreover, we demonstrated an association between early peak of influenza activity and large-scale epidemics mainly occurring because of a major antigenic drift of influenza A(H3N2), which dominantly circulated during these seasons. Although there was an exception in the 2004/2005 season in which influenza activity peaked late though being a large-scale epidemic. In this season, both influenza B and A(H3N2) were co-dominantly circulating and their peak overlapped, which could explain high incidence at peak. In a previous study, we similarly reported that the size of epidemic was correlated to the change in antigenicity and that large epidemics were mostly observed with new antigenic variants of influenza A(H3N2).[15] Furthermore, the greater the number of cases at peak week the shorter was the increasing-to-peak period. Thus, in case of a future pandemic we may expect large number of patients within short period, rapid speed of transmission, and early pandemic peak especially in winter season.

Viboud *et al.*[16] reported that higher morbidity impact of influenza was shown during cold phases of ENSO (La Niña) than in hot phases (El Niño). On the contrary, we found no significant difference in the incidence of influenza-like illness cases among the two phases. Nevertheless, higher incidence of influenza-like illness was found to be associated with a major drift in A(H3N2) (data not shown[15]).

Influenza's association with winter in temperate regions could be partly attributed to the direct influence of cold weather on virus survival or on the defense mechanisms of the upper respiratory tract.[17] We previously showed that influenza-like illness activity peaked first in western-central Japan rather than eastern Japan where the mean temperatures are lower,[10] suggesting a minor role of temperature in triggering peak activity. However, in tropical countries, temperature and humidity were considered to play an important role in driving the timing of influenza epidemics.[18] Different patterns and timings possessed by influenza epidemics in the tropics and temperate areas highlight both its susceptibility to trend modification in response to changing climate and the diversity of its driving factors.

The average peak week for the 25 influenza epidemics investigated in this study was in the winter season (6th week). Therefore, this is in line with the seasonality characterizing influenza epidemics. Yet, our data revealed that the peak of influenza activity was delayed until early spring during the 2000/2001 and 2006/2007 seasons. We have previously demonstrated a shift of peak rotavirus activity in Japan from winter to early spring, which could be related to global warming.[19] In contrast to the gradual shift observed in the case of rotavirus from 1983 through 2003, peak influenza activity showed a prompt shift to early spring during two seasons only. The extent to which global warming might affect the timing of influenza epidemics should be carefully followed up in future studies.

The importance of influenza as a human disease is well established, and concerns about future pandemics are clearly warranted.[2,20,21] Our study provides evidence of association between the timing of the peak influenza-like illness, as a marker of influenza activity, and ENSO and antigenic change of A(H3N2), both which can be forecasted half to 1 year ahead and can therefore serve as tools for predicting early peak activity, and consequently improve our preparedness for annual seasonal epidemics.

Finally, there are clear complexities in trying to understand relations between climate change and disease patterns. Knowing that climate change and ENSO effects on different locations of the globe are not uniform, our data remains specific to Japan. A global study of possible associations between climate change and influenza epidemics is essential. Better understanding of global influenza patterns is paramount to improving our control strategies and mitigating the disease burden.

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Recurrence and Persistence of Fever in Children Who Developed Amantadine-Resistant Influenza Viruses after Treatment

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In recent years, a dramatic increase of amantadine-resistant influenza A has occurred globally, but limited data have been available on the clinical course of patients developed amantadine-resistant viruses. We compared fever reduction between patients who developed resistance or remained sensitive in a pediatric clinic in Niigata, Japan, from 2000 to 2006. A total of 2,802 clinical samples were collected from patients who visited the pediatric outpatient clinic with influenza like illness during the seven influenza epidemic seasons. Patients were divided into 4 groups and analyzed for the fever reduction after amantadine treatment; emerged amantadine-resistant (n = 15); amantadine-sensitive (n = 35); patients administered no antiviral drugs (n = 42); and oseltamivir-treated patients (n = 320), which served as references. All 4 groups showed alleviation of fever up to day 3. The amantadine-resistant group had a significant recurrence of fever on day 4 and/or 5, and as a consequence, the course of illness was prolonged. Considering the pattern of fever, recurrent and persistent patterns were found significantly at higher rates in children with emerged resistant virus compared to other groups, and the age tended to be younger in amantadine-resistant compared to amantadine-sensitive group (3.9 ± 3.0 vs 6.7 ± 4.1 years old, n.s.). Therefore, we concluded that younger children were prone to develop amantadine-resistance after treatment and showed a significant recurrence of fever on day 4 and/or 5, and the course of illness was consequently prolonged. ——— influenza; amantadine; antiviral-resistance; children; recurrence of fever.

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Amantadine and rimantadine are adamantane derivatives, known as M2 channel blockers, which inhibit influenza A virus replication by blocking the M2 protein ion channel activity and thereby preventing viral uncoating and release of free ribonucleoproteins into the cytoplasm of infected cells (Pinto and Lamb 2007). Amantadine has been shown to be effective for treatment and prevention of human influenza A virus infections (Monto and Arden 1992; Oxford et al. 2003). In Japan, the drug was approved for the treatment of influenza A in November 1998.

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During the 1998-1999 through 2005-2006 influenza seasons, the prescription ranged from 0.1 to 2.7 million treatment courses, where one treatment course is equivalent to one dosage of 100 mg for 5 days per person.

Patients who were infected with influenza A virus normally shed drug-sensitive viruses during the early course of treatment. However, patients treated with amantadine or rimantadine shed drug-resistant viruses later (Hayden et al. 1989, 1991; Suzuki et al. 2003), especially after 5-7 days of therapy (Hall et al. 1987). Approximately, one-third of the patients develop such resistance (Hayden and Hay 1992; Saito et al. 2002). It has been reported that influenza A virus becomes resistant to the drug through a single amino acid substitution at positions 26, 27, 30, or 31 within the transmembrane region of the M2 gene (Pinto et al. 1992; Holsinger et al. 1994; Pinto and Lamb 2006). Viral resistance to adamantanes can confer cross resistance to both amantadine and rimantadine (Hay et al. 1986; Belshe et al. 1988).

It is generally accepted that amantadine or rimantadine resistant viruses are not more virulent or transmissible than susceptible viruses (Hayden 2006). Studies in the past documented emergence of rimantadine resistant influenza A virus after treatment (Thompson et al. 1987; Monto and Arden 1992; Saito et al. 2002), but limited data are available on the clinical significance of resistant viruses in treated patients. We therefore conducted a multiple influenza season observational study of fever reduction on patients who shed amantadine-resistant strains after therapy, in comparison with amantadine-sensitive and other treatment cases.

Materials and Methods

Study population and clinical samples

Children who visited Yoiko Pediatric Clinic in Niigata, Japan, with influenza-like illness (ILI), during seven influenza seasons from January 2000 to April 2006 were recruited. ILI was defined as a condition characterized by a sudden onset of fever (≧ 37.5°C) and respiratory symptoms, headache, arthralgia or myalgia. After obtaining written informed consent, two nasopharyngeal swabs or a certain amount of nasal aspirates were collected from patients for screening with influenza rapid tests. An aliquot, which can either be one of the two swabs taken at the same time or remaining aspirates, underwent further laboratory examinations for virus isolation and amantadine susceptibility testing. Influenza rapid test kits used in the study were such as QuickVue Rapid SP influ (DS Pharma Biomedical Co., Ltd., Osaka), Espline Influenza A&B-N (Fujirebio Inc., Tokyo) and Quick S-Influ A/B "SEIKEN" (Denkaseiken Co., Ltd., Tokyo).

Amantadine was administered to patients diagnosed as positive for the influenza A virus by rapid antigen testing. The drug was given within 48 hrs of onset at a dosage of 5 mg/kg body weight/day (maximum dosage of 100 mg/day). Influenza A patients who did not undergo amantadine therapy were given oseltamivir as a reference twice daily at a dosage of 150 mg per day for patients weighing ≧ 37.5 kg, or 4 mg/kg/day for patients weighing < 37.5 kg. The decision on whether to administer amantadine or oseltamivir was left to the discretion of the pediatrician, who considered the background and characteristics of the patients such as the presence of other existing diseases, patient age, and patient preference. Patients' information such as age, sex, body temperature on the first visit, time of onset, history of influenza vaccination, name of antiviral drug administered and treatment period were recorded by the pediatrician. Each patient was given a diary card to record axillary temperature three times daily (9 a.m., 12 noon, and 8 p.m.) at home for up to eight days, and these diary cards were returned by mail or brought to the clinic. Amantadine-treated patients were requested to visit the clinic 3-5 days later and to allow collection of second clinical samples. This study was approved by the Medical Faculty Ethics Committee of the Niigata University Graduate School of Medical and Dental Sciences.

Virus isolation and amantadine susceptibility test

Nasopharyngeal swabs or aspirates from patients were suspended in viral transport media and kept at 4°C, then transferred within 7 days to the Division of Public Health, Graduate School of Medical and Dental Sciences, Niigata University, Niigata City, Japan. Supernatants of nasopharyngeal swabs or aspirates were inoculated into Madin-Darby canine kidney (MDCK) cells for influenza virus isolation. Types of viruses were determined by hemagglutination inhibition tests with influenza vaccine strain antisera for the respective seasons (Masuda et al.

2000). Amantadine susceptibility tests were performed with two series of 10-fold dilutions of viruses from cytopathic effect (CPE)-positive cultures, plated in triplicate in 96-well microplates on MDCK cells, with one dilution series containing 2.0 μg/ml of amantadine in the medium (Masuda et al. 2000). Amantadine-resistant strains were identified when less than 1.0-fold difference in log $TCID_{50}$/0.2 ml titer was observed between series of rows with and without the drug after 48 hrs of incubation at 37.0°C.

PCR (polymerase chain reaction) detection and sequencing of the M2 gene

After viral RNA was extracted from patients' nasopharyngeal swabs or isolates, reverse transcription was performed using random primers to create complementary DNA. Nested PCR was performed using specific primers to amplify the M2 region of influenza A (Masuda et al. 2000). The PCR products were sequenced to examine mutations at positions 26, 27, 30, or 31 in the transmembrane region of the M2 gene that are known to confer resistance. Finally, amantadine resistance was diagnosed from the M2 gene sequencing results.

Analysis of fever reduction

Patients enrolled in this study were divided into three groups by therapy: patients who received amantadine, those who received non-antivirals, and those who received oseltamivir. Furthermore, patients who received amantadine were subdivided into amantadine sensitive and emerged amantadine resistance after therapy. Maximum axillary temperatures on each day were evaluated in the four groups, and reduction of fever was analyzed.

Each clinical course was classified into three patterns: "good response pattern" which was defined as alleviation of the fever by day 5 with a body temperature of less than 37.8°C after starting the therapy; "recurrent pattern" which was rebound fever with a temperature greater than or equal to 37.8°C after reduction of temperature below 37.8°C until day 5; "persistent pattern" was defined by persistence of fever with a body temperature greater than or equal to 37.8°C for more than 5 days. Proportion of recurrent and persistent patterns were calculated and compared in the four study groups.

Statistical analysis

To compare mean values between the two groups, the Student's *t*-test was performed. In case of more than 2 groups, firstly analysis of variance (ANOVA) was employed, then, if statistical significance was determined by ANOVA, the Scheffe's test was performed as an *ad hoc* test. To compare median, the Kruskal Wallis method was performed. To compare proportions, chi-square test was used. p values less than 0.05 were employed to define statistical significance.

Results

Amantadine-sensitive influenza A cases

A total of 2,802 patients who visited the pediatric outpatient clinic with ILI during the seven influenza epidemic seasons were screened. Patients who did not meet the study criteria were excluded from the analysis for reasons as shown in Fig. 1. Among 50 amantadine recipients, resistant strains after treatment were detected from 15 (30.0%) recipients ("resistant group") and sensitive strains from 35 (70.0%) ("sensitive group"). Furthermore, 320 oseltamivir recipients ("oseltamivir-recipient group") and 42 non-antiviral recipients ("non-antiviral group") were included in the analysis.

Demographic details of the study groups

Sex distribution, average age, and body temperature at the first clinic visit did not differ significantly among the study groups (Table 1). Average time to clinic visit in the non-antiviral group was significantly longer than that in the oseltamivir recipient group. Vaccination status varied among the four groups. No significant differences were found in the amantadine treatment period between the sensitive and resistant groups (3.4 ± 0.7 days and 3.4 ± 0.5 days, respectively) and the time from the first to second sampling (3.4 ± 1.3 days and 3.7 ± 0.8 days, respectively).

Analysis of fever reduction in emerged amantadine-resistant cases after treatment

We examined the effectiveness of therapy among the 4 groups (Fig. 2). No significant variation in body temperature was found on day 1. On day 2 and 3, reduction of fever was observed in each group, and the maximum body temperature in the resistant group was higher than that in the sensitive group, although statistical significance

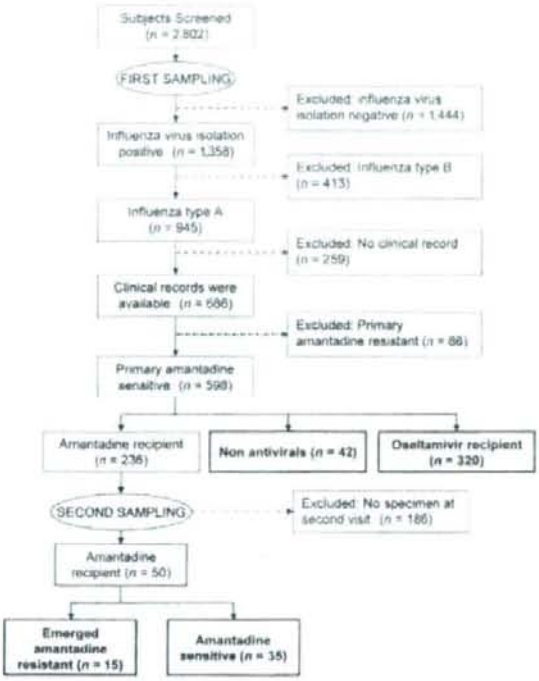

Fig. 1. Flow chart of patients employed in the study. Four study groups were indicated in boldface.

was not shown. The maximum body temperatures in the non-antiviral group (38.2 ± 1.1°C on day 2, and 37.4 ± 1.0°C on day 3) were significantly higher than in the oseltamivir-recipient group (vs 37.7 ± 0.9°C on day 2, $p < 0.05$, and vs 36.9 ± 0.7°C on day 3, $p < 0.01$).

A significant elevation of body temperature was seen in the resistant group on day 4. Average body temperature on day 4 was significantly higher in the resistant group (37.9 ± 0.9°C) than in the sensitive group (vs 37.1 ± 0.9°C, $p < 0.01$), the oseltamivir-recipient group (vs 36.7 ± 0.6°C, $p < 0.01$) and the non-antiviral group (vs 37.1 ± 0.9°C, $p < 0.01$). Fever on day 5 in the resistant group (37.7 ± 1.1°C) was also higher than in the sensitive group (vs 37.2 ± 0.9°C, $p = 0.16$) and the oseltamivir-recipient group (vs 36.7 ± 0.7°C, $p < 0.01$). However, on day 6, body temperature in the resistant group had resolved (37.1 ± 1.0°C) and no difference was found compared to other groups.

We classified all fever records from the patients into three patterns in terms of fever reduction: good response, recurrence, and persistence. Many of the children in the resistant group were classified as recurrent (40.0%) or persistent (26.7%) pattern groups (Fig. 3). In the sensitive, non-antiviral and oseltamivir recipient groups, a recurrent pattern accounted for 22.9%, 16.7%, and 3.1% respectively; and persistent pattern was

TABLE 1. Demographic characteristics of patients' groups

Characteristic	Study groups				
	Emerged amantadine resistant [1] ($n = 15$)	Amantadine sensitive [1] ($n = 35$)	Non antivirals ($n = 42$)	Oseltamivir recipient ($n = 320$)	p value
Sex, no. of male (%)	11 (73.3)	19 (54.3)	21 (50.0)	182 (56.8)	0.47 [2]
Age, mean ± s.d. (years)	3.9 ± 3.0	6.7 ± 4.1	5.5 ± 5.1	5.8 ± 4.2	0.19 [3]
Body temperature at first clinic visit, mean ± s.d. (°C)	38.8 ± 0.8	38.6 ± 0.9	38.6 ± 0.9	38.7 ± 0.8	0.91 [3]
Time from onset to clinic visit, median (range) (hrs)	11.0 (3 - 42)	22.0 (3 - 64)	24.0 (0 - 90)	17.0 (0.5 - 66)	< 0.05 [4]*
Vaccination, no. of patients who received influenza Vaccination in the season (%)	1 (6.7)	0 (0.0)	7 (16.7)	136 (42.5)	< 0.001 [2]
Amantadine treatment period, mean ± s.d. (day)	3.4 ± 0.5	3.4 ± 0.7	-	-	1.00 [5]
Oseltamivir treatment period, mean ± s.d. (day)	-	-	-	3.7 ± 0.6	-
Time from first to second sampling, mean ± s.d. (day)	3.7 ± 0.8	3.4 ± 1.3	-	-	0.33 [5]

[1] Amantadine susceptibility, confirmed with specimens at the second sampling.
[2] Chi-square test was used for comparison among the groups.
[3] Analysis of variance was used for comparison among mean values of each group.
[4] Kruskal Wallis method was used for comparison among median values of each group.
[5] Student's t-test was used for comparison between two groups.
*Statistically significant difference in median values between the non-antiviral group and the oseltamivir recipient group.

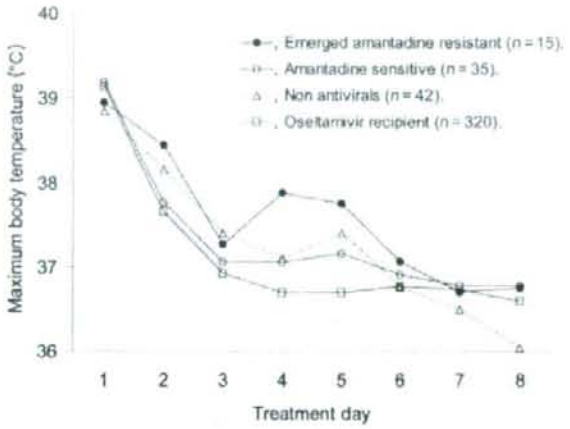

Fig. 2. Daily average maximum body temperatures in the study groups.
The four study groups were as follows: amantadine recipients shedding resistant viruses (●, $n = 15$); amantadine recipients shedding sensitive viruses (○, $n = 35$); patients who received no antivirals (△, $n = 42$); and oseltamivir recipients (○, $n = 320$). On day 4, the emerged amantadine-resistant vs the other three groups, $p < 0.01$, respectively. On day 5, the emerged resistant group vs the oseltamivir recipients, $p < 0.01$. On day 2 and 3, the non-antiviral group vs the oseltamivir recipients, $p < 0.05$ and $p < 0.01$, respectively.

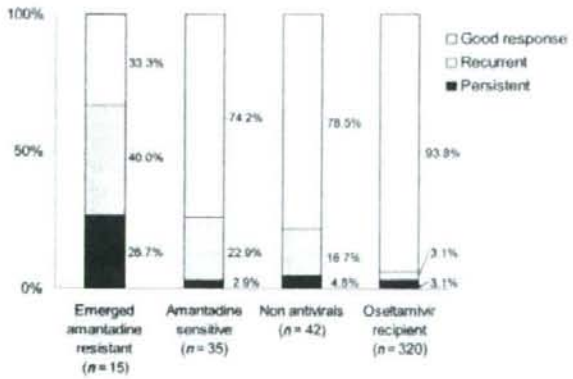

Fig. 3. Proportion of the three patterns of fever reduction among the four groups.
"Good response" is rapid fever reduction group; "recurrence" fever reduction at first but recurrent fever later; and "persistence" group.

2.9%, 4.8%, and 3.1% respectively. Combined proportion of persistent and recurrent patterns in the amantadine-resistant group was significantly higher (66.7% [10 of 15]) than in the sensitive group (vs 25.7% [9 of 35], $p < 0.01$), the non-antiviral group (vs 21.4% [9 of 42], $p < 0.01$), and the oseltamivir-recipient group (vs 6.3% [20 of 320], $p < 0.01$).

Individual fever records showed that in the resistant group, 5 patients (average age 6.4 years

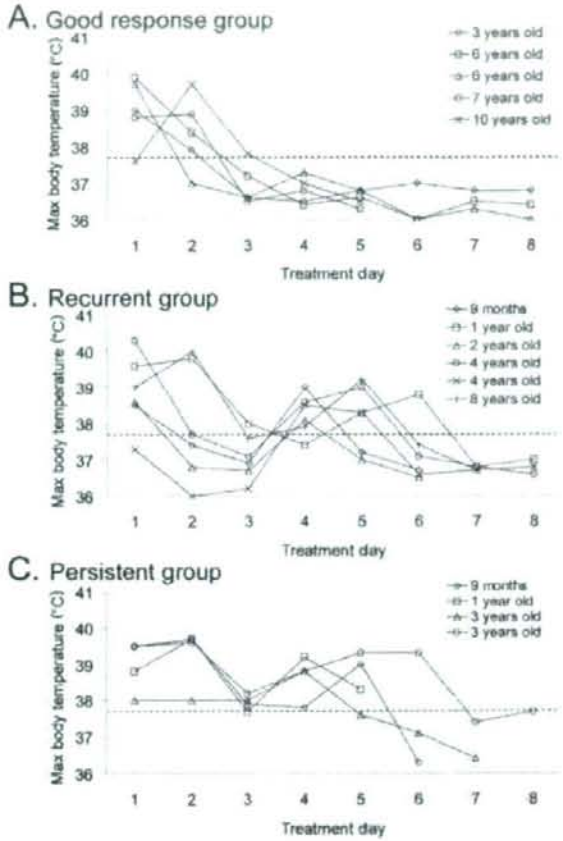

Fig. 4. Individual records of daily maximum body temperature in children who shed amantadine-resistant viruses after treatment.
Good response (panel A) was defined as rapid fever reduction < 37.8°C by day 5. Recurrent group was defined as fever reduction < 37.8°C in the early course of the illness, but a recurrence ≥ 37.8°C by day 5 (panel B). Persistent group was defined as showing persistence of fever ≥ 37.8°C by day 5 without alleviation (panel C). Horizontal dotted lines indicate 37.8°C.

old) showed good response patterns (Fig. 4A), while 6 patients (average age 3.3 years old) exhibited recurrent patterns (Fig. 4B), and 4 children (average age 1.9 years old) showed persistent patterns (Fig. 4C).

Among 50 amantadine treated children (35 amantadine-sensitive and 15 emerged amantadine-resistant), 41 were A/H3N2 subtype and 9 were A/H1N1. Thirteen (31.7%) of 41 A/H3N2 and 2 (22.2%) of 9 A/H1N1 were resistant, but the frequency of resistance between A/H3N2 and A/H1N1 was not statistically significant.

As to timing of recovery, resistant influenza viruses were collected on day 3 from 8 children (53.3%), 4 patients (26.7%) on day 4, and 3 patients (20.0%) on day 5 after starting the amantadine therapy. In the M2 gene sequence analyses, seven (46.7%) out of 15 amantadine-resistant virus had a change at position 31 (serine to asparagine), five (33.3%) at 27 (valine to alanine), two (13.3%) at 30 (alanine to threonine). One strain (6.7%) had dual mutations at 31 (serine to asparagine) and at 27 (valine to alanine). However, no significant difference was observed in clinical pictures by the positions of mutation.

Discussion

In this observational study, the course of influenza illness differed between patients shedding amantadine-resistant and sensitive strains. All four study groups showed reduction of fever during the first few days. The amantadine-resistant group showed a significant recurrence of fever on day 4 and/or 5, and as a consequence, the course of illness was prolonged. In an earlier study (Hall et al. 1987), illness severity which scored late in therapy tended to be higher in rimantadine-treated children who shed resistant viruses compared to those who did not, but statistical significance was not demonstrated. Furthermore, another study (Hayden et al. 1991) indicated that average temperatures did not differ between the rimantadine groups over the first 4 days of treatment, but a non-significant elevation of temperature appeared that shed resistant virus on treatment day 5. Thus, the present study is the first to show significant recurrence and persistence of fever in children who shed resistant influenza viruses after treatment.

Considering the pattern of fever reduction, recurrent and persistent patterns were found significantly at higher rates in children in the resistant group compared to other groups, and the age tended to be younger in the resistant group compared to the sensitive group. These findings are considered as the clinical feature of children who developed amantadine-resistant influenza A viruses. Furthermore, in the emerged resistant group, age of children who showed persistent and recurrent patterns were younger than good response pattern group. In a study of oseltamivir, resistant viruses to this drug appeared more often in young children (Kiso et al. 2004), and it was explained that younger children experiencing their first or second influenza infections typically manifest a prolonged period of illness and virus shedding, and possess higher virus titer. In general, younger children do not have immunological memories in their T and B cells because of no prior exposure of any types of influenza (Ahmed and Rouse 2006; Kalia et al. 2006), and thus, their immune response is slower than that of adults and elderly. Consequently, high viral load in children may allow greater opportunity for selection of resistant viruses after treatment of amantadine.

To reduce the emergence of resistant strains, amantadine therapy is advised to be discontinued as soon as clinically warranted, generally after 3-5 days of treatment, or within 24 hrs after the disappearance of signs and symptoms. The dosage recommended in the United States is 5 mg/kg/day, and should not exceed 150 mg in two divided dosages for children aged 1-9 years. In this study, due to the Japanese regulations, the daily dosage was lower than that in the United States and the duration of treatment was shorter, which was 3 to 4 days. While recurrence of fever was observed on day 4, and most of the emerged amantadine-resistant viruses (84.3%) were recovered until day 4, we may not rule out the supposition that this fever aggravation was caused by the shorter period of treatment. However, this assumption can not explain the fever difference between the resistant and sensitive groups, since both groups pos-

sessed similar treatment durations. Thus, we assume that the fever difference between the two groups is linked with amantadine susceptibility status. Further studies are needed to determine whether recurrence and persistence of fever in individuals shedding drug resistant strains are associated with increased viral load due to development of resistance. In this study, $TCID_{50}$ of the amantadine resistance and sensitive groups at the second sampling were 3.7 and 5.3 (data not shown), respectively. However, these results did not reflect the true viral titers in the original samples, because the virus titer was measured after three passages in MDCK cells. Therefore, further specific study such as quantitative real-time PCR is warranted.

In order to determine the clinical significance of drug-resistant virus from treated patients, reduction of fever and improvement of daily scores for symptoms and severity of illness was used in the previous studies (Hall et al. 1987; Thompson et al. 1987; Hayden et al. 1989, 1991). For this paper, only temperature data but not other symptoms was analyzed since it was the only objective measurement that was not affected by biases from doctors' or participants' feelings or judgments. We used the maximum body temperature data of patients with 3 measurements per day to calculate average maximum body temperatures in each group, so as to avoid possible influence of temporary antipyretic use, which was administered for patients when the fever was too high (*e.g.* > 38°C).

In Japan, more than 90% of influenza cases in children are administered antiviral drugs, mostly oseltamivir and occasionally amantadine (Sugaya et al. 2007). In this study, only 42 of 424 influenza patients (9.9%) did not receive antiviral drug (non-antiviral group). The reasons for no receipt were negative result with rapid test in ambulatory, guardian's will, or more than 48 hrs had passed from onset to clinic visit. Thus, the average time from onset to clinic visit in the non-antiviral group was the longest among the four groups.

In this study, a subset of amantadine recipients (186 of 236) were not included due to lack of a second sample. This might suggest that these patients did not return to the clinic because of adequate recovery from their illness. However, reviewing their clinical records, a combined proportion of the recurrent and persistent pattern rates did not reveal any significant difference between with or without second samples (data not shown). Thus, patients did not return for a second sampling due to unknown reasons not related to their recovery.

Proportion of recovering resistant strains was higher in those shedding A/H3N2 strain than in those shedding A/H1N1 strain although statistically not significant, and these results supported our previous report on a difference of resistant strain appearance by subtype (Saito et al. 2003). Furthermore, we could not find any relationship between clinical pictures and positions of mutation in the M2 gene.

In this study, 88 primary amantadine-resistant cases were excluded, and most of them were A/H3N2 viruses with the S31N mutation in the 2005/06 season reported as a clade N, which was related to a dramatic increase of resistance in communities in Japan (Saito et al. 2006; Saito and Suzuki 2007), Asia, and North America (Barr et al. 2007; Deyde et al. 2007). Further investigations on clinical courses with primary amantadine-resistant viruses are warranted, since available information on concordance or discordance between clinical data and phenotypic/genotypic assays in antiviral resistance is limited.

In conclusion, younger children tended to develop amantadine-resistance after treatment, and these children showed higher incidence of persistence or recurrence of fever on day 4 and/or 5.

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Effectiveness of Oseltamivir Treatment among Children with Influenza A or B Virus Infections during Four Successive Winters in Niigata City, Japan

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Oseltamivir has been used for treatment of influenza A and B infections, but recent reports documented that it was less active against the latter. We compared the effectiveness of oseltamivir in children between laboratory confirmed influenza A and B over 4 influenza seasons from 2001 to 2005 in a pediatric clinic in Japan. Among 1,848 patients screened, 299 influenza A and 209 influenza B patients were administered oseltamivir (treated groups), and 28 influenza A and 66 influenza B patients were assigned as non-treated groups. The duration of fever, defined as period when patients had the maximum temperature higher than 37.5°C in three-time measurements in a day after the clinic visit, was evaluated among the four groups. In uni-variate analysis, the duration of fever was shorter for treated group than non-treated for influenza A (1.8 ± 0.9 days vs 2.6 ± 1.3 days, $p < 0.01$), but it was not significant for influenza B (2.4 ± 1.3 days vs 2.8 ± 1.2 days, $p = 0.9$). The fever duration was longer in treated influenza B than A patients ($p < 0.01$). Multi-variate analysis indicated younger age (< 6 years old) and higher body temperature at the clinic visit prolonged the duration of fever. Adjusted average duration of fever indicated that oseltamivir was effective for both types, but more effective on influenza A, and the benefit increased for younger children. Our data provide evidence that oseltamivir is beneficial for influenza infections, but the effectiveness is differed by type and age.

——— influenza; anti-viral drugs; oseltamivir; children; effectiveness.
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Influenza outbreaks occur annually across the world, causing excess morbidity and mortality (Simonsen et al. 2000; Nicholson et al. 2003; Centers for Disease Control and Prevention 2006). For influenza treatment, there are two types of anti-influenza drug: amantadine and neuraminidase inhibitors (oseltamivir and zanamivir) (Monto 2003; Oxford et al. 2003; Moscona 2005; Oxford 2005; Jefferson et al. 2006). Amantadine is effective for treatment of influenza

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A virus infections while neuraminidase inhibitors are for both influenza A and B (Treanor et al. 2000). Early treatment reduces the severity and duration of influenza illness and associated complications (Nicholson et al. 2000; Aoki et al. 2003; Kawai et al. 2005).

The neuraminidase inhibitors, zanamivir and oseltamivir, interfere with the release of progeny influenza viruses from infected host cells and spread to neighboring cells in the respiratory tract. Clinical efficacy of oseltamivir has been established as treatment for influenza in adults (Hayden et al. 1999; Nicholson et al. 2000; Treanor et al. 2000) and children (Whitley et al. 2001). The neuraminidase inhibitors were tested to be less active against influenza B than A viruses in vitro studies (Boivin and Goyette 2002; Aoki et al. 2003; Mungall et al. 2004). Moreover, increasing evidence suggests that oseltamivir is less effective against influenza B than influenza A infections (Kawai et al. 2006; Sugaya et al. 2007). The present study was conducted to evaluate the efficacy of oseltamivir treatment among children with influenza A and B virus infections during four successive winters in Japan using uni-variate and multi-variate analysis adjusted for various factors that affect the course of illness.

Materials and Methods

Study population and laboratory methods

This study was conducted during 4 influenza seasons from November 2001 to May 2005 at a private pediatric outpatient clinic located at the city center in Niigata City, Japan with a total population of approximately 500,000. This clinic had no bed facility, and approximately 2,300 outpatients visited per month.

Influenza-like illness was defined on the basis of a sudden fever (≧ 37.5°C) and any acute respiratory symptoms and signs, such as, cough, rhinorrhea, sneezing, wheezing, sore throat, headache, nausea, or malaise. Nasopharyngeal swabs or aspirates were examined with rapid antigen test kits for diagnosis of influenza A or B prior to antiviral drug treatment (oseltamivir or amantadine) at the initial office visits. Influenza rapid test kits, such as QuickVue Rapid SP influ (DS Pharma Biomedical Co., Ltd., Osaka), Espline Influenza A&B-N (Fujirebio Inc., Tokyo), and Quick S-Influ A/B "SEIKEN" (Denkaseiken Co., Ltd., Tokyo) were used to screen influenza A or B infections.

Patients were assigned to influenza treatment or non treatment groups, depending whether or not they want to receive antiviral drug medication according to the rapid test results. For patients with influenza A, the decision of whether to receive antivirals (oseltamivir or amantadine) or not was left to patients or their family. For influenza B, patients could choose either oseltamivir or no antiviral therapy. The two drugs were administered twice daily (oseltamivir, 150 mg per day for children ≧ 37.5 kg in weight; or 4 mg/kg for children with < 37.5 kg; amantadine, 1.5-2.5 mg/kg). Both drugs were prescribed for 5 days. For some patients, administration of drugs was discontinued if symptoms were alleviated within 5 days. Single use of antipyretics was allowed when a child had a fever more than 38.5°C.

Written informed consent was acquired from parents of patients to obtain clinical information and specimens for virological investigations upon enrollment to the study. Age, sex, body weight, vaccination status, use of antipyretics, type of drug, the time from the onset of fever to the administration of anti-influenza drug, body temperatures, and the results of rapid antigen test kits were recorded for all patients by the clinician at the time of report to the clinic. The parents were given a diary card to record body temperatures 3 times daily (at 9:00, 12:00 and 20:00 o'clock) and any symptoms such as cough, rhinorrhea, sore throat, fatigue, appetite loss, myalgia, vomiting, or diarrhea, occurring up to 5 days after the therapy started. Parents were requested to return the card by visiting or mailing to the clinic after completion of the course. Time until treatment was defined as days from fever onset until the clinic visit.

Nasopharyngeal swabs or aspirates were collected from the patients, placed in viral transport medium, and then transferred to the Department of Public Health, Niigata University Graduate School of Medical and Dental Sciences. The samples were stored at 4°C for a few days until viral culture, and aliquots were kept at −80°C. For virus isolation, supernatants of specimens were inoculated into Madin-Darby canine kidney cells. Types and subtypes were determined by hemagglutination inhibition tests with type-specific antisera (Masuda et al. 2000). Detection of the influenza genome was performed by reverse transcription-polymerase chain reaction (RT-PCR) (Saito et al. 2002). Briefly, viral RNA was extracted from nasopharyngeal aspirate specimens and reverse transcription reactions were performed for complementary DNA synthesis as described previously

(Masuda et al. 2000). First and nested PCR was performed to detect genetic influenza A, using M2 gene primers (Masuda et al. 2000). Influenza B was detected in separate PCR runs using influenza B hemagglutinin gene primers (Shimizu et al. 1997). In this study, we defined "influenza infections" as PCR or virus isolation positive regardless of rapid test results (Fig. 1). This study was approved by the Medical Faculty Ethics Committee of the Niigata University, Graduate School of Medical and Dental Sciences.

Effectiveness of oseltamivir

Influenza-related fever was defined as body temperature of more than 37.5°C (99.5 F) using the highest body temperature among three different time measurements in a day. The effectiveness was evaluated by the fever duration more than 37.5°C after the first visit to clinic.

Statistical analysis

Statistical comparisons for baseline characteristics among the 4 groups by type of influenza and treatment were made by chi-square test to evaluate the proportions in multiple groups, and one way analysis of variance to compare the mean values. Sheffe's test was used as univariate analysis to compare average values for the duration of fever among the four clinical groups. General linear model was employed as multi-variate analysis to assess independent variable which influenced the duration of fever and to estimate the adjusted average days for duration of fever by type and treatment. All statistical analyses were performed with SPSS 11.0J (SPSS Japan Inc., Tokyo). $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 1,848 individuals with influenza-like illness were screened during the four successive seasons for the study (Fig. 1). Among these, 1,130 (61.1%) patients were positive for influenza with virus isolation or PCR, but nearly half of patients were excluded due to the reasons listed in the Fig. 1. As a result, a total of 602 patients (5 of

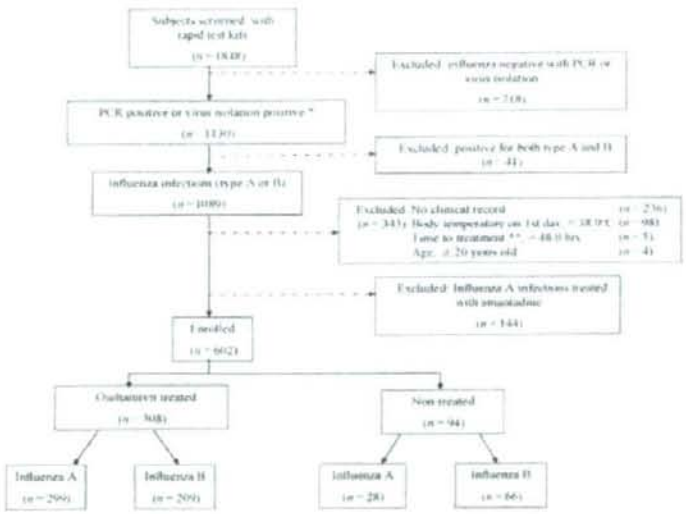

Fig. 1. Flow of participants through the study.
* Subjects were included regardless of rapid test results.
** Time until treatment, the time from the onset of fever to the first dose of treatment.

influenza A/H1N1, 257 of A/H3N2, and 257 of B were identified by virus isolation, and 65 of influenza A and 18 of influenza B by PCR) were enrolled in the study. They were divided into four groups by type of influenza and oseltamivir treatment status: 299 influenza A patients received oseltamivir treatment (treated influenza A), and 28 without treatment (non-treated influenza A), and 209 influenza B patients with treatment (treated influenza B) and 66 without treatment (non-treated influenza B), respectively (Table 1). The mean age and body weights, vaccination status, and the time until treatment did not differ significantly among the four groups. Body temperature at the time of clinic visit was higher in treated influenza A patients than treated influenza B, and younger patients (< 6 years old) had higher temperature than older ones (≧ 6 years old) in all groups.

Effectiveness of oseltamivir treatment for influenza A and B

The duration of fever was shorter in the treatment group as compared to the non treatment in influenza A (1.8 ± 0.9 days vs 2.6 ± 1.3 days; $p < 0.01$), but influenza B did not have statistical significance (2.4 ± 1.3 days vs 2.8 ± 1.2 days; $p = 0.09$) (Table 2). The fever duration was longer for influenza B treatment group (2.4 ± 1.3 days) than influenza A treatment group (1.8 ± 0.9 days; $p < 0.01$). In all four groups, duration of fever was significantly longer in younger (< 6 years old) than older children (≧ 6 years old) (Table 2). For younger group, the duration of fever was statistically shorter in treatment groups than non-treatment for both influenza A (3.1 ± 1.3 days vs 1.9 ± 1.0 days, $p < 0.01$, balance between the two = 1.2 days), and influenza B (3.2 ± 1.1 days vs 2.7 ± 1.3 days, $p < 0.05$, balance between the two = 0.5 days), but not in older children for both influenza

TABLE 1. Demographic details for influenza A and B patients by oseltamivir treatment.

	Influenza A		Influenza B		
	Oseltamivir non-treated ($n = 28$)	Oseltamivir treated ($n = 299$)	Oseltamivir non-treated ($n = 66$)	Oseltamivir treated ($n = 209$)	p value[a]
Season					0.000
2001-2002	7 (25.0)	6 (2.0)	29 (43.9)	38 (18.2)	
2002-2003	6 (21.4)	28 (9.4)	0 (0.0)	6 (2.9)	
2003-2004	2 (7.1)	109 (36.5)	0 (0.0)	4 (1.9)	
2004-2005	13 (46.4)	156 (52.2)	37 (56.1)	161 (77.0)	
Gender					0.036
Male	9 (32.1)	175 (58.5)	39 (59.1)	109 (52.2)	
Female	19 (67.9)	124 (41.5)	27 (40.9)	100 (47.8)	
Age (years)	4.9 ± 4.0	5.8 ± 3.6	5.7 ± 2.3	6.4 ± 2.7	0.044
Body temperature at the clinic visit (°C)	39.1 ± 0.6	39.2 ± 0.6	38.9 ± 0.6	39.0 ± 0.6	0.000
Body weight (kg)	17.8 ± 10.0	22.5 ± 11.6	20.2 ± 6.1	22.4 ± 9.4	0.054
Time until treatment[b] (days)	1.0 ± 1.0	0.8 ± 0.6	0.8 ± 0.7	0.9 ± 0.7	0.208
Vaccination	7 (25.0)	112 (37.5)	17 (25.8)	83 (39.7)	0.115
Use of antifebrile drug	0 (0.0)	9 (3.0)	0 (0.0)	4 (1.9)	0.392

Numbers are mean ± S.D. or n (%).

[a]Chi-square test were employed for multiple rows and column contingency table, and one-way analysis of variance was used to compare means in multiple groups.

[b]Time until treatment, the time from the onset of fever to the clinic visit.

TABLE 2. Average duration of fever compared by uni-variate and multi-variate analysis by type of influenza and oseltamivir treatment.

	Uni-variate						Multi-variate					
	Influenza A			Influenza B			Influenza A patients			Influenza B patients		
	Non-treated	Oseltamivir treated	p	Non-treated	Oseltamivir treated	p	Non-treated	Oseltamivir treated	p	Non-treated	Oseltamivir treated	p
All age	2.6 ± 1.3 (n = 28)	1.8 ± 0.9 (n = 299)	< 0.01	2.8 ± 1.2 (n = 66)	2.4 ± 1.3 [c] (n = 209)	0.09	3.1 (2.5 - 3.6) [c] (n = 14)	2.0 (1.8 - 2.1) [c] (n = 228)	< 0.01	3.2 (2.9 - 3.5) [c] (n = 47)	2.8 (2.6 - 3.0) [b, c] (n = 176)	< 0.05
< 6 years	3.1 ± 1.3 (n = 18)	1.9 ± 1.0 (n = 158)	< 0.01	3.2 ± 1.1 (n = 33)	2.7 ± 1.3 [c] (n = 96)	< 0.05	3.6 (2.8 - 4.3) (n = 9)	2.1 (1.7 - 2.4) (n = 122)	< 0.01	3.5 (3.0 - 4.0) (n = 23)	2.9 (2.5 - 3.3) [c] (n = 80)	0.07
≧ 6 years	2.0 ± 1.2 [a] (n = 10)	1.6 ± 0.7 [a] (n = 141)	n.s.	2.5 ± 1.1 [a] (n = 33)	2.2 ± 1.3 [a, c] (n = 113)	n.s.	2.5 (1.6 - 3.4) (n = 5)	1.8 (1.6 - 2.1) (n = 106)	n.s.	2.9 (2.5 - 3.4) (n = 24)	2.6 (2.4 - 2.9) [c] (n = 96)	n.s.

Sheffe's test was used for uni-variate analysis, and general linear model was applied for multi-variate analysis for comparison of duration of fever ≧ 37.5°C after the first visit to the clinic.

Values indicate mean ± S.D. for uni-variate analysis, and mean with 95% confidence interval in brackets for multi-variate analysis, adjusted for age, sex, season, vaccination status, time until treatment, and the body temperature at the clinic visit.

n.s., not significant.

[a] Oseltamivir treated influenza A vs oseltamivir treated influenza B, $p < 0.01$.

[b] < 6 years vs ≧ 6 years for identical type and treatment group, $p < 0.05$.

[c] Those (n = 137) who were missing more than one of variables were excluded in multi-variate analysis.

Table 3. Effects of influenza type, oseltamivir treatment, time until treatment, and maximum body temperature on the duration of fever analyzed with multi-variate analysis

Factor	β (day)	p value
Influenza B virus infection	0.142	0.659
Oseltamivir treatment	−1.321	0.000
Age less than 6 years old	0.711	0.011
One degree higher body temperature at the clinic visit (°C)	0.550	0.000

General linear model was carried out with 465 patients, adjusted for gender, body weight, season, vaccination status and the time until treatment. Those ($n = 137$) who were missing more than one of variables were excluded from the analysis.

A (2.0 ± 1.2 days vs 1.6 ± 0.7 days, *n.s.*, balance between the two = 0.4 days), and influenza B (2.5 ± 1.1 days vs 2.2 ± 1.3 days, *n.s.*, balance between the two = 0.3 days). However, the fever duration was consistently shorter in treated influenza A than treated B for the two age categories.

We examined independent variable factors influencing the duration of fever using general linear model as multi-variate analysis (Table 3). Of variables analyzed, treatment of oseltamivir was a factor that attributed to the reduction of the fever duration by 1.32 days, whereas influenza B virus infection did not affect the illness duration significantly. Patients who were less than 6 years old exhibited the prolonged duration of fever by 0.71 days, and as well as one degree higher body temperature at the clinic visit by 0.55 days.

Average duration of fever was estimated in the four groups with adjustment for age, gender, body weight, influenza season, vaccination status, time until treatment, and body temperature at the clinic visit. The treatment groups had significantly shorter duration of fever than non-treatment groups for both influenza A (2.0 days vs 3.1 days, $p < 0.01$) and influenza B (2.8 days vs 3.2 days, $p < 0.05$) (Table 2). The duration was longer in treated influenza B than treated influenza A ($p < 0.01$), as in the uni-variate analysis. After stratification by age groups (< 6 years old, or ≥ 6 years old), average duration was consistently longer for all four groups in younger children than older ones (Table 2). In younger children (< 6 years old), the fever duration was significantly shorter in treated groups than non- treated for influenza A (3.6 days vs 2.1 days, $p < 0.01$, balance between the two = 1.5 days), but not for influenza B (3.5 days vs 2.9 days, $p = 0.74$, balance between the two = 0.6 days). In older children (≥ 6 years old), statistical significance was not demonstrated for both influenza A (2.5 days vs 1.8 days, *n.s.*, balance between the two = 0.7 days) and B (2.9 days vs 2.6 days, *n.s.*, balance between the two = 0.3 days). For the two age groups, treated influenza B had consistently longer fever duration than influenza A counterparts.

Discussion

The clinical results in this paper provided evidence that oseltamivir was effective in reducing the duration of fever for both influenza A and B infections, but was less effective for influenza B infections rather than influenza A. Even after adjustment with various underlying factors, or categorization by age groups, the duration of fever in the treatment groups was consistently longer for influenza B than influenza A.

Oseltamivir has been thought to be equally effective against influenza A and B infections (Hayden et al. 1999; Whitley et al. 2001), but growing clinical evidence suggests oseltamivir is less effective against influenza B than influenza A. Our results were basically similar to the previous findings from Japan (Kawai et al. 2006; Sugaya et al. 2007). However, we emphasize that we carried out the study in multiple years, and enrolled sufficient number of non-treated groups for both influenza A and B in order to evaluate the effectiveness of the drug, in comparison with the pre-