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Viral Shedding in University Students Infected by Influenza A(H1N1)pdm09, Nakhon Ratchasima Province, Thailand, June 2011

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Abstract

Oseltamivir is often prescribed to treat influenza patients, yet its effect on viral shedding among Thai young adults infected with influenza A(H1N1)pdm09 virus remained unclear. During May to June 2011, an influenza A(H1N1)pdm09 outbreak was detected in University S, Nakhon Ratchasima Province, Thailand. A prospective observational study was conducted to define duration of viral shedding and immunologic response in infected students undergoing oseltamivir treatment, and identify factors associated with viral shedding. We enrolled all acute respiratory illness (ARI) patients attending the medical center at University S during 3-7 Jun 2011 with laboratory confirmation of influenza A(H1N1)pdm09 infection by real-time reverse transcription polymerase chain reaction (rRT-PCR). Additional throat swabs were collected and tested daily until rRT-PCR results became negative through two consecutive days. Series of serum samples for hemagglutination inhibition (HI) test were also collected from the individuals. Log-rank test was applied in analysis of association between patients' characteristics and duration of viral shedding. Of 29 sick students enrolled, 45% were males. All were prescribed oseltamivir for five days and none of them were hospitalized. Median duration from onset of symptoms to the last day of viral shedding detected was five days (range 3-9 days). Over 80% of the patients had 4-fold rises of HI titer within 2-3 weeks after onset of symptoms. None of the patients' characteristics were significantly associated with duration of viral shedding. However, persons with delayed antiviral treatment tended to have longer duration of viral shedding. Early oseltamivir treatment probably reduced risks of severe influenza in young adult patients. However, guidelines on infection control need to emphasize on strict hygiene and prevention measures in treated patients for nine days in order to minimize the risks of influenza transmission.

Keywords: influenza A(H1N1)pdm09, viral shedding, outbreak, university, Thailand

Introduction

Knowledge on viral shedding from respiratory tract is an important factor in order to minimize transmission in community. In young adults infected by influenza A(H1N1)pdm09 virus with mild symptoms, a study in Singapore showed that mean duration of viral shedding was 6±2 days,[1] while studies in China and the United States reported 4-6 days.[2-4] In Thailand, studies on viral shedding of influenza A(H1N1)pdm09 revealed as five days (range 1-12 days) among military conscripts[5], seven days (range 2-14 days) in a school and 7.5 days (range 3-14 days) in a military camp[6].

Duration of viral shedding of influenza A(H1N1)pdm09 virus in children tended to be longer than that of young adults.[7-9] Moreover, various studies agreed that oseltamivir could effectively reduce the duration of viral shedding and prevent severe complications from the virus.[1,2,10] On the other hand, immunocompromised health status seemed to prolong shedding of respiratory viruses for weeks and even months.[11-13]

Since June 2009, numerous outbreaks of influenza A(H1N1)pdm09 in schools and colleges have been reported in Thailand. On 30 May 2011, influenza A(H1N1)pdm09 outbreak was detected among students in University S, Nakhon Ratchasima Province, the northeastern region of Thailand. In this outbreak, 455 out of 10,515 staff and students were affected, with an attack rate of 4.3% since 22 May 2011. Epidemiological control measures including health education, inhibition of mass gathering and case isolation had been in place on 31 May 2011.[14] All students were informed to visit the medical center of University S for influenza screening by rapid test. If one had positive result by the rapid test, that person was immediately provided with full course of oseltamivir. In Thailand, knowledge on viral shedding of young adult patients infected with influenza A(H1N1)pdm09 and received antiviral treatment remained limited.[5,6] This study was, therefore, conducted among university students infected with influenza A(H1N1)pdm09 and undergoing treatment at the medical center in order to describe characteristics of viral shedding and immunologic response, and identify factors associated with duration of viral shedding.

Methods

Study Design

A prospective observational study was conducted from June to September 2011 among random samples of students from University S. Participants were acute respiratory illness (ARI) cases who had attended the medical center at University S during 3-7 Jun 2011 and had laboratory confirmation of influenza A(H1N1)pdm09 infection by real-time reverse transcription polymerase chain reaction (rRT-PCR). ARI cases were defined as the persons who had any two of four symptoms: fever, runny nose, cough or sore throat. Fever was either measured body temperature of 38°C and above, or self-reported fever. We excluded persons with immunocompromised health status, such as HIV infection, or having steroids or anti-neoplastic drugs. On the enrollment day which was defined as day 0, all participants had a throat swab collected and completed a standardized questionnaire that included demographic characteristics, underlying diseases, height, weight, past history of influenza vaccination, signs and symptoms of respiratory illness, and treatment. Subsequently, throat swabs from each participant were collected daily until two consecutive swabs were negative for influenza A(H1N1)pdm09 virus by rRT-PCR or up to 10 days. Duration of viral shedding was defined as the time period between onset of symptoms and the first date of undetectable RNA by rRT-PCR.

Virological Methods

Throat swab specimens were tested by rRT-PCR for influenza A(H1N1)pdm09. Serum samples were tested for antibodies to influenza A(H1N1)pdm09 virus by hemagglutination inhibition (HI) assay using 0.5% turkey erythrocytes according to the standard protocols.[15] All serum samples were tested using the same batch of turkey erythrocytes to allow precise interpretation of the laboratory results. All the laboratory tests were performed at the Department of Microbiology, Faculty of Medicine, Siriraj Hospital.

Analysis and Statistics

We compared duration of virus shedding by selected characteristics, including gender, body mass index (BMI), underlying diseases, reported symptoms, time of receiving oseltamivir, history of having influenza or influenza vaccine since 2009, and immunologic response. All variables were tested by log-rank test. Moreover, geometric mean titers (GMT) with 95% confidence intervals (95% CI) were calculated from participants' HI titers.

Human Subjects Review

This study was approved by the Ethical Review Committee for Research in Human Subjects, Ministry of Public Health, Thailand (Number 755/2010). Written informed consent was obtained from every person participated in the study.

Results

Twenty nine participants with laboratory confirmed influenza A(H1N1)pdm09 infection by rRT-PCR were enrolled. Among them, 13 (45%) were males and median age was 20 years (interquartile range 19-21 years). Allergy was the most commonly reported underlying condition and was reported in five (17%) patients. There were five (17%) participants with history of having influenza or receiving influenza vaccination since 2009, indicating that they might have gained immunity in the past. Fever (86%) was the most frequently reported, followed by cough (79%), myalgia (72%) and sore throat (69%). Total 20 cases (69%) received antiviral treatment within two days after onset of symptoms while median time from onset of symptoms to treatment was one day (range 0-6 days) (Table 1).

Viral Shedding

Among 29 participants, mean duration of viral detection from onset of symptoms was six days (SD 2

days) and median was five days, with the range of 3-9 days (Figure 1).

Table 1. Characteristics of participants with acute respiratory illness (ARI) in University S, Nakhon Ratchasima Province, Thailand, 3-7 Jun 2011 (n=29)

Characteristic	Number	Percent
Male	13	45
Body mass index (BMI) ≥ 23kg/m^2	14	48
Underlying allergy	5	17
Previously healthy	24	83
History of having influenza or receiving influenza vaccine since 2009	5	17
Self-reported symptoms		
Fever	25	86
Cough	23	79
Myalgia	21	72
Sore throat	20	69
Runny nose	20	69
Headache	20	69
Dyspnea	8	27
Diarrhea	1	3
Treatment		
Received antiviral within 48 hours after onset of symptoms	20	69
Hospitalization or death	0	0

Overall, 83% of participants had undetectable influenza A(H1N1)pdm09 virus after day seven. Comparison on duration of shedding by selected patients' characteristics showed that no characteristics were significantly associated with the duration of viral shedding (Table 2).

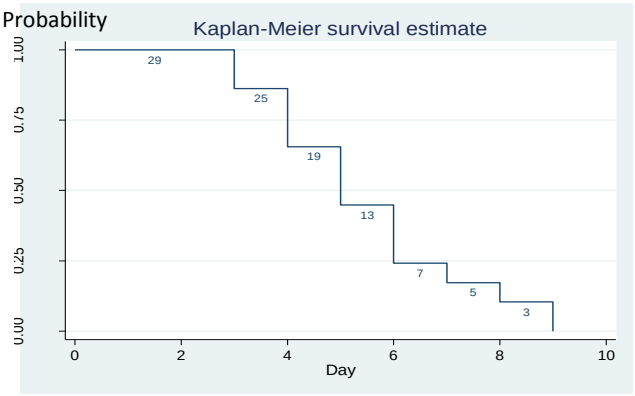

Figure 1. Kaplan-Meier plot showing probability of rRT-PCR positive influenza A(H1N1)pdm09 by days after onset of symptoms in University S, Nakhon Ratchasima Province, Thailand, 3-7 Jun 2011 (n=29)

Geometric Mean of HI Titer (GMT)

All the participants were not enrolled on the same day. Dates of enrollment were 0-6 days after their onset dates. On day 0 or enrollment day, GMT of all participants was 1:6 (95% CI = 1:5-1:7). On day 14 of enrollment, GMT rapidly increased to 1:63 (95% CI = 1:38-1:103), and 4-fold rise was reported on day 14 among 83% of the participants. GMT was 1:69 (95% CI = 1:44-1:106) on day 28 and 1:127 (95% CI = 1:86-1:162) on day 90, which tended to reach a steady stage (Figure 2).

Table 2. Comparison on median duration of viral shedding by selected patients' characteristics in University S, Nakhon Ratchasima Province, Thailand, 3-7 Jun 2011

Variable		Median duration of viral shedding (day)	P-value (Log-rank test)
Gender	Male	5	0.24
	Female	6	
BMI ≥ 23 kg/m^2	Yes	6	0.28
	No	4	
Underlying allergy	Yes	7	0.12
	No	5	
Self-reported fever	Yes	5	0.19
	No	4	
Received antiviral drug within 48 hours	Yes	5	0.09
	No	6	
History of influenza infection or influenza vaccination since 2009	Yes	7	0.44
	No	5	
4-fold rise by HI test within 14 days	Yes	6	0.54
	No	4	

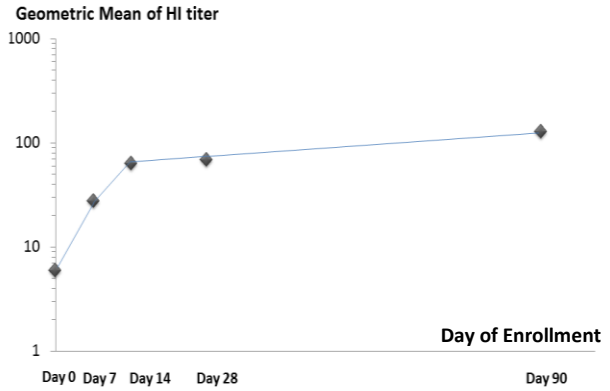

Figure 2. Geometric means of HI titer (GMT) at day 0 (enrollment day), day 7, day 14, day 28 and day 90 (n=29)

Discussions

Median duration of viral shedding of influenza A(H1N1)pdm09 in this university was compatible with the findings from other studies that reported 4-6 days in young adults treated with oseltamivir.[1-4] However, the median duration was shorter than that of the other study conducted in Nakhon Ratchasima Province which revealed as seven days[6]. In comparison of different settings, patients with mild symptoms usually had shorter duration of viral shedding than hospitalized patients.[16,17]

For immunologic response, HI titer rose rapidly 2-3 weeks after the patients were infected by influenza A(H1N1)pdm09. Then, the titer remained constant. By day 90, HI titer increased only slightly from the constant level because titer of some patients (14%) still had not reached 4-fold rise on day 28. In addition, we observed that the patients who had 4-fold rise in shorter time tended to have longer duration of viral shedding probably due to higher viral load. Nonetheless, the test showed no statistical significance and viral load were not measured in this study.

Regarding factors associated with long duration of viral shedding, we observed that duration among those who received antiviral treatment within 48 hours after onset of symptoms was not significantly shorter than those with late treatment (P-value = 0.09). Furthermore, the longest duration of four and six days after their onset dates was observed in two out of three patients receiving late antiviral treatment. On the other hand, viral shedding was extended among those with BMI of 23kg/m^2 and more, underlying allergy and reported fever though the differences were not statistically significant. The statistically insignificant association with patients' characteristics might be due to small sample size.

Even though the actual isolation for seven days could result in effective control of influenza transmission in this outbreak,[14] this study underlined the need to reconsider two additional days to cover 17% of the patients with longer viral shedding.

Limitations

Though we did not obtain the actual first day of viral shedding, this study reported viral shedding as the duration between onset of symptoms and the first date of undetectable RNA virus. Sample size was relatively small since it was not designed to analyze factors associated with long viral shedding. Meta-analysis might be necessary to demonstrate variables associated with viral shedding. Viral load was not measured due to financial limitation. Generalization of the study was limited only to healthy young adult patients receiving oseltamivir. Finally, we were able to follow up all enrolled patients for 28 days. Nonetheless, eight of 29 patients (27%) were lost to follow up for serum collection on day 90.

Conclusions

Duration of viral shedding of influenza A(H1N1)pdm09 among patients treated with oseltamivir in this university was 3-9 days (median five days). Immunologic response was noticed about 2-3 weeks after getting infection and then the HI titer tended to be constant. Early antiviral treatment within 48 hours was identified as a factor that probably reduced the duration of viral shedding, yet not showing any statistical significance. In addition, factors those probably lead to long viral shedding included high BMI, underlying allergy and reported fever. Guidelines on infection control need to emphasize on practice of strict hygiene and prevention measures, such as isolation of treated patients for nine days to minimize risks of influenza transmission, particularly those with obesity or underlying allergy. The study demonstrated the benefit of early antiviral treatment in preventing severe outcome of influenza A(H1N1)pdm09 infection and shortening the viral shedding time.

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Suggested Citation

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