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Pandemic (H1N1) 2009 virus infection: Persistent viral shedding after Oseltamivir treatment

Sawan Kanchana [a], Supannakhon Kanchana [a], Charas Chuntrakul [a], Kumthorn Malathum [b], Slinporn Prachayangprecha [c], Yong Poovorawan [c,*]

[a] *Thungsong Hospital, Nakhon Srithammarat, Thailand*
[b] *Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*
[c] *Center of Excellence in Clinical Virology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

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Summary *Objectives:* To study pandemic (H1N1) 2009 virological outcomes after Oseltamivir treatment in confirmed cases of pandemic (H1N1) 2009 virus infections. A hospital-based cohort study was conducted in south Thailand, between June and September 2009.
Methods: Throat/swab specimens were tested by real-time reverse transcriptase polymerase chain reaction (rRT-PCR) for pandemic (H1N1) 2009. All 357 confirmed cases (122 inpatients, 235 outpatients), whose received a 5-day Oseltamivir treatment. Post-treatment virological follow-up was performed in 91 eligible cases. The NA gene was screened for the H275Y mutation responsible for Oseltamivir resistance.
Results: Thirty-three of 91 patients (36%) had underlying diseases. The duration from the onset of illness to the detection of virus ranged 1–14 days (median 3 days). The rRT-PCR was positive on day 5 of treatment in 24 of 91 patients (26%). Patients with underlying diseases had a higher proportion of post-treatment positive test than those without underlying diseases (15/33 vs 9/58). The rRT-PCR-confirmed viruses detected in all 125 throat swab specimens did not show evidence suggesting Oseltamivir resistance.
Conclusions: Prolonged presence of pandemic (H1N1) 2009 detected by rRT-PCR was found. An extended course of antiviral treatment should be considered in patients with underlying diseases and severe clinical symptoms.

* Corresponding author. Tel.: +66 2 256 4909; fax: +66 2 256 4929.
E-mail address: Yong.P@chula.ac.th (Y. Poovorawan).

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Introduction

Although there are some published reports on the duration of shedding of seasonal influenza virus after treatment with Oseltamivir, data relevant to the pandemic (H1N1) 2009 virus strain in this regard are sparse.[1] In early reports, the median time of pandemic (H1N1) 2009 viral shedding determined by reverse transcriptase polymerase chain reaction (RT-PCR) was 6 days.[2,3] The estimated duration of viral shedding is based upon seasonal influenza virus infection. Infected persons should be assumed to be contagious for up to 7 days from illness onset.[1] Factors associated with duration of viral shedding after antiviral therapy have not been identified. Unlike with seasonal influenza strains, resistance to Oseltamivir has not yet become a major clinical problem. However, as the virus can potentially develop resistance to Oseltamivir at any time, surveillance for Oseltamivir resistance in pandemic (H1N1) 2009 viruses should continue.

In Thailand, the first case of laboratory-confirmed pandemic (H1N1) 2009 virus was found on May 12, 2009.[4] The first case in south Thailand was identified in Thungsong Hospital, Nakhon Srithammarat Province on May 28, 2009. We followed patients seen at this hospital to determine clinical response, duration of viral shedding after treatment, and the development of resistant virus.

Materials and methods

The study was approved by the Ethical Committee of the Thoungsong Hospital. The pandemic (H1N1) 2009 positive patients were chosen from among the patients during the investigation and treatment of influenza like illness (ILI). The objective of the study was informed and written consents were obtained from the participants and/or parents.

Study participants

The hospital-based, prospective cohort study included all patients (outpatients and inpatients) seen at Thungsong Hospital from June to end of September 2009 with at least two symptoms such as fever, cough, sore throat, rhinorrhea, headache, muscle pain, malaise, diarrhea or vomiting and confirmed infected by pandemic (H1N1) 2009 virus by real-time reverse transcription PCR (rRT-PCR) assay. All patients with confirmed pandemic (H1N1) 2009 virus infection received a 5-day course of Oseltamivir treatment. Pandemic (H1N1) 2009 virus infection was defined by World Health Organization criteria.[5] Chest radiography was performed on all patients.

Oseltamivir treatment

All the studied patients were treated with Oseltamivir (The Government Pharmaceutical Organization, Bangkok, Thailand) with the dosage as US-CDC recommendation.[6]

Data collection

The patients were clinically assessed and cared for by doctors and nurses. Basic demographic information, as well as symptoms before and after Oseltamivir treatment, was recorded on a standard data collection form. After discharge, telephone follow-up was used to follow patients until all symptoms disappeared.

Laboratory methods

Throat swabs were collected from patients meeting the clinical case definition. These samples were obtained as part of clinical service and the national public health surveillance for pandemic (H1N1) 2009 in Thailand. A follow-up throat swab specimen was obtained for patients who were positive for pandemic (H1N1) 2009 by rRT-PCR; this second sample was taken the day after completion of the 5-day Oseltamivir treatment course. The samples were transferred into 2 mL of virus transport medium with antibiotics (Penicillin G 2×10^6 U/liter and Streptomycin 200 mg/L) and stored in a biohazard icebox for transportation to the Center of Excellence in Clinical Virology, Faculty of Medicine, Chulalongkorn University. The collected specimens were kept at 4 °C, and processed within 24 h. On a voluntary basis, more frequent serial throat swab samples were collected for rRT-PCR testing and continued every other day until a negative test result was achieved. In addition, the NA gene was tested for the H275Y mutation responsible for Oseltamivir resistance by rRT-PCR with specific TaqMan probes.

RNA extraction

The specimens were processed immediately upon arrival. RNA extraction was performed using the Viral Nucleic Acid Extraction Kit (RBC Bioscience Co, Taipei, Taiwan) according to the manufacturer's protocol. All experiments were performed in a Bio-safety Level 2 plus environment.

Detection and subtyping of influenza virus by rRT-PCR

To detect and sub-type influenza virus, our group performed two separate reactions of single step multiplex real-time RT-PCR (rRT-PCR) based on specific TaqMan probes. The first reaction aimed at detecting the GAPDH gene (internal control), and the matrix genes of influenza A and B virus in order to determine quality of the extracted RNA as well as to discriminate between influenza A and B. The second reaction was designed for detecting seasonal human influenza A virus (subtypes H1 and H3) and avian influenza A virus (sub-type H5).[7] The HA primers and probes used for the detection of pandemic (H1N1) 2009 virus were as described by the CDC.[8] The rRT-PCR was performed using the SuperScript III Platinum One-Step RT-PCR system (Invitrogen, Carlsbad, CA). The reaction comprised 2.0 μL of RNA sample combined with a reaction mixture containing 5 μL of 2-Reaction Mix, 0.2 μL of SuperScript III RT Platinum® Taq Mix (Invitrogen, Carlsbad, CA), each primer and probe at a final concentration of 0.25 μM and 0.125 μM, respectively and RNase-free water in a final volume of 10 μL. One-step multiplex rRT-PCR was performed by a Rotor-Gene 3000 (Corbett Research, New South Wales, Australia). Thermal cycling conditions included a reverse transcription