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Prevalence of Oseltamivir-Resistant 2009 H1N1 Influenza Virus among Patients with Pandemic 2009 H1N1 Influenza infection in NRITLD, Tehran, Iran

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ABSTRACT

Background: *Oseltamivir-resistant cases were reported during the 2009 pandemic influenza outbreak and therefore, widespread emergence of oseltamivir-resistant 2009 H1N1 virus is imaginable. Underlying medical conditions like immunosuppression increase the chance of oseltamivir resistance.*

Materials and Methods: *In a retrospective cross-sectional study, respiratory tract specimens of confirmed cases of 2009 H1N1 influenza referred to the Masih Daneshvari Hospital were analyzed for presence of H275Y mutation.*

Results: *From November 2009 through March 2010, oseltamivir- resistant 2009 H1N1 infection was observed and confirmed in 4 patients (including 2 immunocompromised patients) by performing H275Y mutation molecular testing.*

Conclusion: *Close monitoring of resistance to neuraminidase inhibitors is essential in tertiary care centers. The H275Y mutation (oseltamivir-resistant genotype) could appear in the absence or presence of selective drug pressure.* ***(Tanaffos2011; 10(1): 8-11)***

Key words: *Pandemic 2009 H1N1 virus, H275Y mutation, Oseltamivir-resistance*

INTRODUCTION

The 2009 pandemic influenza A (H1N1) virus that contains a novel combination of genes of swine origin has spread globally since April 2009 (1). On June 11, 2009 due to the global spread of the novel influenza A (H1N1) virus, the World Health Organization (WHO) raised the global pandemic alert level to phase 6 (2). The 2009 H1N1 influenza virus pandemic has highlighted the difficulties faced by clinicians in managing severe influenza A, especially for those at high risk of severe disease.

Oseltamivir, one of the neuraminidase inhibitors (NAIs), has been the drug of choice for treatment of influenza (1). Historically, rapid emergence and

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widespread dissemination of oseltamivir-resistant influenza A viruses have been observed in seasonal H1N1 outbreaks (3-5). Oseltamivir-resistant infection with the 2009 pandemic influenza A (H1N1) virus that is conferred by the H275Y substitution in the neuraminidase (NA) has so far been described in 298 patients by the WHO (5).

***Setting*:** Masih Daneshvari Hospital is the largest tertiary health care center for patients with respiratory diseases in Iran. During the outbreak of pandemic 2009 H1N1 influenza, this hospital was a referral center for H1N1 cases in Tehran and neighboring cities aiming at controlling the pandemic. Diagnosis and virological evaluation of respiratory tract specimens were done in Virology Research Center (VRC).

This study aimed at determining the prevalence of oseltamivir-resistant 2009 pandemic H1N1 virus infection among patients referred to Masih Daneshvari Hospital.

MATERIALS AND METHODS

Study design and study population

A retrospective cross-sectional study was conducted among confirmed cases of 2009 H1N1 influenza referred to Masih Daneshvari Hospital from November 2009 through March 2010. Different types of respiratory tract specimens (including; nasal aspirates, throat swabs, bronchial washings, tracheal washing, bronchoalveolar lavage (BAL), and pharyngeal washing) were analyzed for presence of oseltamivir resistant 2009 pandemic influenza virus.

Laboratory confirmation

Cases of 2009 H1N1 influenza were confirmed by testing their respiratory specimens using a real-time reverse-transcriptase-polymerase-chain-reaction (rRT-PCR) assay at laboratories of Virology Research Center (VRC) according to the protocols recommended by the U.S. Center for Disease Control and Prevention (CDC) (6).

Drug resistance confirmation

The Viral load and H275Y mutation were determined using the Primer Design Quantification of Swine H1N1 Influenza Human Pandemic Strain kit and Primer Design Tami flu® resistance genotyping kit (Primer Design TM Ltd, UK), respectively.

RESULTS

From November 2009 through March 2010, the Virology Research Center (VRC) received 414 respiratory tract specimens from patients with influenza-like illnesses. Out of which, a total of 51 samples were positive for the 2009 H1N1 virus. Approximately half the patients were co-infected with seasonal influenza A. The H275Y substitution was detected in 4 patients as a result of drug resistance genotyping test (Figure 1).

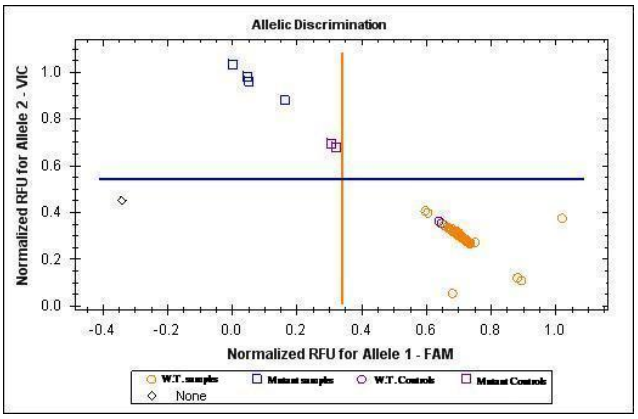

Figure 1. Allelic discrimination of pandemic 2009 H1N1 influenza virus for H275Y mutation (oseltamivir- resistant genotype)

Patients' description:

Patient A: A 50 year-old man (known case of multiple myeloma with a history of bone marrow transplantation rejection 6 months ago) was admitted to the hospital complaining of fever and respiratory distress. He had history of mild fever, cough and myalgia started one week before the admission. On radiological examination, bilateral interstitial and alveolar infiltrations were seen. Treatment with

broad spectrum antibiotics and oseltamivir was initiated after obtaining blood culture and respiratory specimen for 2009 pandemic influenza A (H1N1) virus. The tracheal aspirate was positive for 2009 pandemic influenza A (H1N1) virus and the viral load in the sample was 86,020 copies/ ml. Due to respiratory failure, the patient was intubated and transferred to the ICU. As fever persisted after receiving antiviral treatment, second specimen (tracheal aspirate) was obtained and it was still positive for the 2009 pandemic influenza A (H1N1) virus. The viral load of the sample was 71,944 copies/ml. Treatment with zanamivir was initiated but unfortunately the patient passed away on day 12 after admission due to multiple organ dysfunctions. Drug resistance genotyping test was conducted retrospectively and the H275Y substitution was detected in both clinical specimens. Further studies showed seasonal influenza A co-infection in both his specimens.

Patient B: A 30 year-old man was admitted to the emergency department due to respiratory distress and hemoptysis. Although high grade fever, myalgia and cough were present since a week ago, exacerbation of dyspnea and massive hemoptysis occurred a day before admission. The patient was a known case of common variable immunodeficiency syndrome with irregular medical follow-up. Chest X-ray showed bilateral alveolar infiltration compatible with acute respiratory distress syndrome. He was admitted to the ICU, specimens were obtained for laboratory tests, and treatment with oseltamivir and broad spectrum antibiotics was commenced. The sputum sample was positive for the 2009 pandemic influenza A (H1N1) virus. The viral load in the sample was 10,944 copies/ml. Due to low level of immunoglobulin, intravenous immunoglobulin (IVIG) was prescribed for the patient. After one week of treatment, oxygenation was improved and the patient was transferred to the respiratory infection ward. However, he had intermittent fever and dyspnea on exercise. After two weeks, throat swab (second sample) was still positive for the 2009 pandemic influenza A (H1N1) virus and the viral load in the sample was 25,455 copies /ml. Second course of oseltamivir was prescribed. After one month of admission, the patient was discharged with negative throat specimen for 2009 H1N1 virus in good clinical condition. Subsequent studies revealed that only the second sample had H275Y mutation and contained the oseltamivir resistant virus, as it was positive for seasonal influenza A virus.

Patient C: A 29 year-old man presented to the emergency department of Masih Daneshvari Hospital with dyspnea, hemoptysis and vomiting in November 2009. Because of respiratory distress and hypoxia he needed mechanical ventilation and admitted to the ICU. Dialysis was done due to acute renal failure. Respiratory tract specimen was obtained and antiviral treatment was started immediately. Although he presented with high creatinine level and severe respiratory distress, after receiving antiviral treatment the symptoms improved gradually and he was discharged after 11 days.

The respiratory tract specimen was positive for the 2009 pandemic influenza A (H1N1) virus. Further studies revealed the sample had H275Y mutation and contained the mutant subtype of the virus.

Patient D: A 76 year-old asthmatic woman was presented to ER with productive cough and dyspnea. She was admitted to the ICU due to hypoxemia and loss of consciousness. As she was receiving mechanical ventilation, her tracheal aspirate was obtained and evaluated for 2009 H1N1 influenza virus. Empirical treatment with broad spectrum antibiotics and oseltamivir was initiated immediately. The tracheal aspirate was positive for 2009 pandemic influenza A (H1N1) virus. Fever persisted after receiving antiviral treatment. Thus, the second specimen (tracheal aspirate) was obtained and it was still positive for the 2009 pandemic influenza A (H1N1) virus. Second course of oseltamivir was prescribed. Although she was under treatment with oseltamivir, she passed away on day fifteen of

admission.

Drug resistance genotyping test was conducted retrospectively and the H275Y substitution was detected in the second specimen.

DISCUSSION

Oseltamivir-resistant infection with the 2009 pandemic H1N1 virus has rarely been reported. But by the use of oseltamivir in current pandemic flu, the widespread emergence of oseltamivir- resistant 2009 H1N1 virus (similar to what was happened with seasonal influenza virus) is imaginable (7, 8). To our knowledge, we report the first description of patients infected with oseltamivir-resistant 2009 H1N1 viruses in Iran. First study in early phase of 2009 pandemic influenza in Iran revealed no genetic relation to neuraminidase inhibitor resistance (9).

Some patients with oseltamivir- resistant infection were not receiving oseltamivir when the resistant virus was detected (8,10,11). This study showed the H275Y mutation (oseltamivir-resistant genotype) could appear in the absence or presence of selective drug pressure. However the oseltamivir- resistant virus was detected before initiating oseltamivir treatment in two patients (patient A and patient C), in other patients the resistant virus was detected after prescription of oseltamivir.

The emergence of oseltamivir resistance in 2009 H1N1 viruses highlighted the need for drug resistance genotyping. Especially in tertiary care centers we should consider performing rapid diagnostic tests for drug resistance genotyping for high risk patients. Based on the results of patient testing, appropriate usage of available anti-viral medications may be recommended.

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