Oseltamivir-resistant influenza A(H1N1) pdm09 virus: first reported case from India

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ABSTRACT

Background: Recent studies on antiviral susceptibility from South-East Asia, Europe and the United States have shown sporadic neuraminidase inhibitor (NAI) resistance in A(H1N1)pdm09 viruses. We undertook a study to evaluate NAI resistance in these viruses isolated in India.

Methods: Pandemic influenza viruses, isolated from 2009 to 2013, along with clinical samples were genetically analysed for known resistance markers in the neuraminidase (NA) gene. Clinical samples (n=1524) were tested for H275Y (N1 numbering; H274Y in N2 numbering) mutation by real time reverse transcriptase PCR (rRT-PCR). One hundred and ten randomly selected resistant and sensitive viruses were analysed by phenotypic assay.

Results: All but one of the 2013 A(H1N1)pdm09 isolates were sensitive to oseltamivir. Genetic analysis of this isolate as well as the original clinical material showed that the presence of H275Y mutation was responsible for reduced susceptibility to oseltamivir in the patient. This was confirmed by phenotypic assay.

Conclusion: The emergence of a pandemic influenza strain resistant to oseltamivir emphasizes the need for monitoring antiviral resistance as part of the National Influenza Programme in India.

Key words: pandemic influenza (H1N1) 2009, oseltamivir resistance, influenza surveillance, India

INTRODUCTION

During April 2009, a novel A(H1N1) virus was detected in epidemiologically unrelated cases of influenza-like illness in California, United States of America and Mexico. Its rapid spread prompted the World Health Organization (WHO) to declare a pandemic caused by this virus on 11 June 2009.1 Given the overwhelming demand for testing, WHO guidance in June 2009 recommended a priority focus on reporting fatal and severe cases2 and thus, when the pandemic was declared over in August 2010 the reported numbers of cases and deaths at <1 million and >184 497 respectively3 were far below the real burden due to A(H1N1)pdm09.

The influenza A(H1N1)pdm09 virus was found to be susceptible to neuraminidase inhibitors (NAI) (oseltamivir and zanamivir) and resistant to adamantanes (amantadine and rimantadine).1 NAI resistance in A(H1N1)pdm09 viruses is rare; nevertheless it was first reported in June 20094 and since then a total of 570 confirmed oseltamivir-resistant cases have been reported worldwide.5 All these viruses have substitution H275Y (N1 numbering; H274Y in N2 numbering) in the NA gene, which is a known cause for clinical resistance to oseltamivir but not to zanamivir. In India, an enhanced surveillance system for A(H1N1)pdm09 was set up in April 2009 and continued until 2013. Clinical specimens from various states in India were referred to the National Influenza Center (National Institute of Virology), Pune for diagnosis.

At the start of the pandemic, antiviral drugs were given to all suspected cases in addition to all cases rRT-PCR-positive for A(H1N1)pdm09 virus and their contacts. In the post-pandemic period, the strategy was changed by the Government, and antiviral drugs were given only to symptomatic high-risk patients and severe hospitalized cases. Since antivirals were widely used, it was relevant to screen isolates and clinical samples for susceptibility to oseltamivir. We report one case of oseltamivir-resistant A(H1N1)pdm09 virus infection in Pune.
METHODS

All clinical samples were tested for A(H1N1)pdm09 virus using the United States Centers for Disease Control and Prevention (CDC) protocol. The clinical history of all patients was recorded. Of the 50232 referred specimens, 10035 (19.98%) were positive for A(H1N1)pdm09. Of these, 1557 were processed for virus isolation in the MDCK cell line according to the WHO Manual on Animal Influenza Diagnosis and Surveillance; A(H1N1)pdm09 was isolated from 530 of these samples.

Monthly representative A(H1N1)pdm09-positive clinical samples (n=1524) were selected and screened for the presence of H275Y mutation by allelic rRT-PCR. These included severe, hospitalized recovered as well as fatal cases. Using published primers, 496 isolates were processed for NA and haemagglutinin (HA) gene sequencing to evaluate other possible mutations. 50 ul of clinical specimens or isolates were used for viral RNA isolation using the Ambion MagMAX™ kit as per the manufacturer’s procedures. DNA sequences of NA and HA genes were done using the BigDye® Terminator Cycle Sequencing Kit and an Applied Biosystems® 3730 DNA Analyzer. Sequences were analysed using the Mega Version 5 sequence analysis tool. Whole genome sequence analysis of the resistant virus was done and compared with globally circulating resistant strains.

Phenotypic (neuraminidase inhibition) assay

A fluorescence-based NA inhibition assay was performed for 110 randomly selected pandemic viruses, including resistant strains, to determine the dose of oseltamivir required for 50% inhibition of NA activity (IC50 value). Oseltamivir carboxylate (GS4071) was provided by Hoffmann-La Roche, Basel, Switzerland. The reference viruses and WHO NAI standard operating procedures were provided by the WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia.

RESULTS

Only one clinical sample – from patient X – of the 1524 assayed was not susceptible to oseltamivir. Full NA gene sequencing of the patient X isolate with its companion clinical sample showed H275Y mutation; S182T, N/D248 G and V453 M were also present. Whole genome sequence analysis of this resistant virus revealed no major deviation in signature or pathogenicity markers. Other known amino acid changes responsible for drug resistance in vitro (D199N, I223R, N295S) were not detected in any of the isolates.

In the fluorescence-based NAI assays, oseltamivir susceptible A(H1N1)pdm09 viruses had an IC50 range between 0.5–1.18 nM, whereas the IC50 value of the oseltamivir-resistant A(H1N1)pdm09 virus isolated from patient X was 347 nM.

Patient X was a 42-year-old female resident of a slum area of Pune, with a history of hypertension with seizure disorder and a cerebrovascular event. She presented with high-grade fever for 5–6 days, rhinorrhea, cough, pain in throat, breathlessness, severe headache and was admitted in the hospital on 27 January 2013. A nasopharyngeal swab, obtained before oseltamivir treatment was initiated, was sent to the National Institute of Virology. She had an uneventful recovery and was discharged on 4 February 2013.

By way of proactive vigilance after the oseltamivir-resistant case was detected, hospital staff who had been in close contact with patient X, patients who shared the same ward, family members, and persons with influenza-like illness living in the neighbourhood of the patient, were investigated for pandemic influenza and all tested negative. Influenza patients admitted in other hospitals during the same time period were also investigated; all samples positive for influenza virus were sensitive to NAI.

DISCUSSION

Our findings show a sporadic case of oseltamivir resistance in a patient who recovered uneventfully. This is the first reported case from India. Genetic characterization of the clinical sample and isolate showed resistance to oseltamivir with H275Y substitution in NA and increased IC50 values in the phenotypic assay. This resistant virus could have been the result of a random process of viral drift or could have seeded from outside the country. The resistance observed cannot be attributed to drug-driven selective pressure as H275Y mutation was detected in the clinical specimen collected before administration of oseltamivir. No evidence of transmission of the resistant virus could be found. Whole genome analysis of the resistant virus showed no major deviation in signature/pathogenicity marker mutations. In addition to H275Y mutation, S182T, N/D248 G and V453 M were found in the NA gene. However, the relevance of these mutations in drug susceptibility is unknown.

Our findings demonstrate that oseltamivir-resistant A(H1N1) pdm09 viruses exist in sporadic situations. This suggests that continuous surveillance is required to evaluate the emergence and circulation of drug-resistant A(H1N1)pdm09 viruses and to identify possible potential mutations responsible for drug resistance.

REFERENCES


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