

Comparisons of oseltamivir-resistant (H275Y) and concurrent oseltamivir-susceptible seasonal influenza A(H1N1) virus infections in hospitalized adults, 2008–2009

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In an observational cohort study, we found that adults hospitalized for oseltamivir-resistant (H275Y) seasonal H1N1 influenza ($n = 46$) were older than those infected with oseltamivir-susceptible strains ($n = 31$) [74(IQR 59–83) versus 64(IQR 48–76) years; $P = 0.045$], and most had major comorbidities (78% versus 65%). Disease severity and clinical outcomes were comparable between the two groups: radiographic pneumonia 40–42%, supplemental oxygen use 47–48%, critical

illness 11–13%, median duration of hospitalization 5–6 days, death rate 6–9%. Failure to receive effective antiviral therapy was associated with progression to critical illness (23% versus 0%, $P = 0.016$) and death (20% versus 0%, $P = 0.033$) in hospitalized patients with seasonal H1N1 influenza.

Keywords H275Y, oseltamivir, seasonal influenza A(H1N1) virus.

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Background

The His²⁷⁵->Tyr²⁷⁵ (H275Y, N1 numbering) mutation in neuraminidase (NA) gene of influenza virus is associated with phenotypic resistance to oseltamivir. Earlier studies have suggested that seasonal influenza A(H1N1) viruses harboring the mutation had reduced virulence and infectivity and therefore unlikely to cause widespread transmission.¹ However, starting from the 2007–2008 season, community transmission of an ‘oseltamivir-resistant’ (H275Y) seasonal A(H1N1) virus, which belonged to hemagglutinin (HA) clade 2B (A/Brisbane/59/2007-like) occurred worldwide.² In Hong Kong, the resistant virus was first detected in early 2008, and by year end, it had already replaced (97.8%) the ‘oseltamivir-susceptible’ H1N1 strains and continued to circulate until March 2009.³ Experimental studies using cell culture and ferret models have shown that this newly emerged H275Y mutant virus might have retained its replication fitness,^{4,5} possibly through accumulation of permissive, compensatory

mutations (e.g., R222Q, V234M, and D344N).⁶ However, whether naturally occurring infection by this virus had comparable severity to the ‘oseltamivir-susceptible’ strains and whether antiviral treatment might affect clinical outcomes are unclear.⁷ In this study, we compared the clinical and virological characteristics, disease severity, and outcomes between adults hospitalized for ‘oseltamivir-resistant’ (H275Y) and ‘oseltamivir-susceptible’ seasonal influenza A(H1N1) virus infections.

Study design

An observational cohort study on adults (aged ≥ 18 years) hospitalized for seasonal A(H1N1) influenza virus infections was conducted in two acute-care general public hospitals in Hong Kong between January 2008 and March 2009. The period covered three seasonal peaks: January–March and June–August 2008, and January–March 2009. Locally, the A/H1N1pdm09 pandemic began in June; these patients were reported separately.⁸ Admission, diagnostic

procedures, and antiviral prescriptions for our seasonal influenza patients had been described.⁹ Briefly, nasopharyngeal aspirates (NPAs) were collected at presentation, regardless of perceived etiology/severity, for rapid diagnosis using an immunofluorescence assay. Virus isolation was performed in parallel using MDCK cells. All virus isolates were sent to the National Influenza Centre at the Centre for Health Protection in Hong Kong for H1/H3 virus subtyping. Patients with influenza A(H1N1) were further studied. The original NPAs were subjected to sequencing analysis of the viral NA gene to detect the oseltamivir-resistance-associated H275Y mutation. Viral loads in the NPAs were determined using real-time RT-PCR, targeting the matrix-(M)-gene. Local data indicated that isolates with H275Y mutation were phenotypically oseltamivir-resistant, and nearly all mutants of this lineage (HA clade 2B) were susceptible to adamantanes and zanamivir.³ Isolates negative for such mutation were considered 'oseltamivir-susceptible'; additionally, a segment of the HA gene was sequenced for phylogenetic analysis to determine their virus clade.³ Ethics approval was obtained from our institutional review boards.

Results

Altogether 77 hospitalized patients with seasonal influenza A(H1N1) infections were studied. Their median age was 69 [interquartile range (IQR), 57–81] years. No patient had received antiviral treatment prior to hospitalization. The 'oseltamivir-resistant' (H275Y) virus was found in 46 (60%) patients. Its detection frequency increased from January to March 2008 to June–August 2008 and peaked at January–March 2009 (2/9 [22%], 9/33 [27%] and 35/35 [100%], respectively; $P < 0.0001$). Comparisons of clinical/virological variables between patients infected with 'oseltamivir-resistant' and 'oseltamivir-susceptible' strains are shown in Table 1. Patients with 'oseltamivir-resistant' virus infections were significantly older [74 (IQR, 59–83) versus 64 (48–76) years; $P = 0.045$]; most of them had major comorbidities (78% versus 65%; $P = 0.203$), and nasopharyngeal viral loads at time of presentation were lower [median 5.9 (IQR, 5.3–6.6) versus 6.6 (6.1–7.1) log₁₀-RNA copies/ml, Mann–Whitney, $P = 0.001$; i.e. ~0.7 log lower] (Figure 1). Multivariate linear regression showed that 'oseltamivir-resistant' virus infection was independently associated with a lower viral load (β , -0.715; 95%CI -1.125, -0.305; $P < 0.001$), adjusted for age, comorbidity, time from illness onset, and disease severity. Overall, 59.7% of patients received antiviral treatment upon diagnosis (oseltamivir alone, oseltamivir plus amantadine, or zanamivir alone) (Table 1).

Patients with 'oseltamivir-resistant' and 'oseltamivir-susceptible' seasonal A(H1N1) influenza had similar rates of

pneumonia (40–42%), supplemental oxygen use (47–48%), ICU admission and/or death (11–13%), and duration of hospitalization (median 5–6 days). Altogether, 6 (7.8%) patients died. Fatality occurred exclusively among those who received no antiviral or an antiviral to which the virus was resistant ('oseltamivir-resistant' group: no antiviral, $n = 2$; oseltamivir alone without amantadine, $n = 2$; 'oseltamivir-susceptible' group: no antiviral, $n = 2$) (13% versus 0%, Fisher's exact test, $P = 0.076$). No ICU admission or death was observed among patients in the 'oseltamivir-resistant' group who had received zanamivir or amantadine as part of the antiviral regimen. Among high-risk patients aged >60 years, no antiviral treatment or use of an antiviral to which the virus was resistant was significantly associated with ICU admission/death (23% versus 0%, $P = 0.016$) or death (20% versus 0%, $P = 0.033$).

Discussion

Our data showed that both 'oseltamivir-resistant' (H275Y) and 'oseltamivir-susceptible' seasonal A(H1N1) caused severe and fatal diseases among hospitalized adults. Failure to receive antiviral or an antiviral agent to which the virus was susceptible was associated with adverse clinical outcomes. It is important for clinicians to consider the issue of antiviral resistance while managing severe influenza infections. Real-time surveillance data are essential.

Our findings are consistent with the reports on ambulatory patients which showed that symptoms and complication rates of 'oseltamivir-resistant' and 'oseltamivir-susceptible' seasonal A(H1N1) influenza are generally comparable.^{2,7,10} We add that among hospitalized adults, the major clinical outcomes including length-of-stay, ICU admission, and mortality rates are also similar.¹¹ Notably, we found that the 'oseltamivir-resistant' seasonal A(H1N1) virus had predominantly affected the older, compromised individuals, an observation consistently reported across geographic regions.^{2,7,11} The nasopharyngeal viral loads were also shown to be lower in such patients, although the difference was considered rather small (<1 'log'). These findings remain to be explained, as in cell culture and ferret models this recent H275Y mutant virus does not appear to have impaired replicative capacity and viral fitness.^{4,5} Whether host factors (e.g., innate/adaptive immunity) have played a role in naturally occurring human infections is uncertain. We were unable to analyze the impact of influenza vaccination because of incomplete data; however, the vaccination rate in our hospital cohort, and Hong Kong in general, had been shown to be very low (<20%).^{9,12} Importantly, our data indicated that the 'oseltamivir-resistant' strain is capable of causing severe and even fatal diseases, and in such patients, failure to receive an effective antiviral treatment may lead to adverse

Table 1. Comparisons of 'oseltamivir-resistant' (H275Y) and concurrent 'oseltamivir-susceptible' seasonal influenza A(H1N1) virus infections in hospitalized adults, Hong Kong, 2008–2009

Clinical variables	Seasonal H1N1, oseltamivir-resistant (H275Y) (n = 46)	Seasonal H1N1, oseltamivir-susceptible* (n = 31)	P value
Baseline characteristics			
Age – median (IQR), year	74 (59–83)	64 (48–76)	0.045
Sex, female	22 (48)	14 (45)	1.000
Underlying medical conditions**	36 (78)	20 (65)	0.203
Onset-to-presentation interval, days			
Median (IQR), day	1 (1–2)	1 (0–2)	0.547
Within 2 days after onset	42 (91)	27 (87)	0.707
NPA viral load – median (IQR), log ₁₀ RNA copies/ml***	5.9 (5.3–6.6)	6.6 (6.1–7.1)	0.001
Influenza-related respiratory complications†			
Any	32 (71)	21 (72)	1.000
Pneumonia	19 (42)	12 (40)	1.000
Antiviral treatment††			
Any	31 (67)	15 (48)	0.105
Oseltamivir alone	16 (35)	15 (48)	0.248
Oseltamivir plus amantadine	12 (26)	0 (0)	0.001
Zanamivir alone	3 (7)	0 (0)	0.269
Within 2 days after onset	21 (68)	11 (73)	1.000
Supplemental oxygen use for hypoxemia	21 (47)	14 (48)	1.000
Intensive care unit admission	1 (2)	3 (10)	0.297
Death†††	4 (9)	2 (6)	1.000
ICU admission and/or Death	5 (11)	4 (13)	1.000
Length of hospital stay among survivors – median (IQR), day	6 (4–8)	5 (3–8)	0.224

Data represent numbers of patients with percentages in parentheses, unless otherwise specified. ICU, intensive care unit; IQR, interquartile range; NPA, nasopharyngeal aspirate. Univariate comparisons were performed using Fisher's exact and Mann–Whitney *U* tests, wherever appropriate.

*During the study period, 2 major HA clades of 'oseltamivir-susceptible' seasonal influenza A(H1N1) viruses were co-circulating locally [clade 2B, A/Brisbane/59/2007-like, susceptible to adamantanes (*n* = 7); clade 2C, A/Hong Kong/2652/2006-like, resistant to adamantanes, (*n* = 24)].³

**These included major systemic comorbidities (cerebrovascular, neoplastic, chronic liver and renal diseases, congestive heart failure, ischemic heart diseases, diabetes mellitus, autoimmune disorders, and immunosuppressant therapy) and chronic lung diseases (asthma, chronic obstructive pulmonary diseases, bronchiectasis, and pulmonary fibrosis). Patients could have more than one comorbidities.

***NPA viral load in patients with critical illness (ICU admission and/or death) was lower in the 'oseltamivir-resistant' influenza group [median 5.3 (IQR, 5.1–7.1) versus 7.1 (6.3–8.1) log₁₀ PCR copies/ml, *P* = 0.060].

†These included radiographic pneumonia and acute airway diseases (exacerbation of asthma or chronic obstructive pulmonary diseases, acute bronchitis).

††Antiviral prescriptions in our seasonal influenza cohort had been described.⁹ Oseltamivir was the mainstay of treatment in 2008. Only in late January 2009, oseltamivir plus amantadine or zanamivir was recommended for treatment in the local clinical guidelines. Results on H275Y mutation were retrospective and unknown to clinicians at time of management. None of the patients infected with 'oseltamivir-susceptible' H1N1 viruses had received amantadine treatment.

†††All succumbed patients had comorbidities, mostly chronic lung diseases. Causes of death, 'oseltamivir-resistant' H1N1: chest infection and progressive respiratory failure, received oseltamivir alone (*n* = 2); 'sepsis' and chest infection/respiratory failure, no antiviral received (*n* = 2). 'Oseltamivir-susceptible' H1N1: chest infection/respiratory failure, and 'sepsis'/multi-organ failure, no antiviral received (*n* = 2).

outcomes.^{9,13} In Hong Kong, zanamivir- or amantadine-containing regimen for the 'oseltamivir-resistant' strain was not being considered regularly until late January 2009, when widespread circulation of the virus had already occurred; before the A/H1N1pdm09 pandemic, only about half of the hospitalized influenza patients were treated with antivirals. We acknowledge that our study is limited by a small sample size, but our data do provide support to the use of a susceptible antiviral regimen to treat severe

influenza and highlight the importance of real-time surveillance data on antiviral resistance to assist patient care. Managing severe influenza in the context of oseltamivir-resistance is challenging because: (i) resistance testing result is usually not available at time of treatment initiation and most rapid diagnostic assays do not even differentiate H1 and H3 subtypes; (ii) there are pharmacologic limitations with inhalational zanamivir (e.g., lack of systemic availability); (iii) adamantane susceptibility is

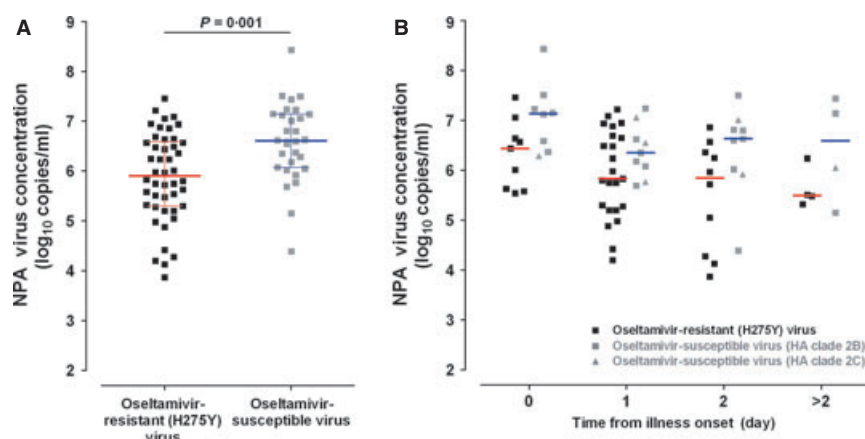


Figure 1. Seasonal influenza A(H1N1) virus RNA concentration in nasopharyngeal aspirates collected at time of presentation, compared between oseltamivir-resistant (H275Y) and oseltamivir-susceptible virus infections: (A) overall comparison, (B) shown according to time from illness onset. Vertical and horizontal bars denote interquartile range and median value, respectively (Mann–Whitney *U*-test was used for statistical comparison).

variable across H1N1 virus clades, its resistance barrier is low and efficacy uncertain; and (iv) there may be cross-resistance to other neuraminidase inhibitors (e.g., with peramivir).¹⁴ As such, continuous surveillance and timely recommendations to clinicians, development of newer tools for rapid detection of antiviral resistance, and clinical trials on newer antiviral agents including the use of combination therapies are urgently required to prepare for the possible emergence and sustained transmission of this and other drug-resistant influenza virus (e.g. oseltamivir-resistant A(H1N1)pdm09 virus which is currently resistant to adamantanes).^{15,16}

Conflict of interest

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Ethical approval

Ethics approval was obtained from institutional review boards of The Chinese University of Hong Kong and the Hospital Authority of Hong Kong.

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