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Original Article

A Prospective Comparison of the Epidemiological and Clinical Characteristics of Pandemic (H1N1) 2009 Influenza A Virus and Seasonal Influenza A Viruses in Guangzhou, South China in 2009

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SUMMARY: Comparisons of the clinical characteristics of contemporaneous pandemic (H1N1) 2009 influenza A virus (A(H1N1)pdm09)- and seasonal influenza viruses-infected patients are important for both clinical management and epidemiological studies. A prospective multicenter observational study was conducted using a preestablished sentinel surveillance system in Guangzhou, China during 2009. In this study, the clinical presentations of patients with either acute respiratory infection or community-acquired pneumonia were recorded, and nasopharyngeal swab samples were collected for detection of respiratory virus strains using cell cultures or real-time reverse transcription/real-time polymerase chain reaction. Comparisons of the clinical features between A(H1N1)pdm09- and seasonal influenza viruses-infected patients were conducted accordingly. Of the 1,498 patients examined, 265 tested positive for A(H1N1)pdm09, 286 were positive for seasonal influenza A viruses, and 137 for influenza B viruses. The predominant virus was influenza B before the emergence of A(H1N1)pdm09 (epidemiological week [EW] 1–EW 21); then, predominantly non-A(H1N1)pdm09 influenza A and, later, A(H1N1)pdm09, which peaked in EW 46. Compared with the common seasonal influenza-infected patients, A(H1N1)pdm09-infected patients were younger, and had a higher proportion of these patients reported prior contact with infected individuals ($P < 0.001$, by χ^2 test). However, few significant differences were observed in clinical symptoms and severity among any of the infections caused by the different influenza A strains. Our hospital-based network served as a useful source of information during A(H1N1)pdm09 monitoring. Viral distribution in Guangzhou was characterized by a sharp rise in A(H1N1)pdm09-infected patients in September 2009. Similar to seasonal influenza A-infected cases, A(H1N1)pdm09 cases had a very small proportion of severe cases.

INTRODUCTION

Guangzhou, one of the largest metropolitan cities in southern China, is situated at 23°6′N and 113°15′E, northwest of Hong Kong, and has a typical subtropical climate with 2–3-peak influenza activities annually (1). In the 20th century, 3 of the 4 influenza pandemics were thought to have originated in southern China (2,3). Moreover, the severe acute respiratory syndrome (SARS) outbreak in 2003 was first reported in Guangzhou, and occasional cases of avian flu were reported later. For the surveillance of acute respiratory infectious diseases, a hospital-based network for sentinel surveillance of patients with flu-like symptoms was established in 2008.

In April 2009, the Centers for Disease Control and Prevention in the United States (4) and the General Directorate of Epidemiology in Mexico (5) identified several human cases of pandemic (H1N1) 2009 influenza A virus (A(H1N1)pdm09) that were characterized by increased hospitalization, severity, and mortality, not only in individuals that had underlying conditions but also in previously healthy individuals.

In response to the potentially high pathogenicity of this novel pandemic influenza strain and the increased reports of severe respiratory illnesses, the sentinel surveillance system mentioned above was redesigned to in-

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clude the detection of A(H1N1)pdm09. The purpose of this study was to compare the clinical characteristics of patients infected with A(H1N1)pdm09 with those of patients infected with common seasonal influenza.

MATERIALS AND METHODS

Study design: This was a prospective, multicenter comparative observational study. The study utilized the surveillance network of patients with flu-like symptoms and attempted to identify cases of SARS or avian flu. Detection of the respiratory virus and follow-up examinations at 1 and 4 weeks post-identification were performed for subjects with suspected SARS or avian flu or confirmed seasonal influenza in order to evaluate the clinical features and outcomes of the infections. This work had been carried out since 2008, before the A(H1N1)pdm09 outbreak was detected. After the outbreak, the study strategy was revised and follow-up cases were increased to include all influenza-positive patients, including the A(H1N1)pdm09-infected cases.

Subjects: Patients were recruited from the outpatient clinical department and inpatient department of the First Affiliated Hospital of Guangzhou Medical College, the outpatient clinic of Hai Yin subsidiary Hospital of the First Affiliated Hospital of Guangzhou Medical College, the emergency department of Guangdong Provincial Hospital of Traditional Chinese Medicine (Guangzhou, China), the emergency department of Ershadao subsidiary Hospital of Guangdong Provincial Hospital of Traditional Chinese Medicine, and the outpatient unit of Fangcun subsidiary Hospital of Guangdong Provincial Hospital of Traditional Chinese Medicine. The inclusion criteria for the study were clinical diagnosis of acute respiratory infection (ARI; defined as feverish with temperature $\geq 37.3°C$, with at least one respiratory symptom such as cough, sore throat, coryza, or shortness of breath) or community-acquired pneumonia (CAP) confirmed by chest X-ray (6). Patients unwilling to participate were excluded from the trial. The study was approved by the ethics committees of the various hospitals and informed consent was obtained from all subjects.

Data acquisition and respiratory virus detection: At the time of enrolment, each subject's clinical characteristics were recorded. In patients with positive viral detection follow-up interviews were conducted 1 week and 4 weeks later via telephone for subjects attending outpatient clinics or face-to-face interview for patients in hospital. All data were recorded by a trained doctor, who was unaware of the results of viral detection.

Prior to June 2009, nasopharyngeal swab samples were collected at enrolment and tested for seasonal influenza virus A and B, parainfluenza virus 1, 2, and 3 (PIV-1, 2, 3), adenovirus (Ad), respiratory syncytial virus (RSV), enterovirus (EV), and herpes simplex virus 1 and 2 (HSV-1, 2) using virus culture techniques (7). After June 2009, the samples were analysed for the presence of these viruses by real-time reverse transcription polymerase chain reaction (rRT-PCR)/real-time polymerase chain reaction (RT-PCR) according to the temporal regulation of the department of health in

Table 1. Clinical features of patients with A(H1N1)pdm09 or seasonal influenza A virus infections

Characteristic	A(H1N1)pdm09 (n = 265)	Seasonal influenza A		P[1)]	Logistic regression[2)]			
		A/H1N1 (n = 117)	A/H3N2 (n = 162)		Seasonal influenza A/H1N1		Seasonal influenza A/H3N2	
					OR (95%CI)	P	OR (95%CI)	P
Demographic features								
Age (y), median (IQR)	21 (16–28)	27 (21–33)	28 (23–39)	<0.001	1.04 (1.02–1.07)	0.001	1.07 (1.05–1.09)	0.001
Male/female	135/130	56/61	76/86	0.690	1.13 (0.69–1.88)	0.618	1.14 (0.72–1.81)	0.571
Sick contact	68 (25.7)	13 (11.1)	16 (9.9)	<0.001	0.46 (0.23–0.90)	0.023	0.44 (0.23–0.83)	0.011
Symptoms								
Temperature >39°C[3)]	85 (32.1)	24 (20.5)	35 (21.6)	0.271	0.66 (0.37–1.15)	0.140	0.75 (0.45–1.25)	0.265
Headache	193 (72.8)	75 (64.1)	106 (65.4)	0.131	0.66 (0.40–1.09)	0.103	0.73 (0.45–1.18)	0.200
Myalgia	157 (59.2)	71 (60.7)	90 (55.6)	0.648	1.14 (0.70–1.86)	0.588	0.91 (0.58–1.42)	0.667
Fatigue	187 (70.6)	74 (63.2)	112 (69.1)	0.359	0.82 (0.49–1.36)	0.437	1.14 (0.70–1.85)	0.610
Coryza	156 (58.9)	65 (55.6)	101 (62.3)	0.517	0.87 (0.54–1.41)	0.581	1.16 (0.74–1.82)	0.522
Sore throat	180 (67.9)	88 (75.2)	131 (80.9)	0.012	1.77 (1.04–3.03)	0.036	2.47 (1.46–4.19)	0.001
Dry cough	114 (43.0)	59 (50.4)	79 (48.8)	0.310	0.85 (0.45–1.64)	0.635	1.00 (0.53–1.91)	0.994
Sputum	113 (42.6)	34 (29.1)	57 (35.2)	0.032	0.46 (0.23–0.93)	0.031	0.66 (0.34–1.29)	0.228
Hemoptysis	2 (0.8)	0 (0)	1 (0.6)	1.000	ND	ND	2.10 (0.17–26.81)	0.567
Chest pain	16 (6.0)	3 (2.6)	4 (2.5)	0.124	0.32 (0.08–1.31)	0.114	0.18 (0.04–0.74)	0.017
Dyspnea	9 (3.4)	3 (2.6)	9 (5.6)	0.379	0.54 (0.10–3.11)	0.493	1.25 (0.31–5.08)	0.760
Gastrointestinal symptoms[4)]	32 (12.1)	8 (6.8)	16 (9.9)	0.293	0.53 (0.22–1.28)	0.156	0.71 (0.34–1.49)	0.365
Pneumonia (diagnosis)	15 (5.7)	3 (2.6)	8 (4.9)	0.423	0.36 (0.07–1.78)	0.209	0.34 (0.09–1.37)	0.131

Data are shown as numbers (%) unless otherwise specified.
1): Comparisons were made among A(H1N1)pdm09, seasonal influenza A (H1N1), and (H3N2) by using ANOVA for quantitative characteristics and Fisher's exact or chi-square test for categorical variables, respectively.
2): Reference category of logistic regression was A(H1N1)pdm09.
3): Denotes the highest body temperature of feverish patients before and on admission to hospital.
4): Gastrointestinal symptoms included nausea, vomiting, diarrhea, abdominal pain, and abdominal distention.
ND, not done.

Table 2. Outcomes and severe cases among pneumonia patients with A(H1N1)pdm09 or seasonal influenza vinuses

Characteristic	A(H1N1)pdm09 (n = 15)	Seasonal influenza A		P[1)]
		A/H1N1 (n = 3)	A/H3N2 (n = 8)	
Hospitalization	10 (66.7)	1 (33.3)	7 (82.5)	0.211
Underlying conditions	8 (53.3)	2 (66.7)	6 (75)	0.723
CURB-65 $\geq$ 2	1 (6.7)	0 (0)	2 (25)	0.502
ICU admission	1 (6.7)	0 (0)	1 (12.5)	1
Requirement for mechanical ventilation	2 (13.3)	0 (0)	1 (12.5)	1
Length of stay in hospital (days), median (IQR)[2)]	9 (7–12)	9	10.5 (7.5–15.8)	
	[10 cases]	[1 case]	[7 cases]	0.785
Complications[3)]	5 (33.3)	0 (0)	4 (50)	0.368
Death within 30 days after onset	0 (0)	0 (0)	1 (12.5)	0.310

Data are shown as number (%) unless otherwise specified.
1): Comparisons were made using Kruskal-Wallis test for quantitative characteristic and Fisher's exact test for categorical variables.
2): Length of stay calculation based on hospitalized patients only (showed in the brackets).
3): Complications include hypoxemia (PaO_2 < 80 mmHg), respiratory failure, cardiovascular failure, shock, renal and/or liver failure, etc.

the local government (8–16). In addition, the A(H1N1)pdm09, human metapneumovirus (HMPV), human bocavirus (HBoV), and coronavirus 229E and OC43 were included in the rRT-PCR/RT-PCR testing.

Statistical analysis: The Statistical Package for the Social Science (SPSS 13.0; SPSS, Chicago, Ill., USA) was employed for statistical analysis. Quantitative data are presented as mean ± standard deviation (SD) or median and interquartile range (IQR). Normally distributed data were compared by analysis of variance (ANOVA); otherwise, the non-parametric Kruskal-Wallis test was used. The categorical variables were reported as frequencies and percentages and were compared using the Fisher's exact or chi-square test. We also computed odds ratios (OR) and 95% confidence intervals (CI) for clinical characteristics using logistic regression. For each regression analysis performed, the dependent variable was defined as influenza type (A(H1N1)pdm09, seasonal influenza A (H1N1) or A (H3N2)), and the independent variables included age, temperature, gender, and potential related factors as shown in Tables 1 and 2, including the patient's underlying conditions, clinical features, disease severity, and outcomes. All hypothesis testing was two-sided and $P < 0.05$ was considered statistically significant.

RESULTS

Patients and etiology: Between January and December 2009, our hospital-based sentinel surveillance system examined 1,498 individuals (with 197 pneumonia cases), and 729 cases tested positive for respiratory viruses. Influenza virus was detected in 695 (48.4%) cases, including 702 influenza strains. Among the influenza-positive patients, 286 (41.5%) had seasonal influenza A virus infection (117 seasonal influenza A (H1N1) virus, 162 had influenza A (H3N2) virus, and 7 could not be subtyped for H1 or H3), 265 (38.1%) had A(H1N1)pdm09 infection, 137 (19.9%) had influenza B virus infection, and 7 (0.9%) had seasonal influenza A and B virus co-infection. The other non-influenza viruses detected included 10 Ad, 1 coronavirus 229E, 9 coronavirus OC43, 12 PIV-1/2/3, 4 HMPV, 3 EV, 3 HBoV, 2 HSV-1, and 1 RSV. As the containment policy for pandemic H1N1, which was initiated by China's Ministry of Health since the onset of pandemic H1N1 until July 9, 2009, this study was divided into three stages: the period before A(H1N1)pdm09 (from epidemiological week [EW] 1–EW 21), the input phase (EW 22–EW 27), and the community pandemic phase (EW 28–EW 52).

The seasonal distribution of respiratory viruses is shown in Fig. 1. The first A(H1N1)pdm09-positive sample was collected on June 9 and then became the predominant strain after EW 34, and seasonal influenza virus became rare. Other non-influenza viruses were sporadically identified throughout the 2009 pandemic year. Viral distribution was further analyzed over three different periods (Fig. 2). During the period before A(H1N1)pdm09, influenza B virus accounted for 61% of all respiratory viruses, whereas the predominant strain gradually shifted to A(H1N1)pdm09 in the pandemic phase with a peak at EW 44–EW46.

Clinical characteristics of influenza patients: The median age of A(H1N1)pdm09-infected patients (median [IQR], 21 [16–28] years) was lesser than that of seasonal influenza A (H1N1) (median [IQR], 27 [21–33] years) and A (H3N2)-infected patients (median [IQR], 28 [23–39] years) ($P < 0.001$, by non-parametric Kruskal-Wallis test). Moreover, comparison of the age distribution of patients in the three groups showed statistical significance ($P < 0.001$, by the χ^2 test) (Table 1 and Fig. 3).

The number of patients who reported previous contact with infected individuals in the A(H1N1)pdm09 group was more than that in the seasonal influenza groups ($P < 0.001$, by χ^2 test) (Table 1). Among the 1,498 patients, 244 (36.3%) had at least one underlying disease such as pulmonary, cardiac, cerebrovascular, hepatic or renal diseases, diabetes mellitus, and hypertension. The underlying conditions in cases infected with three different influenza A strains were compared, however, no statistical significant difference was observed.

The mean highest temperature in seasonal influenza

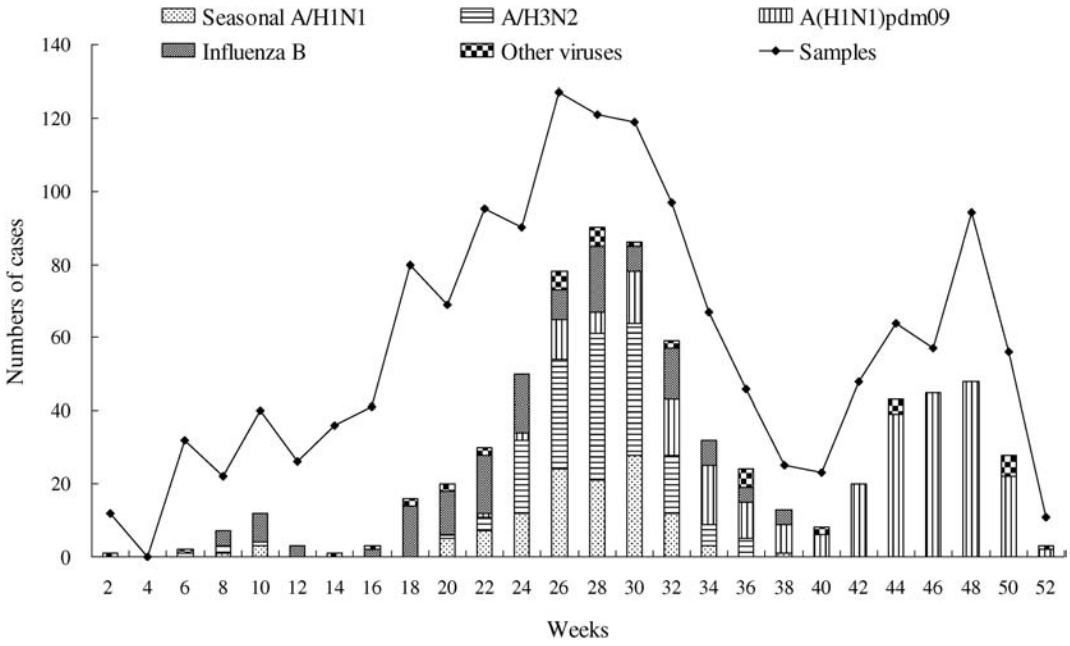

Fig. 1. Viral distribution by epidemiological week in Guangzhou, China between January 1 and December 31, 2009. Other viruses included parainfluenza virus 1, 2, and 3, adenovirus, respiratory syncytial virus, enterovirus, herpes simplex virus 1 and 2, human metapneumovirus, human bocavirus, and coronavirus 229E and OC43.

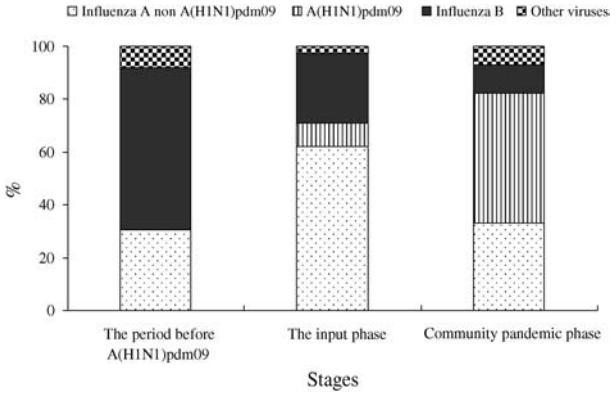

Fig. 2. Viral distribution during the 2009 pandemic, broken into different epidemic periods.

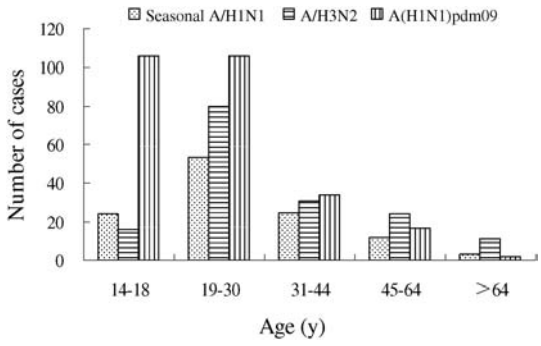

Fig. 3. Age distribution across the three different influenza A strains. Comparison among the three group had statistical significance ($P < 0.001$, by chi-square test).

A (H1N1) patients was lower than that in A(H1N1)pdm09 patients (38.62 ± 0.58°C versus 38.86 ± 0.56°C; $P = 0.001$, by ANOVA). No differences were observed in clinical symptoms, except sore throat, sputum, and chest pain, for the three different influenza A strains (Table 1).

Most influenza patients diagnosed with upper respiratory tract infection recovered in less than a week; approximately 20% of the influenza patients had delayed recovery complicated by continued coughing for up to 3 weeks. This was similar for all three influenza A strains. Comparisons of complications and other characteristics indicated that the severity of illness did not significantly differ among pneumonia patients with A(H1N1)pdm09, seasonal influenza A(H1N1), and A(H3N2) groups (Tables 1 and 2). One pneumonia patient infected with A(H1N1)pdm09 was admitted to the intensive care unit (ICU) and was still in a coma 90 days post-onset of illness. Only three A(H1N1)pdm09 patients (two with pneumonia) received oseltamivir, which was prescribed by the attending doctors. Since a lower number of pneumonia cases occurred in the study population, logistic regression was not performed for a more detailed comparison between these three influenza groups.

DISCUSSION

This was a prospective comparison study of the epidemiological and clinical characteristics of contemporaneous A(H1N1)pdm09 and seasonal influenza A virus infection in the local adolescent and adult population in Guangzhou city during the 2009 pandemic season; the study benefited from our prebuild sentinel surveillance system for suspected SARS or avian flu cases implemented since 2008. The viral distribution in Guangzhou changed after the emergence of A(H1N1)pdm09; it was characterized by a sharp rise in A(H1N1)pdm09-infected patients in adolescents and young adults beginning with the new school semester that started in September. Similar to seasonal influenza,

A(H1N1)pdm09 infection had a very small proportion of severe cases; therefore, this first pandemic wave was characterized by a mild clinical severity in the majority of cases.

The emergence of A(H1N1)pdm09 poses great challenges to the world. Hence, individual governments and healthcare systems have proposed a variety of policies to cope with the situation. Patient's hospital visits have provided an important source of information to this end (17–19). China raised the strength of national surveillance from April 30, 2009 to identify patients with A(H1N1)pdm09 infection (20). Studies have reported the influenza surveillance data, which were mainly limited to patients in the ICU or hospitalized patients or patients with pneumonia (21–23). These data may represent a subgroup of patients with pneumonia and overestimate the average severity of the disease. However, new emerging virus infections usually manifest influenza-like illness (ILI) at their initiation. Hence, a network for sentinel surveillance of patients with flu-like symptoms could provide a more clear understanding of A(H1N1)pdm09 infection. The current study was based on the preestablished sentinel surveillance system implemented since 2008. This sentinel surveillance system focused on patients with acute febrile respiratory illness at initial presentation, including patients at outpatient clinics or undergoing hospitalization, which might cover more cases with mild presentation and represent a more accurate depiction of the infection. This local system responded quickly to the outbreak of A(H1N1)pdm09 and enabled us to conduct a prospective study for newly emergent diseases. All of the epidemiological and clinical information associated with A(H1N1)pdm09 and seasonal influenza infection was collected from this preestablished sentinel hospital-based network. Therefore, the current study might reveal more reliable data on the epidemiology and clinical characteristics of A(H1N1)pdm09 patients, as well as comparison of these characteristics with that of seasonal influenza. Furthermore, this study provided information on the epidemiological and clinical characteristics of A(H1N1)pdm09 infections in Guangzhou city, which is important for establishing policy for the control and clinical management of pandemic of A(H1N1)pdm09.

In some studies, data were retrospectively retrieved from a database (24–27), and A(H1N1)pdm09 and seasonal influenza virus were compared using non-contemporaneous data obtained over different years (20,24–26). Interpretation of such information was challenging because of the limits of a retrospective study, variation in influenza activity from year to year, and the observed populations may not be comparable. In our study, with the preestablished sentinel hospital-based network and standard procedures of data acquisition and follow-up, the whole course of infection was prospectively examined, and the duration of study spanned the entire 2009 pandemic year. Hence, the comparison between A(H1N1)pdm09 and seasonal influenza virus in our study may truly reflect the nature of A(H1N1)pdm09 infection.

In Guangzhou, the viral distribution changed after the emergence of A(H1N1)pdm09. Activities of seasonal influenza in our study were consistent with previous surveillance data from Guangzhou city (1). The pattern of activity of A(H1N1)pdm09 in Guangzhou was also found to be similar to other regions in mainland China (28), but was somewhat later than in other areas, such as the tropical country Peru (EW 25) (29), possibly because South America is near the original outbreak site, Mexico. Moreover, the seasonal pattern of A(H1N1)pdm09 prevalence in Guangzhou was also different from that in other subtropical areas such as Okinawa Prefecture, Japan (July to August) (30) and Hong Kong, which peaked at EW 39 (31). Hong Kong is located 190 km southeast of Guangzhou and has a similar subtropical monsoon climate and influenza activities. Therefore, we think that the difference in the A(H1N1)pdm09 activity between Guangzhou and Hong Kong may be attributed to the A(H1N1)pdm09 containment policy strictly enforced by China's Ministry of Health. This may provide some clues for an appropriate strategy for prevention or containment of pandemics of influenza or other respiratory infectious disease in the community.

Early reports from Mexico (26) showed that more than 70% of the A(H1N1)pdm09-infected cases were severe and that the mortality was 87% even in previously healthy, young or middle-aged subjects. Later reports (24) from various countries such as the US, Spain, Canada, and Australia also showed similar results with an increased mortality of 18% in patients admitted to the ICU. However, these studies were retrospective case series, focusing on cases admitted in the ICU, and did not compare A(H1N1)pdm09 infection to concurrent seasonal influenza. An analysis that includes all A(H1N1)pdm09-infected cases and compares them with contemporaneous non-A(H1N1)pdm09-infected cases will provide accurate values for mortality and proportion of severe cases.

Our study was prospectively designed before the outbreak of A(H1N1)pdm09; this enabled us to compare seasonal with pandemic influenza infection not only in hospitalized patients but also in outpatients. The scope of observation included more cases with mild severity and contemporaneous seasonal influenza. Therefore, we believe that our study provides more accurate information on the proportion of severe cases and mortality of A(H1N1)pdm09 infection. With regards to age distribution in our study, the A(H1N1)pdm09-infected patients were younger than seasonal influenza patients. This result was consistent with previous reports from China (20) as well as other countries (24,26) such as Japan (32). The data discussed above suggests that A(H1N1)pdm09 infections appear to show seasonal variations in different climate zones but display universally similar clinical features.

Our data showed that A(H1N1)pdm09 has the tendency to infect individuals younger than 30 years, especially adolescent populations of less than 18 years. The probable explanation for this finding is the close proximity of adolescents at school, which would increase the probability of influenza transmission. In our present study, A(H1N1)pdm09-infected adolescent cases had a higher rate of contact with infected individuals, and a sharp rise in prevalence was observed after the new semester of school started at EW 36. Another possibility is that older people may have cross-immunoreactivity (32–35) or long-term T cell-mediated immunity (36)

against A(H1N1)pdm09, as a result of prior exposure to influenza virus that was genetically and antigenically closely related to the A(H1N1)pdm09 strain.

An important question was whether A(H1N1)pdm09 infection was associated with a great degree of severity and higher mortality. Our results showed that the majority of A(H1N1)pdm09 infections were mild and similar to common seasonal influenza cases. This results is contrary to previous reports on hospitalized patients (25,27,37–39), which showed that A(H1N1)pdm09 infection was associated with a higher percentage of severe cases and mortality. However, reports from Australia (40), Hong Kong (41), and Japan (32) were consistent with our study. The reason for the difference between different studies may be mainly due to the subjects included in the studies. Another clinical feature was delayed recovery due to continued coughing in some of the non-pneumonia patients; this was also in agreement with a previous study (42). Gastrointestinal symptoms such as diarrhea and vomiting were previously reported to be common symptoms associated with A(H1N1)pdm09, but uncommon in seasonal influenza (43,44). Moreover, it also has been suggested that gastrointestinal symptoms could be useful for discriminating different types of influenza virus infections. However, similar to Cao's study (20) from China, our study showed that gastrointestinal symptoms were uncommon in A(H1N1)pdm09-infected patients. Other clinical features, namely, sore throat, sputum, and chest pain were observed to be statistically different between A(H1N1)pdm09 and seasonal influenza A virus infections. However, these differences were clinically insignificant unless combined with the identification of viruses. Differences in the findings for clinical characteristics among different studies may be related to diverse geographical, cultural, and healthcare environments, although this needs to be confirmed (45).

However, this study has several limitations. First, all patients were older than 13 years and had a fever or were feverish. Recessive infection of A(H1N1)pdm09 with mild symptoms and no fever is highly possible. Obviously, we can not detect these kinds of cases if present. Second, most of the patients were followed-up by telephone interview, so the obtained information may have some bias. Third, seven cases of seasonal influenza A patients could not be further subtyped for H1 or H3 by nucleotide sequencing because of a low cultured viral titer. These factors may lead to statistical bias in comparisons among seasonal influenza virus and A(H1N1)pdm09.

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Conflict of interest None to declare.

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