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LETTER TO THE EDITOR

CLINICAL MANIFESTATIONS SEEMED MORE SEVERE AMONG PATIENTS WITH ANTIBODY 4-FOLD OR A GREATER INCREASE IN TITER BOTH AGAINST pH1N1 AND AGAINST SEASONAL INFLUENZA THAN THOSE WHOSE ANTIBODY 4-FOLD OR GREATER INCREASE IN TITER WAS ONLY AGAINST ONE TYPE OF SEASONAL INFLUENZA

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To the editor

In 2009, after an emergence of pandemic influenza A(H1N1) (pH1N1), one of our serological investigations showed 23 students whose paired serum antibodies simultaneously presented 4-fold or greater increase in titer both against pH1N1 and against seasonal influenza (SI). This caught our attention and in order to further understand clinical features of these cases, a case control study was conducted. From October 2009 to September 2012, we collected the acute and convalescent phase serum samples from patients whose throat swabs were positive for pH1N1 or SI by real-time reverse transcription polymerase chain reaction (RT-PCR)[5]. The patients' information, including clinical symptoms, self-protect measures, and social activities after illness, was also collected through inspecting medical records, and interviewing face to face or by telephone.

Paired blood samples were used to test antibodies against 2009 pandemic A(H1N1) influenza virus and four kinds of seasonal influenza subtype virus (H3N2, H1N1, By, and Bv), which were detected by Hemagglutination inhibition (HI) assays. The influenza viruses used were A/GuangdongLiwan/SWL1538/2009 (H1N1), A/TianjinJinnan/15/2009 (H1N1), A/FujianTongan/196/2009 (H3N2), B/JiangxiXiushui/32/2009 Victoria, and B/Guangdong Xindong/134/2009 Yamagata. The HI assay was performed using a standard technique[2]. Serum samples were treated with receptor destroying enzyme to remove nonspecific hemagglutination. Serum samples were diluted in serial two-fold dilutions from 1:10 to 1:640 and then mixed with chicken red blood cells and the virus strain. HI titer was determined as the highest dilution of serum which showed hemagglutination inhibition.

During the study period, a total of 2,079 paired blood samples were collected, of which 68 cases (3.27%) whose antibodies were simultaneous 4-fold or greater increase in titer against pH1N1 and SI. Among the 68 cases, the sex distribution was 61.76% (N = 42) male and 39.14% (N = 26) female, the age ranged from five to 81 years old (median age = 20), the patients had fevers ranging from 37.5 °C to 40.6 °C, the disease course lasted from two to 11 days. Primary clinical symptoms were a sore throat (65/68, 95.59%), a cough (37/68, 54.41%), and a headache (31/68, 45.59%). The proportion of patients who had arthralgia, nausea, vomiting and diarrhea was 8.82% (6/68), 7.35% (5/68), 4.41% (3/68), and 4.41% (3/68), respectively.

Of those whose antibodies presented 4-fold or greater increase in titer only against one subtype of SI, 136 cases were selected into a control group as a 1:2 match according to the following matching criteria: onset date (+/– 20 days), age (+/– 4 years), and sex. Compared to the control group (136 cases), the proportion of patients with a fever ≥ 38.5 °C, disease course ≥ 5 days, and clinical symptoms ≥ three episodes were significantly higher ($p < 0.05$) among the case group (68 cases) (Table 1).

Table 1
Comparing the clinical characteristics between case group (68 cases) and control group (136 cases)

Clinical symptoms	Case group		Control group		x^2	p
	No.	%	No.	%		
Fever ≥ 38.5 °C	26	38.24	33	24.27	4.30	0.04*
Disease course ≥ 5 days	19	27.94	14	10.29	10.41	0.00*
Cough	37	54.41	66	48.53	0.63	0.43
Sore throat	65	95.59	129	94.85	0.05	0.82
Headache	31	45.59	55	40.44	0.49	0.48
Nasal congestion	23	33.82	40	29.41	0.41	0.52
Rhino rhea	17	25.00	32	23.53	0.05	0.82
Sputum production	14	20.59	25	18.38	0.14	0.70
Fatigue	23	33.82	41	30.15	0.29	0.59
Myalgia	9	13.24	16	11.77	0.09	0.76
Chills	11	16.18	15	11.03	1.08	0.30
Arthralgia	6	8.82	15	11.03	0.24	0.63
Nausea	5	7.35	7	5.15	0.40	0.53
Vomiting	3	4.41	5	3.68	0.07	0.80
Diarrhea	3	4.41	2	1.47	1.64	0.20
Clinical symptoms ≥ 3 episodes above	32	47.06	43	31.62	4.65	0.03*

*$p < 0.05$

Taken together, we reported that of the 2079 influenza patients, 68 cases were found to have simultaneous 4-fold or greater increase in serum antibody titers against pH1N1 and SI, these cases appeared to have more severe clinical pictures, including higher fever, longer disease course and more episodes of clinical symptoms. A possible explanation for this might be that these cases presented with a co-infection. Before emergence of pH1N1, co-infection has been proved to exist in seasonal

influenza. For example, NISHIKAWA *et al.* found a patient who was simultaneously infected with seasonal influenza A(H1N1) and A(H3N2) during the epidemic of 1981[3]. In 2006, TODA *et al.* isolated the A/H3 and B viruses from an influenza patient[4]. In addition, GOKA *et al.* also found co-infection was associated with higher risk of admission to ICU/death[1]. Due to virus isolation not being conducted among our samples, more evidence regarding pH1N1 and SI needs to benefit from molecular virology research in future.

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