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Seroprevalence of Antibodies to Pandemic (H1N1) 2009 Influenza Virus Among Hospital Staff in a Medical Center in Taiwan

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Background: The pandemic (H1N1) 2009 influenza emerged in April 2009 and spread rapidly and broadly all over the world. In addition to specific antiviral agents, massive vaccination is thought to be the most effective way of controlling the transmission. To understand the prevaccination status of certain risk groups, this study compared the baseline seroprevalence of antibodies to the pandemic (H1N1) 2009 influenza virus among hospital staff with different contact risks and that of the general population.

Methods: A total of 295 serum samples from hospital staff and 244 control serum samples from people who came for physical check-up (control group) were collected between October 2009 and November 2009 before the massive vaccination campaign. The hospital staff was divided into first-line risk personnel (group 1) and second-line risk personnel (group 2) according to their potential contact risks. Hemagglutination-inhibition (HI) tests were conducted to determine the individual serological status. The seropositive rate (SPR, defined as the proportion with HI titer $\geq$ 1:40) of antibodies to H1N1 influenza virus and its geometric mean titer (GMT) were calculated and compared among the different groups.

Results: The mean ages and sex ratio (% male) of the hospital staff and control groups were 36.9 $\pm$ 10.6 years and 52.0 $\pm$ 12.6 years, and 24.4% and 57.6%, respectively. The SPR of the antibodies to H1N1 influenza virus of the hospital staff was significantly higher than that of the control group (20.0% *vs.* 2.9%, $p < 0.001$). Furthermore, the SPR antibodies to H1N1 influenza virus of group 1 were significantly higher than that of group 2 (30.8% *vs.* 12.6%, $p < 0.001$). However, the GMT of antibodies to H1N1 influenza virus of the hospital staff was not significantly different from that of the control group ($p = 0.925$).

Conclusion: The SPR of antibodies against the pandemic (H1N1) 2009 virus in the hospital staff was higher than that in the general population, reflecting a higher contact risk. Prevaccination surveillance of the immune status of different risk groups may help to prioritize which groups should be vaccinated first. [*J Chin Med Assoc* 2010;73(2):62–66]

Key Words: hemagglutination-inhibition test, hospital staff, H1N1 influenza virus, vaccination

Introduction

A pandemic novel H1N1 influenza outbreak [the pandemic (H1N1) 2009], starting from the United States and Mexico, has spread worldwide since April 2009.[1–3] A total of more than 1 million confirmed cases with approximately 10,000 deaths have been reported by the World Health Organization (WHO). The sources of the novel rearranged virus are from swine, avian and human influenza viruses.[4,5] Young people are susceptible to the infection, with occasional deterioration of their clinical manifestations.[6,7]

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The first imported case in Taiwan appeared in May 2009, and the epidemic spread to the community in July 2009.[8] Healthcare workers, according to the experience with the severe acute respiratory syndrome (SARS) epidemic, have a higher risk of contracting diseases,[9,10] and belong to a unique risk group. The vaccination campaign for the novel H1N1 influenza virus is a national policy to protect people from viral attack. To determine the prevaccination prevalence of antibody levels against the novel H1N1 influenza, we conducted surveillance of healthcare personnel in a medical center.

Methods

Study design and case recruitment

A total of 295 serum samples were collected from hospital staff, including doctors, nurses, students, and administrative personnel, and 244 serum samples were collected from people who came for physical check-up between October 23, 2009 and November 20, 2009 before the vaccination campaign. The Institutional Review Board of Taipei Veterans General Hospital approved the protocol for this study.

Reagents for the hemagglutination-inhibition (HI) test

The preparation of the test sera was as follows: 100 μL of serum was mixed with 300 μL receptor destroying enzyme (RDE; Denka Seiken Co. Ltd., Tokyo, Japan) at 37°C for 18–20 hours to remove the interfering nonspecific receptors, and the reaction was stopped by incubating the mixture at 56°C for 30 minutes. Then, 600 μL of phosphate-buffered saline (PBS) was mixed with RDE-treated serum to generate a 10-fold diluted solution. Next, 50 μL of packed guinea pig red blood cells (GP-RBC) were added to the solution for 1–2 hours at 4°C to remove nonspecific hemagglutination activity.

The GP-RBC suspension was prepared as follows: approximately 10 mL of guinea pig blood was obtained and thoroughly mixed with a 1:4 ratio of Alsever's solution (anticoagulant). The GP-RBC suspension was filtrated through sterile 2-layered gauze and washed with PBS (pH 7.2) 3 times and then diluted with PBS to form a 10% RBC stock suspension. RBC 0.75% working suspension was prepared for the hemagglutination (HA) or HI tests.

HA and HI tests

Martin-Darby canine kidney (MDCK) cells were cultivated in 5% fetal calf serum (Biological Industries Ltd., Kibbutz Beit Haemek, Israel) and infected with influenza virus [strain A/Taiwan/T1338/2009 (H1N1), A/California/4/2009-like, kindly provided by Dr Yi-Ming A. Chen]; the virus was inactivated before use. The culture supernatant was 2-fold serially diluted with PBS (pH 7.2) in U-plates, followed by adding 50 μL of 0.75% GP-RBC suspension into each well of a 96-well plate that contained equal volume (50 μL) of serially diluted supernatant. The plate was covered and left for 1 hour at room temperature. HA titers were defined as the reciprocal of the highest dilution that showed completely hemagglutination of the GP-RBC. The HI test was conducted by mixing with an equal volume (25 μL) of 2-fold serially diluted RDE-treated serum and 8-HA unit viral antigen in each well of a 96-well plate. After gentle shaking, 50 μL of 0.75% GP-RBC was added into each well of the plate. The plate was covered and left for 1 hour at room temperature. HI titers were defined as the reciprocal of the highest dilution that completely inhibited hemagglutination of the GP-RBC. HI titer ≥ 40 was interpreted as positive. The geometric mean titer (GMT) is defined as the geometric mean of the positive HI titers. The seropositive rate (SPR) is defined as the percentage of HI titers ≥ 40.

Statistical analysis

The χ^2 test was used to compare differences in discrete variables, and Student's *t* test or Mann-Whitney rank sum test was used to analyze the continuous variables such as age and HI titers as appropriate. All analyses were performed with SPSS version 17 (SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered statistically significant.

Results

A total of 295 hospital staff and 244 control cases (control group) were tested. The mean ages and sex ratio of the 2 groups are shown in Table 1. With HI ≥ 40 as the cut-off value for seropositivity, the SPR of the hospital staff was significantly higher than that of the control group (20.0% *vs.* 2.9%, $p < 0.001$) (Table 1).

To further delineate the possible role of close patient contact, the hospital staff were divided into first-line risk personnel (group 1) that included 120 doctors from the Division of Infectious Diseases, and inpatient nurses and staff from the Emergency Department, and second-line risk personnel (group 2) that included 175 general medical doctors, laboratory staff and administrative personnel. The SPR of group 1

Table 1. Seropositivity rate (SPR) of antibodies to H1N1 influenza virus in hospital staff and control group

	Total cases, *n*	Male, *n* (%)	Age (yr), mean ± SD	HI titer ≥ 40, *n* (%)
Control	244	140 (57.6)	52.0 ± 12.6	7 (2.9)*
Hospital staff	295	72 (24.4)	36.9 ± 10.6	59 (20.0)*
Group 1	120	28 (23.3)	34.0 ± 9.0	37 (30.8)*†
Group 2	175	44 (25.1)	38.8 ± 11.2	22 (12.6)*†

*In comparison with the control group for SPR (HI titer ≥ 40): *to control group, $p < 0.001$; †group 1 vs. group 2, $p < 0.001$. HI = hemagglutination-inhibition; SPR = proportion with HI titer ≥ 1:40 as seropositivity rate for antibodies to H1N1 influenza virus.*

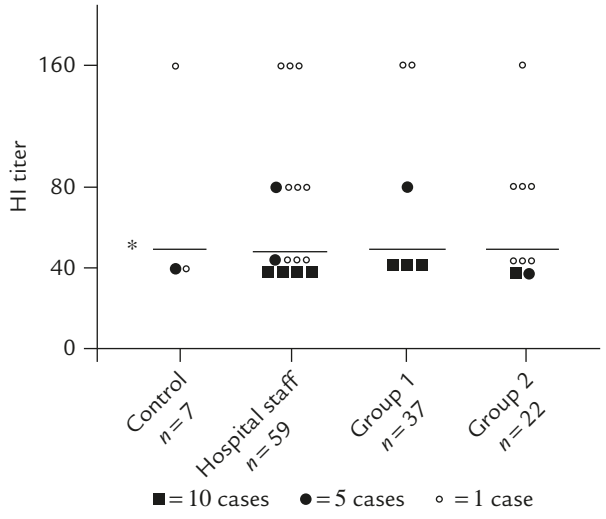

Figure 1. Level and geometric mean titer (GMT) of prevaccination sera against the pandemic (H1N1) 2009 virus among hospital staff and the control group. *Horizontal bars indicate the GMT of antibodies to the H1N1 influenza virus of each group: there were no significant differences among groups. The GMTs of each group are: Control = 48.8; Hospital staff = 47.2; Group 1 = 47.4; Group 2 = 46.8. Samples with hemagglutination-inhibition (HI) titer < 40 were excluded in the calculation.

was significantly higher than that of group 2 (30.8% *vs.* 12.6%, $p < 0.001$) (Table 1).

There was no statistically significant difference in GMT antibodies to H1N1 influenza virus between the hospital staff and control group (47.2 *vs.* 48.8, $p = 0.925$) (Figure 1). There was also no significant difference between groups 1 and 2 (47.4 *vs.* 46.8, $p = 0.962$).

Discussion

Since April 2009, a novel rearranged H1N1 influenza virus (originally referred to as the swine-origin influenza virus) outbreak emerged from Mexico and the United States, with rapid propagation to almost all over the world over several months. The WHO issued a pandemic alarm in June 2009; this pandemic involved 200 more countries, with more than 1 million confirmed cases and 10,000 reported deaths.[1,11] Initially, the case mortality rate was thought to be high (approximately 7% in Mexico), but it now appears that the mortality rate is no more than that of seasonal influenza.[12] The case mortality rate estimated by the Taiwan Center for Disease Control is approximately 1 in 10,000.[8] The decline in the case mortality rate of the novel H1N1 pandemic is not likely to be due to rapid virus evolution as genetic analyses have not shown significant mutations.[5,6] Instead, stringent surveillance for infected people may be identifying a greater number of mild cases. The same phenomenon has been described in another swine-origin influenza outbreak in 1976 in Fort Dix,[13,14] where 230 positive cases were diagnosed by serological survey but there were only 13 cases with clinical symptoms and 1 death. Our results are consistent with the notion that by serological surveillance, seropositive individuals can be identified from certain risk groups, such as hospital staff.

Although there are specific antiviral agents available for treatment, it is generally believed that the most effective approach is through a vaccination campaign.[15] Several studies have demonstrated the efficacy of vaccination to prevent mortality and hospitalization, and recent studies have indicated that the 2009 H1N1 monovalent vaccine is immunogenic in both adults and children.[16–20] The adverse effects associated with vaccination are mild to moderate.[17,18,21] Before initiating a massive vaccination campaign, it is imperative to understand the seroepidemiology of the population. Traditionally, the HI test is a standard to evaluate the antibody response to influenza vaccination. Since the novel H1N1 influenza virus has never circulated among people and previous seasonal vaccination offers limited cross-protection, the SPR should, theoretically, be low even after seasonal vaccination.[22,23] However, there are certain risk groups that may contract the disease. Myers et al found that swine workers are at increased risk of being infected by swine-origin influenza virus through close contact.[24] Healthcare workers are susceptible to infectious diseases and our

results support that notion. Hospital-acquired outbreaks were reported during the SARS epidemic.[9,25,26] For the mild pandemic (H1N1) 2009, our hospital issued guidelines for the use of personal protective equipment, such as the wearing of surgical or higher-level masks, gloves, and facial protection equipment. A self-reporting system was also established to identify symptomatic individuals according to the clinical definition set by the Taiwan Center for Disease Control, i.e. (1) fever > 38°C; (2) influenza-like symptoms, such as cough, headache, general malaise etc.; (3) exclusion of illnesses with definite diagnoses, such as tonsillitis. In this study, the strikingly high SPR among the hospital staff, especially first-line workers, indicates that the symptoms were mostly mild or subclinical. The lack of significant differences in GMTs was not unexpected as the 2 groups had not yet been vaccinated and their immune statuses were presumably similar. However, the relatively low GMT levels indicate that the exposed individuals with mild or vague symptoms did not develop strong immune responses, compared to vaccination which usually elicits a strong immune response. Most of the recent studies on the pandemic (H1N1) 2009 virus focused on the clinical manifestations of symptomatic cases.[6,27] However, the scope of this pandemic may be even more extensive, as admitted cases are likely to be the tip of the iceberg. Most infected individuals, as our data show, probably only have mild or even subclinical symptoms.

Since the infectious control policy is not as stringent as that for the SARS epidemic, it may only prevent severe illness by reducing the amount of viral exposure. Personal protective equipment may not be sufficient for a more virulent influenza virus with equal transmission power.

One of the limitations of this study is that the distribution of age and sex was not comparable between the hospital staff and control group. However, the subjects in the control group were significantly older but with a much lower SPR, suggesting that the hospital staff could be at an even higher risk of infection if they had been selected on an age-matched basis. Another limitation is that this study did not include questionnaires to obtain recent contact or travel history of study subjects.

In the process of establishing vaccination policy and which groups should be vaccinated first, it is imperative to determine the relative risk of contracting the disease for certain risk groups. Our data indicate that hospital staff should be vaccinated as soon as possible against the H1N1 influenza virus as they are at high risk of contracting it. In addition, for prevaccination surveillance, healthcare workers may serve as a target group in whom early seroepidemiologic changes of new emerging infectious diseases may be detected, and vaccine efficacy evaluated. Furthermore, for those who have already developed protective antibodies against the pandemic (H1N1) 2009 virus, whether or not a booster dose of vaccination is needed is debatable. Given that a relatively high proportion of certain groups may have already developed protective antibodies and given the mild nature of the pandemic (H1N1) 2009 virus, the pros and cons of a massive vaccination campaign deserve further investigation.

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