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Factors associated with infection by 2009 pandemic H1N1 influenza virus during different phases of the epidemic

Day-Yu Chao [a], Kuang-Fu Cheng [b], Tsai-Chung Li [b,c], Trong-Neng Wu [c,d], Chiu-Ying Chen [d], Chen-An Tsai [b,c], Jin-Hua Chen [b,c], Hsien-Tsai Chiu [b,d], Jang-Jih Lu [e,f], Mei-Chi Su [f], Yu-Hsin Liao [a], Wei-Cheng Chan [a], Ying-Hen Hsieh [c,d,*]

the CIDER group[g]

[a] Graduate Institute of Microbiology and Public Health, College of Veterinary Medicine, National Chung-Hsing University, Taichung, Taiwan
[b] Biostatistics Center, China Medical University, Taichung, Taiwan
[c] Graduate Institute of Biostatistics, China Medical University, Taichung, Taiwan
[d] Department of Public Health, China Medical University, Taichung, 40402, Taiwan
[e] Graduate Institute of Clinical Medical Science, China Medical University, Taichung, Taiwan
[f] Department of Laboratory Medicine, China Medical University Hospital, Taichung, Taiwan
[g] Center for Infectious Disease Education and Research, China Medical University, Taichung, Taiwan

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SUMMARY

Objective: The focus of this study was to ascertain the factors associated with 2009 pandemic influenza H1N1 (pH1N1) infection during different phases of the epidemic.
Methods: In central Taiwan, 306 persons from households with schoolchildren were followed sequentially and serum samples were taken at three sampling time-points starting in the fall of 2008, shortly after influenza vaccination. Participants who seroconverted between two consecutive blood samplings were considered as having serological evidence of infection. A generalized estimation equation (GEE) with a logistic link to account for household correlations was applied to identify factors associated with pH1N1 infections during the pre-epidemic (April–June) and epidemic (September–October) periods.
Results: The results showed that receiving an inactivated seasonal influenza vaccine (ISIV) and having a hemagglutination inhibition assay (HI) titer of 40 or higher resulted in a significantly lower likelihood of pH1N1 infection during the pre-epidemic period only, for both children and adults (adjusted odds ratio (OR) 0.3, 95% confidence interval (CI) 0.12–0.9). Having a previous infection by pH1N1 with a baseline titer of 20 or higher resulted in a significantly lower likelihood of infection by pH1N1 during the epidemic period (adjusted OR 0.06, 95% CI 0.02–0.16).
Conclusions: Our results provide the first serological evidence to suggest a protection effect from receiving an ISIV against pH1N1 infection only when the HI titer reaches 40 or higher during the pre-epidemic period. This study gives an important insight into the control and intervention measures required for preventing infections during future influenza epidemics.

1. Introduction

On April 24, 2009, a swine-origin H1N1 influenza virus (S-OIV) emerged as a novel influenza A virus and caused widespread illness in many countries worldwide, meeting the World Health Organization (WHO) criteria for a pandemic. This is now termed pandemic influenza H1N1 (pH1N1).[1–3] Reports of antigenicity distinct from that of seasonal human influenza A and differences in the pathogenicity of the virus in animal models increase concerns for a pandemic with major public health consequences.[4,5] At the same time, the incidence of clinically severe cases appears to be similar to that for seasonal flu, with 5011 hospitalizations and 301 deaths in the USA between April 15 and July 24, according to US Centers for Disease Control and Prevention (CDC) estimates.[6] This apparent contradiction highlights the need for better insights into the risk and protection factors behind influenza virus infection.

As the pH1N1 virus spread around the world in late spring 2009, at a time when well-matched pandemic vaccines were not immediately available, the question of providing partial protection with a seasonal influenza vaccine arose. However, the lack of full

* Corresponding author. Tel.: +886 4 22075913; fax: +886 4 22075913.
E-mail address: hsieh@mail.cmu.edu.tw (Y.-H. Hsieh).

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baseline, pre-pandemic immune profiles for recipients of inactivated seasonal influenza vaccines (ISIV) and for unvaccinated individuals of various ages, resulted in inconsistent results when the effectiveness of seasonal influenza vaccination in preventing 2009 pH1N1 morbidity in the general population was evaluated, as previously published.[7–10] As experts in various fields have called for serological investigations to more accurately determine rates of infection, the stored blood bank would be very useful in unveiling the extent of ISIV protection in terms of basic research and public health, which is the focus of our current study.

The government in Taiwan initiated a pandemic H1N1 clinical surveillance system on April 29, and from that date on an increasing number of probable cases was reported, especially after May 15, which correlated with the first laboratory-confirmed imported pH1N1 case on May 19. By the end of June, there were 61 travel-related laboratory-confirmed cases.[11] The first wave of 2009 pandemic H1N1 began around July 1, peaked in the last week of August, and had subsided by the end of September. We utilized the serum banks, initially collected to evaluate the effectiveness of the ISIV, to determine the antibody level of seasonal influenza virus and pH1N1 virus before and after the different phases of the epidemic as the baseline and marker of infection, within a household study design. Our aim was to compare the risk and protection factors associated with infection during different phases of the epidemic.

2. Materials and methods

2.1. Study sites and serum collection

Taiwan has a population of over 23 million. Since 2007, all schoolchildren in grades 1–4 in Taiwan have received a free influenza vaccination (ISIV) annually from the government. In order to evaluate vaccine efficacy, students from two elementary schools located in the urban Taichung City and four schools in the rural Nantou County in central Taiwan were recruited into a 3-year study starting in the fall of 2008. Taichung City is the largest urban city in central Taiwan with a population of more than 1 million and a highly developed socio-economic structure. The two schools selected were located respectively in the north and central districts of the city, with approximately 140 000 total residents. Nearby Nantou County, with a total land size approximately 25 times larger than that of Taipei City, is the second largest county and the only landlocked county on the island of Taiwan, with a population of over 500 000, and is comparatively less developed socio-economically. The four schools in Nantou County were selected purposely from four different administrative districts in the county, namely Nantou City and Tsaotun Township each with around 100 000 residents, and the rural townships of Mingjian and Guoshing with around 40 000 and 20 000 residents, respectively.

Family members of the students were also recruited into the study to further determine the effectiveness of seasonal influenza vaccine in preventing household transmissions. The study protocol based on clinical and laboratory data was established and at least two blood samples were drawn from the study subjects, before and after an influenza season. In the fall of 2008, 454 persons from 147 households were recruited into the study. Among them, 306 persons belonging to 104 households remained in the study in 2009 and underwent all three samplings required for the analysis in this report.

To evaluate the kinetic changes in antibody responses against the influenza H1N1 virus of seasonal vaccine, wild-type, and the 2009 pH1N1 strain, only 306 study subjects who had three complete sequential blood samples taken were selected from the serum bank. The first blood samples were taken in the fall of 2008, about 2 to 3 weeks after influenza vaccination (referred to henceforth as the baseline titer); the second blood samples were taken in April–June of 2009 (referred to henceforth as the pre-epidemic period) after the 2008–09 influenza season; and the third samples were taken in September–October of 2009 (referred to henceforth as the epidemic period) before vaccination with both the 2009–10 seasonal and the 2009 pandemic influenza strains. Venous blood was taken in 5- to 10-ml plain tubes and the serum was collected after centrifugation and stored at −20 °C until use. All subjects gave informed consent to study participation and the study was approved by the Medical Ethics Committee of the China Medical University.

2.2. Data collection

Two questionnaires were conducted by trained interviewers and were used to collect basic demographic and social contact information and whether a seasonal influenza vaccination had been received in the past 2 years. Information regarding underlying diseases, including cardiovascular disease, hypertension, or diabetes mellitus, was also obtained from the adults in the family. Clinical symptoms reviews were carried out using a standardized questionnaire every 2 weeks via telephone interview. Participants were asked to report any newly experienced febrile respiratory symptoms, including fever, sore throats, and headaches during the 2008–09 influenza season. However, the clinical information during the summer season was only obtained retrospectively in December and substantial recall error was expected.

2.3. Laboratory methods

Antibody titers were measured by a hemagglutination inhibition (HI) assay following the standard protocol of the WHO.[12] The virus strain used was originally isolated from a patient infected by S-OIV H1N1, which is antigenically and genetically closely related to A/California/07/2009.

To evaluate cross-reactivity, a vaccine strain of H1N1 (A/Brisbane/59/2007) and a wild-type strain that represented more than 80% of the H1N1 circulating during the 2008–09 influenza season (A/Taiwan/606/2008) were also used. All viruses used in this study were cultured from Madin–Darby canine kidney (MDCK) cells and centrifuged at 1600 rpm, 4 °C to remove cell debris. For the HI assay, serum samples were pre-treated with receptor destroying enzyme and titrated in two-fold dilutions in phosphate-buffered saline (PBS) with an initial dilution of 1:10 and a final dilution of 1:1024. Titers were expressed as the reciprocal of the highest dilution of serum at which hemagglutination was prevented. A four-fold or greater increase in HI titer between the two consecutive serological samples was defined as evidence of H1N1 seroconversion. Samples that were negative by HI were assigned a titer of 1:5 for computational purposes in obtaining a four-fold increase of HI titers.

The HI titers against the individual virus strains used in this study were determined for pH1N1, the seasonal influenza H1N1 vaccine strain (sH1N1 v), and wild-type strain (sH1N1w). Participants who seroconverted between two consecutive blood samplings (either from the first to the second sample, or from the second to the third sample) were considered to have serological evidence of infection during the pre-epidemic or epidemic period, respectively. Geometric mean titers (GMTs) were used to avoid skewed data distribution through a log transformation and were estimated by assigning a value of 5 for titers lower than 10 and a value of 2560 for titers of 2560 or higher.

2.4. Statistical analysis

As the study design was based on the prospective family cohort and the infection status may be non-independent within a