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Seroprevalence of Pandemic H1N1 Antibody among Health Care Workers in Hong Kong Following Receipt of Monovalent 2009 H1N1 Influenza Vaccine

Ying Zhou¹, Diane M. W. Ng¹, Wing-Hong Seto², Dennis K. M. Ip¹, Henry K. H. Kwok², Edward S. K. Ma³, Sophia Ng¹, Lincoln L. H. Lau¹, J. S. Malik Peiris³,⁴, Benjamin J. Cowling¹

¹Infectious Disease Epidemiology Group, School of Public Health, The University of Hong Kong, Hong Kong Special Administrative Region, China, ²Hospital Authority, Hong Kong Special Administrative Region, China, ³Department of Microbiology, The University of Hong Kong, Hong Kong Special Administrative Region, China, ⁴HKU-Pasteur Research Centre, Hong Kong Special Administrative Region, China

Abstract

Background: Healthcare workers in many countries are recommended to receive influenza vaccine to protect themselves as well as patients. A monovalent H1N1 vaccine became available in Hong Kong in December 2009 and around 10% of local healthcare workers had received the vaccine by February 2010.

Methods: We conducted a cross-sectional study of the prevalence of antibody to pandemic (H1N1) 2009 among HCWs in Hong Kong in February–March 2010 following the first pandemic wave and the pH1N1 vaccination campaign. In this study we focus on the subset of healthcare workers who reported receipt of non-adjuvanted monovalent 2009 H1N1 vaccine (Panenza, Sanofi Pasteur). Sera collected from HCWs were tested for antibody against the pH1N1 virus by hemagglutination inhibition (HI) and viral neutralization (VN) assays.

Results: We enrolled 703 HCWs. Among 104 HCWs who reported receipt of pH1N1 vaccine, 54% (95% confidence interval (CI): 44%–63%) had antibody titer ≥1:40 by HI and 42% (95% CI: 33%–52%) had antibody titer ≥1:40 by VN. The proportion of HCWs with antibody titer ≥1:40 by HI and VN significantly decreased with age, and the proportion with antibody titer ≥1:40 by VN was marginally significantly lower among HCWs who reported prior receipt of 2007–08 seasonal influenza vaccine (odds ratio: 0.43; 95% CI: 0.19–1.00). After adjustment for age, the effect of prior seasonal vaccine receipt was not statistically significant.

Conclusions: Our findings suggest that monovalent H1N1 vaccine may have had suboptimal immunogenicity in HCWs in Hong Kong. Larger studies are required to confirm whether influenza vaccine maintains high efficacy and effectiveness in HCWs.

Introduction

In 2009 the first influenza pandemic of the 21st Century was associated with a novel influenza A(H1N1) virus that emerged in North America and rapidly spread around the world [1]. In Hong Kong, the first imported pH1N1 case arrived in Hong Kong on April 30, 2009. Activity of pH1N1 peaked locally in September and had subsided by November [2]. pH1N1 was a notifiable condition throughout the first wave and 36,000 laboratory-confirmed cases were notified including 1,400 HCWs, from a local population of 7 million including 150,000 HCWs [2–4]. Vaccination is considered as the most effective preventive measure and a series of studies found that one dose in adults and two doses in children of monovalent pH1N1 vaccine were sufficient to generate seroprotective levels of antibody against pH1N1 [5–10]. The Hong Kong government purchased 3 million doses of the Panenza monovalent pH1N1 vaccine manufactured by Sanofi Pasteur, which was the only pH1N1 vaccine formulation used in Hong Kong. Local health authorities started to administer the vaccine to members of five target groups including HCWs from December 21, 2009, and extended the vaccination campaign to the general community in January 2010 [11].

We conducted a cross-sectional study of the seroprevalence of pH1N1 antibody among HCWs in Hong Kong following the first epidemic wave. In a separate study we investigated antibody seroprevalence in HCWs who reported that they had not received pH1N1 vaccine [4]. In this study we focus on HCWs who reported...
receipt of pH1N1 vaccine and investigate factors associated with antibody seroprevalence following vaccination.

Methods

Study design
We recruited HCWs between February 11 and March 31, 2010 in 6 public hospitals comprising the Hong Kong West cluster of the local Hospital Authority, with a total workforce of around 7,000 HCWs in one acute care teaching hospital and five non-acute hospitals [4]. We established fixed study locations in each hospital, and participants were invited to participate in our study by open advertisement to all cluster employees. Some participants were approached for recruitment during their regular health check in the cluster staff clinic. HCWs were eligible to participate if they were Hong Kong residents and had worked in the cluster for at least one month. Subjects provided 3 ml of clotted blood, and other information including subject characteristics, history of exposure to influenza infection, and pandemic and seasonal vaccination history was collected by trained research assistants on a short questionnaire. The study protocol was approved by the Institutional Review Board of the University of Hong Kong/ Hospital Authority Hong Kong West Cluster. Written informed consent was obtained from all participants.

Laboratory methods
Serum samples were stored in a refrigerated container at 2–8°C immediately after collection and delivered to the laboratory at the end of each working day for storage at −70°C prior to testing. Serum specimens were tested for antibody responses to A/California/04/2009 (H1N1) by hemagglutination inhibition (HI) and viral microneutralization (VN) assays using standard methods as previously described [4,12,13]. The hemagglutination inhibition (HI) test was carried out in 96 well microtitre plates using reagents provided by World Health Organization (WHO) Collaborating Centre for Reference and Research on Influenza Melbourne or the WHO Collaborating Centre, Centres of Disease Control, Atlanta, GA using standard methods as detailed in the WHO reagent kit and elsewhere. The pandemic H1N1 HA antigen was not included in the WHO reagent kit and was prepared by culture of A/California/04/2009 (H1N1) virus in MDCK cells.

The conventional neutralization test for the A/California/04/2009 was carried out in microtitre plates using neutralization of virus cytopathogenic effect (CPE) in Madin-Darby Canine Kidney (MDCK) cells. Serial serum dilutions in quadruplicate were mixed with 100 tissue culture infectious dose 50 (TCID50) for 2 hours and added to MDCK cells. One hour after infection, serum-virus mixtures were removed and serum free MEM with 2 µg/ml trypsin was added to each well. The plates were incubated and cytopathic effect was observed to determine the highest serum dilution that neutralized ≥50% of the wells. A virus back titration and positive and negative control sera were included in each assay.

Statistical analysis
We compared the differences in the proportion of HCWs with pH1N1 antibody titer ≥1:40 both by HI and VN between groups with chi-squared tests or Fisher’s exact test and used the phi coefficient to compare results between the two assays. We compared antibody seroprevalence over time in our study with the date of administration of pH1N1 vaccines to HCWs in Hong Kong [14,15]. We used Generalized Estimating Equation model to estimate 95% confidence intervals for seroprevalence adjusting for potential clustering within hospitals. Among HCWs who did not report confirmed pH1N1 infection, we used univariable and multivariable logistic regression to explore associations between presence of pH1N1 antibody titer ≥1:40 by HI and VN versus the following factors: sex, age, and seasonal vaccine history in the previous three years. Factors with p-value of 0.2 or lower in univariable analyses were included in the multivariable models. Multiple imputation was used to allow for a small amount of missing data on some background characteristics and make the most use of all available data [16].

Results
We recruited 703 HCWs during the study period, and seroprevalence data for the 599 HCWs who reported that they had not received pH1N1 vaccine have been reported elsewhere [4]. Among the 104 HCWs who reported receipt of pH1N1 vaccine and who are the focus of the following analyses, 56 (53.8%, 95% CI: 44.2%–63.2%) had antibody titer ≥1:40 by HI and 44 (42.3%, 95% CI: 33.2%–52.0%) had antibody titer ≥1:40 by VN. The phi coefficient for the comparison of antibody titers ≥1:40 by HI or VN was 0.75.

Around 10% of HCWs in Hong Kong had received pH1N1 vaccine by January 2010 (Figure 1). We found no evidence of an increase or decrease in antibody seroprevalence over time in participants, and most participants were recruited more than a month after the vaccination campaign had halted (Figure 1). Three HCWs (46/M, 20/F, 55/F) reported having had confirmed pH1N1 infection by reverse transcription polymerase chain reaction [3] in addition to receiving pH1N1 vaccine, and 2/3 had antibody titer ≥1:40 both by HI and VN while the other subject had antibody titer <1:40 by both assays.

Among the 101 HCWs without confirmed pH1N1 infection, the proportion of HCWs with antibody titer ≥1:40 by VN significantly decreased with older age (test for trend, p<0.001), and was significantly lower among HCWs who reported receipt of 2007–08 seasonal influenza vaccine (Table 1). In the logistic regression model, statistically significant differences remained by age while no statistically significant differences remained by seasonal influenza vaccine history (Table 2). There was also a statistically significant difference in the proportion of HCWs with antibody titer ≥1:40 by HI among age groups, but not by seasonal vaccine history (Table 1).

Discussion
Influenza vaccination is recommended as the primary prevention measure against infection, and HCWs are often one of the groups targeted to receive vaccine not only for their direct protection but also to indirectly protect vulnerable patients against nosocomial infection [17,18]. However seasonal influenza vaccine uptake rates are low among HCWs in many countries [19–24], and pH1N1 vaccine uptake was also low in Hong Kong following intense media coverage of a series of adverse events potentially associated with pH1N1 vaccine [11,14,15]. Mandatory influenza vaccination policies for HCWs remain controversial [18,25–27]. Around 15% of HCWs in our study reported receipt of one dose of pH1N1 vaccine, compared to overall vaccine coverage of around 10% of HCWs in Hong Kong. However, among HCWs who reported receipt of pH1N1 vaccine, only 54% had antibody titers at or above the 1:40 level by HI that is conventionally associated with protection against infection [28]. Among 599 HCWs who did not report receipt of pH1N1 vaccine, 12% had antibody titer ≥1:40 by VN during the same time period [4], which was much lower than those who reported receipt of the pH1N1 vaccine. In two studies of non-adjuvanted monovalent inactivated pH1N1 vaccine from the same manufacturer, 93% and 98% of adults achieved antibody titer ≥1:40 by HI within 21 days [29] and...
immunogenicity appeared similarly high for other non-adjuvanted pH1N1 vaccines [5,6,8]. Thus the monovalent pH1N1 vaccine appears to have had only moderate immunogenicity in HCWs in Hong Kong. There are three potential explanations for our findings. Firstly, our results may indicate truly poor immunogenicity of the vaccine specifically in HCWs who have had more repeated annual seasonal influenza vaccine compared with general population; secondly, our results may reflect imperfect effectiveness of influenza vaccine in field condition for all recipients of the vaccine; thirdly, our observed results may be biased by delays from vaccination to serum collection.

While only 42% of HCWs had antibody titers $\geq 1:40$ by VN, which is consistent with the findings of another study of 409 HCWs in Japan who were provided with one dose of pH1N1 vaccine, and just 38% achieved antibody titers $\geq 1:40$ by HI within 21 days of vaccination [30], 90% of confirmed cases achieved this titer within 3–4 weeks of infection using the same assay in the same laboratory [31]. We previously estimated that around 10%–15% of HCWs were infected through the first wave and cumulative incidence of infection was higher in younger HCWs [4] although the majority of infections were not confirmed by laboratory testing [3]. Higher pH1N1 antibody seroprevalence in younger HCWs who reported receipt of pH1N1 vaccine (Table 1) could be associated with a greater risk of infection rather than better immunogenicity [4,12]. In addition, reduced immunogenicity of pH1N1 vaccine in older adults has been reported in some previous studies [5,6,8,10]. The proportion of HCWs with antibody titer $\geq 1:40$ by VN was significantly lower among HCWs who reported receipt of 2007–08 seasonal influenza vaccine (Table 1), while no statistically significant differences remained by seasonal influenza vaccine history after adjustment (Table 2). An early report of the immunogenicity of the same pH1N1 vaccine used in Hong Kong found that geometric mean titer rises between baseline and 21 days after vaccination were significantly reduced in adults who had previously received seasonal influenza vaccines in one or more years between 2004–05 and 2007–08, but the geometric mean titer was not associated with receipt of seasonal vaccine in 2008–09 [10]. Lowered antibody response to pH1N1 vaccine was also associated with prior receipt of 2009–10 seasonal vaccine among pregnant women in Japan [32], among infants and children in Australia [33] and among high school students in Korea [34]. Pre- or co-vaccination with seasonal influenza vaccine was associated with poorer immunogenicity of a pH1N1 vaccine in two randomized trials [35,36], while pre- or co-vaccination with...
Table 1. Characteristics of 101 healthcare workers who reported receipt of monovalent pandemic (H1N1) 2009 influenza vaccine and did not report confirmed pH1N1 infection.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>Proportion with antibody titer ≥1:40 by HI (95% CI)</th>
<th>p-value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Proportion with antibody titer ≥1:40 by VN (95% CI)</th>
<th>p-value&lt;sup&gt;b&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Age 19–24 years</td>
<td>10</td>
<td>100% (69%–100%)</td>
<td></td>
<td>90% (56%–100%)</td>
<td></td>
</tr>
<tr>
<td>25–34 years</td>
<td>16</td>
<td>56% (30%–80%)</td>
<td>0.01</td>
<td>50% (25%–75%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>35–44 years</td>
<td>25</td>
<td>52% (31%–72%)</td>
<td>0.44</td>
<td>44% (24%–65%)</td>
<td></td>
</tr>
<tr>
<td>45–54 years</td>
<td>32</td>
<td>44% (26%–62%)</td>
<td></td>
<td>31% (16%–50%)</td>
<td></td>
</tr>
<tr>
<td>55–64 years</td>
<td>18</td>
<td>44% (22%–69%)</td>
<td>0.02</td>
<td>22% (6.4%–48%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Female sex</td>
<td>75</td>
<td>52% (40%–64%)</td>
<td>0.96</td>
<td>39% (28%–51%)</td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>26</td>
<td>58% (37%–77%)</td>
<td>0.78</td>
<td>50% (30%–70%)</td>
<td>0.44</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Crude odds ratio of titer ≥1:40 (95% CI)</th>
<th>Model 1&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Model 2&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Model 3&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td></td>
<td>Adjusted odds ratio</td>
<td>Adjusted odds ratio</td>
<td>Adjusted odds ratio</td>
</tr>
<tr>
<td>19–24</td>
<td>11.45 (1.25–104.60)</td>
<td>15.58 (1.63–149.34)</td>
<td>10.89 (1.16–102.07)</td>
<td>14.08 (1.39–142.27)</td>
</tr>
<tr>
<td>25–34</td>
<td>1.27 (0.36–4.48)</td>
<td>1.51 (0.41–5.33)</td>
<td>1.42 (0.39–5.14)</td>
<td>1.48 (0.41–5.40)</td>
</tr>
<tr>
<td>35–44</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>45–54</td>
<td>0.58 (0.20–1.72)</td>
<td>0.76 (0.24–2.38)</td>
<td>0.75 (0.24–2.37)</td>
<td>0.74 (0.23–2.34)</td>
</tr>
<tr>
<td>55–64</td>
<td>0.36 (0.09–1.42)</td>
<td>0.52 (0.13–2.17)</td>
<td>0.36 (0.08–1.67)</td>
<td>0.39 (0.08–1.82)</td>
</tr>
</tbody>
</table>

Did not receive seasonal influenza vaccine in 2008–09 | 1.00 | 1.00 |
Received seasonal influenza vaccine in 2008–09 | 0.46 (0.20–1.04) | 0.45 (0.18–1.11) | 0.49 (0.13–1.84) |

Did not receive seasonal influenza vaccine in 2007–08 | 1.00 |
Received seasonal influenza vaccine in 2007–08 | 0.43 (0.19–1.00) | 0.61 (0.24–1.55) | 1.05 (0.27–4.07) |

<sup>a</sup>Some columns do not total 104 due to missing data.
<sup>b</sup>p-values for association calculated by chi-squared tests or Fisher’s exact tests.
<sup>c</sup>Influenza like illness is defined as temperature ≥37.8°C plus cough or sore throat.
doi:10.1371/journal.pone.0027169.t001

Table 2. Univariable and multivariable analysis of the factors associated with antibody titer ≥1:40 to pandemic (H1N1) 2009 by viral neutralization among 101 healthcare workers who reported receipt of pandemic (H1N1) 2009 vaccine.

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<th>Model 2&lt;sup&gt;c&lt;/sup&gt;</th>
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<td>1.48 (0.41–5.40)</td>
</tr>
<tr>
<td>35–44</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
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Did not receive seasonal influenza vaccine in 2007–08 | 1.00 |
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<sup>a</sup>Multiple imputation was used to adjust for a small amount of missing data on some characteristics.
<sup>b</sup>Model 1: adjusted for the variables of age group and vaccination history in 2008–09.
<sup>c</sup>Model 2: adjusted for the variables of age group and vaccination history in 2007–08.
<sup>d</sup>Model 3: adjusted for the variables that had p-value<0.2 in univariable analyses, i.e. age group, vaccination history in 2007–08, and vaccination history in 2008–09.
doi:10.1371/journal.pone.0027169.t002
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