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Title: Immunogenicity of a Monovalent A/H1pdm Vaccine with or without prior Seasonal Influenza

Vaccine Administration

Running titles: A/H1pdm vaccine with or without prior seasonal vaccine

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Abstract

The immunogenicity of A/H1pdm vaccine might be modified by prior seasonal trivalent influenza vaccine (sTIV). We conducted a retrospective analysis of immunogenicity of 243 health-care workers (sTIV+:216, sTIV-:27) by hemagglutination-inhibition. There was no significant difference in the ratio of antibody titers of $\geq 40$ (41.2% versus 48.1%; $P=0.49$) and factor increase in geometric mean titer (3.8 versus 4.5; $P=0.37$). sTIV injected 7-10 days prior to A/H1pdm vaccine did not interfere with the immunogenicity of the latter.

Keywords: Immunogenicity, Influenza A/H1pdm, Vaccine, Interference

Text

Human infections with pandemic A/H1pdm were identified in April 2009. The availability of safe and effective vaccines was a critical component of efforts to prevent A/H1pdm infection and expansion of the pandemic (1). In Chiba University Hospital (CUH), seasonal trivalent influenza vaccine (sTIV) vaccination was conducted 7 to 10 days prior to A/H1pdm vaccination, because the precise date of the initial A/H1pdm vaccine supply was unclear owing to the manufacturer’s production capacity.

There was uncertainty as to whether prior sTIV could possibly interfere with the immunogenicity of A/H1pdm vaccine administered a week later. We undertook a clinical trial with health care workers (HCWs) to examine the immunogenicity of A/H1pdm vaccine (3). This study was a retrospective subgroup analysis of a previous study to evaluate the immunogenicity of a monovalent A/H1pdm vaccine with or without previous sTIV vaccination in HCWs aged between 20 and 39 years. All subjects provided written informed consent. The study was approved by the CUH Research Ethics Committee (G21035).

Vaccine: The A/H1pdm vaccine was monovalent, with a single dose containing 15μg of hemagglutinin antigen. sTIV contained 15μg of hemagglutinin antigen of each seed virus per single dose. Both vaccines were inactivated, split-virus, unadjuvanted ones produced by the same procedure and added with thimerosal as a preservative. Both vaccines were administered subcutaneously into the external upper arm at a single dose.

The Japanese Ministry of Health, Labour and Welfare initiated A/H1pdm vaccination of HCWs with top priority on October 19, 2009. Between Oct 26 and 30, 409 HCWs without prior A/H1pdm infection were enrolled, and peripheral venous blood samples were collected before and 28 days after vaccination. The vaccination was independent of study participation. Of the 409 subjects, 20 were then excluded because 28-day post-vaccination blood samples were not provided. As a result, immunogenicity analysis was performed on 389 subjects. Among these, we selected 243 between 20 and 39 years old for this subgroup analysis.

A/H1pdm (A/California/07/09 (H1N1)) was proliferated in MDCK cells, and then a viral fraction was obtained and inactivated with formalin. Immunogenicity of the A/H1pdm vaccine was evaluated with hemagglutination-inhibition (HI) antibody assay according to standard methods (7), using the inactivated virus as described previously (3).
Statistical analyses were performed with Dr-SPSS II (SPSS Japan Inc, Tokyo). Significance between groups was analyzed by paired *t* test, chi-square test, as well as Wilcoxon signed rank test when appropriate. *P* values of <0.05 were considered significant. We analyzed three factors as follows: 1) the proportion of subjects with antibody titer of ≥40, 2) the proportion of subjects with either seroconversion (pre-vaccination titer <10 with post-vaccination HI antibody titer of ≥40) or an increase by a factor of four or more in antibody titer, 3) the factor increase in geometric mean titer.

At baseline, 6 (3.1%) of 216 subjects with prior sTIV had antibody titer of ≥40 and 1 (2.8%) of 27 subjects without prior sTIV had antibody titer of ≥40. There were no significant differences between the two groups with and without prior sTIV, in the proportions of subjects with a baseline antibody titer of ≥40, or in the baseline geometric mean titers (GMTs) between the two groups (Table).

Post-vaccination titers of ≥40 were observed in 41.2% (95% CI, 34.6-47.8) of the subjects with prior sTIV, and in 48.1% (95% CI, 29.3-66.9) of those without prior sTIV. The proportions of subjects with post-vaccination antibody titer of ≥40 did not differ significantly between the two groups (P=0.49) (Table). Seroconversion or a significant increase in HI occurred in 60.6% (95% CI, 54.1-67.1) of subjects with prior sTIV, and in 59.3% (95% CI, 40.8-77.8) of subjects without prior sTIV, showing no significant difference between the two groups (P=0.89). There was a substantial rise in GMTs after vaccination (sTIV+: *P*<0.001, sTIV-: *P*<0.001), but the value of GMTs and the factor increase in GMTs were not significant between the two groups (P=0.64 and *P*=0.37 respectively).

This study demonstrated that sTIV injected 7-10 days previously did not affect the immunogenicity of A/H1pdm vaccine. Simultaneous administration of sTIV and A/H1pdm vaccine could induce sufficient levels of antibody to both vaccine (8). Ohfuji reported interference with the immune response to A/H1pdm vaccine by Japanese pregnant women who had recently received sTIV (6). Another study also showed lower GMT levels to A/H1pdm vaccine lower among seasonally vaccinated groups of infants and children aged 6 months to less than 9 years (5). Our current study including this subgroup analysis demonstrated lower immunogenicity among healthy HCWs (3) compared with other studies (2, 4, 9). However, we could compare the immunogenicity of A/H1pdm with or without prior sTIV because the samples from both groups were processed by the same protocol.

Regarding the differing results of the former two studies (5, 6), we speculate that the immunogenicity of infants and pregnant women is possibly modified in comparison with healthy HCWs. Since our subjects were healthy, relatively young HCWs that may have different responses to the vaccine, such as the elderly, children, and those with impaired immunity. In the study of Australian infants’ immunogenicity (5), A/H1pdm vaccination was conducted in August and early September, with seasonal influenza vaccination having been conducted more than 2 months earlier. In the study of Japanese pregnant women (6), lower immunogenicity was demonstrated in subjects receiving sTIV vaccination within 19 days prior to A/H1pdm vaccination. The interval between sTIV and A/H1pdm vaccinations was slightly longer than ours, allowing us to speculate that a shorter interval between the two types of influenza vaccines might prevent an interference effect. This study was

...
a retrospective subgroup analysis, and the two groups with and without sTIV were not assigned to this study. The number of sTIV-vaccinated HCWs was 216, and sTIV-unvaccinated ones was 27 respectively. The statistical power for the analysis the difference of the two groups might be insufficient.

In conclusion, sTIV injected 7-10 days prior to a single dose of A/H1pdm vaccine did not interfere with the immunogenicity of the latter according to HI antibody assays.

Acknowledgements

We thank the subjects for their critical role in this study, the staff of Chiba University Hospital, including the following: Dr. Yasunori Sato at the Division of Clinical Research Center, Ms. Kyoko Shoji and Mr. Hitoshi Chiba at the Division of Control and Treatment of Infectious Diseases, Chiba University Hospital, and Drs. Naruhiko Ishiwada, Junko Ogita and Haruka Hishiki at the Department of Pediatrics, Graduate School of Medicine, Chiba University. This study was funded by Chiba University.

None of the authors has any potential conflict of interest relevant to this article.
References


### Table 1 Immune responses after A/H1pdm vaccination as measured by hemagglutination-inhibition antibody assay

<table>
<thead>
<tr>
<th>Prior Seasonal Vaccination</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>216</td>
</tr>
<tr>
<td>Age</td>
<td>30.0±4.7</td>
</tr>
<tr>
<td></td>
<td>P=0.47</td>
</tr>
</tbody>
</table>

**Baseline (before vaccination)**

<table>
<thead>
<tr>
<th>Subjects with HI antibody titer ≥40</th>
<th>6</th>
<th>1</th>
<th>P=0.79</th>
</tr>
</thead>
<tbody>
<tr>
<td>- %</td>
<td>2.8</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(0.6-5.0)</td>
<td>(0.0-10.8)</td>
<td></td>
</tr>
</tbody>
</table>

**Geometric mean titer - value**

<table>
<thead>
<tr>
<th>After vaccination</th>
<th>6.7±1.3</th>
<th>6.1±1.8</th>
<th>P=0.44</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects with HI antibody titer ≥40 - %</td>
<td>41.2</td>
<td>48.1</td>
<td>P=0.49</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(34.6-47.8)</td>
<td>(29.3-66.9)</td>
<td></td>
</tr>
<tr>
<td>Subjects with seroconversion or significant increase in titer</td>
<td>131</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>- %&lt;sup&gt;c&lt;/sup&gt;</td>
<td>60.6</td>
<td>59.3</td>
<td>P=0.89</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(54.1-67.1)</td>
<td>(40.8-77.8)</td>
<td></td>
</tr>
<tr>
<td>Geometric mean titer - value</td>
<td>25.1±5.8</td>
<td>27.9±16.9</td>
<td>P=0.64</td>
</tr>
<tr>
<td>Factor increase in geometric mean titer - value</td>
<td>3.8±2.7</td>
<td>4.5±3.9</td>
<td>P=0.37</td>
</tr>
</tbody>
</table>

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A/H1pdm: pandemic influenza AH1N1 virus, HI: hemagglutination-inhibition, CI: confidence interval

- Hemagglutination-inhibition antibody titer <10 was assigned a value of 5 for the purpose of calculating the geometric mean titer. Plus–minus values are mean ±SD.
- P-values were analyzed by t test, and Wilcoxon rank-sum test was also used where appropriate.
- The proportion of subjects who had either seroconversion (pre-vaccination titer of <10 with post-vaccination HI antibody titer of ≥40) or an increase by a factor of four or more in antibody titer.