IMMUNOGENICITY AND SAFETY OF AN INACTIVATED PANDEMIC H1N1 VACCINE PROVIDED BY THE THAI MINISTRY OF PUBLIC HEALTH AS A ROUTINE PUBLIC HEALTH SERVICE

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Abstract. A prospective study was conducted among 252 participants to study the immunogenicity of unadjuvanted inactivated H1N1 influenza vaccine, using a hemagglutination inhibition (HAI) assay, conducted on Days 0 and 21 following immunization. Adverse events (AEs) were monitored for by interview. The mean age of participants (±SD) was 45 (±11) years. Seventy percent of participants had no history of major medical problems, 28% had a chronic illness and 2% were pregnant women. The HAI assay geometric mean titer (GMT) was 6.9 on Day 0 and 33.4 on Day 21 (4.8 times, $p<0.001$). The proportion of participants who had a HAI assay titers ≥40 was 7% (19/252) on Day 0. Those who had a titer ≥40 and/or a 4-fold rise in their HAI titer on Day 21 was 62% (155/ 252) ($p<0.001$). Fifty-six percent (142/252) had a four-fold increase in their HAI assay titer. Of the 19 subjects with a Day 0 HAI assay titer >40, 10 (53%) had a four-fold increases in their HAI assay titer after vaccination. On multivariate analysis, only "older age" was associated with a lower probability of immune response (OR 0.5; 95%CI 0.3-0.8). No serious systemic AEs were reported. Mild erythema and local reaction on Day 2 were reported in 9% (23 of 252). The antibody response after a single dose of inactivated monovalent H1N1 vaccination in this study was relatively low, especially in the older age group. A booster H1N1 vaccine dose may be needed. The vaccine was safe and well tolerated.

Keywords: H1N1 vaccine, safety, immunogenicity, Thai

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The abstract of this study was presented as a poster presentation at the 44th annual meeting of the 21st European Congress of Clinical Microbiology and Infectious Disease (ECCMID), Milan, 2011.

INTRODUCTION

The 2009 influenza pandemic was an outbreak of a strain of H1N1 influenza virus that contained a combination of gene segments not previously reported in humans (Igarashi *et al*, 2010; Ross *et al*, 2010). High morbidity and mortality have been reported in high-risk population with chronic medical illnesses (Dominguez-Cherit *et al*, 2009; Vaillant *et al*, 2009; Lee

et al, 2010). Thus, initial doses of influenza vaccine should provide to medical personnel, pregnant women, and patients with compromised immunity. The vaccine should boost immunity against H1N1 influenza and help ensure public health as the pandemic evolves.

The efficacy of influenza vaccines depends partly on the immuno-competence of the vaccine recipients. Older populations tend to have a diminished immune response to influenza vaccine compared to younger populations (McElhaney, 2005; Goodwin *et al*, 2006). Inactivated vaccine-related adverse events, include local reactions such as soreness, swelling and redness at the injection site. Vaccine components rarely cause severe allergic reaction (Margolis *et al*, 1990; Govaert *et al*, 1993). Data regarding safety of the influenza vaccine among immuno-compromised recipients, such as HIV-infected patients, is limited. The Thai national vaccination program against the 2009 H1N1 virus was implemented among high risk populations in Thailand. We conducted a prospective cohort study to test the immunogenicity of an unadjuvanted inactivated H1N1 influenza vaccine using a hemagglutination inhibition (HAI) assay obtained on Days 0 and 21 following immunization with 15 g of the vaccine. A survey of adverse events was carried out by interview on Days 2, 7 and 21 post-vaccination.

MATERIALS AND METHODS

We conducted a prospective cohort study of 252 consecutive patients attending the Bamrasnaradura Infectious Diseases Institute or Raj Pracha Samasai Institute, Department of Disease Control, Ministry of Public Health, Thailand, between January 2010 and September 2010. Inclusion criteria were health-care medical services personnel and pregnant women, aged 18-65 years with a chronic medical condition, and who were willing to participate in the study. Chronic medical conditions included cardiovascular diseases, cerebrovascular diseases, chronic lung disease, chronic liver disease, chronic renal failure and HIV-infected patients. Exclusion criteria were having a history of severe reaction to influenza vaccine, having received a live vaccine or live-attenuated vaccine during the 4 weeks prior to enrolment, having received immuno-suppressive drugs during the 6 months prior to enrolment, having received a blood component during the 3 months prior to enrolment, and not being able to follow-up at a second visit.

All participant received a single dose of unadjuvanted inactivated split-virion H1N1 influenza vaccine contained 15 g of hemagglutinin antigen of A/California/7/2009 (H1N1)v-like strain (NYMC X-179A). Vaccine was supplied as a multidose vial containing ten doses and was produced by Sanofi Pasteur, Val de Reuil, France and Sanofi Pasteur-Campus Mérieux, Marcy l'Étoile-France. The pre-vaccine blood samples were collected just prior to the vaccination (Day 0); the post-vaccine blood samples were collected 21 days after vaccination (Day 21). Immunogenicity testing against the vaccine viral strains was performed using a standard hemagglutination inhibition (HAI) assay with goose erythrocytes as an indicator (Kitphati *et al*, 2009). The HAI antibody titer was the reciprocal of the highest serum dilution that completely inhibited the hemagglutination reaction. The immunogenicity end point of the influenza vaccine was assessed by detecting seroprotection on Day 21 (HAI titer ≥1:40) and/or having seroconversion (≥4-fold

increase after vaccination). The geometric mean titer (GMT) was also used to assess immunogenicity in the study. To calculate GMT, an antibody titer <10 was assigned as a titer of 1:5, and a titer ≥1:2,560 was assigned as a titer of 1:2,560.

All patients were monitored for adverse events between visits by telephone interview on Days 2 and 7 post-vaccination, and by encounter interview on Day 21. Solicited injection-site reactions were pain, redness, swelling, induration and ecchymosis. Solicited systemic reactions were fever, headache, malaise, muscle aches and joint aches. Unsolicited adverse events occurring up to Day 21 were recorded. Unsolicited adverse events judged to be related to the vaccine by the investigator were listed as adverse reactions.

Means (±standard deviation) and frequencies (%) were used to describe participant characteristics where appropriate. The chi-square test, paired-samples *t*-test, and Pearson's correlation were used to analyze the data. The binary logistic regression model was used to determine the probability of having adequate immunogenicity at Day 21 by adjusting for confounding factors. A *p*-value <0.1 was used on univariate analysis and included in the multivariate analysis. Age groups were divided into 20-34, 35-49, and 50-65 years. A *p*-value <0.05 was considered statistically significant.

The study was reviewed and approved by the ethical review boards of the Institutes and the Department of Disease Control, Ministry of Public Health, Thailand.

RESULTS

Of the 252 patients who met inclusion criteria, 176 were from Bamrasnaradura Infectious Diseases Institute and 76 were from Raj Pracha Samasai Institute. None of the participants were lost to follow-up during the study period. Baseline characteristics of the patients are shown in Table 1. The mean age ±SD was 45±11years; 65% were females. Twenty-one participants (8%) were HIV-infected patients and all were receiving antiretroviral therapy. The median (IQR) CD4 cell count was 389 (319-500) cells/mm^3.

Fig 1A and 1B show the results of immunogenicity testing; the HAI GMT was 6.9 on Day 0 and 33.4 on Day 21 (4.8 times higher than Day 0; $p<0.001$, by repeated measurement analysis). The proportion of participants who had an HAI titer ≥40 was 7% (19 of 252) on Day 0 and those who had an HAI titer ≥40 and/or a 4-fold rise in HAI titer on Day 21 was 62% (155 of 252) on Day 21, respectively ($p<0.001$). Of the 155 participants (62%) who achieved successful immune responses, 110 had both seroprotection and seroconversion, 32 had only seroconversion, and 13 had only seroprotection. Of 19 subjects who on Day 0 had an HAI titer >40, 10 (53%) had four-fold rise in their HAI titer after vaccination. Day 21 HAI titers were not different among medical personnel, HIV-infected participants and those with other chronic medical conditions ($p>0.05$). Two factors with a *p*-value <0.1 by univariate analysis were included in the multivariate analysis (Table 2). On multivariate analysis, only "older age" was associated with lower probability of adequate immunogenicity ($p=0.002$). The age ranges "20-34", "35-49" and "50-65" years had percentages of participants with HAI titers >40 on Day 0 *vs* Day 21 of 2% *vs* 69% ($p=0.041$), 7% *vs* 50% ($p=0.006$), and 2% *vs* 3.7% ($p=0.132$), respectively. The relationship between Day 21 Log_{10} HAI titers and age is shown in Fig 2. Fig 3 shows the comparison between the mean Log_{10} HAI titers on Day

Table 1
Baseline characteristics of 252 participants and adverse events.

Parameters	*N* = 252
Baseline characteristics	
Age in years, mean±SD	45 ± 11
Female gender	163 (65%)
Medical personnel	177 (70%)
Participants with chronic illnesses	71 (28%)
HIV-infected participants	21 (8%)
Non-HIV infected participants, including diabetics, hypertensives, and asthmatics	50 (20%)
Pregnant women	(2%)
Body weight in kilograms, mean±SD	62 ± 13
Hemoglobin in mg/dl, median (IQR), 135 participants	13.0 (12.3-13.9)
White blood cells in cells/mm^3, median (IQR), *n*=135	6,500 (5,500-7,600)
Serum creatinine in mg/dl, median (IQR), 145 participants	0.7 (0.6-0.9)
Serum alkaline phosphatase in mg/dl, median (IQR), *n*=121	55 (40-69)
Aspatase aminotransferase in mg/dl, median (IQR), *n*=126	18 (13-22)
Alanine aminotransferase in mg/dl, median (IQR), *n*=122	14 (9-24)
Adverse events	
Local reaction on Day 2	23 (9.1%)
Systemic reaction on Day 2	20 (7.9%)
Local reaction on Day 7	0 (0%)
Systemic reaction on Day 7	10 (4.0%)

Table 2
Univariate and multivariate analyses of possible predictive factors for adequate immunogenicity.

	Univariate analysis			Multivariate analysis		
Variables	*p*-value	Odds ratio	95% CI	*p*-value	Odds ratio	95% CI
Increment of every 15 years of age	< 0.001	0.590	0.408-0.852	0.002	0.470	0.291-0.760
Local reactions on Day 2	0.095	2.132	0.877-5.183	0.258	0.510	0.158-1.639

0 and Day 21 for each age interval.

No serious systemic AEs were reported after vaccination. Mild erythema and local reaction on Day 2 was reported in 9% (23 of 252). A systemic reaction on Day 2 was reported by 8% (20 of 252). No unsolicited adverse events related to vaccine were reported.

DISCUSSION

Serum hemagglutination inhibition antibodies and neutralizing antibodies are immune responses after receiving vaccination (Clements *et al*, 1986). High levels of protective antibodies after vaccination may reduce the risk of influenza-related illness (Neuzil *et al*, 2001). The present

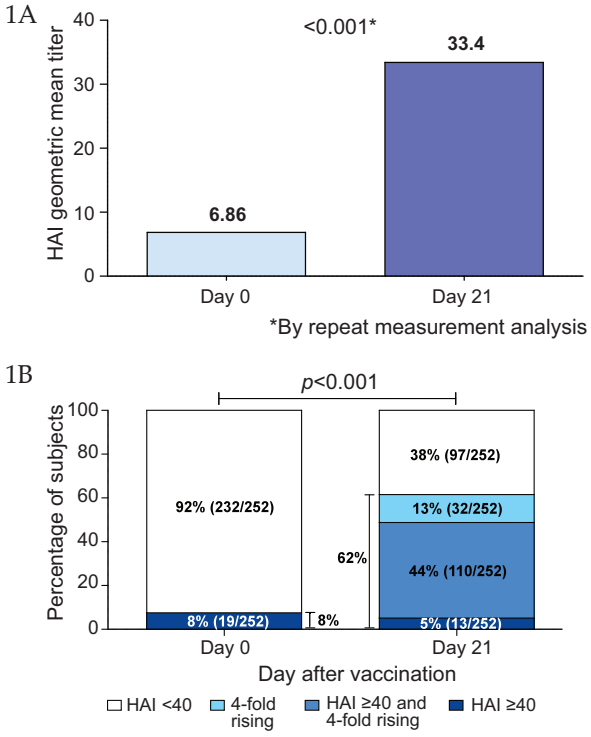

Fig 1–Comparison of HAI GMT titers on Day 0 and Day 21 (1A) and proportion of participants with an immune response on Day 21 compared to those with one on Day 0 (HAI ≥40) (1B).

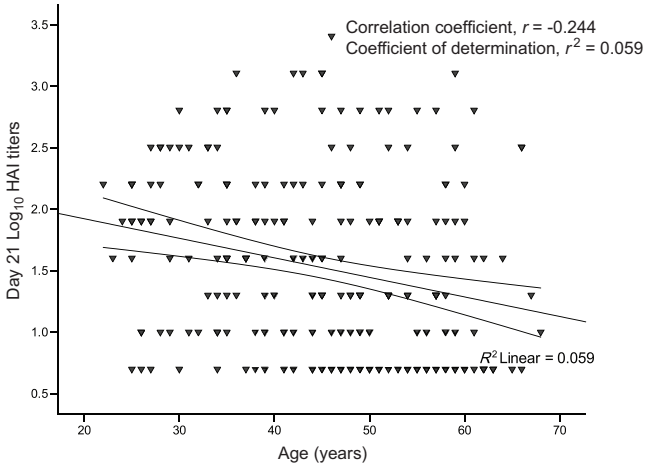

Fig 2–Relationship between Day 21 Log_{10} HAI titers and age. Solid lines represent regression prediction and 95% confidence intervals for the mean. There appears to be a relationship between old age and low Day 21 Log_{10} HAI titers (p <0.001, r = -0.244).

study determined the immune response to a single dose of inactivated pandemic H1N1 vaccine by HAI assay; this was unexpected low (62%) in the study population. Previous studies demonstrated the inactivated pandemic H1N1 vaccine resulted in HAI titers >1:40 in more than 90% of participants protecting adults with a single dose of the vaccine (Greenberg *et al*, 2009; Plennevaux *et al*, 2010). The proportion of participants who had a pre-vaccination HAI titer ≥40 was relatively low (8%) compared to previous studies (Greenberg *et al*, 2009; Plennevaux *et al*, 2010). This substantial difference may be explained by a difference in the prevalence of circulating seasonal H1N1 in each geographic area. It may also be due to circulating seasonal H1N1 affected by cross-reactive protective antibodies to 2009 H1N1 strains (Zimmer *et al*, 2009). Exposure to circulating influenza viruses from the past might provide immunologic benefit. Cross-reactive antibodies from the previous season trivalent influenza vaccine might have played a role because national coverage with an influenza vaccine program was not implemented prior to this study, unlike Western countries.

Middle aged populations with chronic medical conditions are at a high risk for influenza-associated complications (Dominguez-Cherit *et al*, 2009; Lee *et al*, 2010). Thus, an influenza vaccine is an effective preventive strategy. Among the predictive factors examined, only older age had a diminished immune response. For every 15 years incremental increase in age, the odds of having an immune response decreased by nearly 50%. The

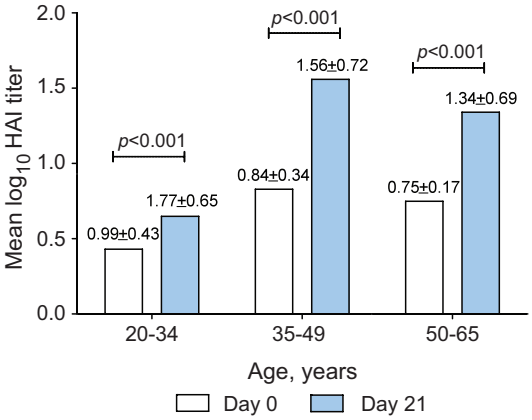

Fig 3–Comparison of mean–$\log_{10}$ HAI titers on Day 0 and Day 21 for each age interval.

period of immunity may be shorter than younger age groups (Goodwin *et al*, 2006). Therefore, we recommend two injections with the influenza vaccine among older Thais. Further study needs to confirm this hypothesis. We also demonstrated this vaccine was efficacious among relatively immuno-competent HIV-infected patients with relatively high CD4 cells who were receiving antiretroviral therapy. This finding corresponds to a previous reports (Huang *et al*, 1987; Staprans *et al*, 1995). Previous studies have shown advanced AIDS patients might not produce protective antibodies and transient virologic rebound of HIV viral load was seen (Ho, 1992; Kroon *et al*, 2000). A meta-analysis showed influenza vaccines are moderately effective in reducing the incidence of influenza among HIV-infected patients (Atashili *et al*, 2006). Monovalent H1N1 vaccination was found to be safe and well tolerated. It commonly caused local reactions, such as soreness, swelling and redness at the injection site, and less often caused fever, muscle and joint aches and headaches. In general, these symptoms were mild and did not need medical attention or interfere with daily activities. This side-effect profile was not different from a previous study (Talbot *et al*, 2008).

A number of limitations should be acknowledged. First, this study was not designed to assess the outcomes. It was designed to examine patients who received our health-care services within a fiscal year period. Future larger studies are needed to confirm these results. Second, a longer study period is needed to examine immune response after Day 21.

In conclusion, the over all antibody response rate after one dose of inactivated monovalent H1N1 vaccination in this study was relatively low, especially among the old age group. A booster H1N1 vaccination is needed. This vaccine was found to be safe and well tolerated.

ACKNOWLEDGEMENTS

The authors wish to thank all the participants in this study. This study was supported by a research grant from the Department of Disease Control, Ministry of Public Health, Thailand.

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