Taiwanese Journal of Obstetrics & Gynecology 56 (2017) 37–40

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

Seropositivity of influenza A H1NI in mothers and infants following maternal vaccination with trivalent seasonal influenza vaccine after the 2009 pandemic

An-Shine Chao [a, *], Yao-Lung Chang [a], Anne Chao [b], Ting-Shu Wu [c], Lan-Yan Yang [d], Reyin Lian [e], Yhu-Chering Huang [e]

[a] Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan
[b] Department of Anesthesiology, National Taiwan University Hospital, Taipei, Taiwan
[c] Division of Infectious Diseases, Department of Internal Medicine, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan
[d] Clinical Trial Center, Chang Gung Memorial Hospital, Taoyuan, Taiwan
[e] Department of Pediatrics, Chang Gung Children's Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan

ARTICLE INFO

Article history:
Accepted 2 August 2016

Keywords:
influenza A (H1N1)
pregnancy
vaccine

ABSTRACT

Objective: To assess H1N1 antibody titers between vaccinated and nonvaccinated maternal and cord blood sera after the 2009 pandemic.
Materials and Methods: Antibody titers were measured in maternal blood and cord sera from three groups of pregnant women in this prospective study. Group 1 comprised women who received a trivalent seasonal influenza vaccine before conception, Group 2 comprised women who received a single injection of monovalent H1N1 vaccine during pregnancy, and Group 3 comprised women who were nonvaccinated. A seropositive or seroprotective hemagglutination inhibition (HAI) assay was defined as titer $\geq$ 1:40.
Results: In this study, 500 healthy women were enrolled, of which 44 women were in the trivalent seasonal influenza vaccine group, 41 women were in the monovalent vaccine group, and 415 women were in the nonvaccinated group. The seropositive HAI titers in the three groups of mothers were 48%, 78%, and 12%, respectively. The HAI titers in the vaccinated groups were significantly higher than those in the nonvaccinated group. The HAI titers of the cord blood samples of the three groups were comparable to their respective maternal samples.
Conclusion: Seroprotection after the 2009 HIN1 pandemic was generally low in pregnant women. Vaccination during pregnancy yielded best seropositivity, whereas receiving a trivalent seasonal influenza vaccine before conception can offer better seroprotection to mothers and newborns than no vaccination.

Introduction

Seasonal influenza vaccine has been safely administered to millions of pregnant women over the past 5 decades and is effective in the mother and infant [1,2]. Passive antibodies formed by the mother reduce the risk of influenza in both the mother and infant by delaying the onset and reducing the severity of the disease [1,3,4]. Routine administration of currently licensed trivalent seasonal influenza vaccines on an annual basis is a strategic approach for protecting mothers and infants against the disease [5–7].

The World Health Organization declared the Influenza A (H1N1) virus to be responsible for the pandemic outbreak from June 2009 to August 2010. Pregnant women, infants, and young adults were susceptible to severe H1N1 infection [2,8,9]. The Taiwan Centers for Disease Control (CDC) and Chang Gung Children's Hospital conducted a survey to analyze the prevalence rate of H1N1 antibody protection and discovered that the seroprotection rate among the general population was approximately 30% [10,11]. Therefore, the Taiwan CDC recommended immunizing either one of the available monovalent vaccines: the inactivated, split-virus vaccine, MF59H-adjuvanted Focetria (Novartis Vaccines and Diagnostics, Sovicille,

* Corresponding author. Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan.
E-mail address: aschao1295@cgmh.org.tw (A.-S. Chao).

http://dx.doi.org/10.1016/j.tjog.2016.08.007

Italy); or unadjuvanted AdimFlu-S (Adimmune Corporation, Taichung, Taiwan) on November 1, 2009 [10,12]. Consequently, a total of 5.68 million doses were administrated between late 2009 and early 2010 (5.14 million doses on AdimFlu-S and 0.54 million doses on Focetria). The prevention and control of the novel H1N1 virus with monovalent vaccines is based on the understanding of seasonal human influenza and consideration of potential modes of transmission [1,13].

After the global H1N1 pandemic, a free-of-charge trivalent seasonal influenza vaccine immunization program was undertaken in autumn 2010 by the Taiwan CDC for a selected population, which included the elderly aged > 65 years, infants and children, medical personnel, and pregnant women. Clinical trial data on the H1N1 antibody response in pregnant women who received a trivalent seasonal vaccine are scarce [5,6]. This prospective clinical trial was initiated in 2010 to evaluate the seroprevalence of the H1N1 virus and antibody response in women without any systemic disease or obstetric complications who had term childbirth and received trivalent seasonal influenza vaccination before conception during 2010–2011. Both the monovalent H1N1 and the trivalent seasonal vaccines are safe and well tolerated and elicit strong immunogenic responses in women of reproductive age, thus meeting the licensure criteria of the US Food and Drug Administration and Center for Biologics [3,5,11,13,14].

Materials and methods

Participants

In this prospective surveillance and consecutive cohort study, 500 healthy pregnant women who had term childbirth in our hospital were recruited during 2010–2011. The study protocol and informed consent form were reviewed and approved by the Institutional Review Board of Chang Gung Memorial Hospital. The women were categorized into vaccinated and nonvaccinated groups. The vaccinated group was subdivided into two groups according to whether they received the monovalent H1N1 vaccine during their pregnancy at least 21 days prior to the delivery or received a single injection of a trivalent seasonal influenza vaccine prior to their conception. The nonvaccinated group did not receive monovalent or trivalent influenza vaccine and had no influenza history. All demographic and obstetric data were recorded. Samples of both maternal venous whole blood and cord blood were collected at the time of delivery for serologic evaluation of H1N1 antibody titers. The exclusion criteria were a known history of systemic diseases or severe allergy, active obstetric problems requiring blood transfusion, preterm delivery, and hypersensitivity.

Babies were followed-up for 2–6 months after birth in the well-baby clinic or by telephone questionnaire regarding any symptoms and signs of influenza and visits to neonatal clinics.

Vaccine

In 2009, the Taiwan CDC provided nonadjuvanted AdimFlu-S (Adimmune Corporation, Taichung, Taiwan) containing 15 mg of the A/California/7/2009 (H1N1)-hemagglutinin antigen and MF59-adjuvanted vaccine containing 7.5 mg of A/H1N1/California/7/2009 strain influenza antigen. A single dose of vaccine was injected by the availability to individuals in the vaccinated group. In 2010, the CDC also had three commercially available trivalent seasonal influenza vaccines, namely AdimFlu-S (Adimmune Corporation, Taichung, Taiwan), Fluvirin (Novartis), and Vaxigrip (Sanofi–Aventis). These vaccines were nonadjuvant, trivalent, inactivated split-virus vaccines, all containing influenza virus antigens of the A/California/7/2009 (H1N1)-like virus, A/Perth/16/2009 (H3N2)-like virus, and B/Brisbane/60/2008-like virus at a concentration of 15 μg/0.5 mL.

Samples

Samples of 5–10 mL of maternal venous blood and cord blood were collected at the time of delivery.

Laboratory assays

a. Whole blood and cord blood were drawn from each woman into vacuum tubes containing preservative-free heparin. Paired samples of maternal and cord serum were prepared.
b. Hemagglutination inhibition assay

A hemagglutination inhibition (HAI) assay was used to determine the H1N1 antibody titers as the standard. The specimens were analyzed in duplicate at the initial dilution of 1:10 and final dilution of 1:640. An HAI titer of 1:40 or greater was considered protective against influenza infection and used as a cutoff for seropositivity and seroprotection. The antibody responses to the 2009 H1N1 (A/Taiwan/126/09) virus were detected using the HAI assay according to the standard laboratory methods [15,16]. Each serum sample was treated with a receptor-destroying enzyme (Sigma–Aldrich, St. Louis, MO, USA) to inactivate nonspecific inhibitors. A sample of positive control serum was procured from a patient at Chang Gung Memorial Hospital with a laboratory-confirmed H1N1 infection (RT-PCR positive) at the convalescent stage. Geometric mean titer (GMT) after vaccination was calculated with a titer less than 1:10 assigned.

Statistical analysis

A reciprocal HAI antibody titer $\geq$ 1:40 from a starting value of < 10 was defined as positive for seroprotection and < 1:40 as negative. The difference between the positive and negative groups was analyzed using the Chi-square test as applicable. All analyses were conducted using the software SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Two-tailed p values < 0.05 were considered statistically significant.

Results

Among 500 women enrolled in the study, 41 (8.2%) received a single injection of monovalent vaccine during pregnancy, 44 (8.8%) received a trivalent seasonal influenza vaccine the year before conception, and 415 women (83%) received neither monovalent H1N1 nor trivalent seasonal vaccination during or after the 2009 pandemic.

All babies, including five sets of twins, were delivered at term. There was no maternal severe adverse vaccination event or fetal anomaly reported in the vaccinated group. Five structural fetal anomalies, including prenatal detection of two facial clefts, an isolated brain cyst, a congenital diaphragmatic hernia, and a newborn with hypospadia, were observed at delivery in the nonvaccinated group. All the babies have been doing well after surgical interventions. The demographic and obstetric data of the enrolled women are summarized in Table 1 showing no significance between the two groups.

Of the 41 women who received monovalent H1N1 influenza vaccinations, 32 (78%) maternal blood samples and 31 (75.6%) cord blood samples of the vaccinated group had an HAI titer $\geq$ 1:40. Of the 44 women who received trivalent seasonal influenza vaccinations, 21 (48%) maternal blood samples and 18 (41%) cord blood samples of the vaccinated group had an HAI titer $\geq$ 1:40. Of the 415

Table 1
Demographics of 500 women in vaccinated and nonvaccinated groups.

	Vaccinated		Nonvaccinated	p
	(n = 44) trivalent	(n = 41) monovalent	(n = 415)	
Maternal age (y)	32.6 ± 3.9	31.0 ± 4.0	31.4 ± 4.2	NS
Gestational age (wk)	38.9 ± 1.4	39.0 ± 1.1	39.1 ± 1.3	NS
Newborn body weight (g)	3091 ± 389	3137 ± 388	3119 ± 340	NS
Gender of newborn (male/female)	25/20[a]	23/18	202/217[a]	NS
Primi/Multipara	23/21	22/19	198/217	NS
Cesarean section	5 (11%)	3 (7.3%)	39 (9%)	NS
Vaginal delivery	39 (89%)	38 (92.7%)	376 (91%)	NS

Data are presented as *n* (%) or mean ± SD.
NS = not statistically significant.
[a] twins.

nonvaccinated women, only 49 (12%) maternal blood samples and their paired cord blood samples had an HAI titer ≥ 1:40. The maternal and cord blood samples of both the vaccinated groups showed significantly higher seroprotection rates than the samples of the nonvaccinated group ($p < 0.05$; Tables 2 and 3). The antibody titers of the paired sera from the cord blood had no significant maternal cord discrepancy in the vaccinated or nonvaccinated groups. GMT in women who received monovalent vaccine during pregnancy was calculated with a value of nearly 4, showing maternal titer at 1:80, which was significantly higher than the titer at 1:40 in women who received trivalent vaccine 1 year prior to the pregnancy (Figure 1).

No major adverse events were reported in the vaccinated women. Three infants did not have follow-up at 6 months. No event of febrile episodes or influenza-related hospitalization of the infants in the vaccinated group was reported during the 6-month follow-up, according to the medical chart review and telephone questionnaire. Only three infants in the nonvaccinated group experienced a mild influenza-like episode requiring no hospitalization.

Discussion

Women aged 22–60 years exhibited 8% preexisting antibodies against the 2009 H1N1 virus, and approximately 40% preexisting antibodies were present in the elderly population before the 2009 pandemic [10,11]. After the 2009 pandemic and a nationwide vaccination program, the overall seroconversion rate increased to approximately 30% in the entire population and 20% in the unimmunized population [10,16]. The present study demonstrated that the prevalence of preexisting antibodies in young pregnant women against the H1N1 virus was only approximately 12%, with no significant increase after the pandemic period. These women with low

Table 2
Distribution of maternal seropositive hemagglutination inhibition titers in vaccinated and nonvaccinated groups.

HAI titer	Vaccinated		Nonvaccinated (n = 415)	p
	(n = 41)	(n = 44)		
	Monovalent (%)	Trivalent (%)		< 0.05
≧:40	32 (78)	21 (48)	49 (12)	< 0.05
1:40	17	11	42	
1:80	7	8	5	
1:160	4	2	1	
1:320	1	0	1	
1:640	3	0	0	

HAI = hemagglutination inhibition.

Table 3
Distribution of cord blood seropositive hemagglutination inhibition titers in vaccinated and nonvaccinated groups.

HAI titer	Vaccinated		Nonvaccinated (n = 415)	p
	(n = 41)	(n = 44)		
	Monovalent (%)	Trivalent (%)		< 0.05
≧:40	31 (76)	18 (41)	47[a](11)	< 0.05
1:40	16	10	41	
1:80	7	7	5	
1:160	4	1	1	
1:320	2	0	0	
1:640	2	0	0	

HAI = hemagglutination inhibition
[a] Two partially clotted samples were excluded.

a seroprotection rate exhibited a low antibody titer in the cord blood, and thus their newborns were susceptible to H1N1 infection.

Few serological studies on pregnant women suggest that the antibody response to influenza vaccine is similar in pregnant and nonpregnant women, and an estimate of 20% had an HAI titer ≧ 1:40 in women without immunization against H1N1 [10]. In the present study, 48% of mothers who received a trivalent seasonal vaccination before conception exhibited a protective H1N1 antibody titer at delivery. The sustained presence of the maternal H1N1 antibody titer was higher than anticipated, and the antibody titer in the cord blood was closely related to the maternal seroconversion rate [5,7,17]. The paired blood samples showed a relevant seroprotection rate, suggesting that approximately 45% of newborns were assured of transplacental protection from the vaccinated mothers in their early life. The GMT of the vaccinated group for the paired samples of maternal and cord blood was double than that of the nonvaccinated group. The GMT for the monovalent H1N1 vaccine during pregnancy in 2009 was also comparably higher than that for the prenatal maternal trivalent seasonal vaccine (Figure 1). Nonetheless, the prenatal trivalent seasonal vaccine offered significantly greater protection to vaccinated women than to the nonvaccinated women. Our results indicated that an annual seasonal vaccination program can benefit women before conception if they conceive in the following year.

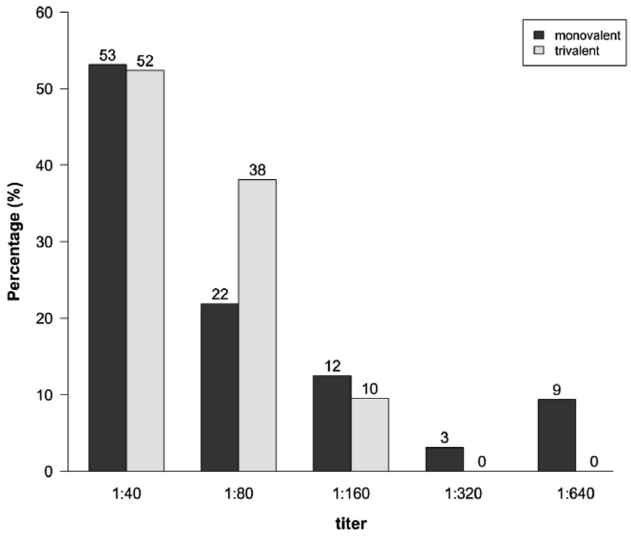

Figure 1. Antibody titers of women at delivery who received a single monovalent vaccine during pregnancy, with a geometrical mean titer of 1:80 versus antibody titers of women at delivery who received a trivalent seasonal vaccine before conception, with a geometrical mean titer of 1:40.

As the H1N1 virus continues to circulate around the world, it is necessary to examine and provide our own data for public education. Safety concerns remain a major barrier for vaccination in obstetrical practice. The 2012–2013 report by the US CDC stated that 51% of pregnant women were vaccinated with a trivalent seasonal influenza vaccine [7,18,19]. In the present study, less than 15% of pregnant mothers were vaccinated with a seasonal trivalent influenza vaccine despite the H1N1 pandemic. This maternal vaccination rate was comparable to that in the 2009 H1N1 pandemic period in which merely 20% of pregnant women received a monovalent H1N1 vaccine [11]. Official recommendation and provision of free-of-charge vaccines are the crucial factors for increasing vaccination rates [19]. Implementing an annual seasonal trivalent influenza vaccination program to general population is an acceptable approach for offering vaccination before conception [18–21]. The study results revealed the efficacy of trivalent seasonal vaccines for the H1N1 virus, and this efficacy was subsequently transferred transplacentally to the infant with beneficial effects on both the mother and the infant. However, a higher baseline or prevaccination serum titer when the same antigen was used in consecutive years [5,22] may result in hyporesponsiveness to repeated immunization in pregnant women [5]. Trivalent inactivated influenza vaccination received at postpartum also exhibited hyporesponsiveness [5].

Five term twin pregnancies were involved in the present study. Despite the small number of participants, with only one woman with seasonal influenza vaccination, the HAI titers of her paired blood samples were comparable for her twins. This small sample size, lacking longitudinal assessment of antibody persistence among women receiving monovalent H1N1 vaccine in 2009, and a lack of data on the modification of antibodies at pregnancy were the limitations to the present study.

In conclusion, our data suggested that trivalent seasonal influenza vaccination protects pregnant women from H1N1 infection without causing any adverse effects on the newborn.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

This study was supported by grants from the National Science Council of Taiwan (NMRPG496041 and 496042) and CMRPG3F1321. The authors thank Ms. Su-Hui Chiu and Shu-Li Yang for their technical support in the laboratory.

References

[1] Zaman K, Roy E, Arifeen SE, Rahman M, Raqib R, Wilson E, et al. Effectiveness of maternal influenza immunization in mothers and infants. N Engl J Med 2008;359:1555–64.
[2] Rasmussen SA, Jamieson DJ, MacFarlane K, Cragan JD, Williams J, Henderson Z. Pandemic influenza and pregnant women: Summary of a meeting of experts. Am J public health 2009;99:248–54.
[3] Steinhoff MC, Omer SB, Roy E, Arifeen SE, Raqib R, Altaye M, et al. Influenza iummunization in pregnancy — Antibody responses in mothers and infants. N Engl J Med 2010;362:1644–6.
[4] Martic J, Savic N, Minic P, Pasic S, Nedeljkovic J, Jankovic B. Novel H1N1 influenza in neonates: from mild to fatal disease. Journal of perinat 2011;31:446–8.
[5] Sperling RS, Engel SM, Wallenstein S, Kraus TA, Garrido J, Singh T, et al. Immunogenicity of trivalent inactivated influenza vaccination received during pregnancy or postpartum. Obstet Gynecol 2012;119:631–9.
[6] Nordin JD, Kharbanda EO, Benitez GV, Nichol K, Lipkind H, Naleway A, et al. Maternal safety of trivalent inactivated influenza vaccine in pregnant women. Obstet Gynecol 2013;121:519–25.
[7] Schlaudecker EP, McNeal MM, Dodd CN, Ranz JB, Steinhoff MC. Pregnancy modifies the antibody response to trivalent influenza immunization. J of infect Dis 2012;206:1670–3.
[8] Mangtani P, Mak TK, Pfeifer D. Pandemic H1N1 infection in pregnant women in the USA. Lancet 2009;374:429–30.
[9] Finberg HV. Pandemic preparedness and response—lessons from the H1N1 influenza of 2009. N Engl J Med 2014;370:1335–42.
[10] Chen CJ, Lee PI, Chang SC, Huang YC, Chiu CH, Hsieh YC, et al. Seroprevalence and severity of 2009 pandemic influenza A H1N1 in Taiwan. PloS one 2011;6:e24440.
[11] Chao A, Huang YC, Chang YL, Wang TH, Chang SD, Wu TS, et al. Seroprevalence of influenza A H1N1 and seroconversion of mothers and infants induced by a single dose of monovalent vaccine. Taiwanese J Obstet Gynecol 2013;52:356–9.
[12] Taiwan Influenza Express. Centers for disease control ROC (Taiwan) 2010: week 15 (4/11–14/17).
[13] Beigi RH, Wiringa AE, Bailey RR, MarieAssi T, Lee BY. Economic value of seasonal and pandemic influenza vaccination during pregnancy. Clin Infect Dis 2009;49:1784–92.
[14] Jackson LA, Patel SM, Swamy GK, Frey SE, Creech CB, Munoz FM, et al. Immunogenicity of an inactivated monovalent 2009 H1N1 influenza vaccine in pregnant women. J of Infect Dis 2011;204:854–63.
[15] WHO information for laboratory diagnosis of pandemic (H1N1) 2009 virus in humans – revised. WHO 2009:1–49.
[16] Chang SC, Chang CM, Huang YC, Chiu CH, Shih SR, Lin TY. Preexisting Antibodies against Pandemic 2009 Influenza A (H1N1) Virus in Taiwan. Clin Infect Dis 2010;51:1465–7.
[17] Ohfuji S, Fukushima W, Deguchi M, Kawabata K, Yoshida H, Hatayama H, et al. Immunogenicity of a monovalent 2009 influenza A (H1N1) vaccine among pregnant women: lowered antibody response by prior seasonal vaccination. J of Infect Dis 2011;203:1301–8.
[18] Lu CY, Shao PL, Chang LY, Huang YC, Chiu CH, Hsieh YC, et al. Immunogenicity and safety of a monovalent vaccine for the 2009 pandemic influenza virus A (H1N1) in children and adolescents. Vaccine 2010;28:5864–70.
[19] Flannery B, Thaker SN, Clippard J, Monto AS, Ohmit SE, Zimmerman RK, et al. Interim estimates of 2013–14 seasonal influenza vaccine effectiveness—-United States, February 2014. MMWR Morb Mortal Wkly Rep 2014;63:137–42.
[20] Couch RB, Atmar RL, Keitel WA, Quarles JM, Wells J, Arden N, et al. Randomized comparative study of the serum antihemagglutinin and anti-neuraminidase antibody responses to six licensed trivalent influenza vaccines. Vaccine 2012;31:190–5.
[21] Rivera L, Mazara S, Vargas M, Fragapane E, Casula D, Groth N. Phase III, randomized controlled trial to evaluate lot consistency of a trivalent subunit egg-based influenza vaccine in adults. Vaccine 2012;30:5285–92.
[22] CDC. Serum Cross-Reactive Antibody Response to a Novel Influenza A (H1N1) virus after vaccination with seasonal influenza vaccine. MMWR Morb Mortal Wkly Rep 2009;58:521–4.