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AOGS MAIN RESEARCH ARTICLE

Antibody response to influenza A(H1N1)pdm09 in vaccinated, serologically infected and unaffected pregnant women and their newborns

RIKKE B. HELMIG[1], RIKKE D. MAIMBURG[1], CHRISTIAN ERIKSTRUP[2], HENRIETTE S. NIELSEN[3], OLAV B. PETERSEN[1], LARS P. NIELSEN[3,4], LONE HVIDMAN[1], JENS E. VEIRUM[5], TINE B. HENRIKSEN[5] & MERETE STORGAARD[6]

[1]Department of Obstetrics and Gynecology, Aarhus University Hospital, Skejby, [2]Department of Immunology, Aarhus University Hospital, Skejby, [3]Department of Microbiological Diagnostics and Virology, Statens Seruminstitut, Copenhagen, [4]Health Sciences, Aalborg University, Aalborg, [5]Pediatric Department, Aarhus University Hospital, Skejby, and [6]Department of Infectious Diseases, Aarhus University Hospital, Skejby, Denmark

Key words
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Correspondence
Rikke Bek Helmig, Department of Obstetrics and Gynecology, Aarhus University Hospital, Skejby, Brendstrupgaardsvej, 8200 Aarhus N, Denmark.
E-mail: rikkhelm@rm.dk

Conflict of interest
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Abstract

Objective. To evaluate the serological response in pregnant Danish women immunized during the 2009 pandemic by serologic infection or by vaccination with influenza A(H1N1) Pandemrix® and describe levels of passively acquired maternal antibody in their offspring. *Design.* Observational cohort study. *Setting.* Department of Obstetrics, Aarhus University Hospital, Skejby, Denmark, October to December 2009. *Population.* Pregnant women and their offspring *Methods.* Serological analysis of antibodies to influenza A(H1N1)pdm09 by hemagglutination inhibition assay in 197 women and their offspring. Blood samples were collected consecutively at delivery from the mother and the umbilical cord. In a subgroup of 124 of the 197 women, an additional blood sample from gestational weeks 9–12 was available for analysis. *Main outcome measures.* Seroconversion, geometric mean titer, geometric mean-fold rise and protective antibodies. *Results.* 33 of the 124 subgroup women (27%) seroconverted during pregnancy, 79% after vaccination and 17% after serologic infection ($p < 0.001$). The geometric mean titer after delivery in non-vaccinated, non-serologically infected women was 17.1 (95%CI 15.7–18.6). The geometric mean titer increased significantly after serologic infection with H1N1 [76.5 (95%CI 51.3–113.9), $p < 0.001$] and after vaccination [589.6 (95%CI 339.3–1024.7), $p < 0.001$]. The geometric mean-fold rise (mother at delivery/mother early pregnancy) was significantly higher after vaccination [2.23 (1.93–2.54)] than after serologic infection [1.73 (1.59–1.87), $p = 0.013$]. In newborns of vaccinated mothers, 89.5% had protective antibody levels compared with 15.8% in newborns of serologically infected mothers ($p < 0.001$). *Conclusions.* Influenza vaccination during pregnancy confers passive immunity to the newborn.

Abbreviations: HAI, hemagglutination inhibition.

Key Message

A positive correlation was found between maternal antibody level and the level of protective antibody in the newborn. Pandemrix® vaccination seems to produce a significantly higher antibody response in the mother than natural influenza infection.

Introduction

All women who are or expect to become pregnant during the influenza season are recommended to have a routine influenza vaccination in the USA (1). The basis for this recommendation is that otherwise healthy pregnant indi-

viduals are at an increased risk of serious complications from influenza (2,3).

Although immunization of pregnant women with trivalent influenza vaccine has been recommended by the World Health Organization since 2005 (4), there has been no tradition in Denmark of following this recommendation. Both general practitioners and obstetricians have been reluctant to recommend influenza vaccination to pregnant women. However, since the 2009 influenza A (H1N1) pandemic, the National Board of Health in Denmark has issued the recommendation for all healthy pregnant women during the 2nd and 3rd trimester of pregnancy. As women with a chronic disease are at increased risk of complications of influenza, those with impaired lung function, severe asthma, diabetes, impaired immune defenses and severe obesity are offered vaccination already in the first trimester (5). The vaccination is free of charge.

The risk of spontaneous abortion is believed to be higher in the first trimester of pregnancy during influenza, but it is not known whether this is due to the fever and inflammation alone (6) or to the specific effects of the influenza virus. Several studies on the effectiveness of influenza immunization in mothers and infants have been carried out. In a randomized study including 340 women from Bangladesh, the inactivated influenza vaccine Fluarix® (GlaxoSmithKline Biologicals, London, UK) containing strains from 2004, reduced proven influenza by 63% in infants up to six months of age and averted approximately one-third of all febrile respiratory illnesses in mothers and young infants (7). In a more recent clinical trial, the immunogenicity of an inactivated monovalent 2009 H1N1 influenza vaccine was tested in 120 pregnant women. The women were randomly assigned to receive two different doses of the vaccine in a two-dose series. The conclusion was that in pregnant women, one dose of an inactivated 2009 H1N1 influenza vaccine containing 25 μg of hemagglutinin elicited an antibody response with hemagglutination inhibition (HAI) titers ≥1:40, typically associated with protection against influenza infection (8).

The purpose of our study was to evaluate the serological response in a cohort of pregnant Danish women immunized by natural infection during the 2009 pandemic, the response of those vaccinated with an influenza A(H1N1) vaccine (Pandemrix®, GlaxoSmithKline) with an adjuvant and to describe the level of passively acquired maternal antibodies in their offspring.

Material and methods

This report adhered to the STROBE statement for observational cohort studies. The study was planned to include the entire influenza A(H1N1) pandemic period. As the pandemic was less extensive than expected, sampling was stopped after three months. The study was conducted in accordance with the guiding principles of the Declaration of Helsinki and approved by the local Scientific Ethics Committee, the Central Denmark Region, Denmark (registered number 23383) and the Danish Data Protection Agency (number 1-16-02-166-11). All participants were informed verbally and signed a written consent form prior to enrollment. Data as well as consent forms are deposited in the department.

Collection of blood samples were carried out from 1 October to 31 December 2009 in the Department of Obstetrics and Gynecology, Aarhus University Hospital, Skejby, Denmark. All women planning to give birth in the hospital were asked to participate and consecutively included at the labor ward. Participants had blood samples taken shortly after birth, and umbilical cord blood was sampled. We collected 197 paired women and offspring samples. For a subgroup of 124 of the 197 women, an additional blood sample taken in gestation weeks 9–12 in the period from 3 March to 2 June 2009 was available, obtained as part of the first trimester screening program (Fig. 1).

The analyses for anti-influenza antibodies were performed at the Department of Virology, Statens Seruminstitut, Copenhagen, Denmark. The serum samples were prepared by centrifugation of the blood samples at 1344 ***g*** for 10 min. The serum was then transferred to a 3.6-mL tube. Serum samples were stored at −80 °C until use. The level of antibodies was measured in the HAI assay against the H1N1pdm strain A/California/07/09,

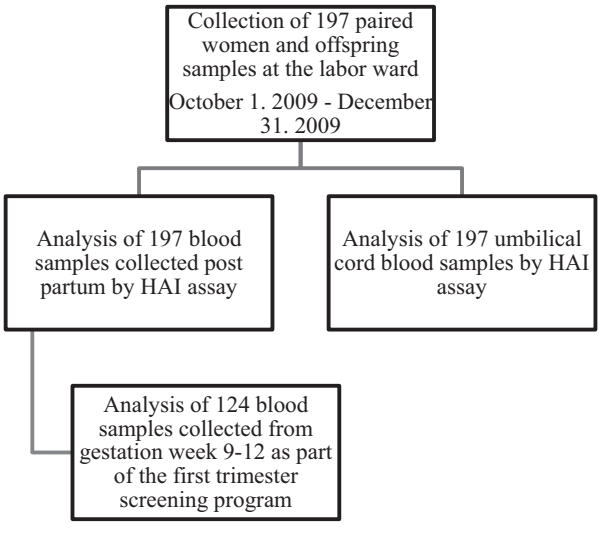

Figure 1. Flow diagram of blood samples collected and analyzed in the study.

essentially as described by Kendal et al. (9). Laboratory personnel were blinded to sample identity. Each serum sample was treated with receptor destroying enzyme (RDE) by diluting one part sample with three parts enzyme and incubating overnight at 37 °C. The enzyme was inactivated by a 30-min incubation at 56 °C followed by the addition of six parts 0.85% physiological saline to a final dilution of 1/10. The HAI assay was performed with a 0.75% guinea pig red blood cell suspension. Samples with an HAI titer ≥1:20 were considered positive. To assess the baseline level of cross-reactive antibodies against influenza A(H1N1)pdm09, stored serum samples (n = 435) from individuals born between 1920 and 1999 and obtained prior to the pandemic (between February 2004 and June 2009) were also analyzed. A baseline prevalence of preexisting cross-reactive antibodies to influenza A(H1N1)pdm09 was found in 9% of the samples. Finally, to confirm the data from the current study, a number of samples were retested in another influenza reference laboratory with similar results (T. Ziegler, pers. comm.).

Unaffected women were defined as women with two available blood samples and no sign of seroconversion or vaccination during pregnancy and women with one blood sample available postpartum with antibody levels <1:40. Serologically infected women were defined as women with two available blood samples, who seroconverted during pregnancy. Vaccinated women were women who received vaccination with Pandemrix® during pregnancy.

Data on vaccination for all participants (date of vaccination, one or two doses of vaccine) with influenza A (H1N1)v (Pandemrix®) were obtained from the Department of Epidemiology, Statens Seruminstitut, Copenhagen, Denmark. This information was valid, as all vaccinations performed with Pandemrix® were registered centrally with name and the unique Danish personal identification number during the 2009 pandemic. However, vaccination using trivalent inactive influenza vaccine was not registered in Denmark at that time.

Information from the medical records of the mother and the newborn as well as data on routine ultrasound scans performed in weeks 12 and 19 as part of the normal surveillance program were available for all participants. Growth restriction was defined as a birthweight less than –2 SD of expected weight for gestational age.

Statistical analysis

Seroprotective levels were defined as HAI titers ≥1:40 (10). Seroconversion was defined as a fourfold increase in HAI titer or a change from being seronegative (<1:20) to a titer ≥1: 40 between two samples. For analysis of the results, titers below the limit of detection were assigned the value of 10. Geometric mean titers (GMTs) were calculated by transforming data to log scale for all computations and comparisons and transforming these results back to the original scale. Comparisons between groups were performed with the use of the t-test. Within- group comparisons were done by paired-sample t-tests. The geometric mean titers with 95% confidence intervals (95% CI) are given. Two-sided probability values (p) are reported if <0.05, which indicated statistical significance. t-tests were used for comparing the equality of the geometric means for mothers postpartum between the groups.

A dichotomous variable described whether the newborn was protected at birth (antibody titer ≥1:40). The association between infection in the mother and protection, and vaccination of the mother and protection, was analyzed by Gamma statistics (11). Gamma statistics was used to show both the strength and the direction of the association between the variables. Gamma is defined as a symmetrical measure of association suitable for use with ordinal variable or with dichotomous nominal variables. It can vary from 0.0 to +/− 1.0 and provides us with an indication of the strength and the direction of the relation between two variables. Analyses were done using SPSS statistics v 21 for Mac.

Results

There was no significant difference in baseline characteristics between the unaffected, serologically infected and vaccinated groups of women and there was no record of comorbidity. Of the women, 47% were nullipara and 86% delivered vaginally. Routine fetal ultrasound examinations in weeks 12 and 19 in these pregnancies were normal except for three cases. None of these had any suspected association with influenza A(H1N1)pdm09. Eight newborns (4.1%) were born preterm before week37^{+0}. The mean gestational age at birth was 39.8 weeks (range 35.4–42.2). The mean birthweight was 3575 g (range 2350–4870 g). Only three (1.5%) of the children were growth-restricted. The mean pH value in cord blood was 7.27 (range 7.03–7.45).

Our local Department of Clinical Microbiology received nine throat swabs for influenza A(H1N1) diagnostics from the 197 included women. One of these was positive. Retrospective record review of all participants showed no records of hospitalization, apart from when giving birth, for any of the mothers or newborns, and thus there was no record of infection or hospitalization from influenza or influenza-associated illness in the mother during pregnancy and postpartum, or for the child in the first half year of life. There was no interview information on influenza or influenza-like disease during pregnancy.

Of the 124 women with two blood samples available, eight women (6.4%) were ≥40 years (40– 42 years) when giving birth. Of the 124 samples from gestation weeks 9–12, 13 (10.5%) showed antibody levels = 1:40 and only three (2.4%) of the samples had antibody levels >1:40.

Antibody titers ≥1:40 were detected in umbilical cord samples from 37 of the 124 newborns (30%). Seroconversion was seen in 33 of these women (27%). With two blood samples available, seroconversion could be proven in 15 of 19 vaccinated women (79%) and in 18 of 105 who received no vaccination (17%) ($p < 0.001$).

The geometric mean titer values from the three groups are presented in Table 1. Among women with seroprotective levels of antibody postpartum, 19 had only one blood sample. As seroconversion cannot be proven, these women and their newborns were excluded from analysis of geometric mean titers in Tables 1 and 2. A highly significant difference was found in the geometric mean titer between unaffected women and serologically infected women ($p < 0.001$) and vaccinated women ($p < 0.001$) and between serological infected and vaccinated women ($p < 0.001$). We also found a significant difference between unaffected, serologically infected ($p < 0.001$) and vaccinated women ($p < 0.001$) with regard to the geometric mean titers between early pregnancy and at the postpartum sampling. When comparing serologically infected and vaccinated women there was a significantly higher rise in the geometric mean titer in vaccinated women ($p = 0.04$). The geometric mean titer ratio (newborn cord blood /mother postpartum) was significantly higher after vaccination than after serological infection ($p = 0.013$).

In Table 2, data on passively acquired antibodies in 178 newborns are presented. The number of newborns with passively acquired maternal antibody levels of ≥1:40 was not higher ($p = 0.588$) in the group of newborns born to serologically infected women than those born to unaffected women. In newborns of vaccinated women, 89.5% had antibody levels in the protective range, which was significantly different from newborns of unaffected women ($p < 0.001$). Twenty-three of the women were vaccinated with Pandemrix® during pregnancy. Of these, 19 were vaccinated more than two weeks before delivery (interval 20–43 days). Seventeen of these (89%) had an antibody titer ≥1:40 postpartum. The remaining four women were vaccinated <14 days before delivery.

Discussion

This study evaluated the serological response in a cohort of pregnant Danish women immunized during the 2009 pandemic either through infection or by vaccination with influenza A (H1N1) Pandemrix®. It showed that vaccination of the pregnant women more than two weeks before

Table 1. Geometric mean titers (antibody titers) of mothers and newborns.

	Mother, geometric mean Early pregnancy		Mother, geometric mean After delivery		Newborn, cord blood Geometric mean		Geometric mean fold rise (Mother post/mother pre)		Geometric mean titer ratio (newborn/mother post)	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Unaffected	15.8 ($n = 90$)	14.2–17.5	17.1 ($n = 140$)	15.7–18.6	20.8 ($n = 140$)	18.5–23.4	1.06 ($n = 90$)	1.01–1.12	1.10 ($n = 140$)	1.05–1.15
Serological infection	12.9 ($n = 19$)	9.6–17.4	76.5 ($n = 19$)	51.3–113.9 *, #	25.4 ($n = 19$)	12.8–50.5	1.73 ($n = 19$)	1.59–1.87 #	0.73 ($n = 19$)	0.63–0.83
Vaccinated	21.9 ($n = 15$)	13.0–36.9	589.6 ($n = 19$)	339.3–1024.7 *, #, ¤	324 ($n = 19$)	143.9–729.3	2.23 ($n = 15$)	1.93–2.54#	0.90 ($n = 19$)	0.81–1.10 **

Comparison of the geometric mean titers between the mothers in early pregnancy and after delivery using paired-samples *t*-test. *$p < 0.001$.
Comparison of the geometric mean rise between the group of women who were neither vaccinated nor infected, and the serological infected and vaccinated group, respectively, using *t*-test. #$p < 0.001$.
Comparison between the serological infected and vaccinated group using *t*-test. ¤$p = 0.04$.
Comparison of geometric mean titer ratio (newborn cord blood/mother postpartum) between vaccinated and serological infected using *t*-test **$p = 0.013$.

Table 2. Passively acquired maternal antibody level in newborns (n = 178).

	Newborn, cord blood Antibody titer				
	<1:40		≥1:40		
Mother	*n*	%	*n*	%	
Unaffected	111	79.3	29	20.7	140
Serological infected	16	84.2	3	15.8	19
Vaccinated	2	10.5	17	89.5*	19

A dichotomous variable describes whether the newborn is protected at birth (antibody titer ≥1:40). Comparison between vaccinated and serologically infected, and between vaccinated and neither infected nor vaccinated using Gamma statistics *$p < 0.001$.

delivery confers passive immunity on the newborn with almost 90% of the newborns having antibody levels in the protective range. It was a strength of this study that we had blood samples from early pregnancy for almost two-thirds of the women, and these samples were taken before the first wave of H1N1 infection was reported in Denmark. To this can be added that all data on vaccination dates and dose, records from the hospital, in pregnancy and postpartum, as well as results from ultrasound scans and microbiological testing on each of the participating women and newborns, were available for analysis.

There are also some limitations to the study. Unfortunately, two blood samples were only available for two-thirds of the study women. In addition, data on vaccination were limited, since only 12% of the pregnant women received the influenza vaccination. Both limitations reduce the power to find rare effects and draw firm conclusions.

When evaluating vaccination response, only those vaccinated more than two weeks before delivery were included for an antibody response to be elicited. Usually, an antibody level ≥1:40 is considered to be clinically relevant and to result in a 50% decrease in symptomatic infection (12). Likewise, an HAI antibody titer of ≥ 1:40 after vaccination is the current standard for licensing influenza vaccine and a widely accepted surrogate for protection against influenza infection (13).

In our study natural influenza infection during pregnancy gave a significant rise in the antibody level of the mothers, but not in the newborn. A possible explanation for this could be maternal infection shortly before delivery. In a fifth of the newborns of unaffected women we found protective HAI titers in the umbilical cord samples. This has been described before and was explained by an active IgG transport system across the placenta, resulting in higher antibody concentration in the fetal compared with the maternal side of the circulation (14).

In 12% of the women from whom we had early pregnancy blood samples, we found HAI titers ≥1:40. This was comparable to our finding of preexisting cross-reactive antibodies to influenza A(H1N1)pdm09 in 9% of stored serum samples. In a recent study on the immunogenicity of an inactivated monovalent 2009 H1N1 influenza vaccine (8), 7% (2–18%) had HAI titers ≥1:40 at baseline before vaccination. As in our study, those authors showed a significant rise in the geometric mean titer after vaccination, with maternal values three weeks after vaccination in the range 259.6–568.6 and values at delivery and up to 150 days after vaccination between 152.1 and 348.7 in cord blood, and 83.0 and 210.3 in the mother. Our values were slightly higher, which may be due to the shorter interval of 20–40 days from vaccination to delivery. Antibody levels are known to decrease with time. In a study where pregnant women were vaccinated in weeks 22–32 with a non-adjuvant H1N1 vaccine, the same significant increase in the geometric mean titer after vaccination and a seroconversion rate of 93% was found (15). At baseline, 19% of these women had an antibody titer of ≥1:40, explained by cross-immunoreactivity with previous seasonal influenza vaccination or subclinical infection with H1N1 infection. This baseline was higher than we found. Cross-reactive antibodies to influenza A(H1N1) were detected before vaccination in 6–9% of individuals aged 18–64 years (16) and, in a study from Australia (17), cross-reacting HAI antibody titers of ≥1:40 were found in a third of individuals aged 60 or older. Thus, cross-reactivity is seen in older individuals, likely representing previous influenza pre-1977 H1N1 infection. This is not a plausible explanation in our study group, as only 6.4% of the women were 40–42 years. In addition, seasonal influenza vaccination is uncommon among healthy young Danes.

Our study was conducted in the early days of the influenza A(H1N1) pandemic, but the first recommendations on the use of vaccination in pregnancy had been issued worldwide before this. Nevertheless, we found that only 11.7% of the women had received the vaccine and thus had followed national guidelines. In the beginning of the pandemic, there was some confusion regarding pregnant women, which could explain this low uptake. During the 2011 and 2012 seasons, the National Board of Health re-issued the same recommendation for pregnant women, but still only a small fraction of pregnant women received the vaccine. In a nationwide register-based cohort study of live born infants between November 2009 and September 2010, only 13.1% had been given the influenza A(H1N1)pdm09 vaccine during pregnancy (18).

In a recent review on the safety of influenza vaccination during pregnancy (19) it was noted that although pregnant women are at a particularly high risk of morbidity and mortality from influenza, and vaccination on this basis is highly recommended, pregnant women have historically had the lowest vaccine coverage rates among adults recommended to receive seasonal influenza vaccination. This is true even though no harmful effects of influenza vaccination on maternal health during pregnancy have been demonstrated in many studies, including a prospective randomized double-blind controlled trial (7).

In conclusion, we found a positive correlation between the maternal antibody level and the level of protective antibody conferring passive immunity to the newborn. It seems that Pandemrix® vaccination elicits a significantly higher antibody response in the mother than natural influenza infection. However, few pregnant women and their doctors followed the vaccination recommendations.

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