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Successful seroconversion against diphtheria and tetanus induced through maternal vaccination in a region of Colombia

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Purpose: This study aims to compare protection against diphtheria and tetanus conferred on the mother and the neonate before and after maternal vaccination against tetanus, diphtheria, and acellular pertussis (Tdap), transfer of antibodies, and the variables that could impact on the protection.

Materials and Methods: The study followed a cohort of 200 pregnant women from a region in Colombia, contacted during prenatal control before vaccination and upon delivery. The work determined immunoglobulin G antibodies against diphtheria and tetanus of pregnant women and umbilical cord. The proportion of protection, the geometric mean of the concentration, and the transfer of maternal antibodies were calculated. The protection profile of the pregnant women was explored by using multiple correspondence analysis.

Results: The concentration of antibodies against diphtheria was significant before and after vaccination of the pregnant women (p=0.000) with proportions of 85.0% and 97.5%, respectively, and of 98.6% in the umbilical cord, with significant antibody correlation (Spearman's coefficient=0.668, p=0.01). Sero-protection against tetanus before vaccination was at 71.0%, after at 92.6%, and in the umbilical cord at 95.9%, with significant antibody concentration before and after vaccination (p=0.000) and antibody correlation (Spearman's coefficient=0.936, p=0.01). Sero-protection was higher when the pregnant women were vaccine 8 to 11 weeks before delivery. Unprotected pregnant women were those not vaccinated during pregnancy.

Conclusion: The high proportion of protection against diphtheria and tetanus and the placental transfer support the need to promote maternal immunization with Tdap.

Keywords: Seroepidemiological studies, Diphtheria-tetanus-acellular pertussis vaccines, Immunization, Colombia

Introduction

In recent decades, maternal vaccination has been promoted to confer direct protection to the mother and indirect protection to the neonate through the placental transfer of antibodies and breastfeeding [1]. During the first decade of the 21st century, vaccination of pregnant women with tetanus, diphtheria, and acellular pertussis (Tdap) was promoted after the global resurgence of pertussis between 2010 and 2013 and acceleration of the vaccine against influenza due to maternal complications reported in 2009 during the AH1N1 influenza pandemic [2].

The Tdap is considered safe for mothers and their infants and immunogenic and ef-

fective against pertussis, according with large-scale clinical trials in different countries [1,3]. Notwithstanding, vaccine coverage has been sub-optimal with persistent social disparity. In the United States, the Center for Disease Control conducted a survey in April 2020, revealing that 56.6% of 463 pregnant women who responded to the survey had received the Tdap vaccine during pregnancy; 72.7% of the pregnant women had received a recommendation or referral to be vaccinated. Nevertheless, vaccination of older pregnant women (35–49 years of age), black or Hispanic, with low educational level, single, unemployed, below the poverty line, residents in non-rural areas, without prenatal insurance was lower in public health insurance [4]. In Latin America and the Caribbean, until 2018, 14 of 49 countries and territories had recommended vaccination of pregnant women with Tdap in their routine vaccination programs and between 2012 and 2018, 12 countries had reported oscillating vaccination coverage [5].

In Latin America and the Caribbean, seroprevalence studies have been conducted on the effect of vaccinating against pertussis [6-8], but as far as we know, immunity conferred against diphtheria and tetanus and the variables that could influence on sero-protection for these diseases has not been studied. Sero-surveillance of diphtheria and tetanus in pregnant women and neonates is especially useful in this region, given the difficulty of having a vaccination card, up-to-data information from population census, and state pregnancy records in the vaccination information system [5,9].

Periodic monitoring of the proportion of sero-protection against tetanus is a critical tool to document the elimination of maternal and neonatal tetanus [10,11]. In spite of the global effort to achieve eliminating maternal and neonatal tetanus (75% of 29 priority countries validated achieving elimination targets in 2018), approximately 47-million women and neonates are not protected against tetanus globally, with low vaccination coverage due to the lack of access to health services, limited healthcare systems, lack of security, social conflict, lack of funding, among others [12].

Seroprevalence against diphtheria in pregnant women and neonate permits verifying the protection conferred and the probability of occurrence of cases. After the social and health crisis caused by the coronavirus disease 2019 (COVID-19) pandemic, global concern exists due to the resurgence of diseases preventable through vaccination after the reduction of infant and maternal vaccination coverage, especially in South America, where before the pandemic there were municipalities with DTwP3 vaccination coverage <80% [13]. Recently, diphtheria outbreaks have been reported between 2016 and 2019 in population affected by migration and social and political conflict (Bangladesh, Yemen, Venezuela); most cases occurred in unvaccinated individuals <15 years of age [14]. Colombia, Haiti, Venezuela, and the Dominican Republic reported confirmed cases between 2018 and 2021 [15,16].

In 1980, Colombia introduced vaccination with DTwP to children <1 year of age (2, 4, 6 months, reinforcement at 18 months and 5 years of age), application of diphtheria and tetanus toxoid (Td) within the neonatal tetanus elimination plan (5 doses, reinforcement every 10 years) and the vaccine with Tdap has been applied to pregnant women since 2013 as part of the Expanded Immunization Program (PAI, for the term in Spanish) [17,18]. The Tdap vaccine coverage in pregnant women has increased gradually until reaching 90% in 2018, but in 2020 it was <80% due to COVID-19 pandemic [5].

This work compared the immunoglobulin G (IgG) antibodies against diphtheria and tetanus before vaccinating with Tdap during the prenatal control, after vaccinating during delivery, and the placental transfer of antibodies in pregnant women residing in Medellín, the second largest city in Colombia (approximately 2.6-million inhabitants) and its metropolitan area. Additionally, the study analyzed the profiles of the pregnant women according to sero-protection status.

This study may be useful for countries in the Americas and other regions of the world, which seek to enhance Tdap maternal vaccination programs and use sero-surveillance to document the control and elimination of these diseases.

Materials and Methods

This study was approved by the ethics committee of the "Héctor Abad Gómez" National Faculty of Public Health at Universidad of Antioquia and of the participating hospitals (act code: 021, February 2019), followed national and international ethical guidelines on research with human beings.

This prospective observational study analyzed anti-diphtheria and anti-tetanus antibodies in a cohort pregnant women and umbilical cord pairs and the profiles of the characteristics of the pregnant women according to the sero-protection status.

The samples were collected from April to October 2016 in two public hospitals in Medellín and its metropolitan area as part of a prior study of the Departmental Sero-surveillance Program of Antioquia [8].

The pregnant women were contacted during prenatal con-

trol before being vaccinated with Tdap and then were monitored until delivery care, when blood samples were taken from the pregnant women and from the umbilical cord, within the regular health care process. The pregnant women attended spontaneously to the vaccination service.

The study did not include women with multiple pregnancy. Upon delivery, it was corroborated that the pregnant women did have not a fever in the previous 72 hours (chorioamnionitis, sepsis) and had no limitations to participate in the study due to requiring care in intensive care unit.

All the pregnant women signed an informed consent upon accepting to participate. Pregnant women <18 years of age signed a consent together with their legal guardian. Blood samples from the pregnant women during prenatal control and delivery were collected through venous puncture. During delivery, a blood sample was also taken from the umbilical cord before clamping it.

These samples were taken by a nurse and the staff in charge of the delivery care, with prior training and standardization. Moreover, a survey was conducted including sociodemographic variables and the state of health of the pregnant women and basic data from the neonate.

The blood samples were frozen at –80°C until use and analyzed in the Departmental Public Health Laboratory of the Sectional Secretary of Health and Social Protection of Antioquia.

Antibodies were determined by using commercial kits. Anti-diphtheria toxin IgG was measured using the Corynebacterium diphtheriae toxin IgG NovaTec ELISA (NovaLisa; NovaTec, Dietzenbach, Germany), following manufacturer's instructions. The results for diphtheria were classified as <0.01 IU/mL as not protected, between 0.01 and 0.09 IU/mL with IgG antibodies of uncertain duration of the protection, and ≥0.1 IU/mL as protected. Anti-tetanus toxin IgG was determined using the NovaTec *Clostridium tetani* toxin IgG ELISA (NovaLisa; NovaTec), following manufacturer's instructions. The results for tetanus were classified as <0.01 IU/mL as not protected, between 0.01 and 0.1 IU/mL with IgG antibodies of uncertain duration of the protection, and ≥0.1 IU/mL as protected. The optical density measurements were converted into IU/mL, using a calibration curve [19].

The IgG antibodies were reported as geometric means with 95% confidence intervals. Placental transfer was measured as the ratio of antibodies from the cord in relation with the maternal antibodies. The study compared the proportion and median of the concentration of paired samples from the pregnant women before and after vaccination and the umbilical cord, using Cochran's Q and Wilcoxon's rank tests, respectively. The correlation between maternal antibodies after immunization and antibodies from the umbilical cord was analyzed, by using Spearman's rank coefficient. A p-value <0.05 was considered statistically significant.

The profiles of the pregnant women according to the seroprotection status against diphtheria and tetanus, categorized as unprotected (<0.099 IU/mL) and protected (≥0.1 IU/mL) was explored through a multiple correspondence analysis according with the sociodemographic characteristics (age group, urban or rural origin, years of schooling, and overcrowding defined as the presence of three or more people in a room of the home) and the antecedent of vaccination with Tdap during the current pregnancy.

Analyses were performed in IBM SPSS Statistics for Windows ver. 21.0 (IBM Corp., Armonk, NY, USA) and Stata ver. 15.0 (Stata Corp., College Station, TX, USA).

Results

The study included 200 pregnant women contacted during prenatal control. From these, there were 162 (81.0%) maternal samples during delivery and 148 (74.0%) umbilical cord samples.

The ages of the pregnant women ranged between 13 and 42 years, with an average of 23 years of age (standard deviation=6.14; median=21; interquartile range [RIQ], 19–26) (Table 1).

The pregnant women resided predominantly in the urban area, were married or in common-law; their homes were of low socioeconomic level, they did not live in overcrowded conditions, studied basic levels of primary and secondary school (median of 9 years of schooling; RIQ, 7–11), and were affiliated to health social security (Table 1).

In all, 11.5% of the pregnant women perceived their state of health as regular or poor (Table 1). The pathological antecedents highlight preexisting hypertension in 21 pregnant women (10.5%). None of the women reported antecedents of pre-eclampsia, diabetes, human immunodeficiency virus (HIV), drug abuse or use of immunosuppressive drugs or immunoglobulin. Delivery took place between weeks 30 and 41 of gestation (median, 39; RIQ, 38–40).

Most of the pregnant women were born after 1980 when infant vaccination with DTwP began. Most received the DTwP vaccine during childhood. Of the total, 33 (16.5%) were

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Table 1. Characteristics of the pregnant women and their neonates

Characteristic	Category	No. (%)
Pregnant women		
Age (yr)	13–21	101 (50.5)
	22–42	99 (49.5)
Birth cohort	Born before 1980	11 (5.5)
	Born 1980+	189 (94.5)
Residence area	Urban	192 (96.0)
	Rural	8 (4.0)
Marital status	Married or common-law union	134 (67.0)
	Single or separated	66 (33.0)
Socioeconomic	Low	155 (77.5)
	High	1 (0.5)
	No data	44 (22.0)
Overcrowding	Yes	7 (3.5)
	No	193 (96.5)
Years of schooling	1–11	181 (90.5)
	>11	18 (9.0)
	No data	1 (0.5)
Social security in health	Contributory	4 (2.0)
	Subsidized	189 (94.5)
	Not insured or linked	7 (3.5)
Self-perceived health status	Good to very good	155 (77.5)
	Fair to bad	23 (11.5)
	No data	22 (11.0)
No. of pregnancies	1	92 (46.0)
	2–9	108 (54.0)
Gestational week at delivery (wk)	30–36	14 (7.0)
	37–41	185 (92.5)
	No data	1 (0.5)
Vaccination with Tdap in the current pregnancy	Vaccinated	167 (83.5)
	Not vaccinated	33 (16.5)
Vaccination with Tdap vaccine (trimester of pregnancy)	Second	84 (42.0)
	Third	91 (45.5)
DTwP vaccination in childhood	Yes	104 (52.0)
	No	8 (4.0)
	Don't know/no data	88 (44.0)
Pregnancy vaccination history	Previous Tdap	9 (4.5)
	Td/TT 1st dose	95 (47.5)
	Td 2nd dose	25 (12.5)
	Td 3rd dose	9 (4.5)
	Td 4th dose	5 (2.5)
	Td 5th dose	5 (2.5)
Newborns		
Delivery type	Vaginal	143 (71.5)
	Caesarean section	55 (27.5)
	No data	2 (1.0)
Sex	Male	109 (54.5)
	Female	89 (44.5)
	No data	2 (1.0)
Birth weight (g)	<2,500	17 (8.5)
	≥2,500	179 (89.5)
	No data	4 (2.0)

not vaccinated with Tdap during the current pregnancy. Those who were vaccinated received the biological between weeks 20 and 39 of gestation (median, 28; RIQ, 26–30). Nine pregnant women (4.5%) had been vaccinated with Tdap during a previous pregnancy and nearly half of the pregnant women had only received one dose of diphtheria or tetanus toxoid (TT) (Table 1). Most of the deliveries were attended vaginally and the neonates weighed ≥2,500 g (Table 1).

Table 2 presents the change in antibodies in the pregnant women before and after vaccination with Tdap and in the umbilical cord. An increase was detected of the proportion of protection of the pregnant women before vaccination, on delivery (after vaccination), and from the umbilical cord, with significant difference for both diseases (p=0.000).

The proportion of seropositivity against diphtheria with certainty of the duration of the protection was 83.5% (n=167) before vaccination with Tdap and 97.5% (n=158) after vaccination. In all the neonates, antibodies were detected in the umbilical cord (seropositivity=98.6%, n=146).

In all the pregnant women and neonates, IgG antibodies were detected against tetanus before vaccination, 71% (n=142) of the pregnant women had protection, and 29% (n=58) had antibodies, but the duration of the protection was uncertain after vaccination. Sero-protection for the pregnant women was at 92.6% (n=150) and at 95.9% (n=142) for the neonates (Table 2).

Regarding antibody concentration, the median difference of antibodies against diphtheria and tetanus before and after vaccination was significant (Z=-4.9 and Z=-5.3, respectively; p=0.000). The antibody correlation in the pregnant women post-vaccination with those from the umbilical cord was significant for diphtheria (Spearman's coefficient=0.668, p=0.01) and for tetanus (Spearman's coefficient=0.936, p=0.01).

Placental transfer of IgG antibodies against diphtheria and tetanus was observed, independent of the vaccination with Tdap during the current pregnancy and the neonate's weight (Fig. 1). The ratio of antibodies from the cord in relation with the maternal antibodies was 1.0 in 140 pairs (70.0%) for diphtheria (minimum=0.66 and maximum=2.13) and in 135 pairs (67.5%) for tetanus (1.0–2.17). In three mother/cord pairs, the ratio of antibodies for diphtheria was <1.0 (Fig. 1).

By age, the mean of antibodies before vaccination was lower in the younger age group (13–21 years) compared with the older age group, for both diseases, although such differences were not significant (Fig. 2).

The proportion of the neonate's seropositivity against diph-

Table 2. Immunoglobulin G antibodies against diphtheria and tetanus from pregnant women and umbilical cord

Variable	Before vaccination (n=200)	After vaccination (on delivery) (n=162)	Umbilical cord (n=148)
Diphtheria			
Protected	167 (83.5)	158 (97.5)	146 (98.6)
Not protected	10 (5.0)	1 (0.6)	0 (0.0)
Had antibodies of uncertain duration of the protection	23 (11.5)	3 (1.9)	2 (1.4)
Geometric means (95% CI)	0.104 (0.09–0.1201)	0.145 (0.139–0.149)	0.146 (0.14–0.149)
Tetanus			
Protected	142 (71.0)	150 (92.6)	142 (95.9)
Not protected	0 (0.0)	0 (0.0)	0 (0.0)
Had antibodies of uncertain duration of the protection	58 (29.0)	12 (7.4)	6 (4.1)
Geometric means (95% CI)	0.754 (0.66–0.845)	0.960 (0.91–0.987)	0.98 (0.96–0.99)

Values are presented as number (%), unless otherwise stated.
CI, confidence interval.

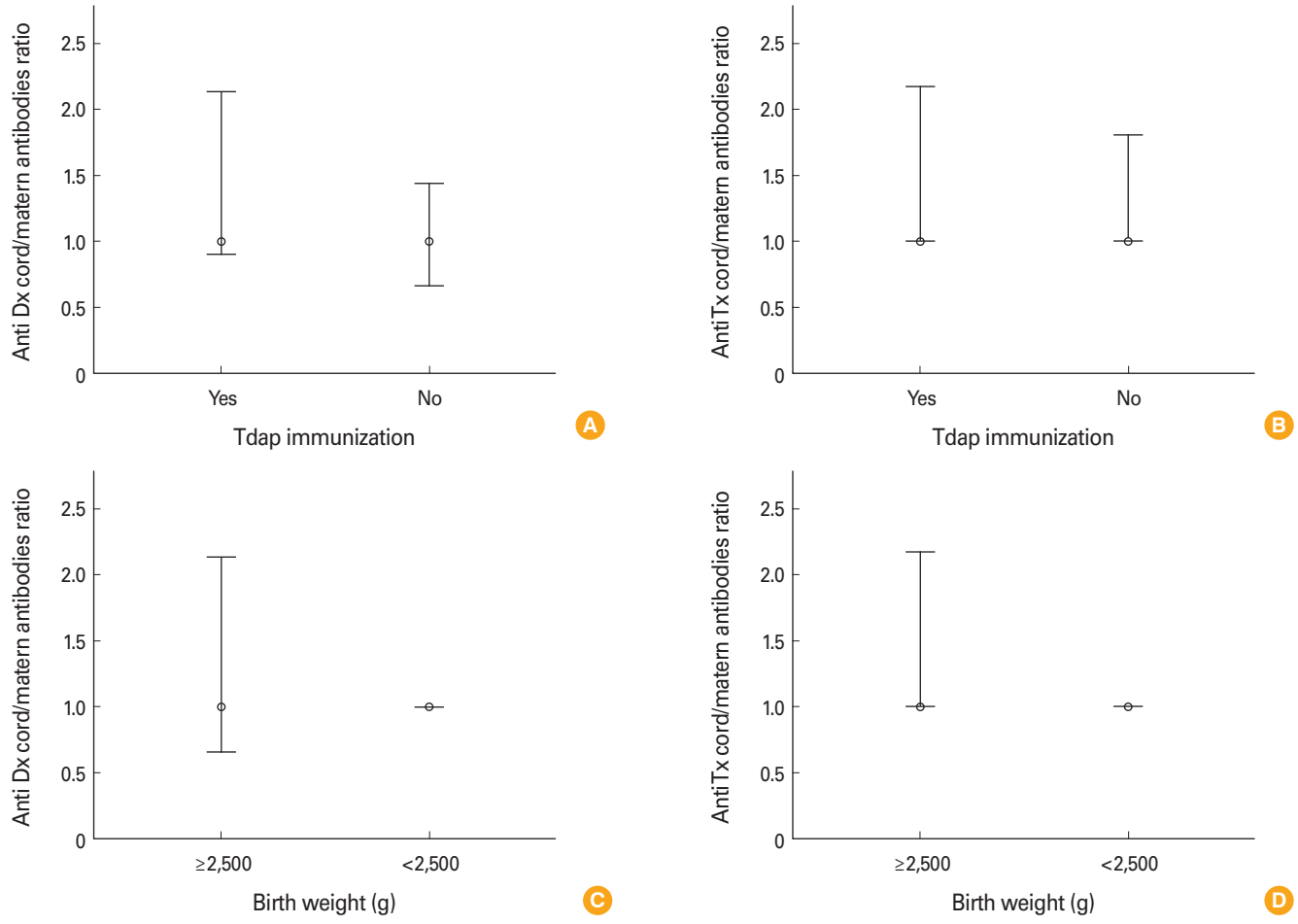

Fig. 1. (A–D) Ratio of immunoglobulin G umbilical cord and maternal antibodies against diphtheria and tetanus (mode, minimum, maximum) according to the tetanus, diphtheria, and acellular pertussis (Tdap) vaccination status during the current pregnancy and birth weight of newborns.

theria and tetanus was higher when the pregnant women were vaccinated with Tdap, 8 to 11 weeks before delivery. The pregnant women with uncertain protection against diphtheria had been vaccinated 12 to 15 weeks before delivery. Those with uncertain protection against tetanus had been vaccinated during these same weeks, but also less than 4 weeks from

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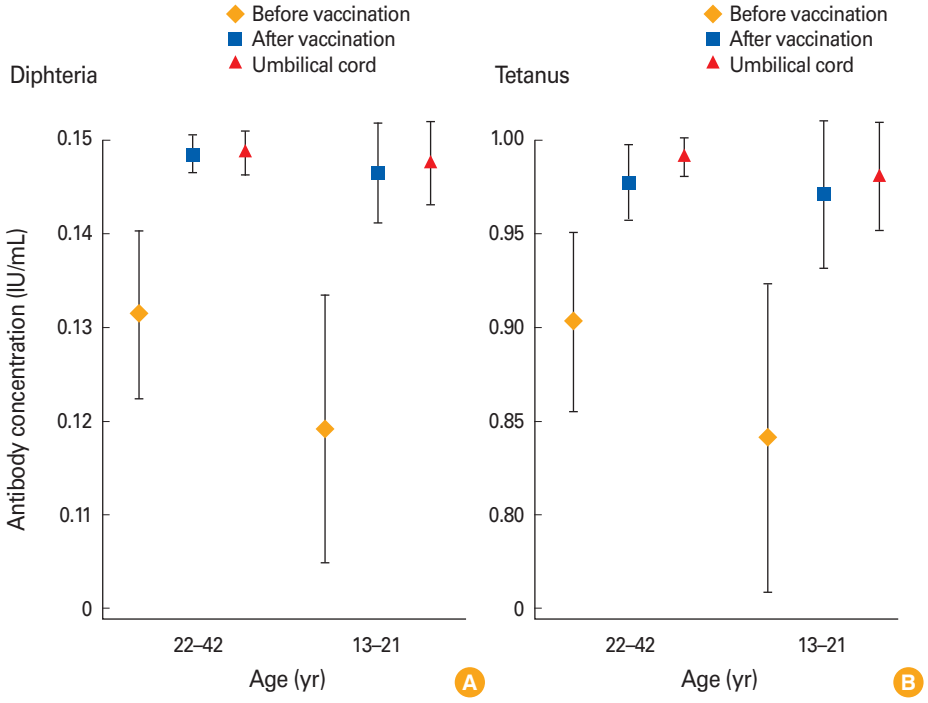

Fig. 2. (A, B) Immunoglobulin G antibodies against diphtheria and tetanus (95% confidence interval) per age group of the pregnant women and umbilical cord.

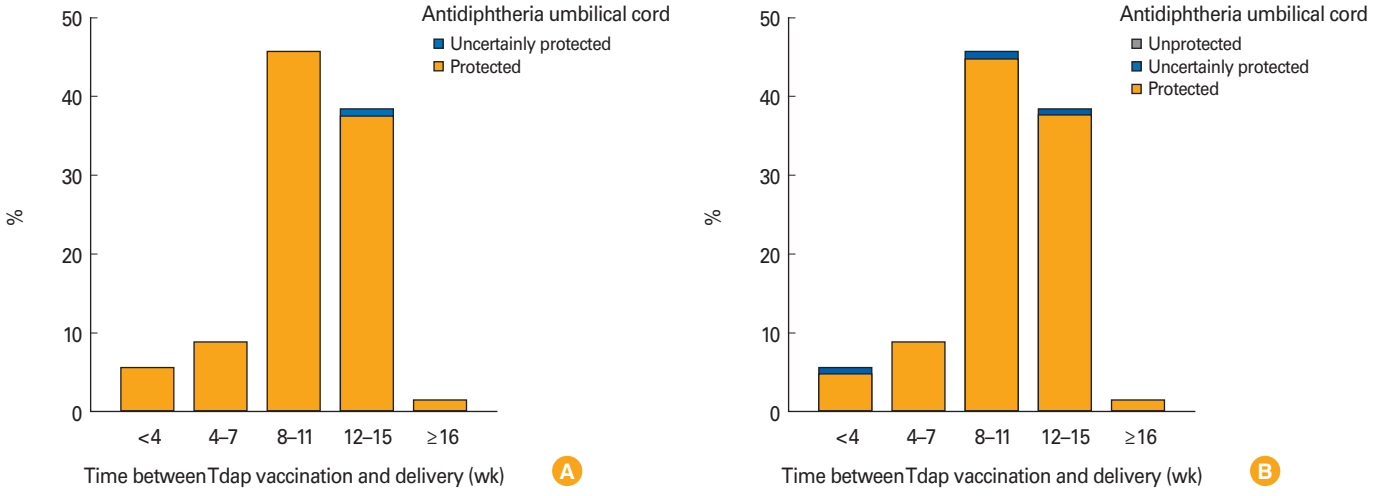

Fig. 3. (A, B) Antibodies against diphtheria and tetanus from umbilical cord according to time (weeks) between vaccination with tetanus, diphtheria, and acellular pertussis (Tdap) during the current pregnancy and delivery.

delivery (Fig. 3).

With respect to the profiles of the pregnant women, it was noted that those unprotected against diphtheria and tetanus after vaccination were those not vaccinated with Tdap during the current pregnancy.

The pregnant women protected against diphtheria had been born after the start of vaccination with DTwP in 1980, resided in the urban zone, their homes did not experience overcrowding by having three or more people per room; they had studied up to 11 years (primary and secondary) and had been vaccinated with Tdap during the current pregnancy (Fig. 4).

Protection against tetanus was observed in pregnant women vaccinated with Tdap, who were 18 to 34 years of age, resided in the urban zone, and had up to 11 years of schooling (primary and secondary) (Fig. 4).

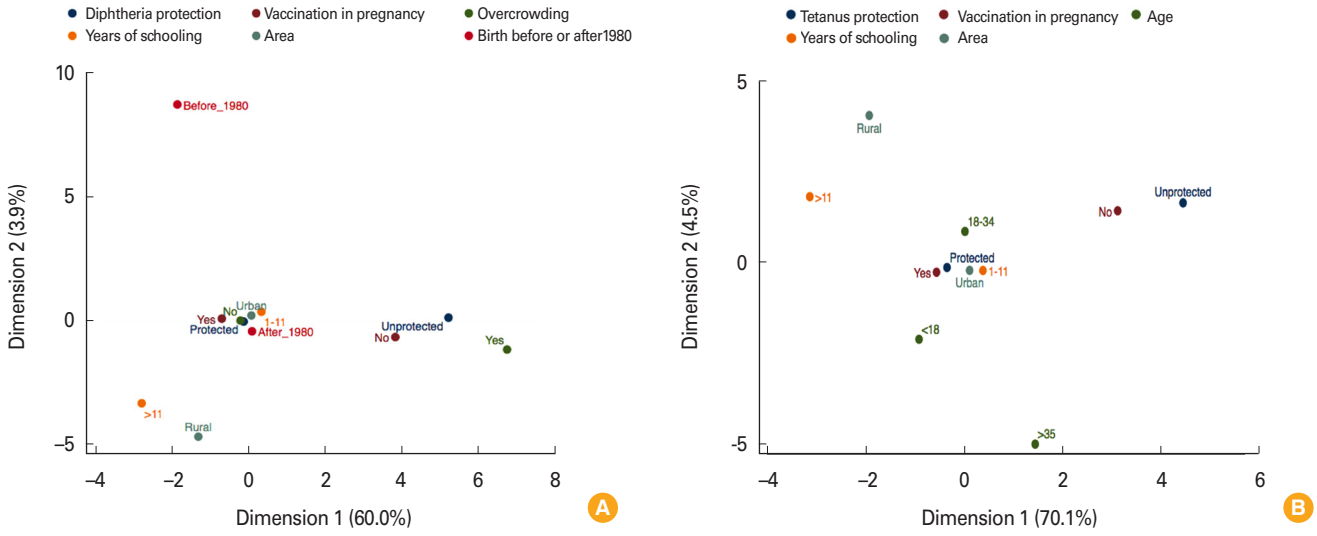

Fig. 4. (A, B) Profile characteristics of the pregnant women, protected and unprotected against diphtheria and tetanus upon delivery.

Discussion

Maternal vaccination with Tdap has been recommended in each pregnancy to prevent primarily the neonate against pertussis. In addition, it prevents maternal and neonatal tetanus and reinforces immunity against diphtheria.

This study detected sero-protection against tetanus at 71% and 29% had antibodies, but the duration of the protection was uncertain, before vaccinating the pregnant women. Importantly, before the vaccination, no pregnant women were detected susceptible to tetanus. After vaccination, maternal and neonatal protection was >90% and a significant transfer of maternal antibodies was corroborated.

These results reflect the impact of vaccinations within the plan to eliminate maternal and neonatal tetanus and of the Expanded Immunization Program in a country that continues with the DTwP vaccine during infancy without changing to the DTaP vaccine, like other developed and developing countries [20]. This study provides the scientific foundation to reinforce the call to continue with maternal vaccination against tetanus, as the only way to sustain elimination, in the absence of herd immunity [21].

In this study, the transfer ratio of antibodies against tetanus was higher upon vaccination at least 8 weeks before delivery, being higher with longer time elapsed between vaccination and delivery, corroborating that proposed in the literature [22]. The transfer ratio was ≥1 even in low-weight neonates and in the pregnant women not vaccinated with Tdap, possibly due to the additional exposure to vaccination with TT and Td.

Regarding diphtheria, although the maternal vaccination with Tdap is not intended to protect the neonate directly against this disease, this study showed significant transfer of maternal antibodies and high humoral response after the maternal vaccination with Tdap. Herein, 83.5% of the pregnant women had sero-protection against diphtheria, but 5% did not have sero-protection, which could support the influence of the accumulation of those susceptible during the resurgence of cases during the last decade, although additional population studies would be needed to corroborate such.

In this study, sero-protection in the pregnant women before vaccination was higher than that reported in other studies whose pregnant women also received the DTwP vaccine during childhood. A study conducted in Turkey, with 91 mother-umbilical cord pairs, detected 50% sero-protection against diphtheria and 58.0% against tetanus in pregnant women not vaccinated with Td [23]. A study in China, with 194 mother and umbilical cord pairs, showed 44.3% sero-protection of pregnant women against diphtheria, using a sero-protection cut-off point of ≥0.1, while the sero-protection against tetanus was 52.6% (0.01–0.1) and 28.1% (>0.1–1.0) [24].

The high maternal transfer of antibodies against diphtheria and tetanus coincides with that reported in a study from the United Kingdom with 31 mother-child pairs. The authors corroborated the significant persistence of antibodies in the children at 7 weeks and at 5 months of age, with higher transfer in the children of pregnant women vaccinated, compared with those not vaccinated [25].

Seroconversion and high sero-protection for both diseases

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in our study could be related with an immune reinforcement due to higher natural exposure, vaccination history of the pregnant women, differences in the formulation of the vaccines used, type of test used, and cut-off points to determine the sero-protection. A study using the same tests and cut-off points as our study detected high sero-protection against diphtheria (70.46%) and against tetanus (94.15%) in 324 children, from 18 to 180 months, who had received the primary series of vaccines from the Polish program [26].

In our study, the proportion of protective antibodies against diphtheria and tetanus transferred to the neonate was higher when pregnant women were vaccinated between 8 and 11 weeks before delivery (29–32 weeks of pregnancy). This coincides with the recommendation to vaccinate with Tdap at the start of the second trimester of pregnancy (≥26 weeks) by the World Health Organization [27], the Colombian Ministry of Health, and our prior study on pertussis [8]. Furthermore, the transfer of antibodies against tetanus is higher with earlier vaccination of the pregnant women, thus, protecting preterm births.

The profile of the pregnant women was primarily characterized by the relation between the lack of protection against diphtheria and tetanus and not having been vaccinated with Tdap during the current pregnancy. In our study, 33 participants (16.5%) were not vaccinated during pregnancy. No studies are known from Colombia on the acceptance of maternal vaccination. In 2020, coverage of maternal vaccination with Tdap in the country was <80% at national and sub-national levels, indicating the need for additional strategies that encourage maternal vaccination, which was affected by the COVID-19 pandemic [28].

Strengths and limitations

This study provides evidence of the sero-conversion of antibodies against diphtheria and tetanus, induced by maternal vaccination with Tdap, in a population that continues using the DTwP vaccine during infancy. Nevertheless, it has various limitations. Inference of the results is limited by the absence of a random sampling and by having conducted the study in two hospitals. It was not possible to measure the antibodies of children before and after the regular vaccination series due to cultural and ethical considerations. Data on vaccine history may be incomplete due to lack of the vaccination card from some pregnant women and possible under-registration of the vaccination information system.

The findings may be extrapolated to pregnant women in similar socioeconomic conditions and access to the DTwP vaccine during childhood and Td, TT, and Tdap during pregnancy. New studies are needed on maternal transfer of antibodies and persistence in children with population samples, including subregions with high exposure to conditions that affect placental transfer (malaria, HIV, etc.).

Conclusions

An increase was observed in the proportion and geometric mean of antibodies against diphtheria and tetanus, before and after vaccination with maternal vaccination with Tdap and placental transfer of antibodies to neonates. The relation was detected between the lack of protection against diphtheria and tetanus of the pregnant women and not having received the Tdap vaccine during the current pregnancy.

The presence of pregnant women unprotected against diphtheria and who were not vaccinated during pregnancy, warn about the need to intensify vaccination during infancy and during prenatal controls, seeking to facilitate access to vaccination with interventions guided according with particular conditions of the pregnant women.

Periodic monitoring of antibodies against diphtheria and tetanus in pregnant women and their neonates through serosurveillance may contribute with documenting progress in eliminating these diseases.

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