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ORIGINAL RESEARCH ARTICLE

Maternal alloimmune antibodies against HPA and HLA class I antigens are associated with reduced birthweight among healthy neonates delivered by Chinese women

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Abstract

Introduction: It is well known that HPA-1a antibodies lead to fetal and neonatal alloimmune thrombocytopenia (FNAIT), and an association with reduced birthweight in boys has been reported. Although it remains unclear whether HLA antibodies cause FNAIT, an association between HLA class I antibodies and reduced birthweight in FNAIT neonates has been observed. The aim of this study was to investigate the incidence of platelet antibodies among Chinese women and the impact of maternal alloimmune antibodies on birthweight among healthy neonates.

Material and methods: In this retrospective observational cohort study, platelet antibody screening was performed among women hospitalized for delivery from March 2019 to November 2020. A portion of each serum sample was used to distinguish HLA class I antibodies from HPA antibodies. Based on neonatal sex, gestational age and maternal age, platelet antibody-negative women who were hospitalized for delivery during the same period were randomly selected as reference groups at a 1:1 ratio for comparisons of the birthweights of healthy neonates delivered by women who were positive or negative for platelet antibodies.

Results: Among 15 156 women, 1008 (6.7%) were positive for platelet antibodies; the incidences of positive platelet antibody were 1.2%, 1.9%, 1.6% and 2.0% among women with 1, 2, 3 and >3 pregnancies, respectively. Among 787 platelet antibody-positive serum samples available for further analysis, 548 (69.6%) were positive for HLA class I antibodies bound to platelets, and 239 (30.4%) were positive for HPA antibodies. The average birthweight of healthy neonates delivered by women who were positive for platelet antibodies, HLA class I antibodies or HPA antibodies was 161–483 g lower than that of neonates delivered by women who were negative for these antibodies ($P < 0.001$). Regarding birthweight reduction, there was no significant

Abbreviations: CI, confidence interval; FNAIT, fetal neonatal alloimmune thrombocytopenia; HLA, human leukocyte antigen; HPA, human platelet antigen; SD, standard deviation.

difference among women who were positive for these antibodies or between boys and girls ($P > 0.05$).

Conclusions: This study is the first to report that maternal HPA and HLA class I antibodies are associated with reduced birthweight among healthy neonates delivered by Chinese women. This finding provides information for the study of the effect of maternal alloimmune antibodies on fetal development.

KEYWORDS
alloimmunization in pregnancy, birthweight of neonates, Chinese women, HLA antibody, HPA antibody, platelet antibody

1 | INTRODUCTION

During pregnancy, platelet antigen incompatibility between the mother and fetus can cause the mother to produce alloimmune platelet antibodies, which commonly include human platelet antigen (HPA) and human leukocyte antigen (HLA) class I antibodies.[1] It is well known that platelet-specific HPA-1a antibodies are the main cause of fetal neonatal alloimmune thrombocytopenia (FNAIT). Between 80% and 90% of FNAIT cases among white people are caused by HPA-1a antibodies[2] and this condition is associated with reduced birthweight in boys and fetal intracranial hemorrhage in neonates.[3,4] Although it remains unclear whether HLA antibodies alone cause FNAIT, an association between maternal HLA class I antibodies and reduced birthweight has been observed among FNAIT neonates[5]; however, the mechanism underlying the antibody-associated reduction in birthweight is unknown. The purpose of this study was to investigate the incidence of HPA and HLA class I antibodies due to pregnancy in Chinese women and explore whether maternal alloimmune antibodies are related to reduced birthweight among healthy neonates.

Key message

Maternal HPA and HLA class I antibodies are associated with reduced birthweight among not only FNAIT neonates but also healthy neonates, suggesting that maternal alloimmune antibodies may affect fetal development.

2 | MATERIAL AND METHODS

2.1 | Population

A total of 15 156 women hospitalized for delivery were included in this study from March 2019 to November 2020; women with a history of blood transfusion or who had FNAIT or small-for-gestational-age neonates were excluded. All clinical information was obtained from medical records. The number of pregnancies included abortion and stillbirths. Gestational age was determined by Naegele's rule. Parity data were not included in this study. Healthy neonates were defined as having a gestational age ≥259 days, birthweight >2500 g and no disease. Neonates small for gestational age (birthweight below the 10th percentile) and those diagnosed with thrombocytopenia (platelet count $<150 \times 10^9$/L) were not included in this study. Blood samples were collected from the participants 1–3 days before delivery. All women were screened for platelet antibodies, and a portion of each platelet antibody-positive serum sample was used to further distinguish between HLA class I antibodies and HPA antibodies. The aim of this study was to investigate the incidence of platelet antibody among Chinese women and compare the birthweights of healthy neonates delivered by women with or without platelet antibodies. Based on neonatal sex, gestational age and maternal age, platelet antibody-negative women who were hospitalized for delivery during the same period were randomly selected as the reference cohort at a ratio of 1:1.

2.2 | Detection of platelet antibody

The monoclonal antibody solid-phase platelet antibody test kit (MASPAT Kit) (Sanquin Reagents B.V.) was used to detect platelet antibodies according to the manufacturer's instructions. Positive results indicated that the serum samples contained HPA and/or HLA class I antibodies. HPA and HLA class I antibodies bound to platelets were further distinguished by using chloroquine-treated HLA-depleted platelets[6] and a Capture-P® analysis kit (Immucor Inc.). In this study, a panel of selected platelets, which covered all HLA-A, B cross-reactive groups (CREG) antigens and all common HPA antigens found in the Chinese population, was used.

2.3 | Statistical analyses

Data are shown as the mean ± SD. The average and 95% confidence interval (CI) of all continuous variables were calculated. The significance level of differences in mean numbers or percentages was estimated by means of the *t*-test. A *P*-value <0.05 was deemed statistically significant. IBM SPSS software Version 19 (IBM Corporation) was used for statistical analysis.

2.4 | Ethics statement

This study was approved by the Medical Ethics Committee of Guiyang Maternal and Child Health Care Hospital on February 20, 2019 (No. 2019–0010). Informed consent was obtained from all participants.

3 | RESULTS

Among 15 156 women, 1008 (6.7%) were positive for platelet antibodies. The incidences of platelet antibodies were 1.2%, 1.9%, 1.6% and 2.0% among women with 1, 2, 3 and >3 pregnancies, respectively. The incidence among women with ≥2 pregnancies was significantly higher than that among women with one pregnancy ($P < 0.001$). Among 1008 platelet antibody-positive serum samples, 787 were available for further analysis; 548 (69.6%) were positive for HLA class I antibodies, and 239 (30.4%) were positive for HPA antibodies. The incidence of HLA class I and HPA antibodies among women with ≥2 pregnancies was significantly higher than that among women with one pregnancy ($P < 0.001$) (Table 1).

Of the 787 antibody-positive women, 38 lacked complete clinical information and were excluded from the following analysis. To compare the birthweights of neonates delivered by women with or without platelet antibodies, two cohorts without antibodies were set up as reference groups. In the first reference group, women were matched for neonatal gestational age at delivery (Table 2). The results showed that there was no significant difference in the average neonatal gestational age between any with the antibody-positive and reference groups ($P > 0.05$). Among the 749 women with platelet antibodies, the average birthweights of 420 boys and 329 girls were respectively 201 g and 292 g lower than those of the reference groups ($P < 0.001$). Additionally, among 749 platelet antibody-positive women, 590 serum samples were available for further analysis: 416 were positive for HLA class I antibodies, and 174 were positive for HPA antibodies. The average birthweights of boys and girls delivered by 416 women with HLA class I antibodies were, respectively, 294 g and 304 g lower than those of the reference groups ($P < 0.001$). Additionally, the average birthweights of boys and girls delivered by 174 women with HPA antibodies were respectively 161 g and 376 g lower than those of the reference groups ($P < 0.001$). There was no significant difference in birthweight reduction between boys and girls ($P > 0.05$). Among the maternal cohorts with platelet antibodies, HLA class I antibodies, and HPA antibodies, there was no significant difference in birthweight reduction ($P > 0.05$) (data not shown).

The second reference group was set up according to maternal age-matching (Table 3). There was no significant difference in the average maternal age between the antibody-positive groups and the reference groups ($P > 0.05$). Among the 749 women with platelet antibodies, the average birthweights of 420 boys and 329 girls were, respectively, 330 g and 354 g lower than those of the reference groups ($P < 0.001$). Additionally, the average birthweights of boys and girls delivered by 416 women with HLA class I antibodies were, respectively, 364 g and 394 g lower than those of the reference groups, ($P < 0.001$). Furthermore, the average birthweights of boys and girls delivered by 174 women with HPA antibodies were, respectively, 297 g and 483 g lower than those of the reference groups ($P < 0.001$). There was no significant difference in birthweight reduction between boys and girls ($P > 0.05$). Among the maternal cohorts with platelet antibodies, HLA class I antibodies and HPA antibodies, there was no significant difference in birthweight reduction ($P > 0.05$) (data not shown).

TABLE 1 Incidence of maternal platelet antibody among Chinese women with different numbers of pregnancies

Antibody detected	Platelet antibody	HLA class I antibody	HPA antibody
No. tested	15 156 *n* (%)	787 *n* (%)	787 *n* (%)
Number of pregnancies			
1	177 (1.2)	98 (12.5)	37 (4.7)
2	287 (1.9)	152 (19.3)	73 (9.3)
3	239 (1.6)	138 (17.5)	60 (7.6)
>3	305 (2.0)	160 (20.3)	69 (8.8)
Total	1008 (6.7)	548 (69.6)	239 (30.4)

Abbreviations: HLA, human leukocyte antigen; HPA, human platelet antigen.

4 | DISCUSSION

In this study, we evaluated the prevalence of antibodies that bind platelets in a population of Chinese women and found lower neonatal birthweights among pregnancies in which the mother was positive for such antibodies. In addition to platelet-specific HPA antigens, the platelet surface membrane contains HLA antigens. Therefore, the maternal platelet antibodies that develop during pregnancy usually contain HPA and HLA antibodies, leading to difficulties in distinguishing HLA antibodies from HPA antibodies.[7] There are numerous published reports on the incidence of platelet antibodies among Chinese women,[8] but reports on the incidence of HPA and HLA antibodies are rare. It is well known that mothers may be immunized by fetal paternal antigens during pregnancy. Since the participants in this study had no history of blood transfusion, the detected platelet antibodies could be considered to have developed due to pregnancy. This study provides a clear profile of the incidence of HPA antibodies and HLA class I antibodies developed due to pregnancy in Chinese women. These data could be used to evaluate the health risk of newborns in China. According to China's seventh census data, in 2020, the national population comprised 1.44 billion people, the birth rate was 0.852%, and the number of newborns was approximately 12 million.[9] Based on the incidence of platelet antibodies in Chinese women reported in this study, it is estimated that approximately 800 000 newborns may have had reduced birthweights due to platelet alloantibodies in 2020.

TABLE 2 Comparisons of healthy neonatal birthweight based on gestational age at delivery

Maternal antibody (*n*)	Characteristics	Boys			Girls		
		Positive[a], *n*	Negative[a], *n*	*P*-value	Positive[a], *n*	Negative[a], *n*	*P*-value
Platelet antibodies (749)		420	420		329	329	
	Gestational age at delivery in days, mean ± SD (95%CI)	268.4 ± 0.5 (267.7–269.7)	268.4 ± 0.4 (267.6–269.2)	>0.05	270.6 ± 0.4 (269.7–271.6)	270.6 ± 0.6 (269.6–271.8)	>0.05
	Birthweight in grams, mean ± SD (95% CI)	3185 ± 24 (3138–3233)	3386 ± 19 (3347–3424)	<0.001	3089 ± 23 (3043–3135)	3381 ± 22 (3338–3423)	<0.001
	Difference in grams, (%)[b]	−201, (5.9)			−292, (8.6)		0.0767[b]
HLA class I antibodies (416)		241	241		175	175	
	Gestational age at delivery in days, mean ± SD (95%CI)	268.0 ± 0.7 (266.2–268.9)	268.1 ± 0.7 (266.8–269.6)	>0.05	271.5 ± 0.6 (270.3–272.8)	271.6 ± 0.6 (270.4–272.9)	>0.05
	Birthweight in grams, mean ± SD (95% CI)	3149 ± 33 (3084–3215)	3443 ± 29 (3383–3500)	<0.001	3114 ± 37 (3041–3188)	3418 ± 25 (3368–3469)	<0.001
	Difference in grams, (%)[b]	−294, (8.5)			−304, (8.9)		0.4431[b]
HPA antibodies (174)		93	93		81	81	
	Gestational age at delivery in days, mean ± SD (95% CI)	268.5 ± 1.1 (266.4–270.8)	268.2 ± 1.0 (266.2–270.2)	>0.05	269.0 ± 1.1 (266.2–270.6)	269.1 ± 1.0 (267.1–271.1)	>0.05
	Birthweight in grams, mean ± SD (95%CI)	3244 ± 52 (3140–3349)	3405 ± 43 (3319–3491)	<0.001	3025 ± 59 (2925–3125)	3401 ± 48 (3305–3498)	<0.001
	Difference in grams, (%)[b]	−161, (4.7)			−376, (11.1)		0.0577[b]

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen; HPA, human platelet antigen; SD, standard deviation.

[a]The positive and negative cohorts represent mothers with and without corresponding antibodies, respectively; negative cohorts serve as a reference group.

[b]The difference in birthweight between the two cohorts was expressed as a percentage of the average birthweight of the reference group. The *P*-value indicates the significance of the percentage difference.

TABLE 3 Comparisons of healthy neonatal birthweight based on maternal age

Maternal antibody (*n*)	Characteristics	Boys			Girls		
		Positive[a], *n*	Negative[a], *n*	*P*- value	Positive[a], *n*	Negative[a], *n*	*P*-value
Platelet antibodies (749)		420	420		329	329	
	Maternal age in years, mean ± SD (95% CI)	30.5 ± 0.2 (29.9–30.9)	30.5 ± 0.3 (29.9–30.9)	>0.05	30.2 ± 0.3 (29.6–30.8)	30.2 ± 0.3 (29.6–30.8)	>0.05
	Birthweight in grams, mean ± SD (95% CI)	3185 ± 24 (3138–3233)	3515 ± 16 (3484–3547)	<0.001	3089 ± 23 (3043–3135)	3443 ± 17 (3409–3477)	<0.001
	Difference in grams, (%)[b]	−330, (9.4)			−354, (10.3)		0.3405[b]
HLA class I antibodies (416)		241	241		175	175	
	Maternal age in years, mean ± SD (95% CI)	30.7 ± 0.3 (30.1–31.4)	30.7 ± 0.3 (30.1–31.4)	>0.05	30.2 ± 0.4 (29.4–30.9)	30.2 ± 0.4 (29.4–30.9)	>0.05
	Birthweight in grams, mean ± SD (95% CI)	3149 ± 33 (3084–3215)	3513 ± 19 (3475–3551)	<0.001	3114 ± 37 (3041–3188)	3508 ± 18 (3471–3545)	<0.001
	Difference in grams, (%)[b]	−364, (10.4)			−394, (11.2)		0.3974[b]
HPA antibodies (174)		93	93		81	81	
	Maternal age in years, mean ± SD (95% CI)	30.0 ± 0.5 (28.9–31.1)	30.0 ± 0.5 (28.9–31.1)	>0.05	30.4 ± 0.6 (29.2–31.6)	30.4 ± 0.6 (29.2–31.6)	>0.05
	Birthweight in grams, mean ± SD (95%CI)	3244 ± 52 (3140–3349)	3541 ± 26 (3488–3595)	<0.001	3025 ± 50 (2925–3125)	3508 ± 31 (3446–3571)	<0.001
	Difference in grams, (%)[b]	−297, (8.4)			−483, (13.8)		0.1280[b]

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen; HPA, human platelet antigen; SD, standard deviation.

[a]The positive and negative cohorts represent mothers with and without corresponding antibodies, respectively; negative cohorts serve as a reference group.

[b]The difference in birthweight between the two cohorts was expressed as a percentage of the average birthweight of the reference group. The *P*-value indicates the significance of the percentage difference.

To obtain reliable results from cohort comparison studies, it is crucial to establish an appropriate reference group. Since neonatal birthweight is closely related to gestational age,[10] neonates matched for gestational age were selected as the first reference group. The results showed that the birthweight of healthy neonates born to women with HPA and HLA class I antibodies was significantly reduced. To confirm this result, a second reference group was set up according to maternal age. The results indicated that the birthweights of both boys and girls delivered by women with antibodies were also significantly reduced. Although we do not have any direct evidence to show the interaction between maternal antibody status and the fetus, this finding suggests that maternal alloimmune antibodies may affect fetal development in some way.

This study has some limitations. In the selection of antibody-negative women as the reference group, maternal age-matching was used as one of the selection criteria. However, it is well known that neonatal birthweight is associated not only with maternal age[11] but also with parity, pre-pregnancy body mass index,[12] dietary patterns[13] and maternal anxiety during pregnancy.[14] Therefore, the reference group based on maternal age was not perfect. In addition, HPA typing and HLA typing for mothers and newborns were not carried out, so it is impossible to identify the specificity of HPA and HLA antigens that stimulate immunization. Additionally, the levels of antibodies were not measured; therefore, the antibody-mediated effect on birthweight could not be evaluated. Furthermore, the findings of this study are based only on the data of women living in Guizhou; therefore, they need to be further confirmed in other female populations in China. Moreover, it is necessary to verify this observation through prospective studies.

5 | CONCLUSION

Among Chinese women, the incidence of alloimmune platelet antibodies due to pregnancy increases as the number of pregnancies increases. The birthweights of boys and girls delivered by women with HPA and HLA class I antibodies were significantly lower than those of neonates delivered by women without these antibodies.

AUTHOR CONTRIBUTIONS

YX designed the study. XZ, ZS and CC performed the experiments. YX, WX and CL collected the data and performed statistical analyses. TZ analyzed the data and wrote this manuscript.

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CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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