Journal of Nanobiotechnology

RESEARCH

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Pre-existing anti-polyethylene glycol antibodies in pregnant women and newborns

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Abstract

Pre-existing anti-polyethylene glycol (PEG) antibodies represent risk factors for reduced efficacy and increased adverse reactions in seropositive individuals, but neither the seropositivities, nor levels nor influencing factors have been investigated in pregnant women or newborns. Herein, maternal and cord blood samples were respectively collected from 256 pregnant women and corresponding 256 newborns at the Women's Hospital, Zhejiang University School of Medicine in China for further determination of pre-existing anti-PEG antibodies, along with questionnaire interviews, demographic and clinical data collections. Our data showed that the seropositivities of total anti-PEG antibodies, anti-PEG IgG1 and IgG2, anti-PEG IgM, and coexistence of anti-PEG IgM and IgG were 19.14%, 2.34%, 7.03%, 10.94% and 1.17%, respectively, in pregnant women, and 5.47%, 2.73%, 2.73%, 0% and 0%, respectively, in newborns. Anti-PEG IgG3, IgG4 and IgE were undetectable in all blood samples. Median anti-PEG IgG1, IgG2 and IgM concentrations were 273.88 ng/mL, 748.35 ng/mL and 175.07 ng/mL, respectively, in pregnant women. Median anti-PEG IgG1 and IgG2 concentrations were 207.92 ng/mL and 336.52 ng/mL, respectively, in newborns. Interestingly, in-depth statistical analyses revealed that maternal age, take-out food consumption and cosmetic use were influencing factors of maternal anti-PEG antibodies, while newborn anti-PEG antibodies were affected by maternal age and cosmetic use. These seroepidemiological characteristics raise concerns over the clinical use of PEGylated drugs in pregnant women and newborns, and provide valuable insight into the induction of risky pre-existing anti-PEG antibodies.

Keywords Pre-existing anti-polyethylene glycol antibodies, Pregnant women, Newborns, Seroepidemiological characteristics, Influencing factors

Introduction

Polyethylene glycol (PEG) is a synthetic polymer comprised of repeating subunits of ethylene glycol and often covalently attached to small molecules, macromolecules and nanomaterials to improve their properties, a process called "PEGylation" [1]. PEGylation has become one of the most preferred methods to enhance the delivery of therapeutic molecules [2]. Currently there are 41 PEGylated medicines approved by the Food and Drug Administration (FDA), including two COVID-19 mRNA vaccines BNT162b2 (Comirnaty®) and mRNA-1273 (Spikevax®), with many other PEGylated agents

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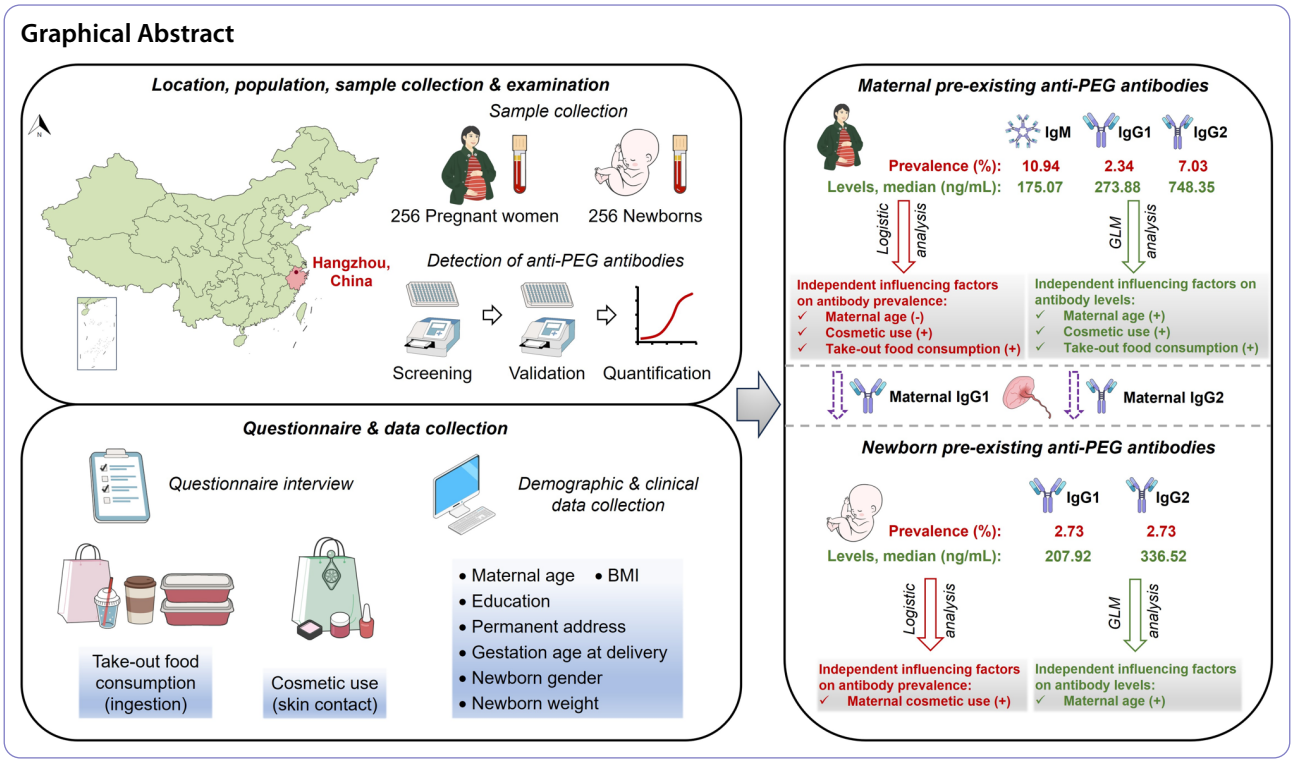


under clinical development [3, 4]. In addition, PEG and its derivatives have been approved for use in cosmetics and personal care products, as well as food additives and packaging materials in processed foods and beverages, etc [5, 6].

Although PEG was initially regarded as non-immunogenic, it has been recognized as a polyvalent hapten [7–9]. Anti-PEG antibodies could be elicited in animals immunized with PEGylated proteins or nanocarriers [7–10] and in patients treated with PEGylated drugs [11–17]. Surprisingly, normal population possesses pre-existing anti-PEG antibodies in the absence of treatment with PEGylated therapeutics [18], which was firstly documented in 1984 [19]. It is noteworthy that the presence of anti-PEG antibodies may represent risk factors for reduced efficacy and adverse reactions in patients requiring treatment with PEGylated drugs [20–22]. Specifically, anti-PEG antibodies may induce the formation of immune complexes with PEG-modified medicines, which could be rapidly cleared via activation of the complement system and subsequent phagocytosis by macrophages, thereby altering the pharmacokinetics and biodistribution [21, 22]. Complement activation may also contribute to the development of infusion-related allergic reactions to PEGylated drugs [21, 22]. For instance, the PEGylated nanoparticles in COVID-19 mRNA vaccine Comirnaty® and Spikevax®, which could induce and/or boost anti-PEG antibodies as recently demonstrated by us and several other teams [23–28], have been suspected to trigger allergic reactions after vaccinations [29].

Up to date, there are seven seroepidemiological studies on pre-existing anti-PEG antibodies in general adults, including Chinese adults in Taiwan of China [30], American [31–34], Austria [32], Japanese [32], Italian [32] and German adults [19, 35], with interesting data obtained (Tables S1 and S2 in Supplementary Appendix; see Supplementary Appendix for all online-only materials, e.g. below Methods S1, Results S1, Table S1, Fig. S1 and Discussion S1). Nevertheless, pregnant women represent a unique population with distinctive and dynamic immunological milieus, and they provide passive immunity to their fetuses/newborns [36]. Hence, parallel investigation on the pre-existing anti-PEG antibodies in pregnant women and their newborns has offered an opportunity to reveal the potential maternal-fetal/newborn disparities in addition to identifying their respective seroepidemiological characteristics. Meanwhile, in some circumstances, pregnant women need to be treated with PEGylated drugs for medical conditions arising during pregnancy [37]. Investigating maternal-fetal/newborn anti-PEG antibodies is thus crucial for assessing the maternal-fetal/newborn safety and efficacy of PEGylated drug treatment.

Motivated by these concerns, 256 pregnant women and their newborns were enrolled in this study, with corresponding maternal and cord blood samples carefully collected and examined for pre-existing anti-PEG antibodies. Using internationally recognized direct ELISA

and competitive ELISA, along with questionnaire interviews, demographic and clinical data collections, and in-depth statistical analysis, the characteristics of pre-existing maternal and newborn anti-PEG antibodies, and the potential influencing factors were investigated.

Results

Study population

A total of 256 pregnant women enrolled had a median (IQR) age of 32 (29–36) years and a median (IQR) BMI of 26.70 (24.80–29.00) kg/m^2. The education levels of participants were as follows: 50 (19.53%) with vocational degrees or below; 61 (23.83%) with associate's degrees; 107 (41.80%) with bachelor's degrees; 38 (14.84%) with master's degrees or above. Moreover, 182 (71.09%) resided in cities while 74 (28.91%) lived in towns. Regarding use of makeup products (herein defined as "cosmetics") commonly containing PEG/PEG derivatives (Table S3; see "Methods"), 205 (80.09%), 31 (12.11%) and 20 (7.81%) respectively reported 0 days, 1–3 days and 4–7 days for each week. In addition, 70 (27.34%), 97 (37.89%), 64 (25.00%) and 25 (9.77%) respectively reported 0 time, 1–3 times, 4–6 times and 7–9 times per week take-out food consumption (ingestion exposure to PEG/PEG derivatives; Table S4; see "Methods"). All newborns were delivered at a mean (SD) gestational age of 38.45 (0.86) weeks, with a mean (SD) birth weight of 3.32 (0.41) kg. Moreover, 127 newborns (49.61%) were males and 129 (50.39%) were females (Table 1).

Table 1 Demographic and clinical characteristics of pregnant women

Characteristic	Participant
Data on pregnant women	(n = 256)
Maternal age, median (IQR), y	32 (29–36)
BMI, median (IQR), kg/m^2	26.70 (24.80–29.00)
Education, No. (%)	
Vocational degree or below [a]	50 (19.53)
Associate's degree	61 (23.83)
Bachelor's degree	107 (41.80)
Master's degree or above	38 (14.84)
Permanent address, No. (%)	
Town	74 (28.91)
City	182 (71.09)
Cosmetic use (per week), No. (%)	
0 days	205 (80.09)
1–3 days	31 (12.11)
4–7 days	20 (7.81)
Take-out food consumption (per week), No. (%)	
0 times	70 (27.34)
1–3 times	97 (37.89)
4–6 times	64 (25.00)
7–9 times	25 (9.77)
Data on newborns	(n = 256)
Gestational age at delivery, mean (SD), wk	38.45 (0.86)
Newborn gender, No. (%)	
Male	127 (49.61)
Female	129 (50.39)
Newborn weight, mean (SD), kg	3.32 (0.41)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); IQR, interquartile range; SD, standard deviation

[a]Elementary school or junior high school (6 cases), high school or technical secondary school (44 cases)

Prevalence and levels of pre-existing anti-PEG antibodies in pregnant women and newborns

By using direct ELISA (Tables S5; Figs. S1-24) and subsequent competitive ELISA (Table S6; Figs. S25-36), anti-PEG antibody seropositive samples were verified and quantified according to standard curves (Figs. S37-42). Our data showed that anti-PEG antibodies were detectable in 49 (19.14%) pregnant women, with 2.34%, 7.03%, 10.94% and 1.17% of all pregnant women respectively positive for anti-PEG IgG1, IgG2, IgM, and both IgG1 and IgM. No pregnant woman was positive for anti-PEG IgG3, IgG4 or IgE. In addition, double-positivity for both IgG1 and IgG2, as well as for IgG2 and IgM, was not detected (Fig. 1A and B; Tables S7-11). Importantly, anti-PEG antibodies were also detected in 14 (5.47%) newborns, with 7 (2.73%) positive for anti-PEG IgG1 and 7 (2.73%) positive for anti-PEG IgG2. No newborn was positive for anti-PEG IgG3, IgG4, IgM or IgE (Fig. 1A and B; Tables S7-11). These data have provided initial characterizations of anti-PEG antibodies in pregnant women (mainly IgM, followed by IgG2 and IgG1) and newborns (IgG1 and IgG2).

Furthermore, anti-PEG antibody levels were defined as low (< 100 ng/mL), moderate (100–500 ng/mL) and high (> 500 ng/mL) levels according to the literatures [31, 33]. When regarding anti-PEG antibody seropositive pregnant women as the whole population (100%), the following distributions of anti-PEG antibodies were revealed: 10 (20.41%) with low levels (IgG2, 4.08%; IgM, 16.33%), 23 (46.94%) with moderate levels (IgG1, 10.20%; IgG2, 10.20%; IgM, 34.69%; both IgG1 and IgM, 4.08%) and 16 (32.65%) with high levels (IgG1, 2.04%; IgG2, 22.45%; IgM, 6.12%) (Fig. 1A, C, D and E; Tables S7-11). Correspondingly, the following distributions were found when regarding 14 seropositive newborns as the whole population (100%): no newborn with low levels, 10 (71.43%) with moderate levels (IgG1, 35.71%; IgG2, 35.71%), and 4 (28.57%) with high levels (IgG1, 14.29%; IgG2, 14.29%) of anti-PEG antibodies (Fig. 1A, C, D and E; Tables S7-11). Moreover, seropositive pregnant women had a median (Range; IQR) anti-PEG IgG1, IgG2 and IgM concentrations of 273.88 ng/mL (183.74–513.90; 191.70–376.08), 748.35 ng/mL (75.54–2604.89; 159.09–1200.81) and 175.07 ng/mL (55.43–23649.14; 95.95–315.19), respectively (Fig. 2A). Seropositive newborns had a median

A No. (%) of pregnant women and newborns with pre-existing anti-PEG antibodies

Variable	IgG1	IgG2	IgG3	IgG4	IgM	IgE	IgG1 & IgG2	IgG1 & IgM	IgG2 & IgM	IgG1, IgG2 & IgM	Total Ab
Pregnant women											
Positive, No. (%)	6 (2.34)	18 (7.03)	0 (0)	0 (0)	28 (10.94)	0 (0)	0 (0)	3 (1.17)	0 (0)	0 (0)	49 (19.14)
Low level, No. (%)	0 (0)	2 (4.08)	0 (0)	0 (0)	8 (16.33)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10 (20.41)
Moderate level, No. (%)	5 (10.20)	5 (10.20)	0 (0)	0 (0)	17 (34.69)	0 (0)	0 (0)	2 (4.08)	0 (0)	0 (0)	23 (46.94)
High level, No. (%)	1 (2.04)	11 (22.45)	0 (0)	0 (0)	3 (6.12)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	16 (32.65)
Newborns											
Positive, No. (%)	7 (2.73)	7 (2.73)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	14 (5.47)
Low level, No. (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Moderate level, No. (%)	5 (35.71)	5 (35.71)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10 (71.43)
High level, No. (%)	2 (14.29)	2 (14.29)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (28.57)

Low level, < 100 ng/mL; Moderate level, 100 - 500 ng/mL; High level, > 500 ng/mL

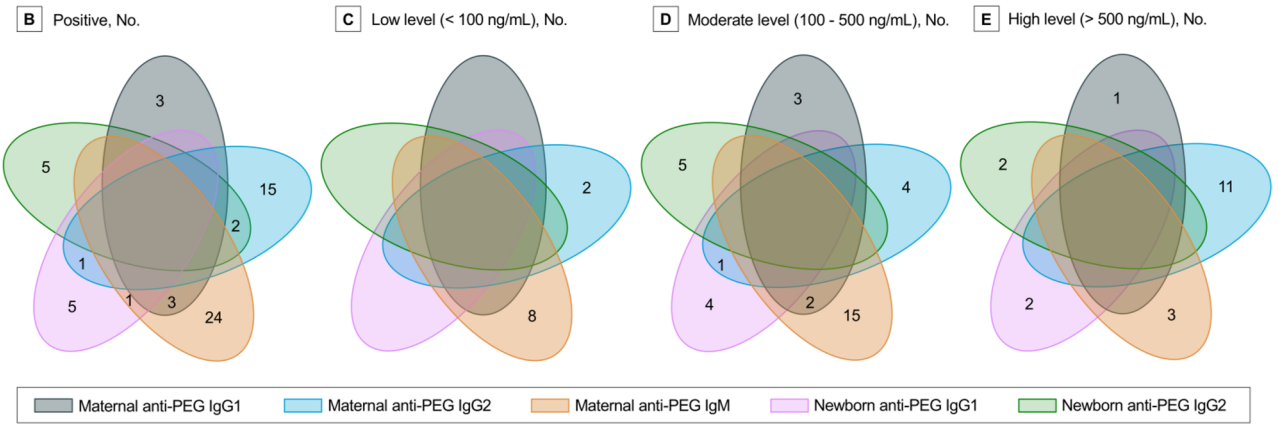


Fig. 1 Prevalence and Levels of Pre-existing Anti-PEG Antibodies in Pregnant Women and Newborns. Total Ab included all known isotypes/subclasses of anti-PEG antibodies (**A**). Venn diagrams display the numbers of cases with or without coexistence of maternal anti-PEG IgG1, maternal anti-PEG IgG2, maternal anti-PEG IgM, newborn anti-PEG IgG1, and newborn anti-PEG IgG2 (**B**), as well as at low level (< 100 ng/mL) (**C**), moderate level (100–500 ng/mL) (**D**) and high level (> 500 ng/mL) (**E**) of these anti-PEG antibodies, respectively. Using the numbers 15 and 2 in Fig. 1B as examples, the number 15 represents the number of cases only seropositive for maternal anti-PEG IgG2 but not any other subclass of anti-PEG antibody, while the number 2 represents the number of cases seropositive for both neonatal anti-PEG IgG2 and maternal anti-PEG IgG2 overlap (2 anti-PEG IgG2-positive mothers with their corresponding newborns positive for anti-PEG IgG2; no other subclass of anti-PEG antibody detected for either mother or her newborn). The number 0 is not displayed in the Venn diagrams

(Range; IQR) anti-PEG IgG1 and IgG2 concentrations of 207.92 ng/mL (120.40–1513.98; 133.97–524.58) and 336.52 ng/mL (100.24–1069.62; 275.80–527.07), respectively (Fig. 2B).

Maternal-newborn differences and associations of pre-existing anti-PEG antibodies

The prevalence and levels of anti-PEG antibodies were further analyzed to uncover the maternal-newborn differences and associations (Figs. 1B and 2C; Tables S7-11). Our data showed that anti-PEG IgG2 was detected in 2 pregnant women and their newborns, with higher antibody levels in mothers (1337.37 vs. 275.80 ng/mL; 2604.89 vs. 100.24 ng/mL). As neither newborns nor fetuses could generate IgG antibodies owing to immature immune systems [36–38], this data suggest a transfer of anti-PEG IgG2 from mothers to their fetuses. Interestingly, although 7 newborns were positive for anti-PEG IgG1, their mothers were negative for anti-PEG IgG1 despite one mother positive for anti-PEG IgG2 and another positive for anti-PEG IgM. Similarly, although 5 newborns were positive for anti-PEG IgG2, their mothers were undetectable for anti-PEG antibodies. Moreover, 6 pregnant women were positive for anti-PEG IgG1, but their newborns were seronegative for anti-PEG antibodies. Sixteen pregnant women were positive for anti-PEG IgG2, but none of their newborns were detectable for anti-PEG IgG2 despite one newborn positive for anti-PEG IgG1. The complexities of maternal-newborn distribution of anti-PEG IgG not only suggest a transmission of anti-PEG IgG antibodies between mothers and fetuses, but indicate dynamic changes in antibody subclasses and

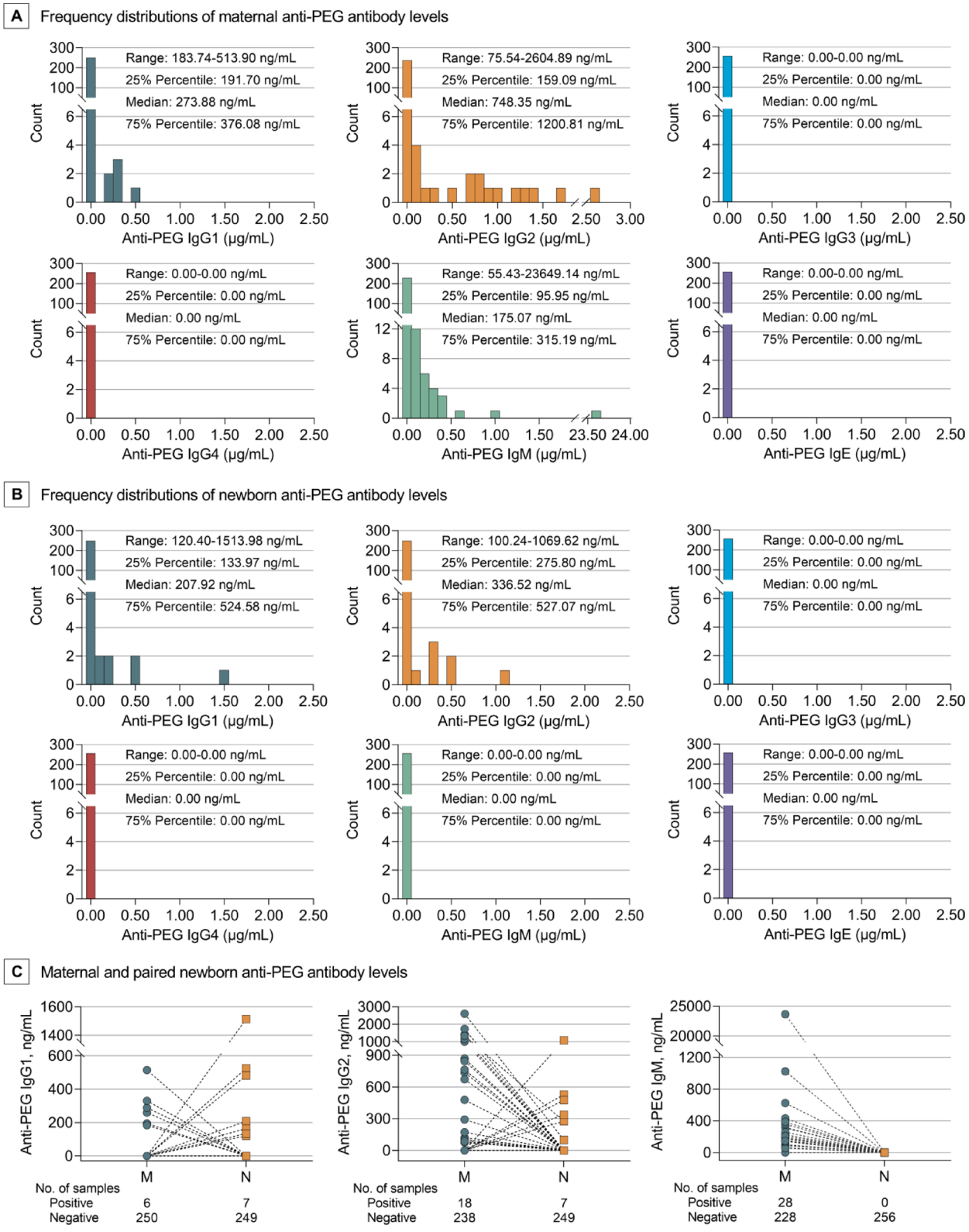


Fig. 2 Frequency Distributions of Maternal and Newborn Pre-existing Anti-PEG Antibody Levels in Seropositive Pregnant Women and Newborns. Concentration range, 25% percentile, median and 75% percentile of each antibody isotype/subclass in seropositive pregnant women (**A**) and newborns (**B**). Specifically, maternal (M) and paired newborn (N) anti-PEG antibody levels were illustrated (**C**). Only detected isotypes/subclasses of anti-PEG antibodies were presented

levels, which may be attributed to varied metabolism and clearance of these antibodies between mothers and fetuses. In addition, 28 pregnant women had anti-PEG IgM, whereas their newborns did not. This data aligns with previous findings that maternal IgM antibodies are unable to cross the placental barrier and reach the fetus [38–40].

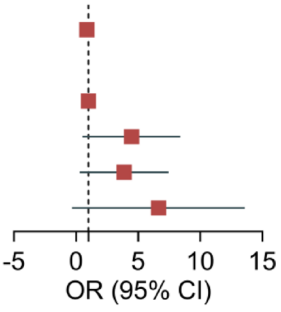
Multivariable logistic regression analysis for the prevalence of pre-existing maternal anti-PEG antibodies

Variables positive in univariable logistic regression analysis (Results S1; Table S12), including maternal age and take-out food consumption, were entered into a multivariable logistic regression analysis to estimate adjusted associations with the prevalence of maternal total anti-PEG antibodies. Our data showed that: with same take-out food consumption, for each 1-year increase in maternal age, the prevalence of maternal total anti-PEG antibodies dropped to 88.3% (95% CI, 0.815–0.958; $P = 0.003$) (Fig. 3 A); with same maternal age, compared with those without take-out food consumption (reference category with odds ratio/OR = 1), the odds of having maternal total anti-PEG antibodies were 3.327 (95% CI, 1.250–8.854; $P = 0.016$) and 4.224 (95% CI, 1.230–14.499; $P = 0.022$) times higher, respectively, for pregnant women with take-out food consumption 1–3

A Multivariable logistic regression analysis for the prevalence of maternal total anti-PEG antibodies

Variable	OR (95% CI)	*P* value
Maternal age	0.883 (0.815-0.958)	0.003
Take-out food consumption (per week)		
0 times	1 [Reference]	NA
1-3 times	3.327 (1.250-8.854)	0.016
4-6 times	2.788 (0.985-7.892)	0.054
7-9 times	4.224 (1.230-14.499)	0.022

Two variables screened from univariable logistic regression analysis for the prevalence of maternal total anti-PEG antibodies, including maternal age and take-out food consumption, were included in the analysis model.



B Multivariable logistic regression analysis for the prevalence of maternal anti-PEG IgG

Variable	OR (95% CI)	*P* value
Cosmetic use (per week)		
0 days	1 [Reference]	NA
1-3 days	2.081 (0.618-7.001)	0.237
4-7 days	4.678 (1.511-14.482)	0.007
Take-out food consumption (per week)		
0 times	1 [Reference]	NA
1-3 times	2.787 (0.570-13.613)	0.205
4-6 times	3.708 (0.734-18.730)	0.113
7-9 times	7.263 (1.268-41.614)	0.026

Two variables screened from univariable logistic regression analysis for the prevalence of maternal anti-PEG IgG, including cosmetic use and take-out food consumption, were included in the analysis model.

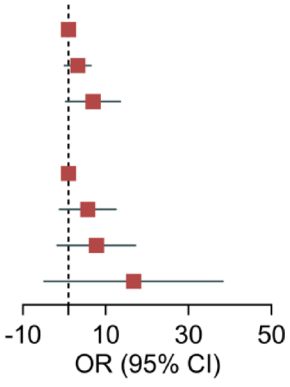


Fig. 3 Multivariable Logistic Regression Analysis for the Prevalence of Pre-existing Anti-PEG Antibodies in Pregnant Women. Abbreviations: NA, not applicable; OR, odds ratio. A, maternal total anti-PEG antibodies in pregnant women ($n = 256$). B, maternal anti-PEG IgG in pregnant women ($n = 256$). OR for maternal age represents the change in the prevalence of maternal total anti-PEG antibodies for each 1-year increase in the age of pregnant women after adjustment for take-out food consumption; and OR for take-out food consumption represents the change in the prevalence of maternal total anti-PEG antibodies for pregnant women who had take-out food 1–3 times, 4–6 times, and 7–9 times per week compared with those without take-out food consumption after adjustment for maternal age (**A**). OR for cosmetic use represents the change in the prevalence of maternal anti-PEG IgG for pregnant women who use cosmetics 1–3 days or 4–7 days per week compared with no cosmetic use after adjustment for take-out food consumption; and OR for take-out food consumption represents the change in the prevalence of maternal anti-PEG IgG for pregnant women who had take-out food 1–3 times, 4–6 times and 7–9 times per week compared with those without take-out food consumption after adjustment for cosmetic use (**B**).

times and 7–9 times per week (Fig. 3 A). These data elucidate that maternal age (inverse association) and take-out food consumption (positive association) are independent influencing factors of the prevalence of maternal total anti-PEG antibodies.

Moreover, variables positive in univariable logistic regression analysis (Results S1; Table S13), including cosmetic use and take-out food consumption, were entered into a multivariable logistic regression analysis to estimate adjusted associations with the prevalence of maternal anti-PEG IgG. Our data indicate that: with same take-out food consumption, compared with no cosmetic use (reference category with OR = 1), the odds of having maternal anti-PEG IgG was 4.678 (95% CI, 1.511–14.482; $P = 0.007$) times higher for pregnant women using cosmetics 4–7 days per week (Fig. 3B); with same cosmetic use, compared with those without take-out food consumption (reference category with OR = 1), the odds of having maternal anti-PEG IgG was 7.263 (95% CI, 1.268–41.614; $P = 0.026$) times higher for pregnant women with take-out food 7–9 times per week (Fig. 3B). These data suggest that maternal cosmetic use (positive association) and take-out food consumption (positive association) are independent influencing factors of the prevalence of maternal anti-PEG IgG.

In addition, univariable logistic regression analysis for the prevalence of maternal anti-PEG IgM only showed that for each 1-year increase in the age of pregnant women, the prevalence of maternal anti-PEG IgM dropped to 88.6% (95% CI, 0.783–0.958; $P = 0.005$) (Results S1; Table S13). That is, requirements for further multivariable logistic regression analysis were not fulfilled for the prevalence of maternal anti-PEG IgM. Similarly, univariable logistic regression analysis for the prevalence of newborn total anti-PEG antibodies only showed that compared with those without cosmetic use (reference category), the odds of having newborn total anti-PEG antibodies were 7.259 (95% CI, 2.159–24.411; $P = 0.001$) times higher for pregnant women with 4–7 days per week cosmetic use (Results S1; Table S14). Hence, multivariable logistic regression analysis was not eligible for the prevalence of newborn total anti-PEG antibodies.

Multivariable generalized linear regression analysis for the levels of pre-existing maternal anti-PEG antibodies

Variables positive in Spearman correlation analysis (Results S2; Fig. S43) and univariable generalized linear regression analysis (Results S3; Table S15), including maternal age and cosmetic use, were entered into a multivariable generalized linear regression analysis to estimate adjusted associations with the levels of maternal total anti-PEG antibodies after $\log_{10}$ transformation among seropositive pregnant women. Our data showed that: with same cosmetic use, for each 1-year increase in the maternal age, the anti-PEG antibody levels increased by 0.047 (95% CI, 0.014–0.079; $P = 0.005$) (Fig. 4 A); with same maternal age, compared with no cosmetic use group, the anti-PEG antibody levels increased by 0.507 (95% CI, 0.148–0.867; $P = 0.006$) for pregnant women with cosmetic use 4–7 days per week (Fig. 4 A). These data revealed that maternal age (positive association) and cosmetic use (positive association) are independent influencing factors of the levels of maternal total anti-PEG antibodies.

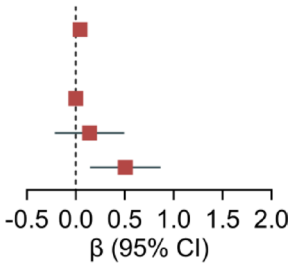
Moreover, variables positive in Spearman correlation analysis (Results S2; Fig. S43) and univariable generalized linear regression analysis (Results S3; Table S16), including maternal age and take-out food consumption, were entered into a multivariable generalized linear regression analysis to estimate adjusted associations with the levels of maternal anti-PEG IgG after $\log_{10}$ transformation among anti-PEG IgG seropositive pregnant women. Our data revealed that: with same take-out food consumption, for each 1-year increase in the maternal age, the anti-PEG IgG levels increased by 0.049 (95% CI, 0.004–0.094; $P = 0.033$) (Fig. 4B); with same maternal age, compared with those without take-out food consumption, the anti-PEG IgG levels increased by 0.591 (95% CI, 0.036–1.145; $P = 0.037$) for pregnant women with take-out food 1–3 times per week (Fig. 4B). These data demonstrate that maternal age (positive association) and take-out food consumption (positive association) are independent influencing factors of the levels of maternal anti-PEG IgG. Interestingly, it is noteworthy that no positive associations were detected between anti-PEG IgG levels with more frequent take-out food consumption such as 4–6 times per week and 7–9 times per week. This data may coincide with previous studies indicating that the positive correlation between anti-PEG antibody level and exposure to a specific antigen was only observed within a certain range of PEG antigen dosages [10, 41].

Furthermore, variables positive in Spearman correlation analysis (Results S2; Fig. S43) and univariable generalized linear regression analysis (Results S3; Table S16), including maternal education and cosmetic use, were entered into a multivariable generalized linear regression analysis to estimate adjusted associations with the levels of maternal anti-PEG IgM after $\log_{10}$ transformation among anti-PEG IgM seropositive pregnant women. Our data indicate that: with same education background, compared with no cosmetic use, the anti-PEG IgM levels increased by 1.395 (95% CI, 0.810–1.981; $P < 0.001$) for pregnant women using cosmetics 4–7 days per week (Fig. 4C). However, anti-PEG IgM levels were not affected by education (with same cosmetic use) (Fig. 4C). These data suggest that cosmetic use is an independent influencing

A Multivariable generalized linear regression analysis for the levels of maternal total anti-PEG antibodies

Variable	β (95% CI)	*P* value
Maternal age	0.047 (0.014-0.079)	0.005
Cosmetic use (per week)		
0 days	0 [Reference]	NA
1-3 days	0.142 (-0.215-0.498)	0.437
4-7 days	0.507 (0.148-0.867)	0.006

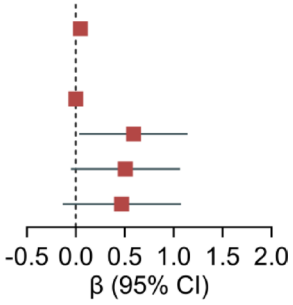
Two variables screened from univariable generalized linear regression analysis for the levels of maternal total anti-PEG antibodies, including maternal age and cosmetic use, were included in the analysis model.



B Multivariable generalized linear regression analysis for the levels of maternal anti-PEG IgG

Variable	β (95% CI)	*P* value
Maternal age	0.049 (0.004-0.094)	0.033
Take-out food consumption (per week)		
0 times	0 [Reference]	NA
1-3 times	0.591 (0.036-1.145)	0.037
4-6 times	0.509 (-0.048-1.066)	0.073
7-9 times	0.471 (-0.133-1.076)	0.126

Two variables screened from univariable generalized linear regression analysis for the levels of maternal anti-PEG IgG, including maternal age and take-out food consumption, were included in the analysis model.



C Multivariable generalized linear regression analysis for the levels of maternal anti-PEG IgM

Variable	β (95% CI)	*P* value
Cosmetic use (per week)		
0 days	0 [Reference]	NA
1-3 days	-0.005 (-0.443-0.434)	0.983
4-7 days	1.395 (0.810-1.981)	< 0.001
Education		
Vocational degree or below	0 [Reference]	NA
Associate's degree	0.173 (-0.189-0.536)	0.348
Bachelor's degree	0.327 (-0.023-0.676)	0.067
Master's degree or above	-0.377 (-1.004-0.250)	0.239

Two variables screened from univariable generalized linear regression analysis for the levels of maternal anti-PEG IgM, including cosmetic use and education, were included in the analysis model.

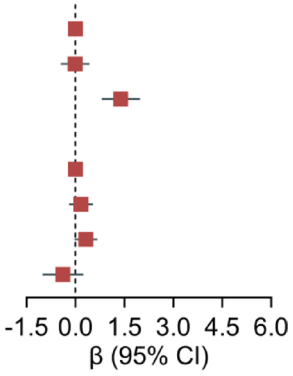


Fig. 4 Multivariable Generalized Linear Regression Analysis for the Levels of Pre-existing Anti-PEG Antibodies in Seropositive Pregnant Women. Abbreviations: NA, not applicable; β, standardized regression coefficient. A, total anti-PEG antibodies in seropositive pregnant women ($n = 49$). B, anti-PEG IgG in seropositive pregnant women ($n = 24$). C, anti-PEG IgM in seropositive pregnant women ($n = 28$). β for maternal age represents the change of total anti-PEG antibody level for each 1-year increase in the age after adjustment for cosmetic use; β for cosmetic use represents the change of total anti-PEG antibody level between cosmetic use at indicated frequency and no cosmetic use after adjustment for maternal age (**A**). β for maternal age represents anti-PEG IgG level for each 1-year increase in the age after adjustment for take-out food consumption; β for take-out food consumption represents the change of anti-PEG IgG level between take-out food consumption at indicated frequency and no take-out food consumption after adjustment for maternal age (**B**). β for cosmetic use represents the change of anti-PEG IgM level between cosmetic use at indicated frequency and no cosmetic use after adjustment for education; β for education represents the change of anti-PEG IgM level between indicated degree and vocational degree or below after adjustment for cosmetic use (**C**)

factor (positive association) of the levels of maternal anti-PEG IgM, while education is not.

Lastly, Spearman correlation analysis for the levels of newborn total anti-PEG antibodies after $\log_{10}$ transformation only showed a positive correlation between the levels of newborn total anti-PEG antibodies and maternal age ($r = 0.711$, $P = 0.006$) (Results S2; Fig. S44), and univariable generalized linear regression analysis for the levels of newborn total anti-PEG antibodies after $\log_{10}$ transformation only showed that for each 1-year increase in the age of pregnant women, the levels of newborn total anti-PEG antibodies increased by 0.065 (95% CI, 0.032–0.098; $P < 0.001$) (Results S3; Table S17). Therefore, no further multivariable logistic regression analysis was conducted for the levels of newborn total anti-PEG antibodies.

Discussion

Despite the challenges of collecting 256 maternal peripheral venous blood samples and corresponding 256 neonatal umbilical cord blood samples during the COVID-19 pandemic, our study is based on a reliable sample size. For instance, the recruited participants are sufficient to achieve all events per variable (EPV) ratios significantly exceeding the commonly recommended threshold of 10 [42]. Followings are several representative EPV values for various analyses conducted in this study: multivariate logistic regression analysis for the prevalence of maternal total anti-PEG antibodies, EPV = 128; multivariate logistic regression analysis for the prevalence of maternal anti-PEG IgG, EPV = 128; multivariate generalized linear regression analysis for the levels of maternal total anti-PEG antibodies, EPV ≈ 25. Nevertheless, future studies with additional representative maternal and fetal/newborn populations and/or with larger sample sizes would be valuable for further validation of our research findings.

It is notable that the low overall seropositivity of pre-existing anti-PEG antibodies (0.2%) firstly reported might be attributed to the low sensitivity of detection method [43, 44]. Meanwhile, it may also reflect the low exposure to PEG antigens in the early 1980 s [43, 44]. Afterwards, the detection method for anti-PEG antibodies has been significantly improved, with the combination of direct ELISA with competitive ELISA being regarded as the most reliable approach [43, 45]. Using this internally recognized method, herein we revealed that the seropositivities of total anti-PEG antibodies in pregnant women and newborns ware 19.14% and 5.47%, respectively. Further profile analysis on anti-PEG IgG1-G4, IgM and IgE showed that only anti-PEG IgM, IgG1 and IgG2 were detectable in pregnant women, with IgG2 as the dominant IgG subclass (Fig. 1; Fig. S45). Previously there was only one literature that determined the anti-PEG IgG subclasses in general adults, in which anti-PEG IgG2 was also most dominant [31]. IgG2 and IgM antibodies are commonly associated with T cell-independent immune responses against non-protein antigens such as lipids, nucleic acids, polysaccharides, and other natural and synthetic polymers [21, 22, 46]. In contrast, protein antigens predominantly induce IgG1 and IgG3 antibodies, with some IgG4 and IgE, by T cell-dependent immune responses [21, 22, 46]. Nevertheless, IgG1 could also arise through a T cell-independent pathway [46]. Therefore, the absence of IgG3, IgG4 and IgE, the presence of IgG1, IgG2, IgM and co-existence of IgG1 and IgM suggest that pre-existing anti-PEG antibodies in pregnant women are more likely generated through T cell-independent mechanisms, supporting the hypothesis that these antibodies mainly stem from exposure to non-protein everyday chemicals containing PEG and its derivatives. Moreover, consistent with five previous studies conducted in general adults [30–34] (Table S1), anti-PEG IgM and IgG exhibited similar seropositivity in pregnant women (10.94% vs. 9.38%).

Importantly, parallel investigation in pregnant women and newborns has provided an opportunity to uncover the potential maternal-fetal/newborn transfer or associations in pre-existing anti-PEG antibodies between two generations (Fig. S45). As the immune system of fetus is immature and unable to produce IgG antibodies autonomously [38–40], the presence of fetal anti-PEG IgG in newborns suggest active maternal-fetal/newborn antibody transfers across the placenta. Nevertheless, several factors such as IgG subclass, maternal antibody titer, gestational age and nature of antigen may affect the placental transfer efficacy of IgG [39, 47]. In addition, as the livers of fetuses are not fully developed and lack some enzymes necessary for antibody metabolism, mothers and fetuses have disparities in antibody metabolism and half-life [48–50]. Specifically, the half-life of antibody in fetuses is generally longer than that in mothers. Therefore, it is understandable to see the discrepancies in prevalence and levels of anti-PEG IgG1 and IgG2 between mothers and their neonates. It is noteworthy that different from IgG, maternal IgM antibodies could not cross the placental barrier to achieve maternal-fetal transfer [39, 51–53]. However, fetuses could produce IgM antibodies by themselves in response to T-independent antigens including PEG and PEGylated derivatives [51–53]. Therefore, the absence of anti-PEG IgM antibodies in newborns may suggest no PEG antigen exposure or degradation of fetal anti-PEG IgM induced by uterus PEG antigen exposure. These findings have deepened our understandings of the pre-existing anti-PEG antibodies and provided initial evidence for their maternal-fetal/newborn transfer. It is noteworthy that large molecular substances including antibodies within the fetus can neither cross the placenta nor enter the maternal circulation, which is crucial for

maintaining the relative independency of maternal and fetal blood and immune systems [54].

Previously, several "inherent" factors such as gender and age have been preliminarily evaluated for their possible influences on pre-existing anti-PEG antibodies in general adults [30–35] (Tables S1 and S2). However, inconsistent findings were obtained across very limited literatures. For instance, both the prevalence and levels of pre-existing anti-PEG IgG exhibited either a negative correlation [30, 31] or no correlation [33] with donor age in general adults, while no correlation was reported between either prevalence or levels of anti-PEG IgM and donor age [30–33, 35]. In addition, the highest prevalence of anti-PEG IgG was observed among American adults aged 18–24 years in one report [33]. These data suggest age-related disparities as well as potential population heterogeneity in pre-existing anti-PEG antibodies. Interestingly, we herein revealed that maternal age exhibited a negative correlation with the prevalence of total anti-PEG antibodies and anti-PEG IgM (but not IgG), and a positive correlation with the levels of total anti-PEG antibodies and anti-PEG IgG (but not IgM). Meanwhile, we discovered that maternal age had a positive correlation with total newborn anti-PEG antibody levels (Fig. S45). Further studies investigating the potential mechanisms mediating these correlations are warranted, and several aspects such as the specific age range of pregnant women, distinctive and dynamic immunological milieus during pregnancy and potential maternal-fetal/newborn crosstalk need to be taken into account.

It has been hypothesized that pre-existing anti-PEG antibodies are associated with exposure to everyday chemicals containing PEG and its derivatives [21, 30, 31, 45]. However, this hypothesis has been rarely tested, and till now only three reports suggesting the association between topical application of lotion products (skin contact) and induction of pre-existing anti-PEG antibodies, including two conducted in mice [55, 56] and one in general adults [57] (Table S18). Impressively, we herein revealed that the frequency of maternal use of makeup products was a positive influencing factor for the prevalence of maternal anti-PEG IgG and newborn total anti-PEG antibodies, and for the levels of maternal total anti-PEG antibodies and anti-PEG IgM. Another important finding is that the frequency of take-out food (ingestion of PEG/PEG derivatives) was found to be a positive influencing factor for the prevalence of maternal total anti-PEG antibodies and anti-PEG IgG, and for the levels of maternal anti-PEG IgG, suggesting that anti-PEG antibodies could be induced by gastrointestinal exposure to PEG antigens (Fig. S45). There are actually four reasons why frequency of take-out food consumption was investigated in this study: (i) PEG/PEG derivatives are commonly contained in food contact packaging materials for take-out food (Table S4), and easily migrate from packaging materials into the food inside under many conditions, such as high temperature and high fat content commonly existed in take-out food; (ii) the present study was conducted during the COVID-19 pandemic when both the consumption of packaged food and food delivery significantly increased in response to external shocks [58, 59], making take-out food consumption a more attractive factor for our investigation; (iii) consumption of take-out food varies significantly among pregnant women due to different health philosophy and lifestyle; (iv) the potential influence of gastrointestinal exposure to PEG antigens on anti-PEG antibody induction has never been investigated, while this study has provided an opportunity to explore this issue.

As a group of anti-drug antibodies (ADA), anti-PEG antibodies have been demonstrated to induce accelerated blood clearance (ABC phenomenon) and increase risks of adverse reactions of PEGylated drugs, including hypersensitivity reactions, in many animal studies [7, 9, 10, 23, 60–71] and in a number of clinical investigations [11, 14–17, 24, 56, 72–74] (Discussion S1; Tables S19 and S20). Particularly, a positive correlation between the concentration of ADA and changes in pharmacokinetics (PK) has been confirmed in clinical studies, with clinical events even induced by certain low level of ADA (30–100 ng/mL) [75, 76]. Considering that moderate to high levels of pre-existing anti-PEG antibodies were observed in a subset of pregnant women and newborns, our findings have naturally raised concerns regarding first-exposure reaction, efficacy and safety over the use of PEGylated drugs in seropositive pregnant women and newborns (Figs. 1 and 2). Certainly, the actual treatment response would be affected by multiple factors, e.g. the level and subclass of anti-PEG antibodies, the type and dose of PEGylated agent, and the frequency and duration of drug treatment, which need further in-depth study [21, 23]. Indeed, our recent work revealed that the occurrence of accelerated blood clearance was associated with the dose of PEGylated lipid nanoparticles injected in a rat model [23].

Finally, it is worthy to note that as passively acquired maternal antibodies in newborns wane throughout 6 to 12 months after birth [38, 77–79], the anti-PEG antibodies detected in newborns may have long-term immunological effects during infancy. Interestingly, a recent report indicates that parental female rats may transmit anti-PEG IgG antibodies to their offspring through milk [80]. Therefore, maternal anti-PEG antibodies might be transferred to newborns/infants through placental transfers (before/upon birth) and breastfeeding (after birth). This is of particular importance as several PEGylated drugs, including COVID-19 vaccines Comirnaty® and Spikevax® [81], Asparlas® (a drug for acute lymphoblastic

leukemia) [82], Neulasta® (a drug for febrile neutropenia) [83] and Revcovi® (a drug for adenosine deaminase severe combined immune deficiency) [84], have been officially approved for use in infants ≤ 12 months old. It is also notable that tests for anti-PEG antibodies have been recently recommended by FDA guidelines in patients for assessing the potential immune response to PEGylated therapies [85].

Conclusion

Sero-prevalence and levels of pre-existing anti-PEG antibodies were revealed in pregnant women and their newborns with this study, which has raised efficacy and safety concerns over the use of PEGylated drugs in seropositive pregnant women and newborns. Moreover, several influencing factors on maternal and newborn pre-existing anti-PEG antibodies were discovered. These findings have provided useful clues for identifying the origins of PEG antigens. Specifically, our data may provide initial evidence that gastrointestinal exposure to PEG and its derivatives could induce risky pre-existing anti-PEG antibodies. It is noteworthy that as a reasonable cost of time was needed for questionnaire interviews, we herein selected several factors that we are particularly interested in for data collection and subsequent analysis. Further evaluations assessing additional factors are warranted to broaden our understandings on seroepidemiological characteristics of pre-existing anti-PEG antibodies.

Methods

Study design and participants

This study was approved by the Ethics Committee of Women's Hospital, Zhejiang University School of Medicine (approval No. IRB-20210139-R) and carefully followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [86]. Pregnant women admitted for delivery at the Women's Hospital, Zhejiang University School of Medicine were approached for enrollment with a survey conducted between May 2021 and May 2022. Eligibility criteria included an age of 20 years or older, a singleton pregnancy, a full-term pregnancy (ranging from 37^{+0} to 41^{+6}), no history of unhealthy lifestyle (e.g. smoking, drinking or drug abuse), no evidence for exposure to PEGylated drugs before and throughout the pregnancy, no contagious disease, no disease requiring drug treatment upon enrollment and a willingness to participate in this study. Pregnant women participating in other clinical studies were excluded. Eligible participants were identified by dedicated clinicians, with written informed consents obtained from all the participants in accordance with institutional requirements and the Declaration of Helsinki. Maternal blood samples were collected from the peripheral vein within 2 days before delivery. Newborn blood samples were collected from the umbilical cord vein immediately after delivery. All blood samples were centrifuged at 1000 × g for 10 min at 4 °C, and the serums were immediately harvested and stored at −80 °C for further determination of anti-PEG antibodies.

Questionnaire interviews and data collections

The questionnaire was designed following the ACCADEMY and CHERRIES guides [87, 88]. A face-to-face questionnaire interview was conducted with each participant by a specially trained physician within three days before or after delivery. It is noteworthy that considering the extremely common application of PEG and its derivatives in makeup products (defined as "cosmetics" in this study; Table S3), as well as food-contact packaging materials (e.g. food-contact paper, paperboard, paper cups; Table S4) in processed foods and beverages [30, 35, 89], weekly frequency of cosmetic use (e.g. mascara, eyeliner, liquid foundation, blemish balm cream, color correcting cream, and other makeup products) and take-out food consumption during pregnancy were evaluated. Specifically, the take-out food referred to most commonly consumed take-out food items packaged in above-mentioned food-contact packaging materials. In addition, demographic and clinical data including maternal age, body mass index (BMI), education level, permanent address, gestation age at delivery, newborn gender and newborn weight were obtained from hospital electronic medical record (EMR) systems.

Screening and quantification of anti-PEG antibodies in serum samples by ELISA

Anti-PEG antibodies in all serum samples were initially screened using a direct ELISA as described in previous studies [30, 35]. Briefly, Maxisorp™ 96-well microplates were coated with 0.05 mg/well NH_2-PEG_{10000}-NH_2 in 100 μL of PBS overnight at 4℃. Subsequently, plates were gently washed with 350 μL of DPBS for three times, followed by incubation with blocking buffer (5% (w/v) skim milk powder in DPBS, 200 μL/well) at room temperature (RT) for 1.5 h. Then the plates were washed three times with DPBS again. Afterwards, 100 μL of each serum sample diluted at 1:10 with sample dilution buffer (2% (w/v) skim milk powder in DPBS), together with six serial dilutions of human anti-PEG IgG1-4, IgM or IgE standards (respectively at 10.3, 30.9, 92.6, 277.8, 833.3 and 2500.0 ng/mL) in standard dilution buffer (10% reference human serum tested negative for anti-PEG antibodies (Methods S1; Figs. S46 and 47), 2% (w/v) skim milk powder in DPBS) were added into corresponding detection plates in duplicate (for serum samples) or triplicate (for anti-PEG antibody standards). Same volumes of sextuplicate standard dilution buffer were used as negative controls. After further incubation for 1 h at RT, and five successive

washes including four with 350 μL of washing buffer (0.05% (w/v) CHAPS in DPBS) and one with 350 μL of DPBS, 50 μL of diluted mouse anti-human secondary antibodies (IgG1 Fc, 1:2500; IgG2 Fc, 1:5000; IgG3 Hinge, 1:5000; IgG4 Fc, 1:500; IgE, 1:10000) and goat anti-human IgM μ-chain secondary antibody (1:10000) were respectively added and incubated for 1 h at RT. Again, unbounded antibodies were removed by five successive washes, followed by incubation with 100 μL of TMB for 30 min at RT in the dark. Finally, the HRP-TMB reaction was stopped with 100 μL of 2 N H_2SO_4 and the absorbance was measured at 450 nm. Detailed information on the materials used in ELISA was listed in Methods S2, and details on the establishment of detection cutoffs of anti-PEG IgG1-4, IgM and IgE were introduced in Methods S3. Serum samples with average absorbance values higher than the detection cutoffs in direct ELISA were further verified with a nanotechnology-based competitive ELISA, in order to confirm the PEG specificity of the antibodies detected and minimize false positives (Methods S4). Only serum samples containing PEG-specific antibodies were ultimately deemed positive for anti-PEG antibodies, and further quantified based on the standard curves established for each batch of the direct ELISA. Detailed information on direct ELISA was described in Methods S5.

Statistical analysis

Continuous variables (maternal age, BMI, gestation age at delivery and newborn weight) were presented as mean with standard deviation (SD) or median with interquartile range (IQR). Categorical variables (maternal education, permanent address, cosmetic use frequency, take-out food consumption frequency and newborn gender) were presented as numbers with percentages (%). To evaluate the correlations between the prevalence of maternal and newborn anti-PEG antibodies with demographic and clinical variables, univariable logistic regression analysis was firstly performed. Then variables with $P < 0.05$ in the univariable logistic regression analysis were re-entered into a multivariable logistic regression analysis to evaluate their potential independent associations with the prevalence of anti-PEG antibodies. Furthermore, for pregnant women and newborns seropositive for anti-PEG antibodies, correlations between antibody levels after $\log_{10}$ transformation and each continuous variable were analyzed using Spearman correlation coefficients (r). Meanwhile, univariable generalized linear regression analysis was conducted to evaluate the correlation of antibody levels after $\log_{10}$ transformation with demographic and clinical variables in seropositive pregnant women and newborns. Then variables with $P < 0.05$ in the univariable generalized linear regression analysis were re-entered into a multivariable generalized linear regression analysis to determine their potential independent associations with maternal and newborn anti-PEG antibody levels after $\log_{10}$ transformation. Multivariable analysis was only performed in case of 10 or more events per variable (EPV) to avoid bias of the regression coefficients [42]. Statistical analyses were performed using GraphPad Prism version 9.0 and IBM SPSS statistics version 29. All statistical tests were based on 2-tailed hypotheses. Differences were considered significant at $P < 0.05$ unless specifically stated. Venn diagrams were used to illustrate the relationships between sets, and was generated using Origin 2021 software.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12951-026-04185-9.

Supplementary Material 1

Acknowledgements

We would like to acknowledge Dr. Xuesi Chen at Chinese Academy of Sciences for insightful discussions.

Author contributions

M.H.S., the principal investigator of the major supporting grants, had full access to all the data in the study and took full responsibility for the integrity of the data and the accuracy of the data analysis. M.H.S., H.Y.W., Y.F. and C.Z.Y. conceived and designed the study. M.H.S. and H.Y.W. drafted the manuscript. All authors revised and edited the manuscript. M.H.S., H.Y.W. and L.Z. analyzed the data. M.H.S., Y.F., L.Z., C.Z.Y., J.C.L. and X.X. provided technical/platform supports. H.Y.W., Y.F. and L.Z. contributed equally.

Funding

This work was supported by the Zhejiang Provincial Natural Science Foundation of China (LZ23E030003 to Dr. Meihua Sui), National Natural Science Foundation of China (21722405 and 22075243 to Dr. Meihua Sui) and Startup Foundation for Hundred-Talent Program of Zhejiang University (to Dr. Meihua Sui).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Women's Hospital, Zhejiang University School of Medicine (approval No. IRB-20210139-R) and carefully followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines. All participants provided informed consent to participate in this study.

Consent for publication

All authors agree to publication.

Competing interests

The authors declare no competing interests.

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Received: 13 July 2025 / Accepted: 10 February 2026
Published online: 18 April 2026

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