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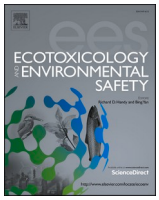

Research Paper

Environmental titanium exposure and reproductive health: Risk of low birth weight associated with maternal titanium exposure from a nested case-control study in northern China

Yu Jin [a,b,1], Zhiyi Li [c,1], Hang An [a,b], Yiming Pang [a,b], Kexin Li [c], Yali Zhang [a,b], Le Zhang [a,b], Lailai Yan [d], Bin Wang [a,b], Rongwei Ye [a,b,*], Zhiwen Li [a,b], Aiguo Ren [a,b]

[a] Institute of Reproductive and Child Health, Peking University/Key Laboratory of Reproductive Health, National Health Commission of the People's Republic of China, Beijing 100191, PR China
[b] Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing 100191, PR China
[c] Institute of Geographic Sciences and Natural Resources Research, CAS, Beijing 100101, PR China
[d] Department of Laboratorial Science and Technology, School of Public Health, Peking University, Beijing 100191, PR China

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ABSTRACT

Titanium (Ti) is commonly used in additives in the form of titanium dioxide (TiO_2). However, our understanding of the effect of Ti on reproductive health remains limited. This nested case-control study, performed in a Ti mining exposure field, investigated the association between maternal blood Ti concentration and the risk of low birth weight (LBW), as well as the potential biological mechanism. A total of 45 women who delivered LBW infants (cases) and 352 women with normal birth weight infants (controls) were included. We collected maternal peripheral blood samples in the first or early second trimester to measure Ti concentration in serum (Ti^{sr}) and blood cells (Ti^{bc}), as well as inflammatory, lipid, and oxidative stress biomarkers thereof. The demographic characteristics of the women included in the study were also obtained. The results showed that the median total blood Ti concentration (Ti^{tb}) in the case group was significantly higher than that in the control group (134 vs. 129 ng/mL, $P = 0.039$). A higher Ti^{tb} level was associated with a greater risk of LBW [odds ratio = 2.62; 95% confidence interval (CI): 1.16–5.90], but no such association was observed for Ti^{sr} or Ti^{bc} after adjusting for potential confounders. The serum lipid biomarkers TC, TG, and total lipids (TL) were all negatively associated with Ti^{sr} and Ti^{tb}. Serum 8-OHdG was positively associated with Ti^{bc}. We concluded that a high Ti^{tb} during early pregnancy may increase the risk of LBW. Lipid metabolism and oxidative stress may play an important role in the adverse health effects associated with Ti exposure. Thus, our results merit more attention to the probable adverse effects of titanium exposure during pregnancy.

1. Introduction

Titanium (Ti) is a highly abundant non-essential trace element that exists ubiquitously in our life (Musial et al., 2020). Its common form, titanium dioxide (TiO_2), was widely used in industrial products, additives of foods, and personal care products (such as sunscreens and toothpaste, etc.) (Musial et al., 2020). Ti could be absorbed by persons through various pathways including oral, skin, inhalation exposure, and implant dissolution. And a person may intake 0.2–0.7 mg of TiO_2/kg daily (approximately 10–35 mg of TiO_2 for a 50 kg adult) through their diet (Weir et al., 2012). A small amount of these Ti would be absorbed but widely distributes in many organs and tissues, especially in the liver, spleen, jejunum, ileum, kidney cortex, and lungs (Bermudez et al., 2004; Hougaard et al., 2010; Golasik et al., 2016; Abukabda et al., 2019; Peters et al., 2020). Thus, Ti exposure has raised great concerns over its health effects on the human body. Increasing numbers of epidemiological studies have shown evidence that Ti exposure may be associated with several adverse health effects including diabetes, colitis, and cardiopulmonary disorders (Ruiz et al., 2017; Yuan et al., 2018; Zhao et al., 2018).

* Corresponding author at: Institute of Reproductive and Child Health, Peking University/Key Laboratory of Reproductive Health, National Health Commission of the People's Republic of China, Beijing 100191, PR China.
E-mail address: yerw@bjmu.edu.cn (R. Ye).
[1] There two authors have equal contribution to this study.

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In pregnant women who were more vulnerable to environmental exposure than the general population (Silver et al., 2018; Freire et al., 2019), Ti exposure was found to be associated with adverse reproductive outcomes including fetal distress, preterm birth, and neural tube defects (Zheng et al., 2014; Li et al., 2016). However, the evidence of maternal exposure to Ti on low birth weight (LBW) —an adverse birth outcome affecting infants' mortality, development, and disease risks throughout the life course with an incidence of 5–6% in China (McCormick, 1985; Gluckman et al., 2008; Tang et al., 2017)—was inconsistent and insufficient. A case-control study in southern China found that a low-level serum Ti was negatively associated with LBW, indicating that a high-level Ti might be a risk factor for LBW (Hou et al., 2019). Some ecological studies performed in the northeastern USA also reported that large-scale environmental exposure to airborne Ti was associated with an increased risk of LBW (Bell et al., 2012; Ebisu and Bell, 2012; Basu et al., 2014). On the other hand, an ecological study performed in California (USA) found no such association after adjusting for confounders (Laurent et al., 2014). These previous studies raised a question about whether Ti exposure could be a risk factor for LBW.

Several in vivo and in vitro experiments have demonstrated that Ti exposure would induce inflammation, oxidative stress, and cytotoxicity, and damage DNA and lipid metabolism (Trouiller et al., 2009; Wang et al., 2011; Schanen et al., 2013; Shinohara et al., 2014; Hong et al., 2017; Zhao et al., 2018; Fadoju et al., 2019). Some of these metabolic processes have also been reported to be associated with LBW risk (Kim et al., 2005; Mridha et al., 2016; Carlson et al., 2018; Gomes et al., 2019). However, few population-based studies further explored the potential pathways of maternal Ti exposure on LBW by detecting or analyzing biomarkers, which could shed light on the connection between Ti and LBW. Therefore, we hypothesized that Ti exposure may increase the risk of LBW by affecting lipid metabolism, inducing inflammation and oxidative stress and examine the potential mechanism thereof by detecting typical biomarkers.

Moreover, as Ti was demonstrated to have different physiological functions in serum and blood cells, the distribution of Ti in different blood fractions could matter when evaluating its biological properties (Oshida, 2013; Saxena et al., 2018). To our knowledge, no study has evaluated the association between Ti concentrations in serum, blood cells, and total blood and the risk of LBW separately. Thus, we conducted a prospective nested case-control study in Shanxi, a high Ti exposure province of industrial mining, to evaluate the association between (and possible mechanisms thereof) Ti in different fractions of maternal blood and LBW, given that the metabolic processes involved may rely on different blood fractions.

2. Materials and method

2.1. Study design and participants

Our nested case-control study was conducted on a prospective cohort (Hao et al., 2019), based on a National Program on Key Basic Research Project (*973* Program), (2007CB5119001). Briefly, 4229 pregnant women from three maternal and child health care hospitals agreed to participate in the cohort study between December 2009 and December 2013. On recruitment at the first prenatal examination, each woman filled out a structured questionnaire under the supervision of local health care workers. We collected maternal blood samples once for each woman between gestational weeks 4–22 in alignment with the time of pregnancy-care examination and followed-up to determine delivery/pregnancy termination status. Ultimately, our cohort comprised 3197 women (75.6%), after excluding those who withdrew ($n = 216$), were lost to follow-up ($n = 83$), terminated their pregnancy ($n = 216$), had an iatrogenic preterm birth ($n = 17$), were recruited at < 4 or > 22 gestational weeks ($n = 18$), had multiple births ($n = 34$), lacked information on fetal number ($n = 16$), date of last menstruation ($n = 3$), or delivery date ($n = 116$), or did not provide blood samples ($n = 313$). Among the participants, 49 women had live births weighing less than 2500 g and were classified into the LBW group (cases). Among the remaining women who delivered normal birth weight infants (2500–4000 g), we selected a larger number of controls to increase statistic power based on the principle of making age, BMI, education, occupation, parity, offspring sex, drugs & folic acid usage, sampling time & fasting status comparable between groups and having high-quality sample collection & experimental records. Serum and blood cells samples were collected separately and stored at −80 °C prior to analysis. Due to some inadequate blood sample volumes, a total of 45 cases and 352 controls were included in our final analysis. This study was approved by the Institutional Review Board of Peking University Health Science Center, and signed consent was obtained from all subjects.

2.2. Blood titanium analysis

The serum was diluted with nitric acid and its Ti concentration (Ti^{sr}) was measured using inductively coupled plasma-mass spectrometry (ICP-MS; ELAN DRC II; PerkinElmer, USA). A standard serum sample (ClinCheck® – Serum Control, Level II; Recipe, Germany) was used for quality control. To determine the blood cell Ti concentration (Ti^{bc}), a 0.2 g sample was transferred into a digestion tank, followed by the addition of 400 μL 50% (v/v) nitric acid and 400 μL ultra-pure water. The mixture was further digested using a microwave digestion system (MARS 6; CEM Co., USA). After 15-fold dilution, the samples were measured using inductively coupled plasma mass spectrometry (ICP-MS; ELAN DRC II, PerkinElmer, USA) method. The lower limit of detection (LOD) for Ti was 0.15 ng/mL. Quantitative analysis was performed at the Central Laboratory of Biological Elements of Peking University Health Science Center, and the protocol was approved by China Metrology Accreditation. The total blood concentration (Ti^{tb}) was calculated based on the serum and blood levels of metals after weighting based on the hematocrit level (Tamada, 2003; Chen et al., 2014). The mean hematocrit level of our cohort was 0.361; therefore, we used the following formula to approximate the Ti^{tb}: Ti in serum (Ti^{sr}) × (1 − 0.361) + Ti in blood cells (Ti^{bc}) × 0.361 (deSilva, 1981).

2.3. Analysis of serum biomarkers

Serum total cholesterol (TC) and triglyceride (TG) concentrations were measured using the oxidative method (InTEC Products, China) following the standard protocol. The optical density was quantified using an automatic biochemical analyzer (AU400; Olympus, Japan). Total lipids (TL) were calculated following the recommended formula (Rylander et al., 2006). Serum concentrations of monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8), and heme oxygenase-1 (HO-1) were measured using ELISA kits (OptEIA™; BD, USA) in accordance with the manufacturer`s protocol, as described previously (Liu et al., 2020). For 8-hydroxy-2 deoxyguanosine (8-OHdG) measurement, serum protein was removed by an ultrafilter with a cut-off molecular weight of 10 kDa (UFC501024; Merck-Millipore, USA) and the 8-OHdG concentration was measured following a method described previously (Chiou et al., 2003).

2.4. Statistical analysis

The case and control groups were compared in terms of age, pre-pregnancy body mass index (BMI, calculated based on the weight and height information recorded using the questionnaires), education, occupation, place of residence, offspring sex, gravidity, parity, spontaneous abortion history, passive smoking, drug usage, folic acid, gestational weeks, and fasting status at sampling using χ^2 tests for unordered categorical data and Wilconox signed-rank tests for ordinal categorical data. Since the Ti concentration showed a non-normal distribution, we calculated the median and interquartile range (IQR) instead of mean and standard deviation for Ti^{sr}, Ti^{bc}, and Ti^{tb} (Hao et al., 2019). The

Mann–Whitney *U* test was used to compare the median values between the case and control groups. Due to the relatively small sample size of the case group, we divided the Ti and biomarker concentrations into two levels (by median) when calculating their association with LBW risk using Logistic regression, and when estimating crude (cOR) and adjusted odds ratios (aOR) with 95% confidence intervals (CIs), with or without adjustment for potential confounders. In the dose-response analysis, we divided the Ti concentration into three levels by tertiles and calculated *P* for trend values using Logistic regression. For robust estimation of the association of blood Ti with biomarkers, we used three general linear models with or without adjustment for confounders. The final results are expressed as the estimated percentage changes in biomarkers (*IQR*% with 95% CIs) associated with an *IQR* increase from the first to third quartile of the log-transformed (to improve normality) blood Ti level, calculated as follows (Wu et al., 2016):

$$IQR\% = \left[\exp^{(IQR\times\beta)} - 1\right] \times 100\% \quad (1)$$

where *IQR*% is the estimated percentage change in biomarkers, *IQR* is the increase in Ti concentration from the first to the third quartile, and β is the correlation coefficient in the general linear models. All analyzes were conducted using R software (version 3.6.1; R Development Core Team, Austria), and the significance level was set at $P < 0.05$ (two-tailed).

3. Results

3.1. Population characteristics

The characteristics of the cases ($n = 45$) and controls ($n = 352$) are summarized in Table 1. Among the 397 women, 193 (48.6%) were aged < 25 years and 255 (64.2%) were within the normal BMI range (18.5–24.9 kg/m^2). Most of the women lived in rural areas (84.4%) and over half (65.7%) had farming-related jobs. All women were of Han ethnicity and not active smokers, although the majority (63.2%) were passive smokers during pregnancy. None of the cases had a history of spontaneous abortion, which was significantly different from the rate among the controls ($P = 0.008$). Thus, the effects of spontaneous abortion history were adjusted in further association analyzes and the results in different models showed consistency which indicated that it has a minor impact on our results (See Table S1 in Supplementary Information). Other characteristics, such as age, BMI, education, occupation, place of residence, gravidity, parity, offspring sex, folic acid, passive smoking, drugs, gestational week, fasting status at sampling time, and recruitment hospitals were comparable between the cases and controls.

3.2. Titanium concentration in maternal blood

The Ti concentrations in serum and blood cell (Ti^{sr}, Ti^{bc}), and the Ti^{tb}, are shown in Table 2 for the cases and controls. The Ti concentration was higher than the LOD in all serum and blood cell samples. The median concentrations of Ti^{sr}, Ti^{bc}, and Ti^{tb} in women were 122 ng/mL (IQR: 113–131 ng/mL), 143 ng/mL (IQR: 132–152 ng/mL), and 130 ng/mL (IQR: 123–138 ng/mL), respectively. The median Ti^{tb} concentration in the cases was significantly higher than that in the controls (134 and 129 ng/mL, respectively, $P = 0.039$). The median Ti^{sr} in the cases was also slightly higher than in the controls (126 and 121 ng/mL, respectively), although the difference was not statistically significant ($P = 0.061$). No significant difference in Ti^{bc} was observed between the cases and the controls.

Table 1
Population characteristics.

a Number of cases (percentage). Total number may not equal to the sum of each sub-level number due to missing data.

b *P was calculated by Wilconox signed-rank Test.*

c *P was calculated by corrected Chi-square Test or Fisher-exact test.*

$P < 0.05$ was shown in red and bold.

Table 2
Titanium concentrations in various blood fractions among low birth weight (LBW) cases and controls.

a Tisr, Titanium in serum; Tibc, Titanium in blood cells; Titb, Titanium in total blood.

b Median value (inter-quartile range), unit: ng/mL.

c Mann–Whitney *U test between cases and controls.*

$P < 0.05$ was shown in red and bold.

3.3. Association between titanium in maternal blood and LBW

We divided the Ti concentrations (Ti^{sr}, Ti^{bc}, and Ti^{tb}) into low and high groups and calculated their association with LBW, with or without adjustment for confounders (Table 3). Overall, we found an increased risk of LBW in the high Ti^{tb} group (aOR = 2.62; 95% CI: 1.16–5.90; $P = 0.02$) in the model adjusted for all potential confounders. However, no significant association between Ti^{sr} or Ti^{bc} and LBW risk was observed. When the blood Ti concentration was divided into tertiles, a positive dose-response relationship was observed between Ti^{tb} and LBW risk (Fig. 1). The cut-off values of maternal titanium levels were shown in Table S2.

3.4. Association between blood titanium and serum biomarkers

In Fig. 2, we evaluated the associations of serum and blood cell Ti concentrations with lipids, inflammatory biomarkers, and oxidative stress biomarkers in the control group ($n = 352$) with adjustment for confounders.

We found that Ti^{sr} was negatively associated with serum TG (*IQR*% = −6.41, 95% CI: −11.00 to −1.59) and TL (*IQR*% = −2.63, 95% CI: −4.76 to −0.46), where inter-quartile increased in Ti^{sr} from the first to third quartile were related to 6.41% and 2.63% decreases in TG and TL, respectively (See Table S3). Ti^{bc} was positively associated with serum 8-OHdG, with an *IQR*% of 19.39 (95% CI: 7.22 ~ 32.93), but not with TC, TG, or TL. Ti^{bc} was negatively associated with MCP-1 in "Model-Adj-I" (*IQR*% = −4.87, 95% CI: −8.91 to −0.64) (See Table S4), but the association disappeared after adjusting for other potential confounders. Ti^{tb} was negatively associated with TC, TG, and TL, with *IQR*% of −2.94 (95% CI: −5.56 to −0.24), −8.06 (95% CI: −13.22 to −2.60), and −4.01 (95% CI: −6.41 to −1.56), respectively (See Table S3). Overall, lipids were negatively associated with Ti^{sr} and Ti^{tb}, but not with Ti^{bc}. Although MCP-1 tended to have negative associations with Ti^{bc} and Ti^{tb}, inflammatory biomarkers were not significantly associated with Ti ($P > 0.05$).

4. Discussion

In this nested case-control study, we investigated the associations of Ti^{sr}, Ti^{bc}, and Ti^{tb} with the risk of LBW. In general, Ti^{bc} was higher than Ti^{sr} in maternal blood. Ti^{sr} and Ti^{bc} were not significantly associated with the risk of LBW, but there was a positive association between Ti^{tb} and the risk of LBW. Moreover, the concentrations of TG and TL were negatively associated with Ti^{sr}, but not with Ti^{bc}. Finally, Ti^{bc} was positively associated with 8-OHdG.

In our study, the median values of Ti^{sr}, Ti^{bc}, and Ti^{tb} were 122, 143, and 130 ng/mL, respectively. Compared to previous studies in humans, the values of Ti^{sr} and Ti^{tb} were higher in our study (Zheng et al., 2014; Yuan et al., 2018; Hou et al., 2019), which probably due to the high Ti exposure of mining around our study field. In the literature, blood Ti levels in different populations range widely from < 2 to > 150 ng/mL (Koller et al., 2018; Li et al., 2019). For populations with Ti implants,

Table 3
Associations of maternal titanium levels in various blood fractions with the risk of low birth weight (LBW).

Group[a]	Levels	Cases ($N = 45$)	Controls ($N = 352$)	COR[b]	AOR[c]	AOR[d]
Ti^{sr}	Low	17	182	Ref.	Ref.	Ref.
	High	28	170	1.76 (0.93–3.34)	1.56 (0.80–3.03)	1.81 (0.83–3.97)
Ti^{bc}	Low	21	178	Ref.	Ref.	Ref.
	High	24	174	1.17 (0.63–2.18)	1.22 (0.63–2.34)	1.17 (0.56–2.43)
Ti^{tb}	Low	16	183	Ref.	Ref.	Ref.
	High	29	169	1.96 (1.03–3.74)*	2.24 (1.12–4.49)*	2.62 (1.16–5.90)*

[a] Ti^{sr}, Titanium in serum; Ti^{bc}, Titanium in blood cells; Ti^{tb}, Titanium in total blood.
[b] Crude odds ratio (COR) with 95% confidence interval (95% CI) by a logistic regression model.
[c] Adjusted odds ratio (AOR) calculated by a Logistic regression model adjusting for age, BMI, education, occupation, place of residence.
[d] AOR calculated by a Logistic regression model adjusting for age, BMI, education, occupation, place of residence, gravidity, parity, spontaneous abortion history, folic acid, passive smoking, drugs, offspring sex, gestational weeks, fasting status.
* $P < 0.05$ was shown.

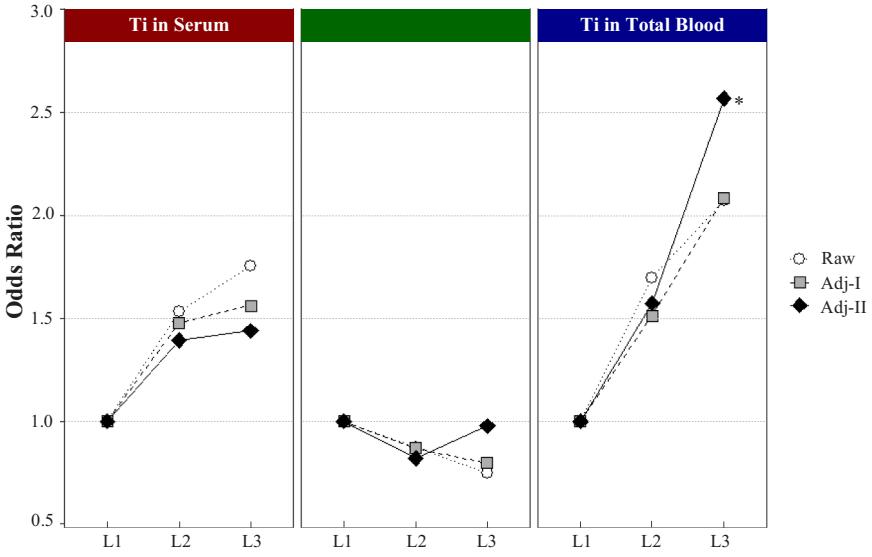

Fig. 1. Dose-response relationships of odds ratios (OR) of titanium concentration with LBW risk in various blood fractions. < 33.3% quantile (L1), 33.3–66.7% quantile (L2), > 66.7% quantile (L3). Three Logistic regression models were used as follows: Model-Raw: without adjustment. Model-Adj-I: with adjusting for age, BMI, education, occupation, place of residence. Model-Adj-II: with adjusting for age, BMI, education, occupation, place of residence, gravidity, parity, spontaneous abortion history, using folic acid, passive smoking, gestational weeks, and fasting status.

and in those with occupational exposure, Ti concentrations of at 300 ng/mL have also been reported (Sarmiento-Gonzalez et al., 2008; Lukina et al., 2016). Wide variations in blood Ti concentrations could be due to differences in area of residence, dietary habits, airborne particulate matter exposure, and metal analysis methods (Koller et al., 2018). In our study, Ti^{bc} was higher than Ti^{sr} (See Table 2), which indicated that Ti may participate in metabolic processes occurring inside blood cells or on the cell membrane. It has previously been speculated that Ti might exist as a labile pool in cells, similar to Fe, which could influence its cellular functions (Saxena et al., 2018). Oshida et al. found that Ti bound to the iron (Fe) transport protein serum transferrin (sTf) on the membrane and promoted cellular uptake via transferrin-directed endocytosis (Oshida, 2013), resulting in a higher Ti concentration in blood cells. The dynamic distribution of the serum Ti may also play a role. A human oral intake experiment revealed the Ti concentration in serum would reach a peak level at 6 h following ingestion (Pele et al., 2015). Besides, evidence in vivo showed TiO_2 mainly accumulated in the liver and spleen and could be retained for over 30 days in these tissues (Xie et al., 2011). This could explain why Ti^{sr} was not different between groups and was lower than Ti^{bc} from another aspect that the serum Ti would spread and accumulated in other tissues.

In our study, a high level of Ti^{tb} was associated with a dose-dependent increase in the risk of LBW risk. Regarding Ti and infant birth weight, several studies have explored their association but reported inconsistent results. For example, Zheng et al. reported a positive association between cord blood Ti and an overall adverse outcome (OR = 2.38; 95% CI: 1.20–4.73) (Zheng et al., 2014), but the risks of preterm birth, fetal distress, macrosomia, and LBW were not evaluated separately. Hou et al. found that the second quartile Ti^{sr} level was associated with a lower risk of LBW compared to the fourth quartile level (OR = 0.51; 95% CI: 0.32–0.81), but the association was not dose-dependent. Moreover, some ecological studies conducted in the US reported that large-scale environmental exposure to airborne Ti was associated with LBW (Bell et al., 2012; Ebisu and Bell, 2012; Basu et al., 2014). However, their exposure data were collected from air pollutant monitors maintained by the US Environmental Protection Agency, thus not reflecting individual exposure level. To the best of our knowledge, our study was the first to investigate both Ti^{sr} and Ti^{bc}, which could provide a unique insight into the relationship between Ti and LBW risk. Our results indicated that LBW risk was positively associated with Ti^{tb}, rather than with Ti^{sr} or Ti^{bc} separately (See Table 3). This interesting result supports the hypothesis of the dynamic exchange of Ti between serum and blood cells (Oshida, 2013; Saxena et al., 2018). According to previous studies, Ti can be absorbed by intestinal epithelial cells and may travel from the gut to the bloodstream via Fe transport routes (Aarabi et al., 2011). During this process, Ti would remain soluble but could be affected by pH changes (where pH ≈ 2 in the stomach) during ingestion, and by gut absorption and transport into the blood (Collins et al., 2005). These changes would lead to extensive changes in the formation and concentration of metal-ligand compounds (Ti citrate and ascorbate) (Bruneel and Helsen, 1988; Silwood and Grootveld, 2005; Tinoco et al., 2016). Eventually, at the pH level of the blood, the Ti in these compounds

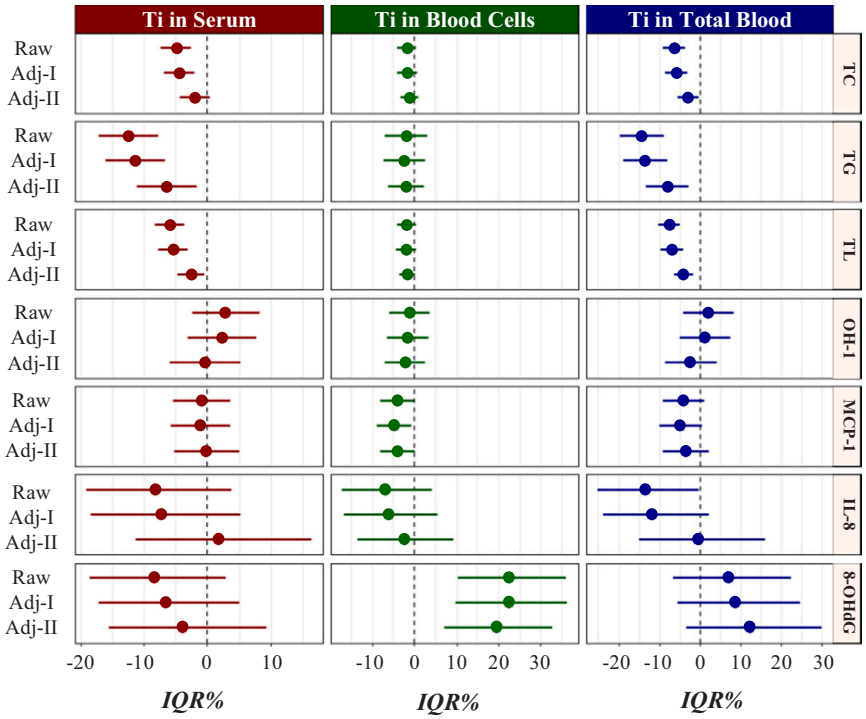

Fig. 2. Association between titanium and biomarkers in control group ($N = 352$) TC, Total cholesterol; TG, Total triglyceride; TL, Total lipid; HO-1, heme oxygenase 1; MCP-1, monocyte chemotactic protein-1; IL-8, interleukin-8; 8-OHdG,8-hydroxy-2 deoxyguanosine. Three general linear models were used as follows: Model-Raw: without adjustment. Model-Adj-I: with adjusting for age, BMI, education, occupation. Model-Adj-II: with adjusting for age, BMI, education, occupation, gravidity, parity, spontaneous abortion history, using folic acid, passive smoking, gestational weeks, and fasting status. *IQR%*, inter-quartile range percentage (with 95%CI), indicating percentage change in biomarkers with an inter-quartile range change in Titanium (Log transformed).

would dissociate and be dynamically exchanged by sTf between serum and blood cells (Buettner et al., 2012). The unstable distribution of Ti between serum and blood cells suggested that the Ti^{tb} provides a more robust estimate of Ti in the bloodstream.

We found a negative association between Ti^{tb} and serum lipids, which has not been discussed in previous studies to the best of our knowledge. However, this was consistent to some degree with previous studies on TiO_2 particles. Two occupational exposure studies in China reported significantly lower TC and TG levels, but higher low-density lipoprotein (LDL), in a TiO_2-exposed group (Xu et al., 2016; Zhao et al., 2018). Some rodent studies have also suggested that exposure to TiO_2 particles can impair liver function and the metabolism of TC and TG (Chen et al., 2009; Duan et al., 2010). Another study suggested that, based on cellular, animal, and epidemiological observation results, Ti might activate the NLRP3 inflammasome and induce a pro-inflammatory response in intestinal epithelial cells, which would affect the absorption of lipids (Ruiz et al., 2017). Thus, Ti might increase the risk of LBW by disturbing lipid metabolism in the maternal body.

In addition, we found that 8-OHdG, a typical marker of oxidative stress, and especially of DNA damage, was strongly associated with Ti^{bc}. This finding was consistent with a series of studies indicating that Ti exposure can induce reactive oxygen species (ROS) production, oxidative stress, and genotoxicity (Shimizu et al., 2009; Wang et al., 2011; Cui et al., 2014; Ruiz et al., 2017). Ti has also been observed to be distributed throughout the cell but has a higher concentration in the nuclear region (Waern et al., 2005). Based on this evidence, it can be inferred that Ti might cause genotoxicity, and thus inhibit the growth and development of infants via oxidative stress pathways (Trouiller et al., 2009; Fadoju et al., 2019).

No association of Ti with inflammatory biomarkers or LBW risk was observed, in contrast to previous in vivo and in vitro studies (Trouiller et al., 2009; Yazdi et al., 2010; Schanen et al., 2013). These results might due to the limitations associated with extrapolating animal experimental results to human populations, where the Ti concentration in our study might have been too low to activate inflammatory mechanisms (Yazdi et al., 2010). When evaluating the association between biomarkers and LBW risk, the ORs were > 1 for TC, TL, MCP-1 and 8-OHdG, and < 1 for TG, HO-1, and IL-8, in all three models (Fig. S3). However, none of these biomarkers were significantly associated with the risk of LBW. One possible explanation is that the fetus owns an independent circulatory system other than the mother. Due to the existence of the placental barrier, the genotoxicity and other potentially deleterious effects may impair maternal health first, rather than harming the fetus grows directly. Although some previous studies found that Ti could accumulate in the placenta or get through into core blood, there would be more complex steps for maternal Ti exposure to play a role in fetus health (Aengenheister et al., 2019; Li et al., 2019). In addition, considering the differences in Ti exposure routes and doses among animal experiments, cell experiments, and real-world scenarios, significant effects of lipids, inflammation, and oxidative stress responses (in the context of pregnancy Ti exposure) on LBW risk may not be evident.

Several limitations need to be considered when interpreting our results. First, the Ti concentrations in our study should be generalized to other populations with caution because of the relatively high level of exposure and the sample we used is serum instead of plasma. Second, due to the relatively small sample size of the cases compared to controls, we only divided the Ti concentration into two levels when calculating the ORs. However, when we divided the Ti concentration into three levels for the sensitivity analysis, a dose-response relationship was observed. Third, we only detected Ti concentration at one time point in early pregnancy which could not evaluate the Ti levels changes during pregnancy. In addition, the exposure pathways and toxicity of the various forms of Ti may differ (Oberdorster et al., 2005). Finally, we did not observe a direct association of LBW with lipids or oxidative stress biomarkers, and the influence on LBW of lipid level reductions and 8-OHdG production in relation to Ti requires further investigation. However, our study also had some advantages. First, it was based on a prospective cohort, such that recall bias was avoided. Second, blood samples collected during early pregnancy reflected the critical periods of offspring development. Third, we collected serum, blood cells, lipids, inflammatory biomarkers, and oxidative stress biomarkers for a detailed exploration of the association between Ti and LBW risk. Finally, we included controls to avoid bias in LBW and other potential confounders when analyzing the association between Ti and biomarkers. A sensitivity analysis of both the case and control groups ($N = 397$) showed consistent results among models adjusted for different confounders, as

detailed in Tables S3–S6, thereby providing robust support for our conclusions.

5. Conclusions

Ti^{tb} during pregnancy was associated with LBW risk at a high level of exposure. Compared to serum and blood cell concentrations, Ti in whole blood may be a more sensitive toxicological biomarker with respect to neonatal birth weight. Lipid metabolism and the oxidative stress induced by 8-OHdG may play important roles in the effects of Ti exposure. Further studies are needed to evaluate the health effects of Ti exposure during pregnancy, and the underlying mechanisms thereof.

CRediT authorship contribution statement

Jin Yu: Conceptualization, Formal analysis, Software, Writing - Original Draft. **Li Zhiyi**: Investigation, Formal analysis. **An Hang**: Data Curation, Validation. **Pang Yiming**: Methodology. **Li Kexin**: Investigation. **Zhang Yali**: Resources. **Zhang Le**: Methodology. **Yan Lailai**: Resources, Methodology. **Wang Bin**: Writing - Review & Editing. **Ye Rongwei**: Conceptualization, Supervision. **Li Zhiwen**: Project administration. **Ren Aiguo**: Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary information

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