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Maternal Lead Exposure and Premature Rupture of Membranes: A Birth Cohort Study in China

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Abstract

Objectives Maternal exposure to lead (Pb) has been suggested to be related with adverse birth outcomes, but evidence of an association between Pb exposure and premature rupture of membranes (PROM) is limited. The aim of our study was to investigate whether maternal Pb exposure was associated with PROM.

Design Prospective cohort study.

Study population This study involved 7290 pregnant women from the Healthy Baby Cohort (HBC) in Wuhan, China, during 2012 to 2014.

Main outcome measures PROM was defined as spontaneous rupture of amniotic membranes before the onset of labor, and was determined with a pH ≥ 6.5 for vaginal fluid. Maternal urinary Pb levels were adjusted by creatinine concentrations and its relationship with PROM was analyzed by logistic regression.

Results The interquartile range of maternal urinary Pb concentrations of the study population was 2.30-5.64 μg/g creatinine with a median of 3.44 μg/g creatinine. Increased risk of PROM was significantly associated with elevated levels of Pb in maternal urine [adjusted odds ratio (OR) = 1.23; 95% confidence interval (CI) = 1.02-1.47 for the medium tertile; adjusted OR = 1.51; 95% CI = 1.27-1.80 for the highest tertile]. A higher risk for preterm PROM associated with Pb levels was observed when compared with the lowest tertile (adjusted OR = 1.24; 95% confidence interval (CI) = 0.80-1.92 for the medium tertile; adjusted OR = 1.73; 95% CI = 1.15-2.60). In addition, the relationship between Pb and PROM was more pronounced among primiparous women than multiparous women (p for interaction < 0.01).

Conclusions Our study found that higher levels of maternal Pb exposure was associated with increased risk of PROM, indicating that exposure to Pb during pregnancy may be an important risk factor for PROM.

Strengths and limitations of this study

- This study was conducted with a large sample size, which included 7290 mother-singleton pairs from a birth cohort study in China.
- All information of the participants was collected from personal interviews and medical records, which allowed us to adjust for other potential risk factors for PROM.
- Although we included many potential confounders for analysis, the potential for residual confounding cannot be ruled out.

Introduction

Premature rupture of membranes (PROM), referred to maternal membranes rupture spontaneously before the onset of labor, accounts for approximately 8%-12% in delivers [1 2]. PROM is related to significant maternal, fetal, and neonatal risk, such as maternal infection, prematurity and neonatal sepsis [3 4]. Rupture begins prior to 37 weeks of gestation is considered as preterm PROM, which appears in 1%-3% of all pregnancies and occurs in one-third of preterm delivery, and thus is a leading cause for perinatal morbidity and mortality [4 5]. The etiology of PROM has been shown to be multifactorial, but increasing evidence regarding exposure to environmental pollutants has been shown as risk factors for PROM [6-8].

Lead (Pb), a ubiquitous non-biodegradable heavy mental that persists in the environment, is widely used in various industries, such as automobiles, paint, batteries, and plastics [9 10]. Due to these industrial processes, Pb has become the most widely scattered toxic heavy metal worldwide [10]. High levels of Pb exposure have been demonstrated to be associated with preeclampsia, pregnancy-induced hypertension, miscarriage, prematurity, congenital abnormalities, and even impaired cognitive function problems in childhood [11-13]. However, the association between maternal Pb exposure and risk of PROM is limited, and the results were inconsistent. Some studies have found significant relationship between the risk of PROM with maternal Pb levels [8 14-16], while a previous study has failed to observe such an association [17].

China is the largest raw and refined Pb producing and consuming country around the

world [18]. Pb pollution poses a significant threat for human health, especially for pregnant women and fetus. Given this background, the present study involving 7290 participants was designed to explore whether Pb exposure during pregnancy could increase the risk of PROM and preterm PROM in Chinese pregnant women.

Materials and Methods

Study population and data collection

The study participants (n = 11311) were enrolled during September 2012 to October 2014 from the Healthy Baby Cohort (HBC) study at Wuhan Medical and Health Center for Women and Children in China, and the eligibility criteria has been described elsewhere [19]. For this study, we excluded women without urine samples (n = 3947), and who delivered an infant with congenital malformation (n = 62), as well as those who reported smoking (n = 7) and drinking (n = 2) during pregnancy. For women who gave birth twice in HBC (n = 3), we excluded the second delivery record (n = 3). Finally, 7290 pregnant women were included for the present study. All participating mothers signed written informed consent at enrollment. The Ethical Committees of Tongji Medical College, Huazhong University of Science and Technology, and the study hospital approved the study protocol.

All participants filled out a structural questionnaire after labor during a face-to-face interview by specially trained nurses. Information on the demographic and socioeconomic background, (e.g., maternal age, educational level, and occupational status), pre-pregnancy body mass index (BMI) (calculated on the basis of

self-reported weight and height before pregnancy), and daily-life habits during pregnancy (e.g., alcohol and tobacco consumption) were collected during this process. Medical/reproductive histories and outcomes (e.g., maternal diseases, complications and infant sex) were gathered from medical records. Last menstrual period (LMP) was used to calculate maternal gestational week.

PROM was defined as spontaneous rupture of amniotic membranes prior to the onset of labor, and was determined with a pH $\geq$ 6.5 for vaginal fluid. Rupture occurred less than 37 weeks' gestation was considered as preterm PROM.

Urine sample collection and lead exposure measurement

Maternal urine samples were collected when they admitted to the hospital while waiting for delivery and were stored immediately in polypropylene tubes at -20 °C for further treatment. The detection method for urinary Pb was introduced previously [20]. Briefly, prior to analysis, urine specimens were thawed at room temperature until treatment. Then, 1 mL of supernatant urine with 4 mL of 3% HNO_3 were added into 15 mL polypropylene tubes for overnight nitrification, and were digested by ultrasound for 1 hour at 40 °C. After that, inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7700, Agilent Technologies, Waldbronn) was used to measure maternal urinary Pb concentrations. Assessment of the instrument performance was conducted by the Standard Reference Material Human Urine (SRM2670a Toxic Elements in Urine, National Institute of Standards and Technology, USA) as external quality control sample in each batch. The detection rate of maternal

urinary Pb concentrations in this study was 99%, and the samples below the limit of detection (LOD) (0.01 μg/L) were replaced by 1/2 LOD. The intra-day coefficient of variation was below 2.0%, and the inter-day coefficient of variation was under 3.0%.

Urine creatinine concentrations measured by an automatic biochemical analyzer (BS-200, Mindray, Shenzhen, China), were used for the adjustment of Pb concentrations to control variable urine dilutions. And the adjusted urinary Pb concentrations were presented as μg/g creatinine.

Statistical analyses

The distribution of Pb concentration was skewed towards right when tested by Kolmogorov–Smirnov normality test. The Wilcoxon signed rank test was used to compare concentrations of Pb between PROM and non-PROM women. To evaluate the association between Pb exposure and PROM, logistic regression analyses were conducted to calculate crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs). Maternal urinary Pb levels were categorized into tertiles, of which the lowest level was used as the reference. We detected the linear trends of Pb with PROM via modeling the median values of tertiles of Pb concentration as a continuous variable, and used the Wald test to evaluate the statistical significance. The adjustment for potential confounders was based on known factors associated with PROM, such as household income, passive smoking, parity, and pregnancy-induced hypertension [3 5 21-23]. Additionally, covariates that altered parameter estimate of Pb for the effect of PROM by over 10% were also included in the final model. Covariates, including

maternal age, family income, pre-pregnancy BMI, parity, passive smoking and pregnancy-induced hypertension, were adjusted for in this analysis. We also performed a sensitivity analysis excluding participants with intrauterine infection during pregnancy in consideration of their potential influence on PROM [24 25]. Besides, we analyzed the ORs for PROM stratified by maternal parity (primiparous vs. multiparous), because of the difference in these variables has been previously reported to be related with PROM [23]. An interaction term was added into the model to assess the effect of Pb and maternal parity on the outcome of PROM.

All data analyses were performed by SAS (version 9.4; SAS Institute Inc., NC, USA), and two-sided *p* values below 0.05 were considered statistically significant.

Results

Table 1 presents the basic characteristics and urinary Pb concentrations of 7290 participants. In this study, the prevalence of PROM and preterm PROM was 12.1% and 2%, respectively. Maternal age at labor ranged 18-46 years with 28 years on average. Most of the mothers were primiparous (84.5%), and had a high educational attainment (> 12 years) (67.2%), high annual family income (≥ 50,000 per year) (56.9%), and normal pre-pregnancy BMI (18.5-23.9 kg/m^2) (66.2%). The average gestational age at delivery was 39.2 weeks. About 22.9 % were passively exposed to smoking during pregnancy. Approximately 3.9% of the pregnant women had hypertension during pregnancy, and 53.4% of the mothers gave birth to a male infant.

The median of maternal urinary Pb concentrations in PROM mothers (3.88 μg/g

creatinine) was higher than that of non-PROM mothers (median = 3.39 μg/g creatinine) ($p < 0.05$). Compared with women without preterm PROM (median = 3.43 μg/g creatinine), the median of maternal urinary Pb concentrations in preterm PROM mothers was also higher (median = 3.96 μg/g creatinine) ($p < 0.05$).

Table 2 shows the relationships of tertiles of creatinine-adjusted Pb levels in maternal urine with PROM and preterm PROM. Compared with the lowest tertile, a significantly positive association of PROM with increasing levels of maternal urinary Pb concentrations was observed (adjusted OR = 1.23; 95% CI = 1.02-1.47 for the medium tertile; adjusted OR = 1.51; 95% CI = 1.27-1.80 for the highest tertile) (p trend < 0.01). A higher risk estimate for preterm PROM in association with Pb levels was found when compared with the lowest tertile (adjusted OR = 1.24; 95% CI = 0.80-1.92 for the medium tertile; adjusted OR = 1.73; 95% CI = 1.15-2.60 for the highest tertile) (p trend < 0.01). In addition, the results of the sensitivity analysis (Supplementary material, **Table S1**) excluding subjects with intrauterine infection during pregnancy demonstrated an essentially unchanged associations of Pb with PROM and preterm PROM.

Table 3 presents the results stratified by maternal parity. Among the 6159 primiparous women, we observed a significant trend in elevated Pb concentrations and increased risk of PROM (adjusted OR = 1.24; 95% CI = 1.03-1.50 for the medium tertile; adjusted OR = 1.52; 95% CI = 1.27-1.83 for the highest tertile) (p trend < 0.01). However, no statistical significant association was found between PROM and Pb in multiparous mothers (adjusted OR = 1.21; 95% CI = 0.65-2.25 for

the medium tertile; adjusted OR = 1.57; 95% CI = 0.87-2.83 for the highest tertile) (p trend = 0.13). The risk estimates for PROM women in relation to Pb levels in those who were primiparous and who were multiparous was significantly different (p for interaction < 0.01).

Discussion

Our study examined the association between maternal urinary Pb exposure before delivery and risk of PROM in Chinese pregnant women, and we found that increased concentrations of urinary Pb were significantly positively related to PROM and preterm PROM. Besides, the results suggested that the relationship between Pb and PROM may vary by maternal parity, since it appeared more pronounced in primiparous women than in multiparous women.

PROM, especially preterm PROM, is the leading factor contributing to preterm birth and neonatal complications, such as perinatal infections, respiratory distress syndrome, umbilical cord compression, intraventricular hemorrhage, sepsis and even death [4 5]. Furthermore, it has been linked with long-term harmful neurodevelopmental consequence for neonatal development [26]. Although there is limited understanding for the cause of PROM currently, there are growing evidences recently suggesting that environmental factors play important roles in inducing PROM by stimulating oxidative stress and inflammation that predispose premature membranes rupture [6 7].

In this study, we observed that maternal exposure to higher levels of Pb before delivery was correlated with increased risk of PROM and preterm PROM. After we

excluded the subjects with intrauterine infection during pregnancy, which has been implicated as one of the major factors in the pathogenesis of PROM [5], we also observed an unchanged significant association of Pb with PROM and preterm PROM. Consistent with our findings, a study including 332 pregnant women in Iran reported that one unit increase in logarithm of maternal blood Pb concentration was associated with a several-fold risk of PROM [8]. Similarly, a study involving 502 pregnant mothers in Columbia found that higher blood levels was associated with an increase in the incidence of PROM [14]. Besides, elevated Pb concentrations in the umbilical cord were reported to be associated with increased PROM risk in a cohort study of 749 mother-infant pairs in a Pb-smelter community in South Australia [15]. Additionally, in a study including 89 mother-infant pairs in the southeast of Spain, Falcon et al. observed a higher placental Pb concentration in the PROM cases than that in the normal deliveries [16]. In contrast, an early study demonstrated that there was no correlation between the risk of PROM delivery with blood Pb concentrations measured in 635 samples from the umbilical cord blood [17]. The discrepancy in results may be due to the differences in Pb exposure levels.

PROM has been reported to be associated with multiple factories, including cigarette smoking, low income, parity, infection, and pregnancy-induced hypertension [3 5 21-23]. In the present study, inclusion of the potential confounding factors for adjustment did not attenuate the association between increased levels of Pb exposure and risk of PROM. In the stratified analysis by parity, our results suggested that there was a significant difference in parity for the risk of PROM related with maternal Pb

exposure. A significantly positive association between PROM and urinary Pb concentrations was observed in primiparous mothers. A similar positive relationship of Pb exposure with PROM delivery was also found in multiparous mothers, though the results were not significant. The possible explanation may attribute to the insufficient sample size of multiparous mothers. Future researches with more multiparous women are warranted to further evaluate this parity difference in the effect of Pb on PROM.

The etiology and mechanism of Pb effect on PROM are not clear. One possible explanation is that Pb can induce toxicity by triggering oxidative stress through the generation of reactive oxygen species (ROS) [9 27], which is responsible for the structural weakness of collagen fibrils and causes the membranes losing strength and elasticity [28 29]. As a result, Pb-induced ROS contributing to membrane rupture occurs via the damage of collagen in fetal membranes [8]. Furthermore, findings have shown that Pb can induce an increase in pro-inflammatory cytokines, like TNF α [30], inflammatory responses may be implicated and predisposed the membranes to rupture by promoting alterations of membrane fluidity and impairing membrane barrier function [31].

Urinary Pb has been favored as a long-term biomarker of exposure, and is widely used in the assessment Pb exposure levels [32 33]. In the present study, maternal urinary Pb concentration [arithmetic mean (AM) = 7.40 μg/g creatinine; geometric mean (GM) = 3.69 μg/g creatinine; median = 3.44μg/g creatinine] was higher than pregnant mothers reported in developed countries, like Australia (AM = 0.87μg/g

creatinine; median = 0.7μg/g creatinine) [34], Japan (GM = 0.48μg/g creatinine) [35],and American (GM = 0.63μg/L) [36]. However, there was also an overlap in the concentration of urinary Pb in our study participants with other countries, like Spain (AM = 5.2 μg/g creatinine; median = 3.9 μg/g creatinine) [37]. In addition, Pb concentration in the present subjects was lower in comparison with pregnant women reported in the developing country, like Nigeria (AM = 28.5 μg/g creatinine) [38]. Although Pb petrol has been phased out since 2000 in China, Pb pollution remains a huge challenge for the environment, since large amounts of Pb pollutants from various sources of Pb consumption increase rapidly, due to the unprecedented development of Chinese economy [39 40]. In the past decades, elevated accumulations of Pb have been reported to be a great threat existed in soil and dust in many provinces of China [18 40]. Pb contamination has become a public health concern for human health in China, since Pb can enter into the body of the humans from atmosphere and soil by drinking water, ingesting food, and inhaling air that contains Pb contaminants [10 40]. As a consequence, excessive Pb emission in China poses serious adverse health effects for humans, especially for the susceptible pregnant women.

The strength of this study is as follows: First, it was conducted with a large sample size, which included 7290 mother-singleton pairs from a birth cohort study in China. We preformed sensitivity analyses and stratified analyses to evaluate the relationship of maternal Pb exposure with PROM and found a significant association between increased levels of Pb exposure and elevated risk of PROM. Moreover, all information about the participants such as demographic characteristics,

socioeconomic status and pregnancy outcomes were gathered from personal interviews and medical records, which made it possible for us to adjust for other potential risk factors for PROM. However, there were still some other potential confounders that we may not be able to control. In addition, urinary Pb collected and measured at labor may fail to accurately reflect the whole period of maternal Pb exposure during pregnancy, further studies from multiple time points and different populations are needed to confirm the observed relationship between Pb and PROM.

Conclusion

In this study, we observed a positive relationship between maternal urinary Pb and risk of PROM, indicating that maternal exposure to Pb may be a potential risk factor for PROM. This finding suggests that appropriate public health measures needed to be taken to control maternal Pb exposure during pregnancy.

Footnotes

SH and WX contributed equally to this work.

Contributors: SH carried out the statistical analyses and drafted the manuscript. WX assisted in the statistical analyses, critically reviewed and revised the manuscript. BZ, TC, SX contributed to the study design and developed the initial protocol. YL contributed to the study design, critically reviewed and revised the manuscript. All authors read and approved the final manuscript.

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Ethics approval: The Ethical Committees of Tongji Medical College, Huazhong University of Science and Technology, and the study hospital approved the study protocol.

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Table 1 Basic characteristics and urinary Pb concentrations (μg/g creatinine) of the 7290 pregnant women.

Characteristics	N (%)	Median (IQR) Pb (μg/g creatinine)
Total	7290	3.44 (2.30-5.64)
Maternal age (years)		
< 25	805 (11.0)	3.37 (2.27-5.52)
25–29	3985 (54.7)	3.49 (2.32-5.77)
30–34	2011 (27.6)	3.40 (2.29-5.47)
≥ 35	489 (6.7)	3.41 (2.15-5.94)
Education background (years)		
≤ 9	1001 (13.7)	3.45 (2.40-5.63)
9–12	1389 (19.1)	3.47 (2.35-5.56)
> 12	4898 (67.2)	3.42 (2.26-5.66)
Missing	2 (0.03)	5.69(3.50-7.90)
Family income(yuan/year)		
< 50,000	3021 (41.4)	3.52 (2.31-5.75)
≥ 50,000	4148 (56.9)	3.39 (2.28-5.60)
Missing	121 (1.7)	3.50 (2.38-5.83)
Parity		
primiparous	6159 (84.5)	3.45 (2.30-5.66)
multiparous	1131 (15.5)	3.38 (2.26-5.59)
Pre-pregnancy BMI (kg/m^2)		
< 18.5	1527 (20.95)	3.47 (2.27-5.77)
18.5–23.9	4832 (66.28)	3.41 (2.29-5.56)
≥ 24	910 (12.48)	3.60 (2.39-6.04)
Missing	21 (0.29)	3.87 (2.07-8.12)
Passive smoking during pregnancy		

Yes	1670 (22.9)	3.30 (2.29-5.32)
No	5620 (77.1)	3.47 (2.30-5.71)
Pregnancy-induced hypertension		
Yes	286 (3.9)	3.26 (2.18-5.21)
No	7004 (96.1)	3.44 (2.30-5.66)
Intrauterine infection		
Yes	987 (13.5)	3.59 (2.41-6.04)
No	6303 (86.5)	3.42 (2.28-5.60)
Gestational age (weeks)		
< 37	291 (4.0)	3.99 (2.62-7.54)
≥ 37	6999 (96.0)	3.42 (2.29-5.60)
Infant gender		
Male	3890 (53.4)	3.48 (2.34-5.78)
Female	3400 (46.6)	3.40 (2.25-5.52)
PROM		
Yes	881 (12.1)	3.88 (2.55-6.59)
No	6409 (87.9)	3.39 (2.27-5.55)
Preterm PROM		
Yes	147 (2.0)	3.96 (2.63-7.67)
No	7143 (98.0)	3.43 (2.29-5.61)

Abbreviation: BMI, body mass index; IQR, interquartile range; PROM, Premature Rupture of Membranes.

Table 2 Risk of PROM and preterm PROM associated with the levels of Pb in maternal urine.

Pb (μg/g creatinine)	case	OR[a] (95% CI)	OR[b] (95% CI)
PROM			
Tertile1 (< 2.65)	241	1.00	1.00
Tertile2 (2.65–4.70)	291	1.24 (1.03, 1.48)	1.23 (1.02, 1.47)
Tertile3 (≥ 4.70)	349	1.52 (1.27, 1.81)	1.51 (1.27, 1.80)
p for trend		< 0.01	< 0.01
Preterm PROM			
Tertile1 (< 2.65)	37	1.00	1.00
Tertile2 (2.65–4.70)	46	1.25 (0.81, 1.93)	1.24 (0.80, 1.92)
Tertile3 (≥ 4.70)	64	1.74 (1.16, 2.62)	1.73 (1.15, 2.60)
p for trend		< 0.01	< 0.01

Abbreviations: OR, odds ratio; CI, confidence interval.

[a] Unadjusted odds ratio.

[b] Adjusted for maternal age, family income, pre-BMI, parity, passive smoking and pregnancy-induced hypertension.

Table 3 Risk of PROM associated with the levels of Pb in maternal urine, stratified by parity.

Pb levels[a]	Primiparous (n = 6159)			Multiparous (n = 1131)			*p* for interaction
(μg/g creatinine)	N	OR[b] (95% CI)	OR[c] (95% CI)	N	OR[b] (95% CI)	OR[c] (95% CI)	
T1	220	1.00	1.00	20	1.00	1.00	< 0.01
T2	268	1.25 (1.04, 1.51)	1.24 (1.03, 1.50)	24	1.21 (0.66, 2.23)	1.21 (0.65, 2.25)	
T3	318	1.52 (1.27, 1.83)	1.52 (1.27, 1.83)	31	1.59 (0.89, 2.84)	1.57 (0.87, 2.83)	
P for trend		< 0.01	< 0.01		0.11	0.13	

Abbreviations: OR, odds ratio; CI, confidence interval; T: tertile;

[a] Pb levels: primiparous, T1 (< 2.66), T2 (2.66-4.71), T3 (≥ 4.71); multiparous, T1 (< 2.61), T2 (2.61-4.61), T3 (≥ 4.61).

[b] Unadjusted odds ratio.

[c] Adjusted for maternal age, family income, pre-BMI, passive smoking and pregnancy-induced hypertension.

Table S1 Risk of PROM and preterm PROM associated with the levels of Pb in maternal urine, after excluding intrauterine infection during pregnancy (n = 6303)

Pb (μg/gcreatinine)	case	OR[a] (95% CI)	OR[b] (95% CI)
PROM			
Tertile1 (< 2.63)	208	1.00	1.00
Tertile2 (2.63–4.65)	257	1.27 (1.04, 1.54)	1.26 (1.04, 1.53)
Tertile3 (≥ 4.65)	302	1.52 (1.26, 1.84)	1.51 (1.25, 1.83)
p for trend		< 0.01	< 0.01
preterm PROM			
Tertile1 (< 2.63)	34	1.00	1.00
Tertile2 (2.63–4.65)	37	1.09 (0.68, 1.74)	1.07 (0.67, 1.71)
Tertile3 (≥ 4.65)	56	1.66 (1.08, 2.55)	1.64 (1.07, 2.53)
p for trend		0.011	0.015

Abbreviations: OR, odds ratio; CI, confidence interval.

[a] Unadjusted odds ratio.

[b] Adjusted for maternal age, family income, pre-BMI, parity, passive smoking and pregnancy-induced hypertension.

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Maternal Lead Exposure and Premature Rupture of Membranes: A Birth Cohort Study in China

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Abstract

Objectives Maternal exposure to lead (Pb) has been suggested to correlate with adverse birth outcomes, but evidence supporting an association between Pb exposure and premature rupture of membranes (PROM) is limited. The aim of our study was to investigate whether maternal Pb exposure was associated with PROM and preterm PROM.

Design Cross-sectional cohort study.

Study population The present study involved 7290 pregnant women from the Healthy Baby Cohort (HBC) in Wuhan, China, during 2012 to 2014.

Main outcome measures PROM was defined as spontaneous rupture of amniotic membranes before the onset of labour and was determined with a pH ≥ 6.5 for vaginal fluid. Maternal urinary Pb level was adjusted by creatinine concentration, and its relationship with PROM was analyzed by logistic regression.

Results The interquartile range of maternal urinary Pb concentrations of the study population was 2.30-5.64 μg/g creatinine with a median of 3.44 μg/g creatinine. Increased risk of PROM was significantly associated with elevated levels of Pb in maternal urine [adjusted odds ratio (OR) = 1.23, 95% confidence interval (CI) = 1.02-1.47 for the medium tertile; adjusted OR = 1.51, 95% CI = 1.27-1.80 for the highest tertile]. The risk of preterm PROM associated with Pb levels was significantly higher when compared to the lowest tertile (adjusted OR = 1.24, 95% CI = 0.80-1.92 for the medium tertile; adjusted OR = 1.73, 95% CI = 1.15-2.60 for the highest tertile). In addition, the relationship between Pb and PROM was more pronounced among

primiparous women than multiparous women (p for interaction < 0.01).

Conclusions Our study found that higher levels of maternal Pb exposure was associated with increased risk of PROM, indicating that exposure to Pb during pregnancy may be an important risk factor for PROM.

Strengths and limitations of this study

- This study was conducted with a large sample size, which included 7290 mother-singleton pairs from a birth cohort study in China.
- All information about the participants was collected from personal interviews and medical records, which allowed us to adjust for other potential risk factors for PROM.
- Although many potential confounders were taken into account for analysis, other confounding factors may remain.

Introduction

Premature rupture of membranes (PROM), refers to maternal membranes rupture more than one hour before the onset of labour, occurs in approximately 5%-15% of deliveries [1-3]. PROM is related to significant maternal, fetal, and neonatal risks, such as maternal infection, prematurity, neonatal sepsis, as well as adverse neurological outcomes [4-6]. When the rupture occurs prior to 37 weeks of gestation, it is considered as preterm PROM. Preterm PROM appears in 1%-3% of all pregnancies and one-third of preterm deliveries, and thus is a leading cause of perinatal morbidity and mortality [5 7]. The etiology of PROM has been shown to be multifactorial, and increasing evidence has regarded exposure to environmental pollutants as risk factors for PROM [8-10].

Lead (Pb), a ubiquitous non-biodegradable heavy mental that persists in the environment, is widely used in various industries, such as automobiles, paint, batteries, and plastics [11 12]. Due to these industrial processes, Pb has become the most widely distributed toxic heavy metal worldwide [12]. High levels of Pb exposure have been demonstrated to be associated with preeclampsia, pregnancy-induced hypertension, miscarriage, prematurity, congenital abnormalities, and even impaired cognitive function problems in childhood [13-18]. However, the association between maternal Pb exposure and risk of PROM is limited, and the results were inconsistent. Some studies have found a significant correlation between the risk of PROM and maternal Pb levels [10 19-21], while another study has failed to observe such an association [22].

China is the largest Pb (raw and refined) producing and consuming country in the world [23]. Pb pollution poses a significant threat to human health, especially for pregnant women and the vulnerable fetuses, who are more susceptible to Pb exposure since Pb can freely cross the placenta [24]. Given this background, the present study involving 7290 participants was designed to explore whether Pb exposure during pregnancy could increase the risk of PROM and preterm PROM in Chinese pregnant women.

Materials and methods

Study population and data collection

The study participants (n = 11311) were enrolled during September 2012 to October 2014 from the Healthy Baby Cohort (HBC) study at Wuhan Medical and Health Center for Women and Children in China, and the eligibility criteria has been described previously [25]. For this study, we excluded women without urine samples (n = 3947), as well as those who delivered infants with congenital malformations (n = 62), which may be caused by an abnormal pregnancy. The number of cases with smoking (n = 7) or drinking (n = 2) during pregnancy were rather small, in line with previous reports [26 27], and were also excluded, as these lifestyles have been shown to have adverse effects on fetal growth. For women who gave birth twice in HBC (n = 3), we excluded the second delivery record and only kept the first one (n = 3). Finally, 7290 pregnant women were included in the present study. All participating mothers signed written informed consent at enrollment. The ethical committees of Tongji

Medical College, Huazhong University of Science and Technology, and the study hospital approved the study protocol.

All participants filled out a structural questionnaire after labour during a face-to-face interview by specially trained nurses. Information on the women's demographic and socioeconomic backgrounds, (e.g., maternal age, educational level, and occupational status), pre-pregnancy body mass index (BMI) (calculated on the basis of self-reported weight and height before pregnancy), and daily-life habits during pregnancy (e.g., alcohol and tobacco consumption) were collected during this process. Medical/reproductive histories and outcomes (e.g., intrauterine infection, maternal diseases, and infant sex) were gathered from medical records. Last menstrual period (LMP) was used to calculate maternal gestational week.

PROM was defined as spontaneous rupture of amniotic membranes prior to the onset of labour and was determined by the visualization of amniotic fluid passing from the cervical canal and pooling in the vagina, plus the nitrazine test of a pH $\geq$ 6.5 for vaginal fluid. The nitrazine test is a simple and rapid bedside method to diagnose PROM and is widely used in Chinese hospitals with a relatively high reliability [28]. The diagnosis of the onset of labour was determined by regular painful contractions and a cervical dilatation of 3 cm or greater. Rupture occurred less than 37 weeks of gestation was considered as preterm PROM. The definition of the clinical diagnosis of intrauterine infection was considered in the presence of maternal fever (> 38 ℃) accompanied by signs or symptoms of maternal and fetal tachycardia, uterine tenderness, foul smelling discharge, maternal leucocytosis or positive amniotic fluid

cultures from an amniocentesis. Clinical vaginitis was defined by the presence of erythema and an exudative discharge that was associated with symptoms of pruritus or pain. Cervicitis was diagnosed based on cervical erosion with purulent discharge from the cervix. Pelvic inflammatory disease was clinically defined by the presence of adnexal tenderness and/or the presence of tender adnexal mass on bimanual pelvic examination. Vaginal bleeding was defined as the presence of bleeding in pregnant women prior to 28 weeks of gestation. Polyhydramnios was defined by having an amniotic fluid index of 24 cm or more. Fetal malposition was defined as occipito-transverse or occipito-posterior position.

Urine sample collection and lead exposure measurement

Maternal urine samples were collected upon their admission to the hospital while waiting for delivery and were stored immediately in polypropylene tubes at -20 °C for further treatment. The detection method for urinary Pb was introduced previously [29]. Briefly, prior to analysis, urine specimens were thawed at room temperature. Then, 1 mL of supernatant urine with 4 mL of 3% HNO_3 were added into 15 mL polypropylene tubes for overnight nitrification and were digested by ultrasound for 1 hour at 40 °C. Next, inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7700, Agilent Technologies, Waldbronn) was used to measure maternal urinary Pb concentrations. Assessment of the instrument performance was conducted using the Standard Reference Material Human Urine (SRM2670a Toxic Elements in Urine, National Institute of Standards and Technology, USA) as external quality control

sample in each batch. The concentrations of the quality controls were measured within the certified range recommended by the manufacturer (5%). The samples were analyzed with an external calibration method using eight standard concentrations ranging from 0 to 500 mg/L. Field and procedure blanks were also included to assess potential contamination, and Pb was not detected in the containers or storage tubes. The detection rate of maternal urinary Pb concentrations in this study was 99%, and the samples below the limit of detection (LOD) (0.01 μg/L) were replaced by 1/2 LOD. The intra-day coefficient of variation was below 2.0%, and the inter-day coefficient of variation was under 3.0%.

Urine creatinine concentrations measured by an automatic biochemical analyzer (BS-200, Mindray, Shenzhen, China), were used for the adjustment of Pb concentrations to control variable urine dilutions. The quality control standards for creatinine were identical to those described previously [19]. The adjusted urinary Pb concentrations were presented as μg/g creatinine.

Statistical analyses

The distribution of Pb concentration was skewed towards the right when tested by the Kolmogorov–Smirnov normality test. The Wilcoxon signed rank test was used to compare concentrations of Pb between PROM and non-PROM women. To evaluate the association between Pb exposure and PROM, logistic regression analyses were conducted to calculate crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs). Maternal urinary Pb levels were categorized into tertiles, with lowest

one used as the reference. We detected the linear trends of Pb with PROM by modeling the median values of tertiles of Pb concentration as a continuous variable and used the Wald test to evaluate the statistical significance. The adjustment for potential confounders was based on known factors associated with PROM, such as household income, passive smoking, parity, and pregnancy-induced hypertension [4 7 30-32]. Additionally, covariates that altered the parameter estimate of Pb effect on PROM by over 10% were also included in the final model. Covariates, including maternal age, family income, pre-pregnancy BMI, parity, passive smoking and pregnancy-induced hypertension, were adjusted for in this analysis. We also performed a sensitivity analysis excluding participants with intrauterine infection (chorioamnionitis), vaginitis, cervicitis, pelvic inflammatory disease, previous vaginal bleeding, polyhydramnios, and fetal malposition in consideration of their potential influence on PROM. As data from NHANES suggested that women usually have low urinary creatinine, and the upper cut-off (3 g/L) remained appropriate for the female population [33], a sensitivity analysis that excluded women with creatinine > 3 g/L was also conducted. In addition, we analyzed the ORs for PROM stratified by maternal parity (primiparous vs. multiparous), because the difference in these variables has been previously reported to associate with PROM [32]. An interaction term was added into the model to assess the effect of Pb and maternal parity on the outcome of PROM.

All data analyses were performed by SAS (version 9.4; SAS Institute Inc., NC, USA), and two-sided *p* values below 0.05 were considered statistically significant.

Patient involvement

No patients were involved in setting the research question or the outcome measures, nor were they involved in the design or implementation of the study. No patients were involved in the interpretation of study results or writing up of the manuscript. There are no plans to disseminate the results of the research to study participants.

Results

The basic characteristics and urinary Pb concentrations of 7290 participants is shown in Table 1. In this study, the prevalence of PROM and preterm PROM was 12.1% and 2%, respectively. Maternal age at labour ranged from 18 to 46 years, with 28 years as the average age. Most of the mothers were primiparous (84.5%) and had a high educational attainment (> 12 years) (67.2%), high annual family income (≥ 50,000 yuan per year) (56.9%), and normal pre-pregnancy BMI (18.5-23.9 kg/m^2) (66.3%). The average gestational age at delivery was 39.2 weeks. About 22.9% were passively exposed to smoking during pregnancy. Approximately 3.9% of the pregnant women had hypertension during pregnancy, and 53.4% of the mothers gave birth to a male infant.

The median of maternal urinary Pb concentrations in PROM mothers (3.88 μg/g creatinine) was higher than that of non-PROM mothers (median = 3.39 μg/g creatinine) ($p < 0.05$). Compared with women without preterm PROM (median = 3.43 μg/g creatinine), the median of maternal urinary Pb concentrations in preterm PROM

mothers was also higher (median = 3.96 μg/g creatinine) ($p < 0.05$).

Table 2 shows the relationship between creatinine-adjusted maternal urinary Pb levels and PROM/preterm PROM. Compared with the lowest tertile, a significantly positive correlation between PROM and Pb concentrations was observed (adjusted OR = 1.23, 95% CI = 1.02-1.47 for the medium tertile; adjusted OR = 1.51, 95% CI = 1.27-1.80 for the highest tertile) (p trend < 0.01). The risk estimate for preterm PROM in association with Pb levels was significantly higher compared to the lowest tertile (adjusted OR = 1.24, 95% CI = 0.80-1.92 for the medium tertile; adjusted OR = 1.73, 95% CI = 1.15-2.60 for the highest tertile) (p trend < 0.01). In addition, the sensitivity analysis (Supplementary material, Table S1) excluding subjects with intrauterine infection, vaginitis, cervicitis, pelvic inflammatory disease, previous vaginal bleeding, polyhydramnios, and fetal malposition demonstrated a similar association between Pb and PROM/preterm PROM. Consistent observation was also made in the sensitivity analysis excluding women with creatinine > 3 g/L in the statistical models (Supplementary material, Table S2).

Results stratified by maternal parity are summarized in Table 3. Among the 6159 primiparous women, we observed a significantly positive correlation between Pb concentrations and PROM risk (adjusted OR = 1.24, 95% CI = 1.03-1.50 for the medium tertile; adjusted OR = 1.52, 95% CI = 1.27-1.83 for the highest tertile) (p trend < 0.01). However, no statistically significant association was found between PROM and Pb in multiparous mothers (adjusted OR = 1.21, 95% CI = 0.65-2.25 for the medium tertile; adjusted OR = 1.57, 95% CI = 0.87-2.83 for the highest tertile) (p

trend = 0.13). The risk estimates for PROM in relation to Pb levels in primiparous and multiparous women were significantly different (p for interaction < 0.01).

Discussion

Our study examined the association between maternal urinary Pb exposure before delivery and risk of PROM in Chinese pregnant women, and we found that urinary Pb concentration was significantly and positively correlated with PROM and preterm PROM incidence. Meanwhile, our study suggested that the effect of Pb on PROM may depend on maternal parity, as the correlation is more pronounced in the primiparous women than in the multiparous ones.

Preterm PROM is the leading cause of preterm birth and neonatal complications, such as perinatal infections, respiratory distress syndrome, umbilical cord compression, intraventricular hemorrhage, sepsis and even death [5 7]. Furthermore, it has been linked with long-term adverse neurodevelopmental outcomes [34]. Although our current understanding about the cause of PROM is limited, accumulating evidence has suggested that environmental factors play important roles in inducing PROM by stimulating oxidative stress and inflammation [8 9].

In this study, we observed that maternal Pb exposure prior to delivery correlated with increased risk of PROM and preterm PROM. After excluding the subjects with complications that are known to cause PROM, or those with creatinine > 3 g/L, significant associations between Pb and PROM/preterm PROM were still observed. Consistent with our findings, a study including 332 pregnant women in Iran reported

that one unit increase in the logarithm of maternal blood Pb concentration was associated with a several-fold risk of PROM [10]. Similarly, a study involving 502 pregnant mothers in Columbia found that higher blood levels of Pb was associated with an increase in the incidence of PROM [19]. Furthermore, elevated Pb concentration in the umbilical cord was reported to be associated with increased PROM risk in a cohort study of 749 mother-infant pairs in a Pb-smelter community in South Australia [20]. Additionally, a study of 89 mother-infant pairs in the southeast of Spain observed a higher placental Pb concentration in the PROM cases than in the normal deliveries [21]. However, despite these findings, an early study demonstrated no significant correlation between PROM risk and blood Pb concentration measured in 635 samples from the umbilical cord blood [22]. The reason to this discrepancy is currently unknown, but it may be, at least in part, due to the differences in Pb exposure levels.

PROM has been reported to be associated with multiple factors, including cigarette smoking, low income, parity, infection, and pregnancy-induced hypertension [4 7 30-32]. In the present study, inclusion of the potential confounding factors for adjustment did not undermine the association between increased levels of Pb exposure and PROM risk. In the stratified analysis by parity, our results suggested that parity status may influence the effect of maternal Pb exposure on the risk of PROM. A significantly positive association between PROM and urinary Pb concentrations was observed in primiparous mothers, whereas a similar positive correlation was also observed in multiparous mothers, despite the unreached statistical significance. One

possible explanation may be the unbalanced sample sizes between primiparous and multiparous mothers (6159 vs. 1131). Future studies enrolling more multiparous women will help to further evaluate this difference caused by parity status.

The etiology and mechanism of the effect of Pb on PROM are not clear. One prevailing mechanistic explanation is that Pb can induce toxicity by triggering oxidative stress through the generation of reactive oxygen species (ROS) [11 35], which is responsible for the structural weakness of collagen fibrils and causes the membranes to lose strength and elasticity, and consequently damage the collagen in fetal membrane [10 36 37]. Furthermore, Pb is shown to induce inflammatory responses via upregulating the expression of pro-inflammatory cytokines, such as TNF-α [38], thus predispose the membrane to rupture by promoting alterations of membrane fluidity and impairment in membrane barrier function [39].

Urinary Pb is favoured for long-term biomonitoring and is widely used in the assessment of Pb exposure level [40 41]. In the present study, maternal urinary Pb concentration [arithmetic mean (AM) = 7.40 μg/g creatinine, geometric mean (GM) = 3.69 μg/g creatinine, median = 3.44 μg/g creatinine] was higher than pregnant mothers reported in several developed countries, such as Australia (AM = 0.87 μg/g creatinine, median = 0.7 μg/g creatinine) [42], Japan (GM = 0.48 μg/g creatinine) [43], and the United States (GM = 0.63 μg/L) [44]. Yet, the urinary Pb concentration in our study also overlapped with other countries, including Spain (AM = 5.2 μg/g creatinine, median = 3.9 μg/g creatinine) [45]. In addition, Pb concentration in the present subjects was generally lower in comparison with pregnant women reported in

developing countries, such as Nigeria (AM = 28.5 μg/g creatinine) [46]. Although Pb-containing petrol has been phased out since 2000 in China, Pb pollution remains a huge environmental challenge, as large amounts of Pb pollutants from various sources of Pb consumption have been increasing rapidly due to the unprecedented economic development [47 48]. In the past decades, elevated accumulations of Pb have been widely spreaded in soil and dust in many Chinese provinces [23 48], raising a major public health concern in China, since Pb can enter human body via intaking Pb-containing food, water, and even air [12 48]. As a consequence, excessive Pb emissions pose serious adverse health effects on humans, especially on the susceptible pregnant women and their fetuses.

The strength of this study is as follows: first, it was conducted with a large sample size, which included 7290 mother-singleton pairs from a birth cohort study in China. We preformed sensitivity analyses and stratified analyses to evaluate the relationship between maternal Pb exposure and PROM, where a significant correlation was observed. Moreover, all information about the participants including demographic characteristics, socioeconomic status and pregnancy outcomes were gathered from personal interviews and medical records, which made it possible to adjust for other potential risk factors for PROM.

Admittedly, there were other potential confounders that we were not able to control. Unfortunately, several important risk factors for PROM, such as drug use, cervical insufficiency, premature contractions, were not collected in the present study but will be included in the future studies. The small numbers of preterm PROM and

multiparous women also limited the power of our study. In addition, urinary Pb collected and measured at labour only reflects plasma Pb level at labour, which may not accurately reflect the dynamic maternal Pb exposure throughout the whole pregnancy and limit the strength to determine the causal effect between maternal urinary Pb level and PROM. Therefore, further studies with urine samples collected at multiple time points and from different populations are needed to confirm the observed relationship between Pb and PROM.

Conclusion

In this study, we observed a positive relationship between maternal urinary Pb and the risk of PROM, supporting that maternal exposure to Pb may be a potential risk factor for PROM. Additionally, the significant association was only present in primiparous women and not in multiparous women. This finding suggested that appropriate public health measures need to be implemented to control maternal Pb exposure during pregnancy.

Footnotes

SH and WX contributed equally to this work.

Contributors: SH carried out the statistical analyses and drafted the manuscript. WX, XS assisted in the statistical analyses, critically reviewed and revised the manuscript. LQ, BZ, TC, SX contributed to the study design and developed the initial protocol. YL contributed to the study design, critically reviewed and revised the

manuscript. All authors read and approved the final manuscript.

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Table 1 Basic characteristics and urinary Pb concentrations (μg/g creatinine) of the 7290 pregnant women.

Characteristics	N (%)	Median (IQR) Pb (μg/g creatinine)	*P* value
Total	7290	3.44 (2.30-5.64)	
Maternal age (years)			0.43
< 25	805 (11.0)	3.37 (2.27-5.52)	
25–29	3985 (54.7)	3.49 (2.32-5.77)	
30–34	2011 (27.6)	3.40 (2.29-5.47)	
≥ 35	489 (6.7)	3.41 (2.15-5.94)	
Education background (years)			0.63
≤ 9	1001 (13.7)	3.45 (2.40-5.63)	
9–12	1389 (19.1)	3.47 (2.35-5.56)	
> 12	4898 (67.2)	3.42 (2.26-5.66)	
Missing	2 (0.03)	5.69(3.50-7.90)	
Family income(yuan/year)			0.37
< 50,000	3021 (41.4)	3.52 (2.31-5.75)	
≥ 50,000	4148 (56.9)	3.39 (2.28-5.60)	
Missing	121 (1.7)	3.50 (2.38-5.83)	
Parity			0.43
primiparous	6159 (84.5)	3.45 (2.30-5.66)	
multiparous	1131 (15.5)	3.38 (2.26-5.59)	
Pre-pregnancy BMI (kg/m^2)			0.10
< 18.5	1527 (20.9)	3.47 (2.27-5.77)	
18.5–23.9	4832 (66.3)	3.41 (2.29-5.56)	
≥ 24	910 (12.5)	3.60 (2.39-6.04)	
Missing	21 (0.3)	3.87 (2.07-8.12)	
Passive smoking during pregnancy			0.07

Yes	1670 (22.9)	3.30 (2.29-5.32)	
No	5620 (77.1)	3.47 (2.30-5.71)	
Pregnancy-induced hypertension			0.08
Yes	286 (3.9)	3.26 (2.18-5.21)	
No	7004 (96.1)	3.44 (2.30-5.66)	
Gestational age (weeks)			< 0.01
< 37	291 (4.0)	3.99 (2.62-7.54)	
≥ 37	6999 (96.0)	3.42 (2.29-5.60)	
Infant gender			0.02
Male	3890 (53.4)	3.48 (2.34-5.78)	
Female	3400 (46.6)	3.40 (2.25-5.52)	
PROM	881 (12.1)	3.88 (2.55-6.59)	0.47
Preterm PROM	147 (2.0)	3.96 (2.63-7.67)	
Term PROM	734 (10.1)	3.87 (2.54, 6.34)	

Abbreviation: BMI, body mass index; IQR, interquartile range; PROM, Premature Rupture of Membranes.

Table 2 Risk of PROM and preterm PROM associated with the levels of Pb in maternal urine.

Pb (μg/g creatinine)	case	OR[a] (95% CI)	OR[b] (95% CI)
PROM			
Tertile1 (< 2.65)	241	1.00	1.00
Tertile2 (2.65–4.70)	291	1.24 (1.03, 1.48)	1.23 (1.02, 1.47)
Tertile3 (≥ 4.70)	349	1.52 (1.27, 1.81)	1.51 (1.27, 1.80)
p for trend		< 0.01	< 0.01
Preterm PROM			
Tertile1 (< 2.65)	37	1.00	1.00
Tertile2 (2.65–4.70)	46	1.25 (0.81, 1.93)	1.24 (0.80, 1.92)
Tertile3 (≥ 4.70)	64	1.74 (1.16, 2.62)	1.73 (1.15, 2.60)
p for trend		< 0.01	< 0.01

Abbreviations: OR, odds ratio; CI, confidence interval.

[a] Unadjusted odds ratio.

[b] Adjusted for maternal age, family income, pre-BMI, parity, passive smoking and pregnancy-induced hypertension.

Table 3 Risk of PROM associated with the levels of Pb in maternal urine, stratified by parity.

Pb levels[a]	Primiparous (n = 6159)			Multiparous (n = 1131)			*p* for interaction
(μg/g creatinine)	N	OR[b] (95% CI)	OR[c] (95% CI)	N	OR[b] (95% CI)	OR[c] (95% CI)	
T1	220	1.00	1.00	20	1.00	1.00	< 0.01
T2	268	1.25 (1.04, 1.51)	1.24 (1.03, 1.50)	24	1.21 (0.66, 2.23)	1.21 (0.65, 2.25)	
T3	318	1.52 (1.27, 1.83)	1.52 (1.27, 1.83)	31	1.59 (0.89, 2.84)	1.57 (0.87, 2.83)	
P for trend		< 0.01	< 0.01		0.11	0.13	

Abbreviations: OR, odds ratio; CI, confidence interval; T: tertile;

[a] Pb levels: primiparous, T1 (< 2.66), T2 (2.66-4.71), T3 (≥ 4.71); multiparous, T1 (< 2.61), T2 (2.61-4.61), T3 (≥ 4.61).

[b] Unadjusted odds ratio.

[c] Adjusted for maternal age, family income, pre-BMI, passive smoking and pregnancy-induced hypertension.

Supplementary Materials

Maternal Lead Exposure and Premature Rupture of Membranes: A Birth Cohort Study in China

Sha Huang[1], Wei Xia[1], Xia Sheng[1], Lin Qiu[2], Bin Zhang[2], Tian Chen[1], Shunqing Xu[1], and Yuanyuan Li [1]

Table of Contents

Table S1 Risk of PROM and preterm PROM associated with the levels of Pb in maternal urine, after excluding intrauterine infection, vaginitis, cervicitis, pelvic inflammatory disease, vaginal bleeding, polyhydramnios, and fetal malposition (n = 6553).

Pb (μg/gcreatinine)	case	OR[a] (95% CI)	OR[b] (95% CI)
PROM			
Tertile1 (< 2.65)	208	1.00	1.00
Tertile2 (2.65–4.71)	245	1.20 (0.99, 1.46)	1.20 (0.98, 1.46)
Tertile3 (≥ 4.71)	308	1.56 (1.29, 1.87)	1.55 (1.28, 1.87)
p for trend		< 0.01	< 0.01
preterm PROM			
Tertile1 (< 2.65)	33	1.00	1.00
Tertile2 (2.65–4.71)	38	1.15 (0.72, 1.85)	1.15 (0.72, 1.84)
Tertile3 (≥ 4.71)	53	1.62 (1.04, 2.51)	1.61 (1.04, 2.51)
p for trend		0.02	0.01

Abbreviations: OR, odds ratio; CI, confidence interval.

[a] Unadjusted odds ratio.

[b] Adjusted for maternal age, family income, pre-BMI, parity, passive smoking and pregnancy-induced hypertension.

Table S2 Risk of PROM and preterm PROM associated with the levels of Pb in maternal urine, after excluding creatinine > 3 g/L (n = 7281).

Pb (μg/gcreatinine)	case	OR[a] (95% CI)	OR[b] (95% CI)
PROM			
Tertile1 (< 2.66)	241	1.00	1.00
Tertile2 (2.66–4.70)	290	1.23 (1.03, 1.47)	1.22 (1.02, 1.46)
Tertile3 (≥ 4.70)	349	1.52 (1.27, 1.81)	1.51 (1.26, 1.80)
p for trend		< 0.01	< 0.01
preterm PROM			
Tertile1 (< 2.66)	37	1.00	1.00
Tertile2 (2.66–4.70)	46	1.25 (0.81, 1.93)	1.24 (0.80, 1.92)
Tertile3 (≥ 4.70)	64	1.74 (1.16, 2.62)	1.73 (1.15, 2.60)
p for trend		< 0.01	0.01

Abbreviations: OR, odds ratio; CI, confidence interval.

[a] Unadjusted odds ratio.

[b] Adjusted for maternal age, family income, pre-BMI, parity, passive smoking and pregnancy-induced hypertension.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-8
Bias	9	Describe any efforts to address potential sources of bias	5-6
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-9
		(*b*) Describe any methods used to examine subgroups and interactions	8-9
		(*c*) Explain how missing data were addressed	No missing data
		(*d*) If applicable, explain how loss to follow-up was addressed	Not applicable
		(*e*) Describe any sensitivity analyses	9
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	Not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10
		(b) Indicate number of participants with missing data for each variable of interest	No missing data
		(c) Summarise follow-up time (eg, average and total amount)	Not applicable
Outcome data	15*	Report numbers of outcome events or summary measures over time	10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11-12
		(*b*) Report category boundaries when continuous variables were categorized	10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11-12
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.