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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 in Wuhan, China, during 2012–2014.

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 analysed by logistic regression.

Results The IQR 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 OR 1.23, 95% CI 1.0 to 1.47 for the medium tertile; adjusted OR 1.51, 95% CI 1.27 to 1.80 for the highest tertile). The risk of preterm PROM associated with Pb levels was significantly higher when compared with the lowest tertile (adjusted OR 1.24, 95% CI 0.80 to 1.92 for the medium tertile; adjusted OR 1.73, 95% CI 1.15 to 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.

INTRODUCTION

Premature rupture of membranes (PROM) refers to maternal membranes rupture more than 1 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 and adverse neurological outcomes.[4–6] When the

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 premature rupture of membranes.
► Although many potential confounders were taken into account for analysis, other confounding factors may remain.

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 aetiology 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 pre-eclampsia, 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=11 311) were enrolled between September 2012 and 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 have been described previously.[25] For this study, we excluded women without urine samples (n=3947) and 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 enrolment.

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 (eg, maternal age, educational level and occupational status), prepregnancy body mass index (BMI) (calculated on the basis of self-reported weight and height before pregnancy) and daily-life habits during pregnancy (eg, alcohol and tobacco consumption) were collected during this process. Medical/reproductive histories and outcomes (eg, intrauterine infection, maternal diseases and infant sex) were gathered from medical records. Last menstrual period 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 visualisation of amniotic fluid passing from the cervical canal and pooling in the vagina, plus the nitrazine test of a pH≥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. A rupture that 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°C) 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 occipitotransverse or occipitoposterior position.

Urine sample collection and Pb exposure measurement
Maternal urine samples were collected on 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 analysed 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 intraday coefficient of variation was below 2.0%, and the interday coefficient of variation was under 3%.

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 ORs and 95% CIs. Maternal urinary Pb levels were categorised into tertiles, with lowest one used as the reference. We detected the linear trends of Pb with PROM by modelling 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, prepregnancy 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 National Health and Nutrition Examination Survey (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 analysed 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 using SAS V.9.4 (SAS Institute), 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 per year) (56.9%) and normal prepregnancy BMI (18.5–23.9 kg/m²) (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 to 1.47 for the medium tertile; adjusted OR 1.51, 95% CI 1.27 to 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 with the lowest tertile (adjusted OR 1.24, 95% CI 0.80 to 1.92 for the medium tertile; adjusted OR 1.73, 95% CI 1.15 to 2.60 for the highest tertile) (p trend<0.01). In addition, the sensitivity analysis (see online 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 (see online supplementary material, table S2).

Results stratified by maternal parity are summarised 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 to 1.50 for the medium tertile; adjusted OR 1.52, 95% CI 1.27 to 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 to 2.25 for the medium tertile; adjusted OR 1.57, 95% CI 0.87 to 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.

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 values
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(¥/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)	
Prepregnancy 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)	

BMI, body mass index; Pb, lead; PROM, premature rupture of membranes.

Preterm PROM is the leading cause of preterm birth and neonatal complications, such as perinatal infections, respiratory distress syndrome, umbilical cord compression, intraventricular haemorrhage, 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]

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

Pb (µg/g creatinine)	Case	OR* (95% CI)	OR† (95% CI)
PROM			
Tertile 1 (<2.65)	241	1.00	1.00
Tertile 2 (2.65–4.70)	291	1.24 (1.03 to 1.48)	1.23 (1.02 to 1.47)
Tertile 3 (≥4.70)	349	1.52 (1.27 to 1.81)	1.51 (1.27 to 1.80)
P for trend		<0.01	<0.01
Preterm PROM			
Tertile 1 (<2.65)	37	1.00	1.00
Tertile 2 (2.65–4.70)	46	1.25 (0.81 to 1.93)	1.24 (0.80 to 1.92)
Tertile 3 (≥4.70)	64	1.74 (1.16 to 2.62)	1.73 (1.15 to 2.60)
P for trend		<0.01	<0.01

*Unadjusted OR.
†Adjusted for maternal age, family income, pre-BMI, parity, passive smoking and pregnancy-induced hypertension.
Pb, lead; PROM, premature rupture of membranes.

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 increase in the 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, although this correlation was not statistically significant. 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 aetiology 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

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

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

*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).
†Unadjusted OR.
‡Adjusted for maternal age, family income, pre-BMI, passive smoking and pregnancy-induced hypertension.
BMI, body mass index; Pb, lead; PROM, premature rupture of membranes; T, tertile.

species[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 proinflammatory cytokines, such as tumor necrosis factor (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 USA (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 low-income and middle-income 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 spread 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 intake of 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 performed 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 and 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.

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