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Prenatal Exposure to Air Pollution and Pre-Labor Rupture of Membranes in a Prospective Cohort Study: The Role of Maternal Hemoglobin and Iron Supplementation

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Background: Exposure to air pollution in prenatal period is associated with prelabor rupture of membranes (PROM). However, the sensitive exposure time windows and the possible biological mechanisms underlying this association remain unclear.

Objective: We aimed to identify the sensitive time windows of exposure to air pollution for PROM risk. Further, we examined whether maternal hemoglobin levels mediate the association between exposure to air pollution and PROM, as well as investigated the potential effect of iron supplementation on this association.

Method: From 2015 to 2021, 6,824 mother–newborn pairs were enrolled in the study from three hospitals in Hefei, China. We obtained air pollutant data [particulate matter (PM) with aerodynamic diameter ≤2.5 μm ($PM_{2.5}$), PM with aerodynamic diameter ≤10 μm (PM_{10}), sulfur dioxide (SO_2), and carbon monoxide (CO)] from the Hefei City Ecology and Environment Bureau. Information on maternal hemoglobin levels, gestational anemia, iron supplementation, and PROM was obtained from medical records. Logistic regression models with distributed lags were used to identify the sensitive time window for the effect of prenatal exposure to air pollutant on PROM. Mediation analysis estimated the mediated effect of maternal hemoglobin in the third trimester, linking prenatal air pollution with PROM. Stratified analysis was used to investigate the potential effect of iron supplementation on PROM risk.

Results: We found significant association between prenatal exposure to air pollution and increased PROM risk after adjusting for confounders, and the critical exposure windows of $PM_{2.5}$, PM_{10}, SO_2 and CO were the 21th to 24th weeks of pregnancy. Every 10-$\mu g/m^3$ increase in $PM_{2.5}$ and PM_{10}, 5-$\mu g/m^3$ increase in SO_2, and 0.1-mg/m^3 increase in CO was associated with low maternal hemoglobin levels [−0.94 g/L (95% confidence interval (CI): −1.15, −0.73), −1.31 g/L (95% CI: −1.55, −1.07), −2.96 g/L (95% CI: −3.32, −2.61), and −1.11 g/L (95% CI: −1.31, −0.92), respectively] in the third trimester. The proportion of the association between air pollution and PROM risk mediated by hemoglobin levels was 20.61% [average mediation effect (95% CI): 0.02 (0.01, 0.05); average direct effect (95%): 0.08 (0.02, 0.14)]. The PROM risk associated with exposure to low-medium air pollution could be attenuated by maternal iron supplementation in women with gestational anemia.

Conclusions: Prenatal exposure to air pollution, especially in the 21st to 24th weeks of pregnancy, is associated with PROM risk, which is partly mediated by maternal hemoglobin levels. Iron supplementation in anemia pregnancies may have protective effects against PROM risk associated with exposure to low–medium air pollution. https://doi.org/10.1289/EHP11134

Introduction

Air pollution is associated with health problems, including pregnancy-associated complications and increased adverse pregnancy outcomes.[1–3] Prelabor rupture of membranes (PROM) affects 3% to 21% of all pregnant women globally[4,5]; it is a serious problem for maternal and infant health and may result in maternal mortality and premature birth.[6–8] Thus, it is essential to identify factors that lead to PROM and their potential pathways.

Few studies have suggested a link between exposure to air pollution and PROM. A study conducted in Australia found that exposure to $PM_{2.5}$ during the second trimester of pregnancy increased the risk of PROM by 3%.[9] The prevalence of PROM has shown a small but significant decline in Australia; however, it continues to increase in China.[9,10] A cohort study conducted in China between 2015 and 2017 indicated that daily $PM_{2.5}$ levels (median concentrations: 61.8 $\mu g/m^3$) were more than 2-fold the limit recommended by the World Health Organization (WHO) (10.00 $\mu g/m^3$ annually)[11,12]; this could partly explain the rise in PROM in China.[11] Because no consistent evidence is available, the association between chronic (during the entire period of pregnancy) exposure to air pollution and an increased risk of PROM needs to be investigated.

Recent studies have focused on the association of PROM with maternal factors, such as low maternal hemoglobin levels and anemia.[13,14] Low maternal hemoglobin concentration may induce infections and predispose women to PROM.[15] Several studies have indicated that maternal anemia may be responsible for the increased risk of PROM.[13,15,16] In addition, few studies have suggested that fine particles might increase the risk of anemia and cause a decrease in hemoglobin levels in children and elder.[17,18] However, evidence of the relationship between exposure to air pollution and hemoglobin levels and anemia in pregnant women is limited.

Therefore, this prospective cohort study aimed to investigate the associations of weekly exposure to air pollution during pregnancy with PROM risk, identify the windows of susceptibility, and calculate the cumulative effect of the window. Furthermore, we examined whether maternal hemoglobin levels have a mediator

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effect on these associations, as well as the potential effect of iron supplementation.

Research Design and Method

Study Participants

Our study was based on a maternal and infant health cohort study in Hefei (MIH-Hefei), China. Details of data collection and recruitment have been described previously.[19,20] From March 2015 to September 2021, a total of 9,320 pregnant women were recruited for the study, provided they were between 13 and 23 gestational weeks, had a single pregnancy, lived in Hefei for at least 2 y, and gave birth at obstetrical examination hospitals.

Subjects who met the following criteria were excluded: participants pregnant using assisted reproductive techniques, participants experiencing serious pregnancy complications (including hyperemesis gravidarum and abnormal heart or liver function), pregnant mothers with uncertain address of residence, and pregnant women whose questionnaires showed missing data on birth outcome. Finally, 6,824 pregnant women were enrolled for the study at three hospitals in Hefei (Hefei First People's Hospital, Anhui Maternal and Child Care Hospital, and the First Affiliated Hospital of Anhui Medical University). The annual outpatient volume of obstetrics in the three hospitals included in our study ranks in the top three in Hefei and accounts for ∼70% of all hospitals in Hefei.[21] Protocols used in our study were approved by the Ethics Committee of Anhui Medical University (number: 2015002).

Exposure to Air Pollution

The ambient air pollution data for $PM_{2.5}$, PM_{10}, SO_2, and CO levels in urban Hefei, estimated at 11 air monitoring points in five air monitoring stations (Shushanqu, Baohequ, Luyangqu, Yaohaiqu, and Xinzhanqu), are available on the Hefei Environment Projection Administration website (http://sthjj.hefei.gov.cn/index.html). We then converted the current address of the participants to map coordinates using Baidu Maps. The distance from the subjects' home addresses to the matching monitor stations was calculated based on the coordinates, and 93.1% (mean: 3.0 km, range: 0.9 to 8.2 km) of participants lived within 5 km of the nearest station. We estimated full gestational and trimester exposure to air pollution by calculating the daily averages and averaging the daily exposures throughout the total gestational and trimester-specific periods. Trimester periods were estimated based on the first day of the mother's previous menstrual cycle and were verified by ultrasound examination of gestational age. The first, second, and third trimesters refer to gestational weeks (GW) 1–13, 14–26, and 27–40 or at birth, whichever was earlier.[22]

Assessment of PROM and Anemia during Pregnancy

Women with PROM (including preterm and term PROM) were identified from clinical and extract this information from the medical records from the three hospitals mentioned above; in our study, PROM was named code O42 and could be found in the *International Classification of Diseases and Related Health Problems, 10th Revision* (ICD-10). Preterm and term PROM is defined as rupture of fetal membranes prior to and at 37 wk or after 37 wk of gestation, respectively.

Maternal hemoglobin levels were measured during GW 24–28 and 32–36 in hospitals. Hemoglobin levels measured during 24–28 wk were used for the diagnosis of anemia according to the WHO guidelines.[23] Women with hemoglobin levels lower than 110 g/L were diagnosed as having anemia during pregnancy according to WHO criteria. Daily iron supplements (120 mg/d of elemental iron) were recommended to pregnant women with anemia.[24] Hemoglobin levels measured between 32–36 wk were used for subsequent analysis.

Confounding Variables

Demographic characteristic covariates were obtained through a face-to-face questionnaire used in a standardized interview of pregnant women at enrollment, including maternal age (<25, 25–34, ≥35 y), education (junior high school, high school, or bachelor's degree and above), average family income [≤3,999, 4,000–7,999, ≥8,000 renminbi (RMB)/month], and parity (primipara, multipara). Self-reported information on lifestyle factors included the frequency of fruit and dessert intake (cake, ice cream, store-bought sweet rolls, etc.), vegetable intake during second trimester, maternal folic acid supplementation frequency (<3, ≥3 d/wk) during first trimester, maternal passive smoking status (ever or never) during second trimester, and maternal iron supplementation frequency (<3, ≥3 d/wk) during third trimester. The International Physical Activity Questionnaire[25] was used to evaluate moderate physical activity (including table tennis, badminton, and vigorous walking) for at least 30 min per day. Pregnant women were divided into groups of <3 or ≥3 d/wk, based on iron supplementation frequency. Health-related characteristics were extracted from medical records during the second and third trimester, including prepregnancy body mass index (BMI) (<18.5 kg/m^2, 18.5–23.9 kg/m^2, or ≥24 kg/m^2), gestational diabetes mellitus (GDM) (yes or no); hypertension during pregnancy, including chronic hypertension (hypertension that preceded pregnancy), and preeclampsia (hypertension along with thrombocytopenia, systemics impairment such as liver function damage, progressing renal insufficiency, etc.), gestational hypertension (hypertension happening after 20 GW without the systemic findings aforementioned or proteinuria),[26] and vaginitis (yes or no). After delivery, further details such as the season of delivery (spring/summer/autumn/winter), gestational weeks of delivery, and preterm status (<37 GW) were collected from the medical records. Information on the daily temperature (°C) in the month before delivery was obtained from the China Meteorological Administration (https://data.cma.cn/).

Statistical Analysis

The demographic characteristics of the participants with and without PROM were summarized using descriptive statistics. Chi-square (χ^2) tests were used to compare the characteristics of women with and without PROM and characteristics of women included and excluded in the analysis. Spearman's correlation analysis was used to examine the associations between $PM_{2.5}$, PM_{10}, SO_2, and CO levels. Confounders related to prenatal air pollution exposure, maternal hemoglobin levels, and PROM were determined using a directed acyclic graph (DAG) to visualize these relationships (Supplemental Figure 1).

Distributed lag nonlinear models were used to examine the sensitive windows of weekly $PM_{2.5}$, PM_{10}, SO_2, and CO exposure throughout GW 1–37 related to PROM in unadjusted and adjusted models.[27,28] Maternal age, education, income, parity, activity, passive smoking, folic acid supplementation, iron supplementation, prepregnancy BMI, hypertension during pregnancy, gestational diabetes mellitus, vaginitis, and temperature were incorporated in the models. We built cross-basis matrices to model the exposure–lag–response association. The exposure–response function was assumed to be linear, and the lag structure was modeled using a natural cubic spline with degrees of freedom (df) based on the Akaike information criterion. We used the distributed lag model (DLM) to calculate the cumulative estimates for the first trimester (GW 1–13), second trimester (GW 14–26), third trimester (after GW 27) by incorporating weekly exposure during

pregnancy. The models were adjusted for several covariates, including maternal age, education, income, parity, activity, passive smoking, folic acid supplementation, iron supplementation, prepregnancy BMI, hypertension during pregnancy, gestational diabetes mellitus, vaginitis, and temperature. We further constructed a logistic regression model to estimate the trimesters-specific exposure with PROM risk. In addition, a logistic regression model was used to estimate the associations of average exposure to air pollutant during the three trimesters with the risk of preterm PROM, term PROM, and PROM in unadjusted and adjusted models. The models were adjusted for several covariates, including maternal age, education, income, parity, activity, passive smoking, folic acid supplementation, iron supplementation, prepregnancy BMI, hypertension during pregnancy, gestational diabetes mellitus, vaginitis, and temperature. The logistic models that additionally included the trimester adjustment were done. Furthermore, we preliminarily evaluated the collinearity of four air pollution concentrations using correlation analysis among four air pollutants. Considering the impact of collinearity on the effect estimate, the copollutant models were used in the sensitivity analysis.

Multiple linear regression was used to estimate the association of each air pollutant (per 10 $\mu g/m^3$ in $PM_{2.5}$ and PM_{10}, per 5 $\mu g/m^3$ in SO_2, and per 0.1 mg/m^3 in CO; per quartile increase) throughout the second and third trimesters with maternal hemoglobin levels in the third trimester. Adjustments for covariates included age, education, income, activity, passive smoking, iron supplementation, prepregnancy BMI, hypertension during pregnancy, gestational diabetes mellitus, and temperature. The odds ratio (OR) of PROM was calculated per unit increase in air pollution exposure (per 10 $\mu g/m^3$ in $PM_{2.5}$ and PM_{10}, per 5 $\mu g/m^3$ in SO_2, and per 0.1 mg/m^3 in CO) stratified by anemia status (110 g/L) according to WHO standards[23] based on logistic regression models. We examined the potential exposure–response relationships between maternal hemoglobin levels in the third trimester and PROM risk using logistic regression models after adjusting for age, education, income, parity, activity, passive smoking, folic acid supplementation, iron supplementation, prepregnancy BMI, hypertension during pregnancy, gestational diabetes mellitus, vaginitis, and temperature. Considering the WHO cutoff points of maternal hemoglobin levels (110 g/L) for the diagnosis of anemia and the sample size, women were classified into nine groups according to 5 g/L change. Women with hemoglobin levels of ≥130 g/L (reference) and <100 g/L were defined as the highest and lowest level groups, respectively.

Principal component analysis (PCA) accounts for interactions between all pollutants and attributes these interactions to a principal component.[29–31] PCA was used as a tool capable of providing an overview of the interdependencies and variability of prenatal air pollutants. After calculating the principal component scores of four pollutants, a mediation analysis was performed using the "mediation" package in R to estimate the role of hemoglobin levels during the third trimester in association with exposure to four pollutants in the second trimester, the third trimester, and throughout the second trimester and third trimester as well as its contribution to PROM risk. The total effect, including the average mediation effect (AME) and average direct effect (ADE), was calculated.[32] The AME referred to the indirect effect of prenatal exposure to four air pollutants on PROM mediated by hemoglobin in the third trimester, and the ADE referred to the effect of prenatal exposure to four air pollutants on PROM, excluding the effect of hemoglobin during the third trimester. The counterfactual framework for mediation analysis was used to examine the causal mediation assumptions. Mediator models (mediator = air pollution + covariates), outcome models (outcome = air pollution + hemoglobin + covariates) were specified, and the "treat" (air pollution) as well as "mediator" variables were specified (Supplemental Figure 1).[33–34] Adjustments for covariates included age, education, income, parity, activity, passive smoking, folic acid supplementation, iron supplementation, prepregnancy BMI, hypertension during pregnancy, gestational diabetes mellitus, vaginitis, and temperature.

Logistic regression models were used to estimate the association between maternal iron supplementation and PROM risk stratified by air pollution in women with anemia. Low-medium air pollution was defined as air pollution concentration <50th percentile. Women with gestational anemia were classified into following four groups: *a*) low–medium air pollution (<50th percentile, P50) and iron supplementation, *b*) low–medium air pollution (<P50) and no iron supplementation, *c*) high air pollution (≥P50) and iron supplementation, and *d*) high air pollution (≥P50) and no iron supplementation. High air pollution (≥P50) and no iron supplementation group was considered as the reference group, and OR for PROM for the other three groups were calculated. The average exposure levels of air pollutants selected at P50 were similar to those seen in previous studies in China.[11] The *p*-trend for maternal hemoglobin levels and the prevalence of PROM across four groups were calculated by general linear regression model and Mantel-Haenszel chi-square test, respectively. Furthermore, we conducted a sensitivity analysis based on the exposure levels of air pollution at P75 (75th percentile).

Statistical significance was considered as a two-sided $p < 0.05$. We performed all analyses in R (version 3.5.0; R Core Development Team) using the R statistical packages "ggplot2," "dlnm," and "mediation" and the SPSS statistical software (Statistical Package for the Social Sciences version 23.0; IBM Corp.).

Results

The characteristics of the study population are summarized in Table 1. Of the 6,824 women who agreed to participate in the study, 1,439 (21.1%) had PROM. Women with PROM were more likely to be younger and to have a lower frequency of folic acid supplementation during pregnancy than those without PROM (Table 1). In comparison with women without PROM, higher proportions of primipara, GDM, gestational anemia, and delivery in winter and spring were observed in women with PROM (Table 1). The excluded women had incomplete covariates on birth outcomes and had a higher proportion of giving birth in winter and spring (Supplement Table 1).

A strong correlation between four air pollutants in three trimesters was observed (Spearman correlation ranged from 0.01 to 0.94; Supplement Table 2). The mean (SD) gestational exposure to $PM_{2.5}$, PM_{10}, SO_2, and CO in the second and third trimester was 54.2 $\mu g/m^3$ (15.1), 83.2 $\mu g/m^3$ (13.5), 11.8 $\mu g/m^3$ (4.0), and 0.9 mg/m^3 (0.2), whereas that in the first trimester was 57.7 $\mu g/m^3$ (18.6), 87.0 $\mu g/m^3$ (17.0), 17.8 $\mu g/m^3$ (4.9), and 0.9 mg/m^3 (0.2) (Table 2).

Distributed lag nonlinear models were used to examine the sensitive windows of weekly $PM_{2.5}$, PM_{10}, SO_2 and CO exposure related to PROM in the unadjusted and adjusted models (Figure 1; Supplement Table 3). The weekly air pollutant exposures were significantly associated with increased PROM risk: The window of susceptibility for $PM_{2.5}$ (per 10-$\mu g/m^3$ increase) was between GW 15 and 37, and the maximum effect was in the 21th–23th GW; for PM_{10} (per 10-$\mu g/m^3$ increase) the susceptibility window was between GW 18 and 32, and the maximum effect was in the 24th GW; for SO_2 (per 5-$\mu g/m^3$ increase) the susceptibility window was between GW 10 and 37, and the maximum effect was in the 21th GW; and for CO (per 0.1-mg/m^3 increase) the window of susceptibility was between GW 13 and 31, and the maximum effect was in the 21th–24th GW. Table 2 depicts the estimated

Table 1. The general characteristics of the study population during 2015 to 2021 in Hefei [*n* (%)].

Characteristics	All ($n = 6,824$)	PROM ($n = 1,439$)	Non-PROM ($n = 5,385$)	p-Value[a]
Sociodemographic characteristics [*n* (%)]				
Age (y)	—	—	—	<0.001
<25	1,666 (24.4)	406 (28.2)	1,260 (23.4)	—
25–34	4,476 (65.6)	912 (63.4)	3,564 (66.2)	—
≥35	682 (10.0)	121 (8.4)	561 (10.4)	—
Education	—	—	—	0.433
Junior high school	941 (13.8)	184 (12.8)	757 (14.1)	—
High school	1,655 (24.3)	348 (24.2)	1,307 (24.3)	—
Bachelor's degree and above	4,228 (62.0)	907 (63.0)	3,321 (61.7)	—
Family income (RMB/month)	—	—	—	0.231
≤3,999	2,149 (31.5)	460 (32.0)	1,689 (31.4)	—
4000–7999	4,132 (60.6)	878 (61.7)	3,254 (60.4)	—
≥8,000	543 (8.0)	101 (7.0)	442 (8.2)	—
Parity	—	—	—	<0.001
Primipara	2,611 (38.3)	642 (44.6)	1,969 (36.6)	—
Multipara	4,213 (61.7)	797 (55.4)	3,416 (63.4)	—
Season of delivery	—	—	—	0.003
Spring	1,756 (25.7)	386 (26.8)	1,370 (25.4)	—
Summer	1,800 (26.4)	331 (23.0)	1,469 (27.3)	—
Autumn	1,730 (25.4)	403 (28.0)	1,327 (24.6)	—
Winter	1,538 (22.5)	319 (22.2)	1,219 (22.6)	—
Enrollment years	—	—	—	0.080
2015–2016	2,385 (35.0)	538 (37.4)	1,846 (34.3)	—
2017–2018	2,569 (37.6)	517 (35.9)	2,052 (38.1)	—
2019–2021	1,870 (27.4)	384 (26.7)	1,486 (27.6)	—
Perinatal health lifestyle factors [*n* (%)][b]				
Vegetable intake (times/week)		—	—	0.982
<3	231 (3.4)	49 (3.4)	182 (3.4)	—
≥3	6,593 (96.6)	1,390 (96.6)	5,203 (96.6)	—
Fruit intake (times/week)		—	—	0.917
<3	470 (6.9)	100 (6.9)	370 (6.9)	—
≥3	6,354 (93.1)	1,339 (93.1)	5,015 (93.1)	—
Dessert intake (times/week)		—	—	0.991
<3	5,633 (82.5)	1,188 (82.6)	4,445 (82.5)	—
≥3	1,191 (17.5)	251 (17.4)	940 (17.5)	—
Physical activity (days/week)	—	—	—	0.281
<3	5,394 (79.0)	1,154 (80.2)	4,240 (78.7)	—
≥3	1,430 (21.0)	285 (19.8)	1,145 (21.3)	—
Folic acid supplementation (days/week)		—	—	0.047
<3	4,387 (64.2)	893 (62.1)	3,494 (64.9)	—
≥3	2,437 (35.7)	546 (37.9)	1,891 (35.1)	—
Iron supplementation (days/week)	—	—	—	0.075
<3	5,847 (85.7)	1,254 (87.1)	4,593 (85.3)	—
≥3	977 (14.3)	185 (12.9)	792 (14.7)	—
Passive smoking	—	—	—	0.113
Never	5,598 (82.0)	1,201 (83.5)	4,397 (81.7)	—
Ever	1,226 (18.0)	238 (16.5)	988 (18.3)	—
Perinatal health status [*n* (%)]				
Prepregnancy BMI (kg/m^2)	—	—	—	0.709
<18.5	985 (14.4)	216 (15.0)	769 (14.3)	—
18.5–23.9	4,816 (70.6)	1,014 (70.5)	3,802 (70.6)	—
≥24.0	1,023 (15.0)	209 (14.5)	814 (15.1)	—
Hypertension during pregnancy	139 (2.0)	37 (2.6)	102 (1.9)	1.106
Vaginitis	783 (11.5)	151 (10.5)	632 (11.7)	0.189
Gestational diabetes mellitus	1,420 (20.8)	255 (17.7)	1,165 (22.1)	0.001
Premature birth	229 (3.4)	111 (7.7)	118 (2.2)	<0.001
Maternal anemia	2,290 (33.6)	591 (41.1)	1,699 (31.6)	<0.001

Note: There were no missing values for covariates. —, no data; PROM, prelabor rupture of membranes; RMB, renminbi.
[a]Based on the chi-square test.
[b]The frequency of vegetable intake, fruit intake, dessert intake, physical activity was during second trimester. The frequency of folic acid supplementation intake was during the first trimester. The frequency of iron supplementation was during the third trimester.

trimester cumulative PROM risk in the DLMs and average models in logistic regression with covariates adjusted, as with the unadjusted model (Supplement Table 4). The estimated cumulative risk of PROM was significantly associated with prenatal exposure to air pollutants throughout the second and third trimesters (Table 2). For example, the cumulative OR for the second and third trimester was 1.13 per 10-$\mu g/m^3$ increase in $PM_{2.5}$ (95% CI: 1.06, 1.22). Similarly, significant associations were observed with per 10-$\mu g/m^3$ increase in PM_{10} [OR = 1.18 (95% CI: 1.10, 1.27)], per 5-$\mu g/m^3$increase in SO_2 [OR = 1.16 (95% CI: 1.06, 1.28)], and per 0.1-mg/m^3 increase in CO [OR = 1.10 (95% CI: 1.04, 1.15)]. DLM estimates were consistent with the results from the average exposure models. We find similar effect estimates in the unadjusted model presented in Supplement Table 4. In addition, adjustment for trimesters air exposure in Supplement Table 5 did not change the pattern of estimates from those in Table 2. The associations between prenatal exposure to air pollution and term PROM and preterm PROM are presented in Supplement Table 6.

Table 2. Cumulative and average effects between air pollutants exposure and PROM risk in distributed lag models and average exposure model.

Pollution	Mean	SD	OR (95% CI) of PROM[d]	
			Distributed lag model[e]	Average exposure model[e]
First trimester				
$PM_{2.5}$ ($\mu g/m^3$)[a]	57.7	18.6	1.01 (0.97, 1.04)	1.01 (0.98, 1.04)
PM_{10} ($\mu g/m^3$)[a]	87.0	17.0	1.02 (0.98, 1.06)	1.02 (0.98, 1.06)
SO_2 ($\mu g/m^3$)[b]	17.8	4.9	1.06 (0.98, 1.16)	1.08 (1.01, 1.14)
CO (mg/m^3)[c]	0.9	0.2	1.03 (0.99, 1.07)	1.04 (1.01, 1.08)
Second trimester				
$PM_{2.5}$ ($\mu g/m^3$)[a]	54.6	18.1	1.11 (1.04, 1.19)	1.14 (1.09, 1.19)
PM_{10} ($\mu g/m^3$)[a]	84.1	16.3	1.14 (1.07, 1.22)	1.17 (1.12, 1.22)
SO_2 ($\mu g/m^3$)[b]	12.1	4.4	1.13 (1.03, 1.24)	1.22 (1.14, 1.31)
CO (mg/m^3)[c]	0.9	0.2	1.10 (1.04, 1.16)	1.13 (1.09, 1.18)
Third trimester				
$PM_{2.5}$ ($\mu g/m^3$)[a]	54.0	19.0	1.04 (1.01, 1.08)	1.06 (1.03, 1.09)
PM_{10} ($\mu g/m^3$)[a]	82.2	16.9	1.05 (1.01, 1.09)	1.07 (1.04, 1.11)
SO_2 ($\mu g/m^3$)[b]	11.5	4.2	1.14 (1.05, 1.25)	1.16 (1.08, 1.24)
CO (mg/m^3)[c]	0.9	0.2	1.06 (1.02, 1.10)	1.08 (1.04, 1.12)
Second and third trimesters				
$PM_{2.5}$ ($\mu g/m^3$)[a]	54.2	15.1	1.13 (1.06, 1.22)	1.11 (1.06, 1.15)
PM_{10} ($\mu g/m^3$)[a]	83.2	13.5	1.18 (1.10, 1.27)	1.14 (1.09, 1.20)
SO_2 ($\mu g/m^3$)[b]	11.8	4.0	1.16 (1.06, 1.28)	1.22 (1.13, 1.31)
CO (mg/m^3)[c]	0.9	0.2	1.10 (1.04, 1.15)	1.11 (1.07, 1.16)

Note: BMI, body mass index; CI, confidence interval; CO, carbon monoxide; OR, odds ratio; PROM, prelabor rupture of membranes; SD, standard deviation.
[a]Per increase in 10 $\mu g/m^3$.
[b]Per increase in 5 $\mu g/m^3$.
[c]Per increase in 0.1 mg/m^3.
[d]Estimated by distributed lag models using weekly mean exposures and by mean air pollution during specific exposure windows (average exposure model).
[e]The models were adjusted for age, education, income, parity, activity, passive smoking, folic acid supplementation, iron supplementation, prepregnancy BMI, hypertension during pregnancy, gestational diabetes mellitus, vaginitis, and temperature.

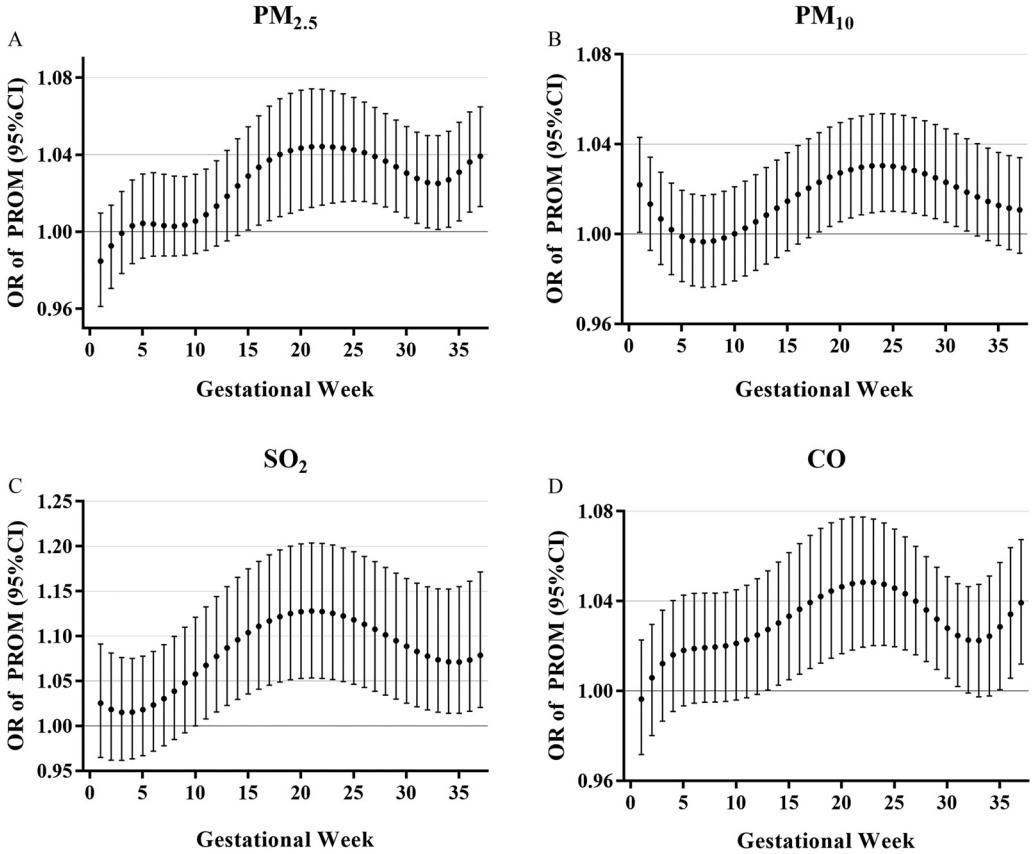

Figure 1. The PROM risk in association with week-specific prenatal air pollution exposure during pregnancy. Week-specific estimates are provided as the OR of PROM (with 95% CI) for a 10-$\mu g/m^3$ increment of $PM_{2.5}$ exposure (A). Week-specific estimates are provided as the OR of PROM (with 95% CI) for a 10-$\mu g/m^3$ increment of PM_{10} exposure (B). Week-specific estimates are provided as the OR of PROM (with 95% CI) for a 5-$\mu g/m^3$ increment of SO_2 exposure (C). Week-specific estimates are provided as the OR of PROM (with 95% CI) for a 0.1-mg/m^3 increment of CO exposure (D). Models were based on a distributed lag (nonlinear) model and adjusted for age, education, income, parity, activity, passive smoking, folic acid supplementation, iron supplementation, prepregnancy BMI, hypertension during pregnancy, gestational diabetes mellitus, vaginitis, and temperature. The numerical results are presented in Supplement Table 3. Note: BMI, body mass index; CI, confidence interval; CO, carbon monoxide; OR, odds ratio; PROM, prelabor rupture of membranes.

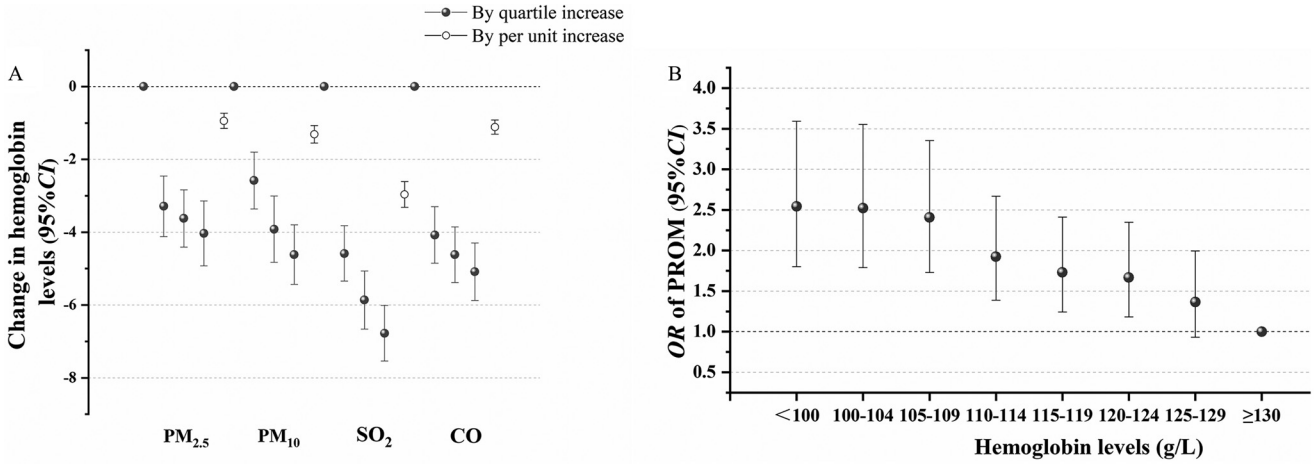

Figure 2. The association among air pollution exposure, hemoglobin levels, and PROM risk. The estimated change in hemoglobin levels was calculated for each quartile and each unit increment in $PM_{2.5}$, PM_{10}, SO_2, and CO during the second and third trimesters in linear regression model (A). The model was based on the line regression model and adjusted for age, education, income, activity, passive smoking, iron supplementation, prepregnancy BMI, hypertension during pregnancy, gestational diabetes mellitus, and temperature. The hemoglobin level per increase in $PM_{2.5}$ and PM_{10} was 10 $\mu g/m^3$, the hemoglobin level per increase in SO_2 was 5 $\mu g/m^3$, and the hemoglobin level per increase in CO was 0.1 mg/m^3. The numerical results are presented in Supplement Table 8. The relationship between hemoglobin levels and PROM (B). The model was based on the logistic regression model and adjusted for age, education, income, parity, activity, passive smoking, folic acid supplementation, iron supplementation, prepregnancy BMI, hypertension during pregnancy, gestational diabetes mellitus, vaginitis, and temperature. Air pollution was in the second and third trimesters. The numerical results are presented in Supplement Table 10. Note: BMI, body mass index; CO, carbon monoxide; PROM, prelabor rupture of membranes.

However, prenatal exposure to air pollution was not associated with preterm PROM. For sensitivity analysis, we further evaluated the correlation in the copollution models during same exposure period, and the results are presented in Supplement Table 7. Results of copollution models for estimating the effect for $PM_{2.5}$, PM_{10}, CO, and SO_2 were generally consistent with those of the single-pollutant model exposure to same period.

As presented in Figure 2A and Supplement Table 8, effect of each air pollutant (per 10 $\mu g/m^3$ in $PM_{2.5}$ and PM_{10}, per 5 $\mu g/m^3$ in SO_2, and per 0.1 mg/m^3 in CO) throughout the second trimester and third trimester was negatively associated with low maternal hemoglobin levels in third trimester [β with 95% CI for $PM_{2.5}$: −0.94 g/L (95% CI: −1.15, −0.73); β for PM_{10}: −1.31 g/L (95% CI: −1.55, −1.07); β for SO_2: −2.96 g/L (95% CI: −3.32, −2.61); and β for CO: −1.11 g/L (95% CI: −1.31, −0.92)] upon adjustment for age, education, income, activity, passive smoking, iron supplementation, prepregnancy BMI, hypertension during pregnancy, gestational diabetes mellitus, and temperature. Significant associations with per quartile increase for each air pollutant were observed in Figure 2A. The results from the copollution models showed similar negative associations with the effect estimation for $PM_{2.5}$, PM_{10}, SO_2, and CO in the single-pollutant model in Supplement Table 9. We further examined the potential exposure–response relationships between maternal hemoglobin levels during the third trimester and PROM risk (Figure 2B; Supplement Table 10). After adjusting for age, education, income, parity, activity, passive smoking folic acid supplementation, iron supplementation, prepregnancy BMI, hypertension during pregnancy, gestational diabetes mellitus, vaginitis, and temperature, higher risk of PROM was observed in women with hemoglobin below 100 g/L (OR = 2.44; 95% CI: 1.73, 3.46), women with a hemoglobin range of 100–104 g/L (OR = 2.42; 95% CI: 1.72, 3.41), women with a hemoglobin range of 105–109 g/L (OR = 2.40; 95% CI: 1.73, 3.34), women with a hemoglobin range of 110–114 g/L (OR = 1.97; 95% CI: 1.42, 2.73), women with a hemoglobin range of 115–119 g/L (OR = 1.77; 95% CI: 1.27, 2.45), women with a hemoglobin range of 120–124 g/L (OR = 1.72; 95% CI: 1.23, 2.42), and women with a hemoglobin range of 125–129 g/L (OR = 1.52; 95% CI: 1.05, 2.21) when compared with that in women in the conference group (hemoglobin higher than 130 g/L).

The OR of PROM was calculated with per unit increase in exposure to air pollution (per 10 $\mu g/m^3$ in $PM_{2.5}$ and PM_{10}, per 5 $\mu g/m^3$ in SO_2, and per 0.1 mg/m^3 in CO) stratified by anemia status (Figure 3). Our results showed a decreased risk of PROM associated with per unit increase in exposure to $PM_{2.5}$, PM_{10}, SO_2, and CO in women without anemia, in comparison with that in women with

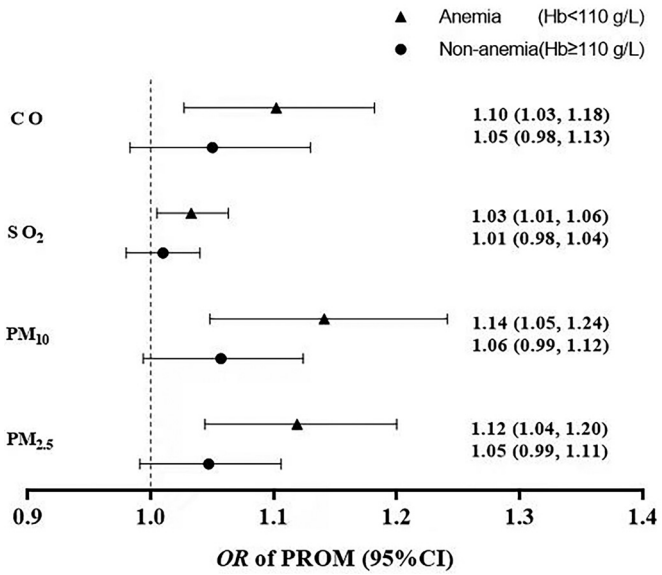

Figure 3. The relationship between air pollution and PROM risk in different hemoglobin levels. Air pollution was in the second and third trimesters. The hemoglobin level per increase in $PM_{2.5}$ and PM_{10} was 10 $\mu g/m^3$, the hemoglobin level per increase in SO_2 was 5 $\mu g/m^3$, and the hemoglobin level per increase in CO was 0.1 mg/m^3. Models adjusted for age, education, income, parity, activity, passive smoking, folic acid supplementation, iron supplementation, prepregnancy BMI, hypertension during pregnancy, gestational diabetes mellitus, vaginitis, and temperature. Note: BMI, body mass index; CO, carbon monoxide; Hb, hemoglobin; PROM, prelabor rupture of membranes.

Table 3. Mediation effect by hemoglobin levels on third trimester air pollution associated with PROM risk.

Exposure window	AME β (95% CI)	*p*-Value	ADE β (95% CI)	*p*-Value	Proportion (%)[a]
Second trimester	0.02 (0.01, 0.03)	<0.001	0.08 (0.02, 0.10)	<0.001	17.31
Third trimester	0.02 (0.01, 0.04)	<0.001	0.06 (0.01, 0.08)	0.044	24.26
Second and third trimester	0.02 (0.01, 0.05)	<0.001	0.08 (0.02, 0.14)	0.008	20.61

Note: Meditation effect by hemoglobin levels was calculated per 1 g/L. Air pollution was based on the total score of principal components of four air pollutant exposures (PM_{10}, $PM_{2.5}$, SO_2, and CO). ADE, average direct effect; AME, average mediation effect; BMI, body mass index; CI, confidence interval; CO, carbon monoxide; PROM, prelabor rupture of membranes.
[a]Proportion (%): The extent to which the association between four air pollution exposures and PROM was mediated through hemoglobin in the third trimester. The models were adjusted for age, education, income, parity, activity, passive smoking, folic acid supplementation, iron supplementation, prepregnancy BMI, hypertension during pregnancy, gestational diabetes mellitus, vaginitis, and temperature.

anemia. For example, for $PM_{2.5}$, PROM risk in women without anemia [OR = 1.05 (95% CI: 0.99, 1.11)] was lower than that in women with anemia [OR = 1.12 (95% CI: 1.04, 1.20)].

In PCA, the calculated eigenvalue of the first principal component (PC1) was 3.615 (>1), providing 90.38% composite information. PC1 is mainly driven by CO, PM_{10}, $PM_{2.5}$, and SO_2 (Supplement Table 11). Maternal hemoglobin levels in the third trimester mediated 20.61% (AME = 0.02; 95% CI: 0.01, 0.05) of the contribution to the association of principal component of exposure to air pollutants throughout the second and third trimesters with PROM after adjusting for age, education, income, parity, activity, passive smoking, folic acid supplementation, iron supplementation, prepregnancy BMI, hypertension during pregnancy, gestational diabetes mellitus, vaginitis, and temperature (Table 3).

The 50th and 75th percentiles for the exposures during second and third trimester was 52.8 $\mu g/m^3$ and 66.5 $\mu g/m^3$ for $PM_{2.5}$, 82.4 $\mu g/m^3$ and 92.3 $\mu g/m^3$ for PM_{10}, 11.9 $\mu g/m^3$ and 14.9 $\mu g/m^3$ for SO_2, 0.90 mg/m^3 and 1.03 mg/m^3 for CO, respectively. (Table 4; Supplement Table 12). Data presented in Table 4 show that the risk of PROM was significantly attenuated by iron supplementation during pregnancy in women with gestational anemia under low–medium exposure to air pollution in adjusted and unadjusted models. Iron supplementation in women with low–medium exposure to air pollution resulted in a significantly lower incidence of PROM than that seen in women without iron supplementation. For $PM_{2.5}$, our analysis indicated a pattern toward lower incidence of PROM in women who were taking iron supplements in comparison with that in women not taking iron supplements under low–medium air pollution (16.0% vs. 18.5%) and high air pollution (21.4% vs. 23.4%). The decreasing trend toward PROM incidence in the above-mentioned groups persisted with exposure to PM_{10} concentration of 10 $\mu g/m^3$, SO_2 concentration of 5 $\mu g/m^3$, and CO concentration of 0.1 mg/m^3. Results from the sensitivity analysis using the cutoff point of exposure to air pollution at P75 remained robust, as shown in Supplement Table 12. [For example, incidence of PROM under low–medium $PM_{2.5}$ pollution was 17.9% and 19.5% for women with iron supplementation, without iron supplementation; high pollution (21.7% vs. 23.6%)] (Supplement Table 12). We did not observe discrepancy in the results of unadjusted model using cutoff point of P50 in Table 4. [For example, OR = 0.65 (95% CI: 0.51, 0.83) for women with iron supplementation under low–medium $PM_{2.5}$ pollution; OR = 0.76 (95% CI: 0.56, 1.03) for women without iron supplementation under low–medium $PM_{2.5}$ pollution; OR = 0.90 (95% CI: 0.56, 1.43) for women with iron supplementation under high $PM_{2.5}$ pollution] or P75 in Supplement Table 12 [OR with 95% CI: 0.70 (0.51, 0.98) for women with iron supplementation under low- to medium $PM_{2.5}$ pollution; OR = 0.78 (95% CI: 0.58, 1.06) for women without iron supplementation under low–medium $PM_{2.5}$ pollution; OR = 0.90 (95% CI: 0.55, 1.47) for women with iron supplementation under high $PM_{2.5}$ pollution].

Table 4. The association between iron supplementation and PROM risk stratified by air pollution levels in women diagnosed with anemia.

				Hemoglobin[d]	PROM[d]		
Pollutions[a]	Exposure level	Iron supplementation (days/week)	*n*	Mean ± SD, g/L	*n* (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)[e]
$PM_{2.5}$	<P50[f]	≥3	531	115.3 ± 10.3[b]	85 (16.0)[c]	0.65 (0.51, 0.83)	0.62 (0.49, 0.79)
		<3	817	113.2 ± 11.1	151 (18.5)	0.76 (0.56, 1.03)	0.74 (0.53, 1.01)
	≥P50	≥3	309	112.1 ± 10.3	66 (21.4)	0.90 (0.56, 1.43)	0.87 (0.54, 1.40)
		<3	633	110.2 ± 10.1	148 (23.4)	1.00[f]	1.00[f]
PM_{10}	<P50	≥3	555	115.3 ± 10.3[b]	90 (16.2)[c]	0.69 (0.52, 0.92)	0.68 (0.50, 0.93)
		<3	821	112.7 ± 10.9	156 (19.0)	0.79 (0.59, 1.06)	0.75 (0.56, 1.01)
	≥P50	≥3	285	111.6 ± 10.1	60 (21.1)	0.92 (0.65, 1.30)	0.90 (0.62, 1.27)
		<3	629	110.5 ± 10.5	144 (22.9)	1.00[f]	1.00[f]
SO_2	<P50	≥3	587	115.9 ± 9.7[b]	95 (16.2)[c]	0.68 (0.51, 0.91)	0.66 (0.50, 0.87)
		<3	790	113.8 ± 10.7	149 (18.9)	0.75 (0.53, 1.05)	0.72 (0.51, 1.02)
	≥P50	≥3	253	109.6 ± 10.7	50 (19.8)	0.80 (0.56, 1.14)	0.77 (0.54, 1.12)
		<3	660	109.1 ± 10.3	156 (23.6)	1.00[f]	1.00[f]
CO	<P50	≥3	596	115.1 ± 10.3[b]	103 (17.3)[c]	0.72 (0.54, 0.95)	0.74 (0.55, 0.99)
		<3	843	112.7 ± 10.7	158 (18.7)	0.79 (0.61, 1.02)	0.81 (0.62, 1.05)
	≥P50	≥3	244	111.5 ± 10.3	52 (21.4)	0.93 (0.65, 1.34)	0.93 (0.65, 1.34)
		<3	607	110.4 ± 10.8	137 (22.6)	1.00[f]	1.00[f]

Note: CI, confidence interval; OR, odds ratio; PROM, prelabor rupture of membranes; SD, standard deviation.
[a]Air pollution was in the second and third trimesters.
[b]The *p* for trend of hemoglobin levels across four groups was <0.001, <0.001, <0.001, <0.001, respectively.
[c]The *p* for trend of PROM prevalence across four groups was <0.001, 0.006, 0.012, 0.004. respectively.
[d]The test for *p*-trend was performed using general linear regression model and Mantel-Haenszel chi-square test in hemoglobin levels and PROM prevalence across the above four groups.
[e]Models adjusted for age, education, income, parity, activity, passive smoking, folic acid supplementation, iron supplementation, prepregnancy BMI, hypertension during pregnancy, gestational diabetes mellitus, vaginitis, and temperature. The 50th percentile for the exposure during second and third trimester was 52.8 $\mu g/m^3$ for $PM_{2.5}$, 82.4 $\mu g/m^3$ for PM_{10}, 11.9 $\mu g/m^3$ for SO_2, and 0.90 mg/m^3 for CO, respectively.
[f]Reference group.

Discussion

A positive association between exposure to air pollution and a statistically significance showed in this multicenter, prospective cohort study. Our mediation analysis suggested that maternal hemoglobin levels in the third trimester could partly mediate the association between prenatal exposure to air pollution and PROM. In addition, the potential association of iron supplementation on PROM risk was observed in women with gestational anemia under low–medium levels of air pollution. Therefore, iron supplementation during pregnancy could possibly affect PROM associated with prenatal exposure to air pollution in women with anemia.

The relationship between elevated air pollution and PROM has been examined but with inconsistent results.[35–37] Contrasting results presented in these studies may be attributed to apparent differences in levels of air pollution, inconsistent domestic conditions, and differences in population characteristics. A prospective cohort study conducted in Wuhan, China, indicated that $PM_{2.5}$ levels during pregnancy increased the risk of PROM, and the estimated $PM_{2.5}$ exposure concentration was 61.8 $\mu g/m^3$ during the entire pregnancy, which is similar to the finding in our current study.[11] In recent years, some researchers have proposed that considering longer exposure periods does not take into account the potential windows of exposure that may span different periods of pregnancy, resulting in deviations in inferred susceptible windows of exposure. Therefore, it is necessary to further refine the exposure period to identify the window of the effect of exposure to air pollution more accurately.[38] Results of the present study showed that the time windows for the maximum effect of $PM_{2.5}$, PM_{10}, CO, and SO_2 were in the 21th to 24th week of pregnancy. Our study confirmed the association between a higher prenatal exposure to air pollution and PROM during pregnancy and more accurate time windows of maximum effects of exposure to pollutants. Accelerated fetal membrane aging and higher levels of immune responses from early- to mid-pregnancy could account for the sensitivity time windows of exposure to prenatal air pollution associated with risk to PROM.[22,39,40]

Anemia during pregnancy (hemoglobin <110 g/L) is associated with poor maternal outcomes (such as postpartum infections) and infant outcomes (such as neonatal perinatal and mortality).[41,42] Lower levels of hemoglobin have been considered as a marker in the condition of subclinical infections and inflammation.[15] In our study, we found that exposure to air pollution during mid- to late pregnancy was negatively associated with maternal hemoglobin levels. This evidence indicates that changes in hemoglobin levels with increased prenatal exposure to air pollution could be an indicator of increased risk of adverse pregnancy outcomes. These findings are consistent with prior results reported for women with an average age of 69.6 y.[17] Honda et al. reported that mean concentrations of $PM_{2.5}$ exposure and hemoglobin levels were 10.39 $\mu g/m^3$ and 13.5 g/dL, respectively. They found that a 3.9-$\mu g/m^3$ increase in annual-average $PM_{2.5}$ was associated with a 0.81-g/dL decrease in hemoglobin levels. In accordance with our findings, these results indicate a significant negative association between air pollution and hemoglobin levels. Studies in Ethiopia have suggested that indoor air pollution is associated with anemia during pregnancy.[43] Consistently, our study emphasized that exposure to air pollution during pregnancy is related to lower levels of maternal hemoglobin, thus contributing to prenatal anemia. Furthermore, our study found a significant correlation between anemia and an increased risk of PROM in pregnancy. Mediation analysis suggested that maternal hemoglobin levels could represent a potential factor contributing to increased risk of PROM posed by prenatal air pollution exposure. The assumption of unmeasured exposure–mediator confounding (e.g., wearing N95 masks for mitigating inhaled particulate air pollution, etc.),[44] unmeasured mediator–outcome confounding (maternal diet, etc.), and unmeasured exposure–outcome confounding (genetic risk, etc.) could bias our results, although major covariates were adjusted for in our analyses.[33,34,41]

Iron deficiency may result from dramatically increased demands for iron in pregnancy to meet rapid fetal growth, as well as a decrease in serum iron triggered by inflammation, increased numbers of red blood cells, and increased plasma volume.[45] Such factors may predispose pregnant women to anemia. Iron deficiency also accounts for adverse pregnancy and birth outcomes. In our study, we found a possible association of iron supplementation on the correction of anemia and PROM risk. Furthermore, in a stratified analysis using air pollution levels, the possible association of iron supplementation on PROM risk was detected only at low- to medium air pollution levels, showing that the potential positive effect of iron supplementation on PROM risk because of prenatal exposure to air pollution in women with anemia was limited. Our study suggests that PROM risk posed by exposure to low- to medium air pollution could be attenuated by maternal iron supplementation to some extent, especially in women with gestational anemia.

The mechanism through which hemoglobin may mediate the association of prenatal air pollution on PROM is unknown. We hypothesize the following potential mechanisms: Air pollutants cause a systemic inflammatory response and directly affect bone marrow function, and inflammation decreases renal erythropoietin secretion and increases bone marrow endogenous erythropoietin resistance, leading to the reduction in erythrocyte and hemoglobin production.[46,47] In addition, air particles may cause hemolysis and destruction of red blood cells. Low hemoglobin levels is a known marker of inflammation,[48] and therefore is associated with impaired immune function as well as dysregulation of functions of natural killer cells, T-cells, and neutrophils. Furthermore, impaired immune function has been identified in anemia, contributing to a higher susceptibility to bacterial infection, and it has been hypothesized to be associated with air pollution–related PROM.[14]

In the present study, the rates of preterm birth among included vs. excluded participants are 3.4% vs. 4.1%, respectively. The overall preterm rate in this study (3.4%) is lower than the rates in the overall China population[49] (6.1%) but is similar with the rates in Anhui province[50] (4.1%). Although several studies in China showed similar incidences of PROM from Nanjing[5] (20.8%) and Shanghai[51] (22.0%), the incidence of PROM in our study (21.0%) appears to be higher in comparison with that of the United States[52] (12.0%). It could be explained by the discrepancies in air pollution levels, race, and socioeconomic factors.[10,15,53] Moreover, considering the lower rate of preterm birth in this study, the proportion of preterm PROM (7.7%) of all PROM appears to be relatively low.

Our study is significant for several reasons. Our study could add to the growing body of literature pertaining to demonstrate the relationship between chronic ambient exposure to air pollution and PROM mediated by maternal anemia as well as decreasing hemoglobin levels based on results from our large-scale prospective cohort of pregnant women. Moreover, our finding that iron supplementation possibly alleviates the association of air pollution on PROM may drive further studies in this field. Second, we used data from our multicenter, prospective cohort study with adjustment for several confounding variables. Third, we simultaneously observed the influence of maternal anemia and possible association of iron supplementation. Finally, a more accurate time window for the effect of exposure of air pollution on PROM was identified in our study.

Nevertheless, this study has several limitations. First, we did not consider indoor air pollution, such as indoor dust, kitchen cooking oil fumes, indoor cigarette smoke, and sleep air quality. We failed to assess traffic-related air and noise pollution during

pregnancy, which was demonstrated to be related to PROM.[54] Second, we performed an observational study rather than a randomized controlled trial to elucidate the effect of iron supplementation. Moreover, the residual confounding and uncontrolled risk factors, such as prior history of PROM, could bias our results. In addition, given the cumulative effect of secular trends in air pollution before conception on hemoglobin levels in women, our study possibly overestimated the effect of air pollution exposure during pregnancy. Information on the change of addresses during pregnancy was not available, which may cause some bias in the effect estimates. Finally, the exposure data were assigned to the nearest air quality monitor rather than the estimated personal exposure to air pollution, which could provide incorrect results.

Conclusion

Prenatal exposure to ambient air pollution ($PM_{2.5}$, PM_{10}, SO_2, and CO), especially in 21th to 24th week of pregnancy, is positively associated with PROM risk, partly mediated by maternal hemoglobin levels. Iron supplementation in anemia pregnancy potentially has a positive association on low- to medium air pollution-related PROM. Screening and treatment of gestational anemia could provide novel insights into the prevention of low-to medium air pollution–related PROM.

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