Nitrogen dioxide exposure during pregnancy and risk of spontaneous abortion: a case-control study in China

Beiying Wang, Wei Hong, Qingjing Sheng, Zhiping Wu, Li Li and Xiaocui Li

Department of Obstetrics and Gynecology, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai, PR China

ABSTRACT

Background: Evidence on the relationship between prenatal exposure to NO2 and CO and spontaneous abortion (SAB) is insufficient. We investigated whether there is an association between maternal exposure to nitrogen dioxide (NO2) and carbon monoxide (CO) before and during pregnancy and SAB.

Methods: We conducted a case-control study using medical records of 2445 pregnant women who admitted for abortion prior to 20 weeks of gestational age from January 2014 to December 2019 at a tertiary-care hospital in Shanghai, China. Of the 2445 participants, 1075 were SAB cases and 1370 were healthy controls (underwent elective abortions). Maternal exposure to NO2 and CO before and during pregnancy was estimated using daily air pollution concentration data. Multivariable logistic regression models were constructed to quantify the relationships between maternal exposure to NO2 or CO and the risk of SAB while controlling for potential confounders.

Results: NO2 exposure levels during pregnancy were significantly higher in SAB cases than in healthy controls (42.26 vs. 40.67, \( p < .01 \)). NO2 exposure during pregnancy was positively associated with the risk of SAB. An interquartile range (16 \( \mu g/m^3 \)) increase in NO2 exposure was associated with 68% increase in the odds of SAB (OR = 1.68, 95% CI, 1.28, 2.21). Analyses of associations by quartile of NO2 exposure showed that elevated NO2 exposure during pregnancy was associated with increased odds of SAB in linear dose–response manners. Compared with the lowest quartile of NO2 exposure, the odds of SAB in the fourth quartile of NO2 exposure increased 61% (OR = 1.61, 95% CI, 1.03–2.53). No associations of CO exposure with SAB risk were observed.

Conclusions: Our study suggested that exposure to NO2 during early pregnancy was associated with increased risk of SAB. Further studies are needed to confirm our results and explore the potential biological mechanism underlying these associations.

Introduction

Ambient air pollution is the most serious environmental threat and represents a major mortality risk factor worldwide [1]. According to a recent global diseases burden estimates, exposure to ambient air pollution from particulate matter (PM) caused about 4.2 million deaths and 103.1 million disability-adjusted life-years (DALYs) in 2015 [2].

The cardiovascular and respiratory effects of air pollution have been well documented [3,4]. Recently, its reproductive and developmental toxicity has raised concern. Numerous epidemiological studies have reported significant associations between prenatal exposure to air pollution and increased risk of adverse pregnancy outcomes [5]. Those adverse pregnancy outcomes included low birth weight (LBW), fetal growth restriction (FGR), preterm birth (PTB), and birth defects [6–8].

Spontaneous abortion (SAB) or miscarriage, a common and serve complication of pregnancy, defined as the spontaneous loss of a pregnancy before 20 weeks of pregnancy [9]. At least 25% of all women experience one or more sporadic miscarriages [10]. Detrimental effects of air pollution on SAB have been previously suggested with exposure to PM and some gaseous pollutants [11]. Most of the previous studies reported strong associations of high PM [12–15], sulfur dioxide (SO2) [16,17], and ozone (O3) [12,15,18] exposure with increased risk of SAB. However, evidence regarding nitrogen dioxide (NO2) and carbon...
monoxide (CO) are insufficient and less inclusive, suggesting more studies are warranted to better understand their associations with SAB risk [11].

In this study, using clinical records of abortions in a tertiary hospital in Shanghai, China, we conducted a case-control study to examine whether maternal exposure to NO2 and CO before and during pregnancy was associated with increased risk of SAB.

Methods

Study design and subjects

A retrospective review of the medical records of abortions was conducted at Shanghai First Maternity and Infant Hospital, which is a tertiary-care hospital serving approximately 45,000 inpatients per year in Shanghai, China. The electronic medical record system of our hospital was used for data collection. From January 2014 to December 2019, a total of 2445 singleton pregnant women, aged between 14 and 48 years, admitted for abortion prior to 20 weeks of gestational age (GA). Of the 2445 pregnant women, 1075 were diagnosed as having missed abortion and were enrolled as SAB cases. While 1370 women with normal pregnancies who requested induced abortion due to unplanned or unwanted pregnancy were enrolled as healthy controls.

Maternal demographic characteristics including maternal age (MA), menstrual cycles, weight, and height, the use of assisted reproduction technology (ART) and reproductive history were collected. GA was calculated based on the last menstrual period (LMP) of pregnant women and confirmed by ultrasound. The study protocol was approved and monitored by the medical Ethics Committee of the Shanghai First Maternity and Infant Hospital (no. KS2008). Since all data were from medical record and used anonymously, there was no informed consent.

Air pollution exposure assessment

Daily (24-h) NO2 and CO concentration data for each of the 16 administrative districts in Shanghai city, from 1 May 2013 to 30 December 2019, were obtained from the database of the Shanghai Environmental Monitoring Center (SEMC). The daily concentrations of NO2 and CO in each district were averaged from the available data of all fixed-site monitoring stations. Ambient NO2 and CO measurements in each monitoring stations relied on a chemiluminescence detection method (API 200e, Thermo 42i) and a gas filter correlation method (API 300e, Thermo 48i), respectively. All ambient measurements of NO2 and CO were operated under the China National Quality Control Automated methods ([GB3095-2012] and [HJ/T 193-2005]) for ambient air quality monitoring. In order to adjust for the potential confounding effects of weather on SAB, we also obtained daily 24-h mean temperature and relative humidity from the database of the Shanghai Meteorological Bureau.

To explore the critical exposure time windows of NO2 and CO on SAB, we examined four exposure time windows for each subject (phases 1–4). The four exposure windows were defined as follows: phase 1, from the first day of LMP to the date of abortion; phase 2, 30 days before the first day of the LMP; phase 3, 60 days before the first day of the LMP; phase 4, 90 days before the first day of the LMP. The phase-averaged values of daily concentrations of NO2 and CO during various phases in the district where the subject’s residence is located were calculated.

Statistical analyses

In descriptive analysis, the demographic characteristics of all participants were shown as mean ± standard deviation (SD) or percent (%). Parametric t-tests and χ2 tests were used to compare the difference in demographic characteristics between SAB cases and healthy controls. As NO2 and CO exposure levels were not normally distributed, medians (25th–75th percentile) were presented to characterize their distribution in descriptive analysis. Mann–Whitney’s U-test was used to examine the potential differences in NO2 and CO exposure between SAB cases and healthy controls.

Multivariate logistic regression models were used to examine the association between SAB risk and exposure to NO2 and CO with adjustment for potential confounders. According to the data accessibility and literature studies [15,19], we denoted the following factors to be covariates: GA, MA, body mass index (BMI), maternal parity, marital status, the use of ART, temperature, and relative humidity. We first conducted single pollutant model with only NO2 or CO in the multivariate logistic regression models. Then, we conducted two-pollutants model to examine the joint effects of NO2 and CO exposure on SAB. We reported the odd ratio (OR) and 95% confidence interval (CI) in association with an interquartile range (IQR) increase in NO2 or CO concentrations.

In order to explore the potential dose-response relationships between NO2 exposure and risk of SAB, the distribution of NO2 exposure levels was divided into quartiles and an OR and 95% CI was calculated.
for each higher quartile compared with the lowest quartile using multivariate logistic regression analysis.

All statistical analyses were performed by using SPSS16.0 software (SPSS Inc., Chicago, IL) and a two-sided \( p < 0.05 \) was considered statistically significant.

**Results**

**Population characteristics**

Table 1 presents the demographic characteristics of all study participants (\( N = 2445 \)). The average MA of all participants was 33.18 (5.34) years at enrollment. The average GA and BMI was 72.73 (23.29) days and 22.20 (3.31) kg/m\(^2\), respectively. Of the 2445 participants, 1075 were SAB cases and 1370 were health controls. SAB cases and healthy controls differed with regard to GA, BMI, marital status, menstrual cycles, parity, and the use of ART. SAB cases and health controls did not differ by MA (33.15 ± 4.98 vs. 33.20 ± 5.60, \( p = .819 \)).

**NO\(_2\) and CO exposure levels**

Table 2 presents the distribution of NO\(_2\) and CO exposure levels during each specific exposure window (phases 1–4). The median (25th–75th percentile) NO\(_2\) exposure levels for phases 1–4 were 41.46 (33.52, 49.52), 40.34 (31.85, 50.81), 41.00 (32.24, 50.76), and 41.65 (32.73, 50.11) μg/m\(^3\), respectively. The median CO exposure levels were 0.68 (0.60, 0.78), 0.69 (0.59, 0.79), 0.69 (0.60, 0.80), and 0.69 (0.61, 0.79) mg/m\(^3\), respectively. Exposure levels of NO\(_2\) were moderately correlated with CO during each phase, with the correlation coefficients ranging from 0.633 to 0.680 (Figure 1).

Analyses of potential differences in NO\(_2\) and CO exposure levels during each exposure window between SAB cases and health controls were conducted using the Mann–Whitney U-test. As shown in Table 2, NO\(_2\) exposure levels during phase 1 (from the first day of LMP to the date of abortion) were significantly higher in SAB cases than in healthy controls (42.26 vs. 40.67, \( p < .01 \)). While there was no significant difference in CO exposure during each exposure window.

**Associations of NO\(_2\) and CO exposure with SAB risk**

The associations of NO\(_2\) and CO exposure with SAB risk were investigated. ORs and 95% CIs for the risk of
SAB in relation to NO2 or CO exposure are presented in Table 3. Higher NO2 exposure during phase 1 was significantly associated with increased risk of SAB. In single-pollutant model, an IQR (16 μg/m3) increase in NO2 exposure during phase 1 was associated with a 53% (OR = 1.53, 95% CI, 1.19–1.97) increase in the odds of SAB. A similar result was observed in two-pollutant models with a 68% (OR = 1.68, 95% CI, 1.28, 2.21) increase in the odds of SAB per IQR increase in NO2 exposure during phases 1. However, no significant association was found between SAB risk and CO exposure during any phases.

Given NO2 exposure during phase 1 was found to be significantly associated with increased risk of SAB, we examined the dose–response associations between NO2 exposure during phase 1 and the risk of SAB. As shown in Figure 2, higher NO2 exposure was associated with increased odds of SAB in linear dose–response manners. In single-pollutant model, compared with the lowest quartile of NO2 exposure, being in the fourth quartile of NO2 exposure was significantly associated with increased odds of having SAB (OR = 1.55, 95% CI, 1.01–2.38). Similar results were observed in two-pollutant models. Compared with the lowest quartile of NO2 exposure, the odds of

Table 3. Associations of NO2 and CO exposure with spontaneous abortion risk.

| Pollutants | Single pollutant modela OR (95% CI) | Two-pollutants modelsb OR (95% CI) |
|------------|-----------------------------------|-----------------------------------|
| NO2        |                                   |                                   |
| Phase 1    | 1.53 (1.19, 1.97)                 | 1.68 (1.28, 2.21)                 |
| Phase 2    | 0.95 (0.76, 1.19)                 | 0.96 (0.75, 1.23)                 |
| Phase 3    | 0.99 (0.76, 1.30)                 | 1.01 (0.76, 1.35)                 |
| Phase 4    | 0.95 (0.71, 1.27)                 | 0.98 (0.71, 1.36)                 |
| CO         |                                   |                                   |
| Phase 1    | 0.99 (0.86, 1.14)                 | 0.88 (0.76, 1.03)                 |
| Phase 2    | 0.98 (0.85, 1.12)                 | 0.99 (0.85, 1.15)                 |
| Phase 3    | 0.98 (0.85, 1.13)                 | 0.98 (0.84, 1.15)                 |
| Phase 4    | 0.96 (0.84, 1.10)                 | 0.97 (0.83, 1.13)                 |

Notes: aAdjusted for GA, MA, BMI, marital status, maternal parity, ART, temperature, and relative humidity. bAdjusted for GA, MA, BMI, marital status, maternal parity, ART, temperature and relative humidity, and NO2 or CO.

Phase 1: form the last menstrual period to the date of missed abortion, phase 2: 30 days before pregnancy, phase 3: 60 days before pregnancy, and phase 4: 90 days before pregnancy.

Figure 1. Plots of the correlations between NO2 exposure levels and CO exposure levels. Scatterplots depict the correlation of NO2 exposure levels (y-axis) and CO exposure levels (x-axis), with the Spearman correlation coefficient (r) provided. All Spearman’s correlation coefficients were significant at p < .01. Phase 1: form the last menstrual period to the date of missed abortion, phase 2: 30 days before pregnancy, phase 3: 60 days before pregnancy, phase 4: 90 days before pregnancy.
SAB in the fourth quartile of NO2 exposure increased 61% (OR = 1.61, 95% CI, 1.03–2.53). The trend tests for the above dose–response relationships were statistically significant (single pollutant model, p-trend = .059; two-pollutants model, p-trend = .044).

Discussion

SAB is one of the most frequent traumatic life events a woman may encounter. It is associated with various negative psychological outcomes including depression, grief, anxiety, and marital conflict [20]. The causes of SAB are likely to be multifactorial [9,21,22]. Along with genetic and socioeconomic factors, environmental pollution, including air pollution, may possibly play a role in the development of SAB.

In this study, on the basis of a case-control study design, we investigated the adverse effects of NO2 and CO exposure on SAB. We found that exposure to NO2 during pregnancy was associated with increased risk of SAB. Moreover, we observed significantly linear dose–response relationships between NO2 exposure and SAB risk. The case-control study design, however, may not support a causal relationship. Nevertheless, the association we observed is unlikely to be due to chance. Our findings indicate that pregnant women who exposed to higher levels of NO2 during pregnancy might be at higher risk for SAB.

Some studies have investigated the associations between maternal exposure to NO2 and SAB. Most of those reported studies used fixed air monitoring station data to estimate individual NO2 exposure and reported significant positive associations between NO2 exposure and SAB risk [18,19,23,24]. Our findings were consistent with the results of those previous studies. Moreover, one prospective cohort study found that pregnant women living within 50 m of a road with higher annual average daily traffic was statistically significantly associated with increased risk of SAB [25]. The results of this prospective cohort study also supported our findings since vehicle emissions is a major source of ambient NO2.

For air pollutant of CO, only three studies examined the association of maternal exposure to CO with the risk of SAB. In agreement with findings from this presented study, both a time-series study and a prospective cohort study did not find any association between SAB risk and CO exposure [15,26]. However, a recent study failed to support our findings. Zhang et al. examined the records of 255,668 pregnant women from 2009 to 2017 in Beijing, China, and quantified the link between CO exposure and SAB risk. They found long-term exposure to CO before pregnancy was associated with significant increased risk of SAB [17]. Inconsistent results may mainly be ascribed to different study designs and exposure ranges.

The mechanisms of action of NO2 exposure on SAB are not fully understood. Some studies demonstrated that exposure to NO2 during pregnancy could induce structural or chromosomal anomalies, which are relevant for SAB [27]. Moreover, some previous studies have shown that NO2 exposure could induce oxidative stress and inflammatory responses [28–30], which have been hypothesized to play a role in the development of SAB [31].

Some limitations should be acknowledged. First, since women who have very early SAB usually do not visit hospitals, we were unable to enroll them in our study. Second, we estimated maternal exposure to NO2 and CO based on fixed-site monitoring station data and ignored the spatial distribution of those two pollutants, which may cause exposure misclassification.
Third, due to the data inaccessibility, we were unable to collect information about some potential risk factors for SAB and thus could not rule out the role of those unmeasured confounders.

In summary, our findings suggest that maternal exposure to NO\textsubscript{2} during early pregnancy is associated with increased risk of SAB. Further studies are needed to confirm our finding and to explore the biologic mechanisms underlying this association.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**ORCID**

Xiaocui Li [http://orcid.org/0000-0002-9770-1940](http://orcid.org/0000-0002-9770-1940)

**References**

[1] Brunekreef B. Air pollution and health. Ned Tijdschr Geneeskd. 2018;2018:162.

[2] Cohen AJ, Brauer M, Burnett R, et al. Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015. Lancet. 2017;389(10082):1907–1918.

[3] Dominici F, Peng RD, Bell ML, et al. Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA. 2006;295(10):1127–1134.

[4] Mohammed MO, Song WW, Ma WL, et al. Potential toxicological and cardiopulmonary effects of PM2.5 exposure and related mortality: findings of recent studies published during 2003–2013. Biomed Environ Sci. 2016;29(1):66–79.

[5] Klepac P, Locatelli I, Korosec S, et al. Ambient air pollution and pregnancy outcomes: a comprehensive review and identification of environmental public health challenges. Environ Res. 2018;167:144–159.

[6] Guo LQ, Chen Y, Mi BB, et al. Ambient air pollution and adverse birth outcomes: a systematic review and meta-analysis. J Zhejiang Univ Sci B. 2019;20(3):238–252.

[7] Li X, Huang S, Jiao A, et al. Association between ambient fine particulate matter and preterm birth or term low birth weight: an updated systematic review and meta-analysis. Environ Pollut. 2017;227:596–605.

[8] Xiong L, Xu Z, Wang H, et al. The association between ambient air pollution and birth defects in four cities in Hunan province, China, from 2014 to 2016. Medicine. 2019;98(4):e14253.

[9] Garcia-Enguidanos A, Calle ME, Valero J, et al. Risk factors in miscarriage: a review. Eur J Obstet Gynecol Reprod Biol. 2002;102(2):111–119.

[10] Rai R, Regan L. Recurrent miscarriage. Lancet. 2006;368(9535):601–611.

[11] Grippo A, Zhang J, Chu L, et al. Air pollution exposure during pregnancy and spontaneous abortion and stillbirth. Rev Environ Health. 2018;33(3):247–264.

[12] Di Ciaula A, Bilancia M. Relationships between mild PM10 and ozone urban air levels and spontaneous abortion: clues for primary prevention. Int J Environ Health Res. 2015;25(6):640–655.

[13] Perin PM, Maluf M, Czeresnia CE, et al. Effects of exposure to high levels of particulate air pollution during the follicular phase of the conception cycle on pregnancy outcome in couples undergoing in vitro fertilization and embryo transfer. Fertil Steril. 2010;93(1):301–303.

[14] Perin PM, Maluf M, Czeresnia CE, et al. Impact of short-term preconceptional exposure to particulate air pollution on treatment outcome in couples undergoing in vitro fertilization and embryo transfer (IVF/ET). J Assist Reprod Genet. 2010;27(7):371–382.

[15] Ha S, Sundaram R, Buck Louis GM, et al. Ambient air pollution and the risk of pregnancy loss: a prospective cohort study. Fertil Steril. 2018;109(1):148–153.

[16] Hou HY, Wang D, Zou XP, et al. Does ambient air pollutants increase the risk of fetal loss? A case-control study. Arch Gynecol Obstet. 2014;289(2):285–291.

[17] Zhang LQ, Liu WW, Hou K, et al. Air pollution-induced missed abortion risk for pregnancies. Nat Sustain. 2019;2(11):1011–1017.

[18] Moridi M, Ziae S, Kazemnejad A. Exposure to ambient air pollutants and spontaneous abortion. J Obstet Gynaecol Res. 2014;40(3):743–748.

[19] Green RS, Malig B, Windham GC, et al. Residential exposure to traffic and spontaneous abortion. Environ Health Perspect. 2009;117(12):1939–1944.

[20] Schwedtfeiger KL, Shreffler KM. Trauma of pregnancy and other health challenges. Environ Res. 2018;167:144–159.

[21] Zheng D, Li C, Wu T, et al. Factors associated with spontaneous abortion: a cross-sectional study of Chinese populations. Reprod Health. 2019;16:113.

[22] Zhou H, Liu Y, Liu L, et al. Maternal pre-pregnancy risk factors for miscarriage from a prevention perspective: a cohort study in China. Eur J Obstet Gynecol Reprod Biol. 2016;206:57–63.

[23] Leiser CL, Hanson HA, Sawyer K, et al. Acute effects of air pollutants on spontaneous pregnancy loss: a case-crossover study. Fertil Steril. 2019;111(2):341–347.

[24] Enkhmaa D, Warburton N, Javzandulam B, et al. Seasonal ambient air pollution correlates strongly with spontaneous abortion in Mongolia. BMC Pregnancy Childbirth. 2014;14(1):146.

[25] Kouroumztoglu MA, Raz R, Wilson A, et al. Traffic-related air pollution and pregnancy loss. Epidemiology. 2019;30(1):4–10.

[26] Dastoorpoor M, Idani E, Goudarzi G, et al. Acute effects of air pollution on spontaneous abortion, premature delivery, and stillbirth in Ahvaz, Iran: a time-series study. Environ Sci Pollut Res. 2018;25(6):5447–5458.

[27] Vrijheid M, Martinez D, Manzanares S, et al. Ambient air pollution and risk of congenital anomalies: a systematic review and meta-analysis. Environ Health Perspect. 2011;119(5):598–606.
[28] Patel MM, Chillrud SN, Deepti KC, et al. Traffic-related air pollutants and exhaled markers of airway inflammation and oxidative stress in New York City adolescents. Environ Res. 2013;121:71–78.

[29] Lanki T, Hampel R, Tiittanen P, et al. Air pollution from road traffic and systemic inflammation in adults: a cross-sectional analysis in the European ESCAPE Project. Environ Health Perspect. 2015;123(8):785–791.

[30] Viehmann A, Hertel S, Fuks K, et al. Long-term residential exposure to urban air pollution, and repeated measures of systemic blood markers of inflammation and coagulation. Occup Environ Med. 2015;72(9):656–663.

[31] Gupta S, Agarwal A, Banerjee J, et al. The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: a systematic review. Obstet Gynecol Surv. 2007;62(5):335–347.