



Ambient PM_{2.5} and clinically recognized early pregnancy loss: A case-control study with spatiotemporal exposure predictions

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ABSTRACT

Background: Experimental research suggests that ne particulate matter (PM_{2.5}) exposure might a ect embryonic development. However, only few population-based studies have investigated the impact of maternal exposure to PM_{2.5} on the early pregnancy loss.

Objectives: To estimate associations between clinically recognized early pregnancy loss (CREPL) and exposure to ambient PM_{2.5} at individual residences during peri-conception periods, with the aim to identify susceptible exposure time windows.

Methods: CREPL cases and normal early pregnancy controls (of similar age and gravidity presenting within one week, a total of 364 pairs) were recruited between July 2017 and July 2018 among women residing in Tianjin, China. Average ambient PM_{2.5} concentrations of ten exposure windows (4 weeks, 2 weeks and 1 week before conception; the rst, second, third and fourth single week, the rst and second 2-week periods, and the entire 4-week period after conception) at the women's residential addresses were estimated using temporally-adjusted land use regression models. Associations between PM_{2.5}

physiological systems, and also is the most susceptible stage to environmental chemicals exposure (Cooke, 2014). However, there is less information on the effects of maternal exposure to $PM_{2.5}$ on human embryonic development during early pregnancy. Relevant research is needed to investigate the exposure-response relationships and exposure-effect mechanisms, and to provide evidence for adopting appropriate interventions to prevent adverse pregnancy outcomes and possible fetal exposure-related adulthood diseases.

Early pregnancy loss (EPL), also known as miscarriage or spontaneous abortion, is a typical indicator which reflects severe abnormalities of embryonic development. It is one common type of adverse pregnancy outcomes, with an incidence of approximately 10% of all clinically recognized pregnancies (The American College of Obstetricians and Gynecologists, ACOG, 2015). EPL is defined as a nonviable, intrauterine pregnancy with either an empty gestational sac or a gestational sac containing an embryo (prior to 8 weeks post-conception) or fetus (beyond 8 weeks post-conception) without fetal heart activity within the first trimester (ACOG, 2015). The occurrence of EPL is mainly related to embryonic chromosomal abnormalities, endocrine factors, reproductive immune dysfunction or prethrombotic state, but the upstream factors and pathogenic mechanisms are not completely clear (Practice Committee of the American Society for Reproductive Medicine, 2012; Royal College of Obstetricians and Gynaecologists, RCOG, 2011). Recent studies have suggested that EPL was related to oxidative stress and inflammation in maternal systemic and maternal-fetal interface (Cross et al., 2015; Lyu et al., 2013; Wu et al., 2016; Zhang et al., 2016; Zhu et al., 2017).

Exposure to $PM_{2.5}$ may induce human systemic inflammation and oxidative damage (Feng et al., 2016; Hassanvand et al., 2017; Liu et al., 2015; Moller and Loft, 2010; Pope et al., 2016). Maternal exposure to ambient $PM_{2.5}$ during preconception and specific periods of pregnancy has been associated with intrauterine inflammation indicated by placental pathology at delivery (Nachman et al., 2016). Additionally, animal experimental studies have found that $PM_{2.5}$ exposure can increase oxidative stress in peripheral blood of pregnant mice and induce placental inflammation and increased absorbed blastocysts in rats (Liu et al., 2017; Liu et al., 2016). Accordingly, we hypothesize that maternal $PM_{2.5}$ exposure during early pregnancy might be associated with EPL, likely through increasing oxidative stress and inflammation.

The epidemiological evidence for effects of $PM_{2.5}$ on pregnancy loss is still limited (Grippio et al., 2018). Based on our latest literature retrieval until 1 August 2018, there were only three relevant population-based studies. A retrospective study identified associations between ambient $PM_{2.5}$ and spontaneous abortion by examining fetal deaths per calendar month based on medical records and monthly average $PM_{2.5}$ concentrations measured at administrative monitoring stations (Enkhmaa et al., 2014). Another retrospective study assessed the relationship between cases of EPL diagnosed in the emergency department and regional daily $PM_{2.5}$ at 3-day and 7-day lags (Sawyer et al., 2018). Recently, a prospective cohort study of 343 pregnancies (including 97 pregnancy losses occurred before 18 weeks of gestation) provided evidence that $PM_{2.5}$ concentrations averaged over the entire pregnancy (chronic exposure) were associated with faster time to pregnancy loss. Ambient $PM_{2.5}$ at two acute exposure windows (2 weeks before ovulation and the last 2 weeks of pregnancy) was also estimated, but was unrelated to risk of pregnancy loss (Ha et al., 2018).

Effects on the pregnant women, the placenta and embryo/fetus from toxic exposures depend on the exposure time window. EPL is a gradual process, and symptoms do not always appear immediately after embryonic or fetal death. It is therefore difficult to determine the specific time that EPL occurred. Estimating $PM_{2.5}$ exposure during the entire pregnancy or for periods in the latter part of pregnancy, seemed not to be sensible. To some extent, a number of chemical components of $PM_{2.5}$ are also teratogen or at least have some toxicological effects. According to the U.S. Food and Drug Administration (FDA), teratogen exposures during the first 2 weeks after conception are not known to cause

congenital anomalies; however, such exposures may interfere with implantation of the blastocyst or cause spontaneous abortion. Further, the embryo is most easily disrupted by teratogen exposures during organogenesis (3 to 8 weeks post-conception) (U.S. FDA, 2005).

Although prospective cohort is considered the most perfect design in environmental epidemiological studies, it is unsuitable for subjects' recruitment and exposure assessment of specific disease as EPL. Firstly, portions of EPL do not end up in medical institutions. Sometimes women may not even notice the EPL, and may interpret the EPL as the next menstrual period. Secondly, women usually do not realize that they are pregnant until a menstrual period is missed, by which time the ability to monitor exposure in the peri-conception period would have passed. Thus, to identify specific periods of heightened vulnerability, we conducted this case-control study to estimate spatiotemporal $PM_{2.5}$ exposures using temporally-adjusted land use regression models, focusing on the short-term exposure windows before and after conception. To the best of our knowledge, this is the first environmental epidemiological study to estimate associations between clinically recognized early pregnancy loss (CREPL) and acute exposures to $PM_{2.5}$ during several peri-conception (especially post-conception) exposure time windows.

2. Methods

2.1. Study participants

This study used a matched case-control design with pre-designed interviewer-administered questionnaire. CREPL cases and normal early pregnancy controls were recruited from The Second Hospital of Tianjin Medical University and Tianjin Central Hospital of Gynecology and Obstetrics, between July 2017 and July 2018, among women residing in six central districts or four adjacent suburban districts (total area 2084 km²) of Tianjin who had not changed residences during the previous year. Tianjin is a megacity in northern China that experiences high levels of air pollution. In 2017, the annual average concentration of $PM_{2.5}$ was 62 g/m³ (Ministry of Ecology and Environment of the People's Republic of China, 2018). The two hospitals have the two largest family planning departments in Tianjin. A total of about 10,000 induced abortions are performed each year in the two departments, including unintended pregnancies and CREPL cases requiring surgical evacuation to terminate the pregnancy.

Case and control volunteer study participants provided written informed consent and completed an interviewer-administered questionnaire. The questionnaire provided information on participant residential address, the pregnancy and demographic characteristics. Where information was unclear, telephone follow-up was conducted shortly after recruitment. Fasting peripheral blood of pregnant women and early pregnancy chorionic villus (the same as chorionic villi) tissues were collected on the day of surgical evacuation. The study was approved by the Medical Ethics Committee of the Second Hospital of Tianjin Medical University (No.KY2017K003).

2.2. Definition of cases and controls

The diagnostic criteria of CREPL were based on a thorough medical history, physical examination, ultrasonography and serum β -human chorionic gonadotropin (β -hCG) testing, in line with the Society of Radiologists in Ultrasound guidelines for transvaginal ultrasonographic diagnosis of EPL (ACOG, 2015; Doubilet et al., 2013). The inclusion criteria of CREPL cases were: pregnancy < 13 weeks gestation; with regular menstrual cycle (the range of variation was ± 1 day during the previous year); clinical diagnosis of EPL with transvaginal ultrasonography demonstrating crown-rump length of ≤ 7 mm and no heartbeat, mean sac diameter of ≤ 25 mm and no embryo, or absence of embryo with heartbeat ≥ 11 days after a scan that showed a gestational sac with a yolk sac (Doubilet et al., 2013; Morin et al., 2016). The

inclusion criteria of controls were: pregnancy < 13 weeks gestation; with regular menstrual cycle; ultrasonography showing an intrauterine pregnancy with an embryo or fetus with heartbeat; no vaginal bleeding during the present pregnancy; no history of pregnancy loss, fetal malformation, preterm birth or delivered a newborn with low birth weight.

The exclusion criteria for cases and controls were pregnancy complications including reproductive tract infections, uterine malformations or uterine myoma or systemic diseases (e.g., hypertension, diabetes, or thyroid disease) or change of residential address in the previous year. Because maternal exposure to PM_{2.5} might be associated with fetal DNA damage or placental DNA hypomethylation and altered gene expression (Janssen et al., 2013; Qin et al., 2017; Teng et al., 2016; Winckelmans et al., 2017) which could mediate the effects of PM_{2.5} on EPL, we did not routinely screen for chromosomal abnormalities in the participants or include chromosomal abnormalities as exclusion criteria.

After enrollment of each CREPL case, a corresponding control was recruited consisting of a woman with a normal early pregnancy of similar age (± 5 years) and gravidity (± 1 number of pregnancies) presenting within one week of the corresponding case. The matching criteria were determined due to maternal age is the most common risk factor of EPL, and gravidity is an important indicator of female fertility (ACOG, 2015; RCOG, 2011). Controls were also pregnant woman requesting an induced abortion to allow collection of biological samples, especially chorionic villus, for subsequent biomarker measurements. Parity was not considered as one of the matching variable, because in China, a couple can have two children at most, and the induced abortion is legal. Therefore, parity was more closely related to social factors, rather than physiological factors.

2.3. Exposure estimation

Our spatiotemporal exposure predictions were based on a land use regression (LUR) model with high spatial resolution (1 km) as described previously (Chen et al., 2017), combined with temporal adjustment. In brief, the same procedures as used in European Study of Cohorts for Air Pollution Effects (ESCAPE) were followed for model development and validation (Eeftens et al., 2012; ESCAPE, 2009). PM_{2.5} concentration data were collected from 28 routine monitoring sites operated by the Tianjin Environmental Monitoring Center in 2014. The geographic predictor variables included in the final PM_{2.5} LUR model were population density, road length within a 1 km buffer, industrial land area within a 2 km buffer and distance to the coast. Model predictive accuracy was very good with a leave-one-out cross-validation (LOOCV) R² of 0.73 (Chen et al., 2017).

To estimate spatial exposure, all subjects' residential addresses were geocoded and spatially linked to the gridded outputs from the LUR models. Google Maps was used to obtain longitude and latitude of all residential addresses (available on <http://www.gpsspg.com/maps.htm>). ArcGIS 10.2 software (ESRI Inc., CA, USA) was used to integrate the layers of longitude and latitude, administrative districts map of Tianjin and the raster map of the LUR model. In order to effectively avoid the exposure misclassification due to maternal residential mobility, we recruited the subjects who had not changed residences during the previous year.

To temporally-adjust the PM_{2.5} exposure during different exposure time windows, date of ovulation of each subject was estimated by obstetricians based on the last menstrual period (LMP) and menstrual cycle, and combined with transvaginal ultrasonography if necessary. Because fertilization usually occurs within 24 h after ovulation, we assumed the ovulation date to be the date of conception. To be specific, we used the following equation to estimate the date of ovulation, based on the fact that the interval from ovulation to the next menstruation (luteal phase length) is relatively fixed at 14 days (Crawford et al., 2017; Lam et al., 2011; Practice Committee of the American Society for Reproductive Medicine, 2015):

$$\text{Date of ovulation} = \text{LMP} + \text{menstrual cycle} - 14 \text{ days}$$

We collected reports of transvaginal ultrasonography of all subjects. For each of the controls, the crown-rump length (CRL) of the embryo was generally in line with the corresponding gestational age. We used the above-mentioned methods to estimate the timing of conception of all controls. In the vast majority of cases, due to EPL, the CRL of the embryo or mean sac diameter was less than gestational age. We also used the above-mentioned methods to estimate the timing of conception. But for 13 cases, the CRL of the embryo or mean sac diameter was significantly larger than its normal size at the gestational age. After further inquiries, the cases recognized that the last menstrual volume was obviously less than usual. Actually, it was just the vaginal bleeding after pregnancy (threatened abortion). Therefore, we plugged the previous menstrual period, i.e., the real LMP, into the above-mentioned equation, to estimate the timing of conception.

Ten exposure windows included three pre-conception exposure windows (4 weeks, 2 weeks and 1 week before conception) and seven post-conception exposure windows (the first, second, third and fourth single week, the first and second 2-week periods, and the entire 4-week period after conception; i.e., days 0–7, 8–14, 15–21, 22–28, 0–14, 15–28 and 0–28 after conception).

We obtained daily individual-level estimates of ambient PM_{2.5} from the LUR residential annual exposure estimates utilizing daily PM_{2.5} data during the study period from the Tuanbowa background monitoring station in Tianjin. Spatiotemporal exposure estimates were made using the ratio method procedure from ESCAPE for extrapolation back in time (ESCAPE, 2009). The daily spatiotemporal estimates of each subject were calculated as the residential exposure estimate multiplied by the ratio of daily to annual 2014 average PM_{2.5} concentrations at the background monitoring station. Daily spatiotemporal estimates were then averaged over each peri-conception exposure time window (Dadvand et al., 2013; ESCAPE, 2009; Schembari et al., 2014). This assumes that the daily to annual ratio on any day is the same across the study area (Dadvand et al., 2013; Eeftens et al., 2011; Schembari et al., 2015).

2.4. Statistical analysis

Descriptive characteristics and estimated PM_{2.5} exposures within the matched case-control sets were compared using the paired-t-test for normal continuous variables, the Wilcoxon signed rank test for non-normal continuous variables, the McNemar test for binary variables, and the marginal homogeneity test for ordered categorical variables. Pearson correlation coefficients between the PM_{2.5} predicted concentrations at each exposure time window of interest were calculated.

Unadjusted and adjusted ORs for associations between CREPL and spatiotemporal PM_{2.5} estimates were obtained from single-variable and multivariable conditional logistic regression models. We calculated ORs and 95% confidence intervals (CIs) for a 10 g/m³ increase in PM_{2.5} exposures. Separate regression models were first used to estimate associations with average PM_{2.5} exposures for each peri-conception exposure window. Since exposures in each time window were correlated with those of other time windows, in order to estimate independent exposure time window effects, a single regression model that included an exposure variable for each mutually exclusive one-week time window was estimated.

The adjusted conditional logistic regression models were adjusted for the following 9 potential confounders: body mass index (BMI), parity (0, 1, 2, 3), maternal education (high school or lower, college, higher than college), economic status from family monthly income per capita (< 5000 ¥, 5000–7500 ¥, > 7500 ¥), interior renovation either of home or work (> 1 year ago, < 1 year ago), occupational exposure (exposure to potential toxic or hazardous substances at work such as building decoration materials, printing, textile dyeing, plastic molds, metal packaging, chemical or electronics factory, etc. - yes/no), alcohol

consumption during pregnancy (yes/no), active smoking during pregnancy (yes/no) and passive smoking during pregnancy (second-hand smoke exposure either at home or work - yes/no). Estimated PM_{2.5} exposures and BMI were entered into the models as continuous variables while all other covariates were entered as categorical variables.

Sensitivity analyses were performed by: a) excluding cases and controls who actively smoked during pregnancy and their corresponding controls or cases, even if they did not actively smoke during pregnancy, n = 80 (11%); b) excluding cases and controls who drank alcohol during pregnancy and corresponding controls or cases, even if they did not drink alcohol during pregnancy, n = 206 (28%). Sample size for conditional logistic regression was estimated roughly as 20 times the number of variables (Norman et al., 2012), resulting in estimated required sample sizes of cases and controls, respectively, of at least 200 subjects. All statistical analyses were conducted using SPSS version 22.0 (IBM Corporation, NY, USA). Statistical significance was defined as a two-tailed level of 5%.

3. Results

3.1. Study participants

During the course of the study, 734 participants (367 CREPL cases and 367 normal early pregnancy controls) were recruited. Three cases and corresponding controls were excluded because of a missing geocoded address, leaving 364 cases and controls for the final analysis. Addresses of the cases and controls both spread all over the ten districts of Tianjin. The distribution of addresses of the 728 subjects is shown in the Supplemental Material (Fig. S1). The recruitment time of cases in our study ranged from 33 to 69 days (4⁺⁵ to 9⁺⁶ weeks) after conception, and the recruitment time of controls ranged from 31 to 47 days (4⁺³ to 6⁺⁵ weeks) after conception. Twenty-three percent of the cases had history of CREPL for once or more times. All cases had no history of fetal malformation, preterm birth or delivered a newborn with low birth weight.

By virtue of the pre-designed interviewer-administered questionnaire and telephone follow-up, there were no missing data for each variable of interest. Descriptive statistics of the characteristics of cases and controls are presented in Table 1. Maternal age ranged from 19 years to 46 years. Maternal age and gravidity were well matched within case-control sets. Compared with controls, the cases had higher BMI, less parity, higher proportion of interior renovation during the previous year, and lower proportion of alcohol consumption and active smoking during pregnancy. There was no significant difference in maternal education, family income, occupational exposure or passive smoking.

3.2. Exposure estimates

CI: 1.03, 1.23; $p=0.010$) and for the entire four-week period after conception (OR = 1.22; 95% CI: 1.02, 1.46; $p=0.027$). Differences between the unadjusted and adjusted models were not notable.

Unadjusted and adjusted ORs (95% CIs) for associations between CREPL and $PM_{2.5}$ estimates (for a $10 \mu\text{g}/\text{m}^3$ increase in $PM_{2.5}$) during each one-week period exposure window adjusted for all other mutually exclusive time windows are shown in [Table 4](#). Estimated effects from

4.2. Estimation of maternal exposure

Measurement of actual personal exposure to air pollution over the course of pregnancy is not feasible. Also, women usually do not realize that they are pregnant until a menstrual period is missed, by which time the ability to monitor exposure in the peri-conception period would have passed. By necessity, then, pollutant concentration data from ambient air quality monitoring stations have been most frequently used to estimate exposures in epidemiological studies of birth outcomes (DeFranco et al., 2016; Dugandzic et al., 2006; Heck et al., 2014; Wilhelm and Ritz, 2005). However, there is legitimate concern that this approach results in substantial exposure measurement error (Kumar, 2012).

Exposure prediction models such as LUR models were developed to attempt to reduce exposure measurement error and its impact on health effect estimates. LUR models have now been employed to generate predicted exposure variables in many epidemiological studies, including numerous studies of the association between maternal exposures to PM_{2.5} and adverse pregnancy outcomes (Brauer et al., 2008; Estarlich et al., 2016; Poirier et al., 2015; Pedersen et al., 2013; Rudra et al., 2011; Stieb et al., 2016). One advantage of LUR models is the high spatial resolution of the pollutant concentration predictions, with predictions made at specific points in space such as individual study subject addresses (Methery et al., 2008). The performance of LUR models in urban areas is typically better or equivalent to geo-statistical methods such as kriging or conventional dispersion models (Hoek et al., 2008).

Standard LUR models do not produce time-varying predictions. Temporally-adjusted LUR models were developed to allow short-term or specific period air pollution exposure predictions to be made. This extension of LUR models made use of adjustment factors derived from

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other pollutants as well as sources of $PM_{2.5}$. Such studies would help point the way to focus efforts in preventing exposure-related effects on embryonic development.

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