Environment International 120 (2018) 34–42

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Urinary concentrations of phthalate metabolites associated with changes in clinical hemostatic and hematologic parameters in pregnant women

Minmin Jiang[a], Yuanyuan Li[a], Bin Zhang[b], Aifen Zhou[b], Yingshuang Zhu[a], Jiufeng Li[c], Hongzhi Zhao[c], Li Chen[a], Jie Hu[a], Chuansha Wu[a], Yang Peng[a], Jiaqiang Liao[a], Zhiguo Xia[b], Zongwei Cai[c], Xi Chen[a], Bing Xu[a], Wei Xia[a,*], Shunqing Xu[a,*]

[a] Key Laboratory of Environment and Health, Ministry of Education & Ministry of Environmental Protection, and State Key Laboratory of Environmental Health (Incubation), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, People's Republic of China
[b] Wuhan Medical and Healthcare Center for Women and Children, Wuhan, Hubei, People's Republic of China
[c] State Key Laboratory of Environmental and Biological Analysis, Department of Chemistry, Hong Kong Baptist University, Hong Kong, People's Republic of China

ARTICLE INFO

Handling editor: Lesa Aylward

Keywords:
Phthalate
Hematologic parameters
Coagulation function
Anemia
Pregnancy

ABSTRACT

Background: Exposure to phthalates, one kind of widely used plasticizers, has been demonstrated to be associated with some clinical hematological changes in circulatory system from animal studies and in vitro experiments, but their relations to hemostatic and hematologic changes in human are unknown.
Objectives: We explored the relationships of urinary phthalate metabolites with clinical hemostatic and hematologic parameter changes in pregnant women.
Methods: The present study population included 1482 pregnant women drawn from an ongoing prospective birth cohort study in Wuhan, China. Eight urinary phthalate metabolites and eight blood clinical parameters, including activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), fibrinogen (Fg), total white blood cell counts (WBC), red blood cell counts (RBC), hemoglobin (Hb), and platelet counts (PLT) were measured in the late third trimester. The associations between phthalate metabolites and blood parameters were analyzed using general linear model. The odds ratios (ORs) for anemia during pregnancy associated with phthalates were also explored by using logistic regression models.
Results: After adjustment for false discovery rate, a significantly negative association between ln-transformed urinary mono-ethyl phthalate (MEP) concentration and blood Fg, and a positive association between urinary mono-butyl phthalate (MBP) and APTT were found in this study. Higher concentrations of mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP) were associated with lower Hb concentrations. In addition, higher levels of MEHP, MEOHP and MECPP were also associated with increased likelihood of anemia. No significant associations were found between phthalates and other hematologic parameters.
Conclusions: Higher urinary phthalate metabolites in late third trimester were associated with prolonged blood clotting time, decreased Hb concentrations, and increased likelihood of anemia in pregnant women. Further research is needed to replicate the observed findings and clarify the potential biological mechanism.

1. Introduction

Normal pregnancy is accompanied by physiological changes in maternal coagulation and fibrinolytic systems and hematology, which are aimed to allow adequate nutrients transfer from mother to fetus and minimize intrapartum blood loss (Yeomans and Gilstrap, 2005). Compared to nonpregnant women, significant hematological changes in pregnancy include expanded blood volume, relatively decreased hemoglobin (Hb) concentration, increased red blood count (RBC), white blood cell count (WBC) and fibrinogen (Fg) and decreased platelet count (PLT) (Hill and Pickinpaugh, 2008).

In population-based studies, some environmental and occupational contaminants, such as particulate matter, O_3 and SO_2, were associated with plasma hemostatic markers, including Fg, factor VIII-C and platelet aggregation (Liao et al., 2005; Rudež et al., 2009). Arsenic, lead, benzene and 1,1-dimethydrazine were reported to have toxic effects on

* Corresponding authors at: School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, People's Republic of China.
E-mail addresses: xiawei@hust.edu.cn (W. Xia), xust@hust.edu.cn (S. Xu).

https://doi.org/10.1016/j.envint.2018.07.021
Received 1 March 2018; Received in revised form 27 June 2018; Accepted 14 July 2018

hematopoietic system and cause anemia (Choudhary and Hansen, 1998; Heck et al., 2008; Jain et al., 2005; Rinsky et al., 2002). In addition, abnormal coagulation function in pregnancy is a risk factor for obstetric hemorrhage, and a massive obstetric hemorrhage is responsible for one quarter of the maternal deaths worldwide (McLintock and James, 2011). Furthermore, maternal anemia is also a significant public health problem in pregnant women and fetuses in developing countries (Levy et al., 2005). Pregnancy is accompanied by many physiological hematological changes (Yeomans and Gilstrap, 2005), and environmental pollutants were reported to be associated with certain blood parameters. Thus, it is possible that pregnant women may be more prone to be influenced by environmental factors, leading to hematologic abnormalities.

Phthalates are a group of chemical compounds that have been extensively used as plasticizers to impart the flexibility of synthetic polymers, polyvinyl chloride in particular (Rahman and Brazel, 2004). Phthalates are widely detected in water, soil, food, indoor and ambient air, as well as human biological specimens, because the chemicals can be easily released from various plastic products or plastic containers, such as inflatable toys, food packaging bags, blood product storage bags and personal care products (CDC, 2009). Phthalates are considered endocrine disrupting chemicals that pose a great risk on human health (Latini et al., 2004). The reproductive and developmental toxicity of phthalate esters attracts a major concern among public (Kay et al., 2013; Witorsch and Thomas, 2010). Besides, animal and in vitro studies have provided some evidence that phthalates could induce hematologic changes, including elevated activated partial thromboplastin time (APTT) (Zhong et al., 2013), PLT (Poon et al., 1997) and WBC (Ahbab et al., 2017), and decreased RBC, Hb (Gray et al., 1977; Poon et al., 1997) and coagulation factor IX (Zhong et al., 2013). However, to our current knowledge, no human study has investigated these associations.

In the present study we aimed to investigate the associations between urinary phthalate exposures and certain hematologic changes in late third trimester, including hemostatic and hematologic parameters, in a population of pregnant women in Wuhan, China. We further assessed the associations of phthalate exposures with the likelihood of anemia in pregnant women.

2. Methods

2.1. Study population

The study population was recruited between December 2013 and October 2015 from the Wuhan Medical and Healthcare Center for Women and Children in Wuhan, China. The pregnant women, who came to the center for delivery were eligible to participate in this study if they satisfied the following conditions: 1) singleton pregnancy; 2) residence in Wuhan and with an expectation to reside in Wuhan for the foreseeable future; 3) donated urine sample before delivery; 4) completed clinical routine blood tests and coagulation function tests; 5) completed the face-to-face interview questionnaire. A total of 1642 pregnant women were enrolled in the study period. Among these women, we excluded 28 women who had a history of medication in the third trimester due to infection (respiratory infection, urinary tract infection and vaginal infection, etc.) because medicine or infection could influence the hematological parameters. We further excluded 132 women who had intravenous fluids or urine catheterization within two weeks before the urine samples were collected because phthalates from medical soft plastic tube may contaminate urine sample. Finally, we included 1482 eligible subjects who had complete urine phthalates measurement data and blood tests in the late third trimester.

2.2. Ethics approval

This study was approved by the ethics committee of Tongji Medical College, Huazhong University of Science and Technology and Wuhan Medical and Healthcare Center for Women and Children, and all mothers provided written informed consent after a full understanding of the research protocols.

2.3. Data and sample collection

Demographic characteristics, pregnancy health care, lifestyle habits and personal/family history of disease of the pregnant women were obtained from a face-to-face questionnaire interview after delivery. Menstrual and pregnancy history and birth outcomes were collected from medical records. Anemia in the population was defined as Hb concentration < 110 g/L in third trimester (WHO, 2008).

The blood and urine samples from participants were collected on the same day after admission into hospital and before delivery. A sample of venous blood from pregnant women was drawn in siliconized vacutainer tube with 1:9 volumes 3.8% sodium citrate for coagulation test. Another venous blood sample was drawn in a tube anticoagulated with edetic acid for routine blood test. Blood samples were tested for relevant clinical hematologic parameters within 2 h after collection. A random spot urine sample (~30 mLs) was collected and transferred into 5 mL polypropylene cups. Urine samples were stored at -20 °C until further analysis for phthalate metabolites.

2.4. Urinary phthalate analysis

The urinary concentrations of phthalate metabolites were measured by solid phase extraction coupled with ultra-performance liquid chromatography–tandem mass spectrometry (SPE-UPLC–MS/MS). Details of the analytical method have been described in our previous article (Zhu et al., 2018). In brief, urine samples (0.5 mL) were mixed with isotope-labeled internal standards (25 uL), β-glucuronidase (25 uL) and 250 μL of ammonium acetate buffer (pH = 6.5), incubated at 37 °C for 90 min in a thermostatic shaking water bath. Then, a mixture of 20 μL formic acid and 500 μL ultrapure water was added and the analytes were extracted by loading onto Hypersep Retain Pep SPE cartridges (3 mL, 60 mg) (Thermo Fisher Scientific Inc., USA). SPE cartridges were conditioned with 2 mL acetonitrile and 2 mL 0.5% formic acid before use. Then urine samples passed through the cartridges and eluted with 2 mL 0.5% formic acid, 2 mL acetonitrile and 1 mL ethyl acetate. The eluents were evaporated under nitrogen to dryness, and the residues were then redissolved in 500 μL acetonitrile/water (1:9, v/v), filtered with 0.22 μm organic filters and transferred to vials. Phthalates were chromatographed on an Acquity UPLC® BEH C18 (1.7 μm, 100 mm × 2.1 mm, Waters, Milford, MA, USA) column. Analysis was performed on an Acquity TQD mass spectrometer equipped with an Acquity UPLC system (Waters, Milford, MA, USA) in negative-ion electrospray ionization mass spectrometry and multiple reactions monitoring mode. Eight phthalate metabolites were targeted for measurement, including mono-methyl phthalate (MMP), mono-ethyl phthalate (MEP), mono-butyl phthalate (MBP), mono-benzyl phthalate (MBzP), mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP).

For each batch of thirty urine samples, we included calibration standards, reagent blanks, field blanks and quality control of high and low concentrations. The average recovery of isotope-labeled internal standard was 95.8 ± 14.4% for D_4-MMP, 92.9 ± 10.3% for D_4-MEP, 89.9 ± 7.6% for D_4-MBP, 84.9 ± 5.3% for D_4-MBzP, 84.8 ± 7.5% for D_4-MEHP, 83.7 ± 5.3% for $^{13}C_4$-MEHHP, 87.2 ± 3.8% for $^{13}C_4$-MEOHP and 87.9 ± 2.7% for $^{13}C_4$-MECPP, respectively. Recoveries of all target compounds corrected by internal standard ranged from 88.2 ± 5.2% to 105.9 ± 3.9%. The limits of detection (LOD) were 0.5 μg/L for MMP, MEP, MBP and MEHP, 0.1 μg/L for MBzP, 0.2 μg/L for MEHHP, MEOHP, and MECPP. The intraday precision for all phthalates were between 2.5% and 9.9%, and interday precision were between 7.0% and 13.7%.