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Perfluoroalkyl substances and endometriosis-related infertility in Chinese women

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ABSTRACT

Endometriosis is one of the main causes for female infertility. Previous studies suggested that perfluoroalkyl substances (PFASs), a group of ubiquitous environmental chemicals with properties of endocrine disruption and reproductive toxicity, were risk factors for endometriosis but there lacks direct evidence on the possible role of PFASs in endometriosis-related infertility. To fill this gap, we examined the association between PFASs and endometriosis-related infertility among Chinese reproductive-age women in a case-control study, which comprised 157 surgically confirmed endometriosis cases and 178 controls seeking infertility treatment because of male reproductive dysfunction in 2014 and 2015. Blood specimens were collected at the enrollment and analyzed for ten PFASs. Logistic regression was utilized to estimate the adjusted odds ratios (OR) and 95% confidence intervals (CI) for individual PFAS compound. Plasma concentrations of perfluorobutane sulfonic acid (PFBS) were associated with an increased risk of endometriosis-related infertility (second vs. lowest tertile: OR = 3.74, 95% CI: 2.04, 6.84; highest vs. lowest tertile: OR = 3.04, 95% CI: 1.65, 5.57). This association remained consistent when we restricted to subjects with no previous pregnancy (second vs. lowest tertile: OR = 2.91, 95% CI: 1.28, 6.61; highest vs. lowest tertile: OR = 3.41, 95% CI: 1.52, 7.65) or to subjects without other gynecologic pathology (second vs. lowest tertile: OR = 4.65, 95% CI: 2.21, 9.82; highest vs. lowest tertile: OR = 3.36, 95% CI: 1.58, 7.15). Plasma concentrations of perfluoroheptanoic acid (PFHpA), perfluorohexane sulfonic acid (PFHxS) and perfluorononanoic acid (PFNA) were inversely associated with endometriosis-related infertility, but the associations were attenuated in the sensitivity analyses. Our preliminary evidence suggests that exposure to PFBS may increase the risk of female infertility due to endometriosis. Future prospective studies are necessary to confirm these findings.

1. Introduction

Perfluoroalkyl substances (PFASs) are synthetic fluorinated chemicals that have been used in a variety of consumer and industrial products such as surfactants, household cleaning products, textiles, paints, fire-fighting foams and food packaging due to their unique hydrophobic and lipophobic nature (Lehmler, 2005). PFASs, especially perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), are commonly detected in wildlife and human bodies (Houde et al., 2006). PFASs are highly stable and resistant to degradation, allowing them to bioaccumulate in food chain, which is considered as the primary source of human exposure to PFASs (Lindstrom et al., 2011; Vestergren and Cousins, 2009). In 2009, PFOS and its precursors were listed as "restricted use" compounds by the Stockholm Convention on Persistent Organic Pollutants (POPs) (UNEP, 2009). However, a large amount of these chemicals are still being manufactured and used in China (Paul et al., 2009).

There is an increasing concern about the potential adverse developmental and reproductive effects of PFASs. Experimental studies demonstrate that PFASs have estrogenic properties in vitro and can adversely affect the reproductive system in laboratory animals by disrupting the function of nuclear hormone receptors, interfering with steroidogenesis, and altering the expression of endocrine-related genes (Henry and Fair, 2013; Du et al., 2013a, 2013b; Kraugerud et al., 2011). Thus, there is biologic plausibility that PFASs may affect hormone-dependent diseases such as endometriosis.

Endometriosis is a common gynecologic disorder characterized by the growth of endometrial glands and stroma outside the uterine cavity. Approximately 6% to 10% of women at reproductive age suffer from endometriosis and associated clinical symptoms including chronic pelvic pain, dysmenorrhea and infertility (Missmer and Cramer, 2003). Endometriosis is one of the leading causes of infertility, accounting for 30% to 50% of female infertility (Macer and Taylor, 2012). Although its etiology

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remains unknown, a growing body of evidence supports the role of environmental chemicals such as dioxins, organochlorine pesticides, polychlorinated biphenyls and phthalates in the development of endometriosis (Heilier et al., 2005; Louis et al., 2005; Cooney et al., 2010; Reddy et al., 2006). Despite its ubiquity, limited research has focused on PFASs as a potential risk factor for endometriosis. Louis et al. (2012) first reported that PFOA and perfluorononanoic acid (PFNA) were associated with endometriosis. Another study conducted in a nationally representative sample of the U.S. population also observed positive associations of PFOA, PFNA, and PFOS with endometriosis (Campbell et al., 2016). Likewise, several epidemiologic studies (Vélez et al., 2015; Fei et al., 2009), but not all (Vestergaard et al., 2012; Whitworth et al., 2012), found that higher levels of exposure to some PFASs were associated with an increased risk of reduced fecundity.

The current study aimed to examine the association between PFAS exposure and endometriosis-related infertility among Chinese women, where common PFASs such as PFOS and PFOA are still being manufactured and used in a large quantity and new generation PFASs are increasingly present.

2. Material and methods

2.1. Study population

This study was based on a case-control study assessing the relationship between environment pollutants and female infertility in China. Women who were 20–45 year old and came to the Women's Hospital Affiliated to Zhejiang University School of Medicine for treatment of infertility from 2014 to 2015 were potentially eligible for the study. Those who had chromosome abnormalities were excluded. A total of 173 women who were diagnosed of endometriosis-related infertility were selected as cases. Infertility was defined as having unprotected intercourse for >12 months and cannot conceive spontaneously. The diagnosis of endometriosis was confirmed by surgical visualization using laparoscopy. 196 women who had no reproductive endocrine disorders seeking infertility treatment because of male reproductive dysfunction during this period were selected as controls. There were no specific matching criteria for controls. 16 cases and 18 controls failed to provide blood samples, leaving 335 subjects (157 cases and 178 controls) for final analysis. None of the participants had hysterectomy at time of recruitment. A written informed consent was obtained from each participant. The study protocol was approved by the Ethics Committees of Shanghai Xinhua Hospital affiliated with Shanghai Jiao Tong University School of Medicine and Zhejiang University School of Medicine.

2.2. Data collection

Participants were given an in-person interview by a trained interviewer using a standardized questionnaire to collect a range of information, including demographic factors (age, occupation, education, family income), reproductive and menstrual history (age at menarche, history of pregnancy, menopausal status, and gynecologic surgeries), family history, and lifestyle behaviors (including current alcohol consumption and smoking). Medical information such as anthropometric variables (height and weight), history of contraception, gynecological examination and chromosome analysis was obtained from medical records.

A 10 mL blood sample was drawn from each participant. All blood specimens were centrifuged at 4000 rpm for 10 min afterwards. The plasma was then separated and stored at −80 °C until analysis.

2.3. Plasma PFAS measurements

Plasma samples were analyzed for ten PFASs: perfluorododecanoic acid (PFDoA), perfluoroundecanoic acid (PFUA), perfluorodecanoic acid (PFDA), PFNA, perfluorooctane sulfonamide (PFOSA), PFOS, PFOA, perfluoroheptanoic acid (PFHpA), perfluorohexane sulfonic acid (PFHxS), perfluorobutane sulfonic acid (PFBS). The analytical method was described in detail previously (Wang et al., 2016). Briefly, the plasma samples were extracted using the protein precipitation extraction method with $^{13}C_4$-PFOS and $^{13}C_4$-PFOA spiked as internal standards. Calibration standards were prepared by spiking blank fetal bovine serum with the standard mixture of 10 analytes and isotope labeled internal standards.

Analyte separation was quantified using liquid chromatography system coupled with tandem mass spectrometry (HPLC-MS/MS, Agilent 1290-6490, Agilent Technologies Inc., USA). A 2-μL aliquot of the sample was injected into a ZORBAX Eclipse Plus C18 column (2.1 × 100 mm, 1.8 μm; Agilent, USA) maintained at 35 °C and equipped with a C18 pre-column (2.1 × 5 mm, 1.8 μm). The instrument was operated in the electrospray ionization (ESI) negative mode with multiple reaction monitoring (MRM). Quantification was performed using the internal-standard method. The calibration curve covering the concentrations ranging from 0.5 ng/mL to 100 ng/mL with six points exhibited good linearity with correlation coefficients ≥0.99 for all the compounds. Procedural blank analysis was conducted using newborn fetal bovine plasma for each batch composed of 20 samples. Trace levels of PFNA were detected in procedural blanks. Therefore the sample concentrations for this compound were subtracted from blank values. The accuracy (% mean recovery) and precision tests were evaluated by replicating analysis of samples at a low (1.6 ng/mL) and a high concentration (80 ng/mL). The recoveries of PFASs ranged from 80% to 110%, and intra- (n = 5) and inter-day (n = 15) calibration variations were <10% for all the analytes (see QA/QC results from Wang et al., 2016). The limit of detection (LOD), defined as a signal-to-noise ratio of 3, ranged from 0.009 ng/mL (PFBS) to 0.12 ng/mL (PFOSA) for the selected PFASs. Due to the low detection rate (<20% of the samples), PFOSA was not included in the analysis.

2.4. Statistical analysis

Descriptive statistics and comparisons of baseline characteristics by case status were performed using the Chi-square test and Wilcoxon rank-sum test. We summarized the distribution of PFAS compounds in the cases and controls using the median and interquartile range (IQR). The values below the LOD for PFBS were set to a value of LOD divided by the square root of 2 (Hornung and Reed, 1990). Differences in the plasma concentrations of PFASs were compared between cases and controls with Wilcoxon's nonparametric test.

To investigate the relationship between plasma PFAS levels and endometriosis-related infertility, unconditional logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI). Given that the exposure-disease relationship may not be monotonically linear, PFAS concentrations were categorized into tertiles to model each PFAS analyte as a set of indicator variables, with the lowest tertile serving as the referent. Based on findings from previous studies and our univariate analysis, we identified the following variables as potential confounders and adjusted for in the multivariable logistic regression: age, body mass index (BMI), household income, and education. The vast majority of women were non-smokers and non-drinkers, and these two variables were not considered for adjustment. Given the purpose of infertility treatment, none of the participants used oral contraceptive in recent periods, thus oral contraceptive use was not adjusted as a confounder. Since parity may be a consequence rather than a determinant of endometriosis, we did not include parity as a covariate in order not to induce potential over-adjustment (Louis et al., 2012). To test the trend across categories of PFAS concentrations, a continuous variable coded as the median tertile concentration of each exposure category was included in the adjusted logistic regression model. Each PFAS compound was modeled separately.

Household income and education, two covariates adjusted in the statistical models, were missing in a large percentage of participants. To propagate the uncertainty in missing data estimations, multiple

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