The association of bisphenol A exposure with premature ovarian insufficiency: a case–control study

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ABSTRACT

Background: A few epidemiological investigations and animal studies have demonstrated that bisphenol A (BPA) may affect female reproductive health. However, no epidemiologic study has investigated the relationship between BPA exposure and the risk of premature ovarian insufficiency (POI).

Methods: In this case–control study, urinary concentrations of BPA and serum levels of reproductive hormone were measured. Associations between BPA concentrations and the risk of POI and POI-related hormone levels were estimated.

Results: Among BPA quartiles, no obvious association was found between BPA levels and the risk of POI (p = 0.603). Although the adjusted odds ratio (OR) of POI was slightly increased for participants in the highest BPA concentration quartile, the association was not statistically significant (OR = 1.282, 95% confidence interval [CI] 0.615–2.049 for the highest vs. lowest quartile, p = 0.508). Although follicle stimulating hormone (FSH) and anti-Mullerian hormone (AMH) levels showed no tendency of an association with BPA (p = 0.941 and p = 0.876 for FSH and AMH, respectively), the highest quartile of luteinizing hormone was significantly positively associated with BPA levels (OR = 1.333, 95% CI 0.986–1.803, p = 0.042).

Conclusions: The urinary concentrations of BPA determined in this study were consistent with the range of exposure currently observed in Chinese women. However, BPA exposure at a relatively low level is not associated with POI in Chinese women. Further epidemiological studies are needed to confirm our findings.

Introduction

Bisphenol A (BPA) is extensively used as a synthetic plasticizer in the manufacture of polycarbonate plastics, epoxy resins, and polystyrene. Over 3.5 million tons of BPA are produced globally, with more than 100 tons released into the environment every year. Under exposure to ultraviolet light, basic or acidic solutions, and heat, BPA can leach into food and beverages of consumers, resulting in pervasive human exposure to BPA. Indeed, BPA was detected in more than 95% of urine samples from the general US population. It has also been detected in the ovarian follicular fluid (an average of 2.0 ng/ml), which indicates that oocytes may be exposed to BPA during folliculogenesis.

BPA has been recognized as a typical endocrine-disrupting chemical due to its hormone-like activities. Several recent epidemiological studies have shown that BPA exposure is associated with a variety of reproductive dysfunctions and endocrine abnormalities in women. For example, serum BPA concentrations were found to be more than 30% higher in women with polycystic ovary syndrome (PCOS), and correlated positively with total testosterone, free testosterone, androstenedione, and dehydroepiandrosterone sulfate levels, suggesting that BPA may be involved in the pathogenesis of PCOS. BPA concentrations above the limit of detection were also reported to be significantly more represented in the infertile female population than in healthy women. Moreover, high urinary concentrations of BPA are associated with inverse oocyte maturation, low antral follicle count, peak estradiol, and a reduction in the number of oocytes retrieved. These findings raise concerns that environmental BPA exposure may adversely affect ovarian function, although the magnitude of the actual risk remains unknown.

Previous animal studies have reported that BPA mimics estrogen action and interferes with hormonal homeostasis, resulting in a spectrum of adverse reproductive outcomes. For instance, BPA exposure caused a significant decrease in primordial follicles and an increase in primary, secondary, and antral follicles in mouse ovaries. Lee et al. observed that 17β-estradiol synthesis was decreased.
via downregulation of steroidogenic acute regulatory protein and aromatase cytochrome P450. BPA exposure also limited expansion of the primordial germ cell population and reduced the primordial follicle pool in the fetal mouse ovary16, with a consequent decrease in the primordial follicle reserve and an increased incidence of premature ovarian insufficiency (POI) in adult female mice17. POI, a common heterogeneous endocrine disorder that affects 1% of women, is characterized by a loss of ovarian function before the age of 40 years and is considered one of the leading causes of female infertility18. Although the pathophysiology of POI is still unclear, it appears to be complex and multifactorial. Environmental factors are thought to contribute to the risk of POI19,20.

In this case–control study, urinary concentrations of BPA and serum levels of POI-related hormones (follicle stimulating hormone [FSH], luteinizing hormone [LH], and anti-Mullerian hormone [AMH]) were measured in 159 POI patients and 186 healthy control women. Associations of BPA concentrations with the risk of POI and reproductive hormone levels were estimated using logistic regression models.

Methods

Study population

In the present study, 159 women were enrolled among those who visited the Department of Gynecology at Women’s Hospital of Zhejiang University School of Medicine in Hangzhou, China for irregular menstruation or amenorrhea and were diagnosed with POI from January 2015 to September 2018. POI was diagnosed according to the European Society of Human Reproduction and Embryology guidelines21, including the following: under 40 years of age at first diagnosis; oligomenorrhea or amenorrhea for at least 4 months; and an increased FSH level >25 IU/l on two occasions >4 weeks apart. Patients with explicit causes of POI (such as karyotypic abnormalities, previous chemotherapy and/or radiotherapy, ovarian surgery, or autoimmune diseases) and hormone therapy for at least 3 months were excluded.

A total of 186 controls were selected from women who visited our hospital for routine check-up with long-term regular menstrual cycles and were matched for age and body mass index. Women with endocrine system diseases (such as hypothyroidism, hyperthyroidism, hyperprolactinemia, PCOS, or thyroid disease) or taking any hormonal therapy in the previous 6 months were excluded.

The Ethics Committee of Women’s Hospital of Zhejiang University approved this case–control study. Informed consent was obtained from all participants.

Data and specimen collection

All participants were interviewed by trained medical staff using a standardized questionnaire about basal information including sociodemographic characteristics (e.g. age, occupation, education, house income), gynecological situation (menarche age, menstrual and reproductive history), lifestyle factors (organic food intake, bottled drinks), and medical histories (history of radiotherapy, chemotherapy, surgery, contraception, chromosome analysis).

A single-spot urine sample was collected upon entry into the study before any medical interventions. The samples were stored in clean glass containers (without BPA) at −80°C until analysis. Venous blood of POI patients was collected at first diagnosis; for controls, to evaluate basal levels of FSH, LH, and AMH, collection occurred during the early follicular phase of the menstrual cycle. The serum was placed in a polypropylene tube and stored at −80°C for further analysis.

Urinary BPA analysis

The urine concentration of BPA was used as an index of exposure to BPA. The chemical analysis procedure was performed as described in previous studies, with slight modifications22–24. Briefly, β-glucuronidase was added to 2 ml of urine sample with isotopically labeled BPA (Cambridge Isotope Laboratories, Inc., Tewksbury, MA, USA) to liberate conjugated metabolites. After liquid–liquid extraction with ethyl acetate, the concentrated extracts were reconstituted in 200 μl of a water and acetonitrile (80:20, v/v) mixture. The extract was analyzed using an ultra-performance liquid chromatography–triple quadrupole mass spectrometry system (Xevo TQ-S; Waters, Milford, MA, USA) with a Waters BEHC18 column (2.1 mm × 50 mm, 1.7 μm). The mobile phase was water and acetonitrile containing 0.05% ammonia at a constant flow rate of 0.3 ml/min. The limit of detection (LOD) was 0.014 μg/l, which was calculated as the 3:1 signal versus noise value. The mean recoveries for BPA ranged from 69.0% to 120.5%. Artificial urine was used in each batch to monitor potential contamination during sample analysis for quality control and quality assurance. The entire sample analysis procedure was carried out in a glass container to avoid BPA contamination. The concentration of creatinine in the urine was used to adjust the effects of urine dilution. Urinary creatinine levels were measured using a commercial diagnostic enzyme kit according to the manufacturer’s instructions (Oxford Biomedical Research, Rochester Hills, MI, USA). If the creatinine concentration was below 0.1 mg/dl, the urine sample was considered too dilute to be analyzed accurately.

Reproductive hormone measurement

Serum levels of LH, FSH, and AMH were determined using an automated Roche Modular Analytics E170 immunoassay system (Roche Diagnostics, Mannheim, Germany). The LODs were 0.10 IU/l for LH, 0.10 IU/l for FSH, and 0.001 ng/ml for AMH. The inter-assay and intra-assay coefficients of variation for all of the tested hormones were 6.25% and 8.33%, respectively. All hormone assays were repeated three times.

Statistical analyses

All statistical analyses were conducted using R 3.6.2 software. Demographic statistics are presented to describe the basic
Table 1. Demographic characteristics of POI cases (n = 159) and controls (n = 186).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n = 186)</th>
<th>POI (n = 159)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at enrollment (years)</td>
<td>33.58 ± 5.72</td>
<td>34.48 ± 6.35</td>
<td>0.17</td>
</tr>
<tr>
<td>Age at menarche (years)</td>
<td>13.77 ± 1.23</td>
<td>14.00 ± 1.76</td>
<td>0.18</td>
</tr>
<tr>
<td>Menopausal age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16–29</td>
<td>26 (17.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30–39</td>
<td>111 (75.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40–45</td>
<td>10 (6.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>23 (12.4)</td>
<td>14 (8.9)</td>
<td>0.561</td>
</tr>
<tr>
<td>18.5–24.0</td>
<td>135 (73)</td>
<td>121 (76.6)</td>
<td></td>
</tr>
<tr>
<td>&gt;24.0</td>
<td>27 (14.6)</td>
<td>23 (14.6)</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Elementary school</td>
<td>22 (11.8)</td>
<td>48 (31)</td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>18 (9.7)</td>
<td>53 (34.2)</td>
<td></td>
</tr>
<tr>
<td>College and beyond</td>
<td>146 (78.5)</td>
<td>54 (34.8)</td>
<td></td>
</tr>
<tr>
<td>Annual family income (yuan)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5000</td>
<td>0 (0)</td>
<td>12 (7.7)</td>
<td></td>
</tr>
<tr>
<td>5000–30,000</td>
<td>7 (3.8)</td>
<td>33 (21.3)</td>
<td></td>
</tr>
<tr>
<td>30,000–100,000</td>
<td>51 (27.4)</td>
<td>58 (37.4)</td>
<td></td>
</tr>
<tr>
<td>&gt;100,000</td>
<td>128 (68.8)</td>
<td>52 (33.5)</td>
<td></td>
</tr>
<tr>
<td>Bottled drinks</td>
<td></td>
<td></td>
<td>0.067</td>
</tr>
<tr>
<td>Never</td>
<td>13 (7)</td>
<td>23 (14.6)</td>
<td></td>
</tr>
<tr>
<td>&lt;1 bottle per week</td>
<td>156 (83.9)</td>
<td>115 (73.2)</td>
<td></td>
</tr>
<tr>
<td>1–2 bottles per week</td>
<td>8 (4.3)</td>
<td>11 (7)</td>
<td></td>
</tr>
<tr>
<td>3–5 bottles per week</td>
<td>8 (4.3)</td>
<td>5 (3.2)</td>
<td></td>
</tr>
<tr>
<td>&gt;5 bottles per week</td>
<td>1 (0.5)</td>
<td>3 (1.9)</td>
<td></td>
</tr>
<tr>
<td>Organic food</td>
<td></td>
<td></td>
<td>0.908</td>
</tr>
<tr>
<td>Never</td>
<td>15 (8.1)</td>
<td>13 (8.5)</td>
<td></td>
</tr>
<tr>
<td>Sometimes</td>
<td>129 (69.4)</td>
<td>102 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Always</td>
<td>36 (19.4)</td>
<td>34 (22.2)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6 (3.2)</td>
<td>4 (2.6)</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as mean ± standard deviation or N (%). BMI, body mass index; POI, premature ovarian insufficiency.

characteristics of the POI cases and controls. Values of BPA below the LOD were set as LOD/√2. Most urinary BPA data were clustered in the lower range, with only a few having large values. Therefore, the distributions of BPA were described as a central tendency (geometric mean [GM], median), spread (interquartile range), and extreme (95th percentile). Due to the skewed nature of the data, differences in BPA and reproductive hormone levels between the two groups were examined using the Mann–Whitney U-test. Univariate analysis was conducted to select candidate variables with p < 0.2 in the association with risk of POI using a binary logistic regression model. The associations between the risk of POI and urinary BPA were assessed using both categorized and log-transformed BPA to solidify the conclusion. Multinomial logistic regression was performed to evaluate the relationship between reproductive hormone quartiles and urinary BPA concentrations while controlling for the adjusted factors selected earlier. All tests were two-sided, and a α level of p < 0.05 was used to indicate statistical significance.

Results

Characteristics of participants and urinary concentrations of BPA

The baseline demographic characteristics of the case and control groups are presented in Table 1. Urinary BPA concentrations were measured in 159 POI women and 186 control women. There was no significant difference in the mean age and distribution of body mass index or the intake frequency of organic food and bottled drinks. However, compared with POI cases, most of those in the control group has a college education, and 68.8% of them lived a life with an annual household income over 100,000 yuan, which was twice the percentage for the POI cases.

The serum reproductive hormonal parameters of the case and control groups are shown in Supplementary Table S1. As expected, all participants in the POI case group had a lower median level of the control group was 6.648 IU/l (p < 0.001). Women with POI also had significantly higher LH (p < 0.001) and lower AMH (p < 0.001) levels.

Table 2 summarizes the distribution and percent detection of urinary BPA concentrations (both unadjusted and adjusted for creatinine). BPA was detected at frequencies of 78.62% in the POI case group and 74.19% in the control group. The GM of urinary BPA for the POI case samples was 0.236 μg/l (0.355 μg/g creatinine), and the control samples had slightly lower BPA concentrations (0.213 μg/l and 0.322 μg/g creatinine). However, there was no significant difference between the POI cases and controls (Mann–Whitney U-test, p = 0.991 and p = 0.899, respectively, for volume-based and creatinine-adjusted BPA concentrations).

Univariate analysis was conducted to select candidate variables in the association with risk of POI using a binary logistic regression model (Supplementary Table S2). Age at enrollment, age of menarche, higher education, and annual household income were observed to correlate significantly with POI risk.

Association between BPA exposure and POI

The association of BPA exposure with POI risk was assessed using binary logistic regression (Table 3). When compared with the lowest quartile of urinary BPA concentrations, there was no significant association between BPA exposure and the risk of POI in the unadjusted model, despite a slight increase in the odds ratio (OR) from the second to the highest quartile (OR = 1.259, 95% confidence interval [CI] 0.691–2.304 for the second quartile; OR = 1.261, 95% CI 0.694–2.3 for the third quartile; OR = 1.121, 95% CI 0.615–0.2049 for the highest quartile; p for the trend = 0.943). After adjustment, the 95% CIs of the ORs still straddled the value of 1, suggesting that no increased risk of POI was associated with a higher quartile of BPA (OR = 1.161, 95% CI 0.691–2.304 for the second quartile; OR = 1.176, 95% CI 0.694–2.3 for the third quartile, OR = 1.282, 95% CI 0.615–2.049 for the highest quartile; p for the trend = 0.603). Besides, we also re-performed the logistic model using the log-transformed BPA, which was considered a continuous variable. However, no significance was observed.

Correlation between BPA concentrations and serum hormone levels

Next, the association of urinary BPA levels with reproductive hormones was examined using multinomial regression...
models (Table 4). FSH, LH, and AMH were stratified in these analyses due to their skewed distribution in the whole study population. According to the ORs, FSH and AMH levels exhibited no tendency to be associated with urinary BPA concentrations ($p$ for the trend in the unadjusted model $= 0.678$ and 0.77 for FSH and AMH, respectively; $p$ for the trend in the adjusted model $= 0.941$ and 0.876 for FSH and AMH, respectively), whereas LH approached marginal significance for being positively associated with urine BPA levels after adjustment for confounders ($p$ for the trend $= 0.086$). In the adjusted model, compared with the lowest quartile of LH levels, there was a 33.3% induction in odds of being in the highest quartile of LH levels per unit increase in urinary BPA concentrations, with a significantly positive association (OR $= 1.333$, 95% CI 0.986–1.803, $p = 0.042$).

### Discussion

Numerous studies have assessed urinary concentrations of BPA in the general population worldwide. The GM urinary concentration of BPA measured in our control participants (0.322 µg/g creatinine) was very similar to that detected among 952 general Chinese population participants without occupational exposure (GM $= 0.38$ µg/g creatinine)\(^25\). However, the GM of urine BPA in our subjects was approximately four times lower than that of a group of 210 females from the Third National Health and Nutrition Examination Survey (NHANES III) callback cohort in the USA (1.35 µg/g creatinine)\(^4\) and was approximately three times lower than that of a study consisting of 188 early-pregnancy Mexican-American women (1.1 µg/g creatinine)\(^26\). In addition, the urinary BPA level among a Korean female cohort aged 19–40 years was 1.05 µg/g creatinine\(^27\), which was much higher than our results. In general, these data suggest the existence of geographic differences in exposure to BPA, with different diet and lifestyle. Chinese women may have a lower overall exposure to BPA than people in developed countries\(^25\).

To date, this is the first epidemiologic study to assess POI in relation to BPA exposure. Previous epidemiological studies have mainly explored the association of BPA and ovarian function in women, but with contradictory results. Much of the available human research conducted to date consisted of women seeking assisted reproductive technologies. One epidemiological study of women undergoing in vitro fertility treatments indicated that urinary BPA levels were not associated with ovarian volume but correlated negatively with antral follicle counts, accelerated follicle loss, and reproductive aging\(^9\). However, another human study did not find an association between BPA levels and the number of oocytes retrieved per cycle in individuals undergoing infertility treatments\(^28\). Animal experimental data on the effects of BPA exposure on ovarian function have also shown conflicting results. For example, exposure of female rats to BPA initiated excessive premature activation of primordial follicles not only in immature animals but also in mature mouse ovaries via the PTEN signaling pathway\(^29\).

Administration of BPA to female rats stimulated both initial recruitment of the primordial follicle pool and subsequent follicular development, with
accelerated folliculogenesis, resulting in a significantly decreased number of primordial follicles, which is the main phenotype of POI. BPA exposure at relatively higher doses and different time points also affected ovarian follicle numbers and sex steroid levels in rats. Conversely, another recent animal experiment indicated few or no effects on reproductive function among rats administered BPA in the diet. In addition, oral exposure of adult female mice to BPA at 50 μg/kg body weight per day for the first three reproductive cycles (12–15 days) did not impact any parameters related to ovulation. Similarly, oral administration of low-dose BPA to superovulated female mice did not affect oocyte retrieval, meiotic maturation, or aneuploidy, and low-dose BPA administered to mice on postnatal days (22–28 days) did not reduce germinal vesicle breakdown. These results indicate that BPA appears to affect ovarian function only at relatively high concentrations of exposure and not at low concentrations. Therefore, the lack of association in our study may have resulted from the relatively low concentrations of BPA in women, as the urinary concentration of BPA in our study population was significantly lower than the reported values for women in many developed countries. It is possible that BPA contributes to the risk of POI only at concentrations higher than those observed in our population.

BPA is known to have weak estrogenic activities. It has been suggested that BPA may affect hypothalamic–pituitary–ovarian axis function. FSH and LH are gonadotropin hormones produced by pituitary gonadotropes and are essential for the development of antral follicles. AMH, a hormone produced by ovarian follicles, is a biomarker of ovarian reserve. This study quantitatively assessed the relationship between urinary BPA concentration and ovarian function-related hormones in women. In agreement with our observations, a previous study found that BPA exposure was associated with increased LH in healthy women. Among women seeking fertility treatment or with ovarian dysfunction in two small studies (n = 41 and n = 74), BPA exposure was found to not be associated with FSH levels. Another study reported that BPA was negatively associated with AMH and FSH levels, but neither reached statistical significance in women among infertility with PCOS. These findings suggest that BPA does not have significant effects on ovarian function-related hormones in women.

The present study has some strengths. First, this was a case–control study with a relatively large sample size considering that the morbidity of POI was approximately 1%. Previous epidemiological studies usually included only a few dozen POI cases. Second, the availability of detailed questionnaire information enabled the adjustment for potential confounding factors. Nonetheless, there are also some limitations worth mentioning. The post-diagnostic assessment of exposure levels is a limitation that is characteristic of all retrospective case–control studies of biomarkers and disease. Namely, the mode of action of BPA in some reproductive tissues may be highly dependent on the window of exposure, and it would have been better to evaluate BPA exposure prior to disease onset than at the time of diagnosis. Moreover, a single measurement design may be ineffective at detecting an association between POI and BPA exposure because BPA is a non-persistent chemical with a urinary elimination half-life of less than 6 h. Regardless, previous studies have proven that the single spot-sampling approach may adequately reflect the average exposure of the population to BPA.

In conclusion, the results of this study suggest that the current BPA burden may not be associated with the risk of POI in Chinese adults. Further evaluation in population-based studies conducted in occupational or environmental settings is needed to assess associations with greater BPA exposure.

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Potential conflict of interest No potential conflict of interest was reported by the authors.

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et al.


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