

A case–control study of bisphenol A and endometrioma among subgroup of Iranian women

Batool Hossein Rashidi, Massoud Amanlou¹, Tahere Behrouzi Lak², Mahya Ghazizadeh, Bita Eslami

Vali-e-Asr Reproductive Health Research Center, Tehran University of Medical Sciences, ¹Department of Medicinal Chemistry, Faculty of Pharmacy, Drug Design and Development Research Center, Tehran University of Medical Sciences, Tehran, ²Department of Obstetrics and Gynecology, Reproductive Health Research Center, Urmia University of Medical Sciences, Urmia, Iran

Background: Endometriosis is a multifactorial hormonally related complex disease with unknown etiology. Epidemiologic data were suggested the possible effects of endocrine disrupting chemicals such as bisphenol A (BPA) on endometriosis. BPA is similar to endogenous estrogen and has the ability to interact with estrogen receptors and stimulate estrogen production. Our aim was to evaluate the relationship between urinary BPA concentrations in women with endometrioma. **Materials and Methods:** This case–control study consisted of fifty women who have been referred to gynecology and infertility center with endometrioma and were candidates for operative laparoscopy and ovarian cystectomy as cases. Fifty women who had not any evidence of endometrioma in clinical and ultrasound evaluation and came to the same clinic for routine check-up were selected as controls. One-time urine sample was collected after receiving informed consent before surgery and medical intervention. Total BPA in urine was measured with high-performance liquid chromatography method and detection limit was 0.33 ng/mL. **Results:** Percentage of urine samples containing BPA was 86% of cases and 82.4% of control. Urinary BPA showed a right-skewed distribution. The mean concentration of BPA was 5.53 ± 3.47 ng/mL and 1.43 ± 1.57 ng/mL in endometriosis and control group, respectively ($P < 0.0001$, Mann–Whitney U-test). The logistic regression showed that the odds ratio of the BPA was 1.74 (95% confidence interval: 1.40–2.16) after adjustment of age, parity, body mass index <30 , and educational status. **Conclusion:** This study showed a positive association between urinary BPA concentrations and endometrioma. However, further large-scale studies are needed to confirm this hypothesis.

Key words: Bisphenol A, endometrioma, high-performance liquid chromatography

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INTRODUCTION

Endometriosis is a multifactorial hormonally related complex disease, characterized by endometrial-like tissue growing outside of the uterus. This ectopic endometrium responds to hormonal signaling and is manifested by dysmenorrhea. One of the most common complications of this disease is reduced fertility and infertility. Although it has been estimated to affect 5% of general population, its prevalence may be as high as 30% in infertile women.^[1] Endometrioma usually presents as a pelvic mass arising from the growth of ectopic endometrial tissue within the ovary. The pathogenesis of endometriosis is still unknown.

Different theories were described the endometriosis pathogenesis. The latest advances in the pathogenesis of endometriosis were based on the embryological theory. The similarity of the presence of ectopic endometrium in female fetus in the same anatomical locations found in the adult patient with endometriosis suggests embryogenetic theory of endometriosis pathogenesis.^[2,3]

In the last decades, epidemiologic data found some evidence that environmental chemicals may effect on reproductive endocrine system. These chemicals are called endocrine disrupting chemicals (EDCs), which have been described as “exogenous chemical substances or mixture that alters the structure of function(s) of the endocrine system and causes adverse

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Address for correspondence: Miss. Bitā Eslami, PhD Candidate, Vali-e-Asr Reproductive Health Research Center, Second Building of Vali-e-Asr, Imam Khomeini Hospital, Keshavarz Blv., Tehran, Iran. E-mail: b-eslami@razi.tums.ac.ir

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effects."^[14] Among the EDCs, bisphenol A (BPA) is one of the highest volume chemicals produced worldwide and used by the manufacturers of plastics and epoxy resins which are pervasive in our environment and our daily lives.^[5] They are used for polycarbonate bottles and containers, food and drink cans, medical devices and dental fillings. It seems that the human exposure to BPA can be through the diet by leaching from plastics containers and lining in cans.

BPA is similar to endogenous estrogen and has the ability to interact with estrogen receptors, stimulate estrogen production, and also alter gonadotropin hormone secretion.^[6-8]

The Endocrine Society suggested the strong evidence of EDCs for adverse reproductive outcomes.^[6,9] Meanwhile, several studies confirmed the effects of BPA on reproductive indices.^[10-13]

Animal study by Signorile *et al.* was reported the presence of endometriosis-like phenotype in female offspring of mice exposed to BPA in the uterus,^[14] and it supports the embryogenetic pathogenesis of endometriosis.

Previous epidemiological studies evaluating the relationship between urinary or serum BPA concentrations and endometriosis had equivocal results^[15-18] due to critical data gaps and short elimination half-life of BPA.

The possible action of BPA as a toxicant and actual risk for human remains unclear. Hence, further studies are needed. Especially the lack of data about EDCs in women in Iran was the motivation for doing this study.

The aim of our investigation was to evaluate the relationship between BPA levels in urine samples of women with endometrioma who were candidates for operative laparoscopy in comparison to controls.

MATERIALS AND METHODS

Study design and population

This case-control study was approved by the Institutional Review Board of Tehran University of Medical Sciences (No. 18630). We received informed consent from all participants. They were informed that participation included an interview, pelvic and clinical examination, and transvaginal ultrasound.

All cases were selected from women who were referred to gynecology and infertility center with ovarian endometrioma from other centers and were candidates for operative laparoscopy and ovarian cystectomy.

The control group was selected from women who had clinical file in the center due to previous problem and came for routine checkup and Pap smear.

The study samples were interviewed by a trained midwife about basal information and symptoms of endometriosis. All participants had a pelvic examination and transvaginal ultrasound in lithotomic position (HS-2600, Honda Electronic Co., Ltd., Japan) with 12.5 MHz.

After confirmation of the endometrioma in cases, they entered into our study. The control group had no symptom of current or previous endometrioma in clinical and sonographic examination.

Meanwhile, if both groups had no characteristics such as polycystic ovarian syndrome, uterine fibroma, diabetes mellitus, metabolic and endocrine disorders, history of cardiovascular disease, blood pressure more than 140/80 mmHg, renal failure, body mass index (BMI) >30, neoplastic disorders, and smoking, they included in the study.

The participants were asked to collect a first morning urine sample before surgery and any medical intervention. The samples were transferred into a special tube (without BPA compound) within 1 h and stored at -70°C until analysis.

For sample size calculation, we used the Upson *et al.*'s study^[18] that was about the evaluation of BPA and risk of endometriosis. The proportion of controls above the median value was about 50% and we estimated in cases will be about 75%. Therefore, we calculated that fifty samples would be required in each group with a power of 80% and $\alpha = 0.05$ using the Epi Info website (www.cdc.gov/epiinfo). We collected fifty samples in each group from volunteers living in Tehran (capital city of Iran) from June 2013 to September 2014.

Urinary bisphenol A analysis

The samples were analyzed at Pharmaceutical Science Research Center of Tehran University of Medical Sciences. Total BPA (conjugated and free) in urine was measured using high-performance liquid chromatography (HPLC) based on the modified methods of Yang *et al.*^[19] and He *et al.*^[20] In brief, the reaction mixtures of phosphorous acid buffer, β -glucuronidase (Sigma), and sample aliquots in glass tubes were incubated for hydrolyzation at 37°C and then were extracted twice with ether (HPLC grade, Merck). The supernatants were collected and evaporated with stream of nitrogen gas. The residue was dissolved in 60% acetonitrile (HPLC grade, Merck) and analyzed by HPLC on the following parameters: a KNAUER

liquid chromatograph (KNAUER, Germany) with RF-20A prominence fluorescence detector with excitation wavelength 275 nm and emission wavelength 300 nm and a ChromGate software version 3.3 (KNAUER, Germany) used for data processing. Column, Chromolith® Performance RP-18e, 5 µM, liquid chromatography column 100 × 4.6 mm; 20 µl injection loop, mobile phase A and B, acetonitrile/water (40:60, v/v), equivalent grade; and flow: 1.0 mL/min. HPLC water was from Millipore Super-Q Plus Water Purification System (Bedford, MA, USA). The limit of detection (LOD) was calculated with the method recommended by the Environmental Protection Agency (2004). The LODs of BPA in urine were 0.33 µg/L.

Statistical analyses

Statistical analyses were performed using the SPSS software (SPSS, version 16, SPSS, Inc., IL, USA). To estimate the relationship between urinary BPA concentrations and endometriosis, we estimated the odds ratio (OR) and 95% confidence interval (CI) using logistic regression. We perform analysis with adjustment for age, BMI, parity, and education. These variables were selected prior to inclusion on the basis of evidence of an association from the literature. We substituted the concentration of BPA below the LOD by a value equal to the LOD divided by two. Urinary BPA concentration had no normal distribution and showed a right-skewed distribution. Data are expressed as arithmetic and geometric mean ± standard deviation (mean ± SD) and number (percentile). Statistical significance for differences was tested by Student's *t*-test or Mann–Whitney U-test depends on normal distribution of continuous data. Categorical data differences were compared by Chi-square test. A $P < 0.05$ was considered statistically significant.

RESULTS

The participants were aged 22–45 years (median, 32). Descriptive analysis of the group characteristics in Table 1 shows that total characteristics of both groups were similar ($P > 0.05$).

BPA was detected in 86% (43/50) of cases and 82.4% (42/51) of control samples. Based on Table 2, the mean concentration of BPA was 5.53 ± 3.46 ng/mL and 1.42 ± 1.56 ng/mL in endometrioma and control group, respectively. The differences were statistically significant ($P < 0.0001$, Mann–Whitney U-test). Geometric mean and geometric SD, median, and interquartile range are manifested in Table 2.

Logistic regression analysis showed a positive association between endometrioma and BPA level with OR 1.74 (95% CI: 1.40–2.16) after adjustment for age, BMI, parity, and education status. The result of crude OR and adjusted OR was fairly similar [Table 3].

Table 1: Total characteristics of study participants

Variables	Endometrioma group (n=50)	Control group (n=50)	P
Age at enrollment (years)	32.22±5.34	33.20±5.46	0.37
Age at menarche (years)	13.28±1.40	13.20±1.67	0.80
Menstrual cycle length (days)	30.09±4.56	29.80±4.43	0.76
Bleeding duration (days)	6.35±1.68	5.84±1.54	0.12
BMI (kg/m ²)	25.07±4.00	25.18±4.06	0.91
Parity			0.80
Nulliparous	40 (80%)	41 (82%)	
Multiparous	10 (20%)	9 (18%)	
Education			0.06
Diploma and lower	38 (76%)	45 (90%)	
Higher diploma	12 (24%)	5 (10%)	

Values reported as Mean±standard deviation, or number (%). BMI=Body mass index
P value refers to student *t*-test and Chi-squared when appropriate

Table 2: Comparison of BPA urinary level between endometrioma and control group

Group	Mean±SD	GM±GSD	Median	Inter-quartile range
Case	5.53±3.46	3.14±0.34	6.41	2.86-7.89
Control	1.42±1.56	0.72±0.23	1.10	0.19-1.98

All values are reported as ng/mL

Table 3: Results of logistic regression analyses for the relationship between urinary BPA and endometrioma

	Crude OR (95% CI)	P	Adjusted OR (95%CI)*	P
BPA level	1.75 (1.41-2.17)	<0.001	1.74 (1.40-2.16)	<0.001

*Analysis with adjustment for age, body mass index (BMI), parity and education

DISCUSSION

In this study, the BPA urinary concentrations among women with endometrioma were statistically higher in compared with control group. Meanwhile, it seems that the confounding factors have not any effect on the result of logistic regression analyses.

The results of the study by Cobellis *et al.*^[17] which was accordant to our result suggested a positive relationship between bisphenol and endometriosis based on the measurement of serum samples. They reported that at least one of the bisphenols (A or B) was found in 63.8% of sera of 58 patients with endometriosis and they did not find BPA in serum samples from healthy women.

However, in the present study, the detection rate of the total urine BPA, in both groups (86% vs. 82.4%), was higher than the study by Cobellis *et al.*^[17] maybe due to differences of type of samples (urine vs. serum).

This supports the hypothesis that BPA is metabolized quickly and excreted in urine without evidence of accumulation within body.^[21] Hence, we believe that measurement of BPA

in urine sample provides a better estimation of exposures than measurement of these compounds in serum. This is because of short-lived nature of BPA in serum or plasma and risk of contamination arising during sample collection or analysis.

A major advantage of the study by Cobellis *et al.*^[17] was selection of cases and controls after operative laparoscopy in patients who had evidence of ovarian cysts or chronic pelvic pain and dysmenorrhea. Therefore, study groups' selection was more reliable.

Two previous epidemiologic studies which were measured the BPA in urine samples reported no statistically significant association of urinary BPA concentrations and endometriosis^[15,16] and their results are contradictory with our findings. These differences are due to some factors such as evaluation all type of endometriosis based on their severity without the separation of results based on ovarian and nonovarian endometriosis, laboratory analyses error, and intra-individual variation in BPA exposure.

In a recent study by Upson *et al.*, a statistically significant association between total urinary BPA concentrations and nonovarian endometriosis but not ovarian was reported.^[18] These findings are in contrast with our results that show significant association between urinary BPA and endometrioma or ovarian endometriosis.

There is evidence that BPA has adverse effects on the maturing oocytes and meiotic cell division machinery^[22-24] and also may have adverse impact on survival of oocytes, follicle dynamics, ovarian reserve, and infertility in general.^[25] However, animal study did not find any endometriosis lesion in ovaries of mice treated with BPA respect to the control.^[26]

Our data support the idea that exposure to BPA can cause reproductive disorders in women, being the ovary one of the first targets.

As we mentioned, a research group has recently demonstrated the presence of ectopic endometrium in human fetus at different gestational ages and another study revealed that the prenatal exposure of mice to BPA elicits an endometriosis-like phenotype in female offspring.^[3,14] Therefore maybe, exposure to chemicals such as BPA in pregnant women and their fetus causes endometriosis during organogenesis and it manifested during reproductive age. Hence, evaluation of BPA in serum samples of pregnant women and possible transferring to the fetus through placenta should be investigated in a future study.

The results of the present study as the first evaluation of BPA in women's population in Iran manifested higher exposure in endometriosis patients in compared with another study.^[15,16] Hence, more restrict regulation must be designed in our country to prevent the uncontrolled increasing usage of such chemicals, and further investigation is needed to find the origin of these chemicals.

We should also consider that endometriosis is a multifactorial disease with unknown pathogenesis and it is not reliable to conclude only environmental factors such as BPA are the main causative agents.

Our study had some limitations: First, it is possible to confirm ovarian endometriosis by clinical and pelvic examination accompanied by transvaginal ultrasound. However, nonovarian endometriosis can be detected accurately by surgery. We confirmed nonovarian endometriosis in the case group during surgery, but in control selection, it was not feasible to surgically confirm the absence of nonovarian endometriosis because of ethical issue. Second, we did not measure urinary creatinine to evaluate the daily exposure to BPA and urine sample dilution by measuring creatinine has more accurate result. Third, BPA is a nonpersistent chemical with a urinary elimination half-life <6 h^[21] and we measured BPA in only one-time urine sample and it should be better to collect 24-h urine sample. More importantly, it would have been better to evaluate BPA exposure before disease onset than at the time of diagnosis or surgery.

Meanwhile, it should be better to evaluate the relationship between environmental chemicals and endometriosis based on severity. Hence, further large-scale studies with more defined population and innovative methodologies are required to be designed to evaluate the association of other category of endometriosis based on the severity and stages.

CONCLUSION

Although the results of this study manifested the significant relationship between BPA concentrations and ovarian endometriosis in subgroup of Iranian women, the exact role of BPA on the reproduction is still not clear and epidemiologic data on human health effects are limited.

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Conflicts of interest

There are no conflicts of interest.

AUTHORS' CONTRIBUTION

BHR contributed in the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. MA contributed in the conception of the work, experimental analysis, drafting and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. TBL contributed in the conception of the work, conducting the study, literature search, data acquisition, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. MGH contributed in the conception of the work, conducting the study, data acquisition, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. BE contributed in the conception of the work, conducting the study, analysis, writing paper, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

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