The Association Between Bisphenol A and Polycystic Ovarian Syndrome: A Case-Control Study

Batool Hossein Rashidi[1], Massoud Amanlou[2], Tahere Behrouzi Lak[3], Mahya Ghazizadeh[1], Fedyeh Haghollahi[1], Maryam Bagheri[4], and Bita Eslami[5]

[1] *Vali-e-Asr Reproductive Health Research Center, Tehran University of Medical Sciences, Tehran, Iran*

[2] *Department of Medicinal Chemistry, School of Pharmacy, Drug Design and Development Research Center, Tehran University of Medical Sciences, Tehran, Iran*

[3] *Department of Obstetrics and Gynecology, Reproductive Health Research Center, Urmia University of Medical Sciences, Urmia, Iran*

[4] *Department of Reproductive Health, School of Nursing and Mediwifery, Tehran University of Medical Scinces, Tehran, Iran*

[5] *Breast Diseases Research Center (BDRC), Tehran University of Medical Sciences, Tehran, Iran*

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Abstract- Polycystic ovarian syndrome (PCOS) is an endocrine, metabolic disorder with unclear etiopathogenesis among reproductive-age women. Evidenceshows genetic susceptibility and environmental factors were associated with PCOS. The aim of this study was to find the association between urinary concentrations of Bisphenol-A as an endocrine disrupting chemical (EDC) and PCOS. A case-control study was conducted in 51 samples in each group. All cases were selected from women who diagnosed with PCOS at gynecology and infertility center. The control group was selected from women who had clinical file in the center due to the previous problem and came for routine check-up and pap smear. The participants were asked to collect a first-morning urine sample before any medical interventions. Total BPA in urine wasmeasured by High-Performance Liquid Chromatography (HPLC) method. Comparison of BPA level between two groups shows the significantly higher level in PCOS group compared with control group (3.34±2.63 vs. 1.43±1.57 ng/mL, P<0.001). Using logistic regression analysis, BPA as the main dependent variable was significantly associated with PCOS with adjusted Odds Ratio (OR) equal to 1.53 (95% CI: 1.14-2.05, P=0.004). The results of this study indicated that BPA might play a major role in the PCOS pathogenesis. Further investigations with better design are necessary to confirm this association.

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Keywords: Polycystic ovarian syndrome; Bisphenol A; Case-control study; HPLC

Introduction

Polycystic ovarysyndrome (PCOS) is an endocrine, metabolic disorder that affects 7.1-14.6% of reproductive-aged women of different geographic regions of Iran using different criteria (1). Women with PCOS are at increased risk of infertility (2), endometrioma carcinoma, hyperinsulinemia, and type 2 diabetes (3). PCOs characterized by two of the following three criteria by the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM): clinical and/or biochemical hyperandrogenism, chronic oligomenorrhea and/ or anovulation or the present of polycystic ovaries on transvaginal ultrasonography (4). There is some evidence that genetic susceptibility has been associated with PCOS and environmental factors such as environmental pollutants, diet and geography have important role in the expression of those genetic traits (5,6). Endocrine disrupting chemical (EDCs) are defined by the Environmental Protection Agency (EPA) as: "exogenous agents that interface with the synthesis, secretion, transport, metabolism, binding action, or elimination natural blood-borne hormones that are present in the body and responsible for homeostasis, reproduction and development process" (7). 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 (8).

Corresponding Author: B. Eslami
Breast Diseases Research Center (BDRC), Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 61192509, Fax: +98 21 66581526., E-mail address: dr.bes.96@gmail.com

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 gonadotrophin hormone secretion (9-11). The pathophysiology of PCOS is still not clear and seems to be multifactorial. Clinical and experimental evidence indicates that BPA may play an important role in the PCOS pathogenesis via several pathways and may also cause epigenetic changes which in certain environmental predispose to the development of hormonal disturbances typical for PCOS (12). Data from animal studies have been proven that BPA can directly stimulate androgen synthesis in the ovarian theca-interstitial cells (13) and can also interact with the human sex hormone binding globulin (SHGB) and displace sex steroids from SHGB and therefore increase the free testosterone level (14). Meanwhile, the urinary BPA, as well as serum BPA concentrations in women with PCOS, was associated with obesity and insulin resistance (15), and animal studies reported that BPA could also increase insulin secretion (16). Meanwhile, the neonatal exposure to high doses of BPA in rats was associated with increased testosterone and estradiol levels, reduced progesterone in adulthood and development of polycystic ovarian syndrome (17). However, more studies about human exposure are needed to determine the relevance of these finding to human health.

Therefore, recently many researchers focus on finding the relationship between gynecologic and endocrine disease and environmental factors such as BPA. Some evidence found positive association between serum concentrations of BPA and PCOS (18-20). However, another study that was investigated urinary BPA level did not find any association between BPA level and PCOS (21) may be due to the difference of evaluated matrix.

Therefore, the aim of the present study was to investigate an association between urinary concentration of BPA and PCOS among subgroup of Iranian women.

Materials and Methods

Study design and population

Institutional Review Board of Tehran University of Medical Sciences (No: 25278) was approved this case-control study. Informed consent was obtained from all participants. They were informed that participation included an interview, pelvic and clinical examination and transvaginal ultrasound evaluation.

All cases were selected from women who diagnosed with PCOS at Gynecology and infertility center. The control group was selected from women who had clinical file in the center due to the previous problem and came for routine check-up and pap smear.

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

After confirmation the PCOS in cases they entered to our study. The control group had not any symptoms of current or previous PCOS in the clinical and sonographic examination.

Both groups included in the study if they had not reported any complications such as endometriosis, uterine fibroma, diabetes mellitus, history of cardiovascular disease, blood pressure more than 140/80 mmHg, renal failure, neoplastic disorders,and smoking.

The participants were asked to collect a first-morning urine sample before any medical interventions. The samples were transferred into a special tube (without bisphenolA compound) within 1 hour and stored at -70º C until analysis.

For sample size calculation, we used the Kandaraki *et al*., 2011 paper (19) that was evaluated the serum level of BPA in women with PCOS. By considering the mean and standard deviation in both groups, we calculated that 44 samples would be required in each group with a power of 90% and α=0.05 by using the Epi Info Web site (www.cdc.gov/epiinfo). We collected 51 samples in each group from volunteers living in Tehran (capital city of Iran) from September 2013 to September 2014.

Urinary BPA analysis

The samples were analyzed at Pharmaceutical Science Research Center of Tehran University of Medical Sciences. Total BPA (conjugated and free) in urine were measured with High-Performance Liquid Chromatography (HPLC) based on the modified methods of Yang *et al*., (22) and He *et al*., (23). In brief, the reaction mixtures of the phosphorous acid buffer, β-glucuronidase (Sigma) and sample aliquots in glass tubes were incubated for hydrolyzation at 37º 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, was used for data processing. Column, Chromolith® Performance RP-18e, 5 µM, LC Column 100 x 4.6 mm; 20 µl injection loop,mobile phase A and B, acetonitrile/water (40:60, v/v), equivalent grade; flow: 1.0 mL/min. HPLC water was from Millipore Super-Q Plus water purification system (Bedford, MA).The limit of detection (LOD) was calculated with the method recommended by EPA (EPA, 2004). The LODs of BPA in urine was 0.33 µg/L.

Statistical analyses

Statistical analyses were performed using the SPSS software (SPSS, version 16, SPSS, Inc., IL, USA). In order to estimate the relationship between urinary BPA concentrations and PCOS, we estimated the Odds ratio (OR) and 95% confidence interval (CI) using logistic regression. We perform analysis with adjustment for age, body mass index (BMI), parity, menstrual irregularity, history of abortion, and education. These variables were selected prior for inclusion on the basis of bivariate analyses and 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 not 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 of differences we tested by student's t-test or Mann-Whitney U-test depends on thenormal distribution of continues data. Categorical data differences were compared by Chi-square test. A *P* less than 0.05 were considered as statistically significant.

Results

Table 1 represented the baseline characteristics of two groups. As we expected BMI was significantly higher in PCOS group in comparison with control group. Meanwhile, in PCOS group menstrual cycle length is longer than thecontrol group (*P*<0.001). Control group was older than PCOS group (*P*=0.01).

Table 1. Basal characteristics of participants

Variables		PCOS (N = 51)	Control (N = 51)	*P* *
Age (yrs)		29.80 ± 7.02	32.96 ± 5.58	0.01
BMI (kg/m^2)		26.45 ± 3.72	24.86 ± 3.91	0.04
Age at menarche (yrs)		13.45 ± 1.24	13.22 ± 1.65	0.42
Menstrual cycle length (days)		37.36 ± 14.58	29.67 ± 4.52	0.001
Bleeding duration (days)		6.20 ± 1.43	5.82 ± 1.53	0.21
Education	**Diploma and lower**	43 (84.3%)	46 (90.2%)	0.39
	Higher Diploma	8 (15.7%)	5 (9.8%)	
Parity	**Nulliparous**	37 (72.5%)	43 (84.3%)	0.23
	Multiparous	14 (27.5%)	8 (15.7%)	

**P* refers to student t-test or chi-square when appropriate

Detection rate (value higher than LOD) was 88% and 82% in PCOS and control group, respectively. Comparison of BPA level between two groups shows the significantly higher level in PCOS group (3.34±2.63 vs. 1.43±1.57 ng/mL, *P*<0.001) in compared with control group. Geometric mean (GM) and SD were significantly higher in PCOS patients, too (1.79±8 vs. 0.81±2.92, *P*<0.001). Table 2 shows someone with irregular menstrual cycle had higher level of BPA (*P*=0.02). Meanwhile, BPA levels is higher in younger and more educated women. However, the difference was not statistically significant. The results manifested the BPA level was related to BMI. Correlation by Spearman's rho test showed BPA level was significantly correlated with BMI (r=0.26, *P*=0.008 (Data are not shown).

Table 3 shows, OR and 95% CI of BPA in relation to PCOS with using logistic regression analysis. As the main dependent variable, BPA manifested with OR equal to 1.53 (95% CI: 1.14-2.05, *P*=0.004), which was

significantly associated with PCOS. As we expected, menstrual irregularity has significant relation with PCOS, too.

Table 2. Determinants of BPA in the study sample

Variable		N	Mean±SD	*P**
Age (yrs)	**18-25**	18	3.03 ± 2.19	0.23
	26-34	53	2.21 ± 2.42	
	35≥	31	2.30 ± 2.37	
BMI (kg/m^2)	**<18.5**	3	1.31 ± 0.47	0.08
	18.5-24.9	43	1.82 ± 2.10	
	25-34	54	2.83 ± 2.49	
	35≥	2	4.15 ± 4.04	
Parity	**Nulliparous**	80	2.33 ± 2.42	0.36
	Multiparous	22	2.58 ± 2.16	
Education	**Lower Diploma**	51	2.26 ± 2.42	0.68
	Diploma	37	2.48 ± 2.25	
	Higher Diploma	13	2.77 ± 2.59	
Menstrual Regularity	**Yes**	55	1.66 ± 1.47	0.02
	No	47	3.24 ± 2.89	
History of Abortion	**Yes**	20	2.16 ± 2.06	0.66
	No	82	2.44 ± 2.44	

**P* refers to mann whitney U-test and kruskal wallis test

Table 3. The results of logistic regression analysis

Crude Analysis		ß	SE	OR	95% CI	*P*
BPA level		0.45	0.12	1.56	1.24-1.97	<0.001
***Adjusted Analysis**	**BPA level**	0.42	0.15	1.53	1.14-2.05	0.004
	Menstrual Irregularity	2.36	0.52	10.16	3.83-29.38	<0.001

*Analysis with adjustment for age, BMI, parity, education, menstrual irregularity and history of abortion. ß = constant, SE = Standard Error, OR = Odds Ratio, CI = Confidence Intervals

Discussion

The results of the present study manifested BPA as endocrine disrupting chemicals maybe are one of the new risk factors for the development of PCOS.

A few studies have investigated the relationship between BPA and PCOS. The results of our study were consistent with other studies those were evaluated the BPA in serum samples, and they found serum BPA level is significantly higher in PCOS women compared to women without PCOS (19,24). However, our results were not similar to another study which was evaluated the urinary BPA level to find the association with PCOS (21).

These differences may be due to analysis technique. In this study, we analyzed the urine samples with HPLC method, whereas they measured urinary concentrations of this toxicant using the gold standard detection technique, isotope dilution mass spectrometry (25). In other studies which evaluated the serum levels, they used ELISA kit with inadequate analytical selectivity and specificity (19,24). Based on some investigation, ELISA is not suitable method for quantitative determination of BPA in clinical specimens (20).

BPA is metabolized quickly and excreted in urine without evidence of accumulation within the body (26).

So 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.

Additionally, urinary BPA levels positively correlated with BMI in the present study, which was similar to another study (18). Our investigation shows the urinary BPA level was statistically significant in women who had irregular menstrual cycle. This may be due to the effects of BPA on hormonal levels such as total and free testosterone level, androstenedione, and dehydroepiandrosterone sulfate (DHEA-S) (18). Unfortunately, we have not complete data of hormonal levels of participants, and we cannot evaluate the correlation between hormonal level and urinary BPA concentrations. Although one study reported polychlorinated biphenyls (PCBs) as an endocrine disrupting chemical, was associated with menstrual cycle abnormalities (27).

We should also consider that PCOS is a multi-factorial disease with unknown pathogenesis and it is not reliable to conclude only environmental factors such as BPA is the main causative agent. Human is exposed to many chemicals at once. It is therefore difficult to evaluate the true association between environmental pollutants and disease.

Our study had some limitations: firstly; we did not measure urinary creatinine to evaluate the daily exposure of BPA and urine sample dilution by measuring creatinine has more accurate result. Secondly; BPA is a non-persistent chemical with a urinary elimination half-life less than 6 hours (21). Therefore, a single measurement design may be ineffective in detecting the association between PCOS and pollutants that metabolized quickly, and it should be better to collect 24-hour urine sample. Thirdly; we have not sufficient data about thehormonal profile of patients. So, it was not possible to analysis the correlation between hormonal levels and BPA. More importantly, it would have been better to evaluate BPA exposure prior to disease onset than at the time of diagnosis.

In conclusion, although the results of this study manifested the significant relationship between BPA concentrations and PCOS 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. Menwhile, 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.

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