Incrimination of Bisphenol A in Egyptian females diagnosed with Polycystic Ovary Syndrome

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Abstract

Background: Bisphenol A (BPA) is an organic compound that is widely used in the production of plastic substances. It is thought to play a role in the pathogenesis of many diseases, including Polycystic Ovary Syndrome (PCOS).

Aim: Determination of the association between urinary BPA level and PCOS in Egyptian females.. **Subjects and methods**: The current study included 120 female subjects, 60 patients diagnosed with PCOS based on Modified Rotterdam's Criteria and 60 healthy controls. All included females were subjected to full history taking, clinical examination and pelvi-abdominal ultrasound examination. Panel of laboratory assay included FSH, LH, fasting blood glucose, fasting insulin level, HOMA-IR, DHEAs and free testosterone. Urinary BPA was measured in all subjects using UHPLC in-house method. **Results:** Body mass index was significantly higher in PCOS than control group (p value <0.01). Regarding laboratory findings, LH, LH/FSH ratio, HOMA-IR, DHEAs and free testosterone all showed significantly higher levels in PCOS compared to healthy controls. Urinary BPA was significantly higher in PCOS than control group whether adjusted to urinary creatinine or not with p values 0.04 and 0.17 respectively. **Conclusion**: Levels of BPA were significantly higher in PCOS cases than controls.

Keywords: PCO, BPA, H

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Introduction

Polycystic ovary syndrome (PCOS) is considered to be the most common endocrine disorder in women of reproductive age. It is a multifactorial condition with serious impacts on emotional, physical, and mental health [1]

The prevalence of PCOS is about 15-20% according to the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine (ESHRE/ASRM) diagnostic criteria [2].

Polycystic ovary syndrome is characterized by increased androgen levels, ovulatory dysfunction and polycystic ovarian morphology. Clinical features of PCOS may include obesity, hirsutism, alopecia, acne, irregular menses, infertility and hypertension [3].

The environmental role in PCOS pathogenesis has recently gained more attention and environmental endocrine disruptors (EEDs) have been proposed to play a role in the pathogenesis as they have the ability to interfere with hormone sensitivity [4].
Bisphenol A (BPA) is one of the most common and strong EEDs in the environment. It is utilized in the production of polycarbonate plastics and epoxy resins, which are employed in various everyday products such as water bottles, containers, food and beverage can resin linings, electronic equipment, dental sealants and many other substances [5,6].
Bisphenol A levels were reported to be higher in females with PCOS compared to healthy individuals and could possibly be involved in one of many underlying causes of this disorder [7].
This case control study tried to evaluate whether urinary BPA levels in PCOS patients have a relation to the occurrence of the disease or not.

Material and methods

Study design and population

This was a prospective case control clinical study carried out at Clinical and chemical pathology Department Kasr Alainy Hospitals, Faculty of Medicine, Cairo University. The study was conducted on 120 females attending to Assisted Fertility Clinic, Kasr Alainy Hospitals, 60 patients of mean age 26.8 ± 4.2 years (range 16–36 years) diagnosed with polycystic ovary syndrome were included in this study and 60 apparently healthy females without menstrual cycle disturbances of average age 28.08 ± 3.55 (range 19–34 years) as control group.
PCOS was diagnose based on modified Rotterdam criteria [8] all patients had at least two of following three criteria symptoms: clinical and/or biochemical hyperandrogenism, chronic anovulation and ultrasound picture of polycystic morphology of the the ovary. Clinical hyper-androgenemia was diagnosed based on the Prescence of acne, hirsutism [modified Ferriman–Gallwey score (mFG ≥6)].Biochemical hyperandrogenemia was diagnosed by elevated levels of free testosterone (FT) and/or dehydroepiandrosterone sulfate (DHEA-s). Chronic anovulation was defined by menstrual cycle of < 21 or > 35 days, simultaneously with the progesterone levels ≤6 ng/ml estimated within days 20–23of the ovarian cycle. The presence of more than 12 follicles on classic ultrasonography or ovarian volume more than 10 ml indicates presence of polycystic ovarian morphology. Exclusion criteria included Patients diagnosed with congenital adrenal hyperplasia (CAH), thyroid dysfunction, Cushing's syndrome, hyperprolactinemia and androgen-secreting tumors. All patients included in the study had serum 17-OH progesterone, thyroid stimulating hormone and prolactin measured to exclude late onset CAH, thyroid dysfunction and hyperprolactinemia respectively.
All subjects participating in the study signed written consent, and the study was approved by the Ethical Committee of the Faculty of Medicine, Cairo University. and has given it serial number; (MD-65-2020).

Methods:

Anthropometric parameters such as weight, height, and waist circumference were determined in all women. Body mass index (BMI) was calculated in each subject being expressed as kg/m2.

Fasting blood and urinary samples were collected in an early follicular phase of menstrual cycle (between days 2–5of the menstrual cycle, or whenever in those with amenorrhea). Serum levels of the follicle-stimulating hormone (FSH), luteinizing hormone (LH), free testosterone (FT), dehydroepiandrosterone sulphate (DHEAS) were evaluated in all women diagnosed with PCOS. In addition fasting serum glucose and insulin were measured in all subjects. HOMA IR was assessed by count: HOMA-IR=(glucose × insulin)/405. Hormonal levels of FSH (reference range 1.4–9.9 IU/l), LH (reference range 1.7–15 IU/l), DHEAS (reference range 35–380 µg/dl) and insulin (reference range 2–25 µIU/ml) were determined using chemiluminescent immunoassay (**Siemense Atellica IM module[1]**). Free testosterone was assayed by radio-immuno assay using (**DIA source[2])**. Reference range for FT 0 –2.85 pg/ml.

Urine specimens were collected from all included subjects in clean containers marked with the subject coding number, where blood containing samples or turbid samples were excluded. Afterward, 5 ml urine from each sample was taken in a plain glass tube and preserved at -20 C to time of BPA extraction.1 ml urine from each sample was taken for urinary creatinine assay on **Siemense Atellica chemistry module**.

Urinary total BPA was assayed using A high-pressure isocratic system consisting of a **Thermofisher Dionex UltiMate 3000 UHPLC**[3], pump, autosampler, column compartment and fluorescence detector, Chromatographic column reversed-phase 150mm× 4.6 mm C18, particle size 5u.

Extraction Phase and sample injection:

500 µl of each urine sample were added to a 15 ml glass tube, then 30 µl of 2.0 M sodium acetate (pH 5.0) were added to each urine sample. β-glucuronidase was added to each urine sample to analyze total urinary BPA. After incubation, HCl was added and the mixture was extracted with ethyl acetate that included 20 ng/mL bisphenol B as internal standard. After the extraction, the supernatant was transferred to a new glass tube and the solution was evaporated by Eppendorf concentrator. Several modifications were tried to improve resolution by changing mobile phase relative composition, mobile phase flow rate and the liquid used for reconstituting the sample residue after evaporation. The mobile phase was prepared by mixing acetonitrile, tetrahydrofuran and water (35:35:130, 70:35:95) in the gradient mode. Column temperature 25°C, injection time 5 minutes and flow rate 1.5 ml/min. Detection was done by fluorescent detector with excitation at 275 nm and emission at 300 nm. Methanol was injected after each sample for column wash.

Siemens Healthcare Diagnostics Inc.511 Benedict Avenue. Tarrytown, NY 10591 USA[1]
DIA source Immunoassay S.A.– Rue du bosquet, 2–b 1348 louvain-la-Neuve, Belgium[2]
Thermofisher Im Steingrund 4-6, 63303 Dreieich. Germany [3]

Validation of the method of BPA:

The calibration curve for the determination of BPA levels in urine was tested up to 50ng/ml and was linear over the range of 0.5-50 ng/mL. Precision was determined in five replicates of three concentrations (0.5, 1.5 and 5 ng/mL), the largest coefficient of variation (CV%) of the assays was 12% for 1.5 ng/mL. Linearity was assessed using linear regression analysis as well

as spiked urine samples containing BPA at various concentrations (calibrators) within the 0.50–50 ng/mL range for BPA.

The calibration curve was constructed by plotting the ratio of the (area under the curve (AUC) of BPA to the AUC of IS) against BPA concentrations. The slopes and intercepts were calculated with the data's least square linear regression analysis. Seven different concentrations (0.5, 1, 1.5, 2, 5, 10, and 50 ng /mL) of urine-based calibrators were injected into the UHPLC column after applying all the current modifications, and a calibration curve was plotted (figure 1).

The limit of detection (LOD) was 0.50 µg/L, which was comparable with previous studies. The levels of BPA were adjusted for urinary creatinine concentration – BPA ug/g creatinine.

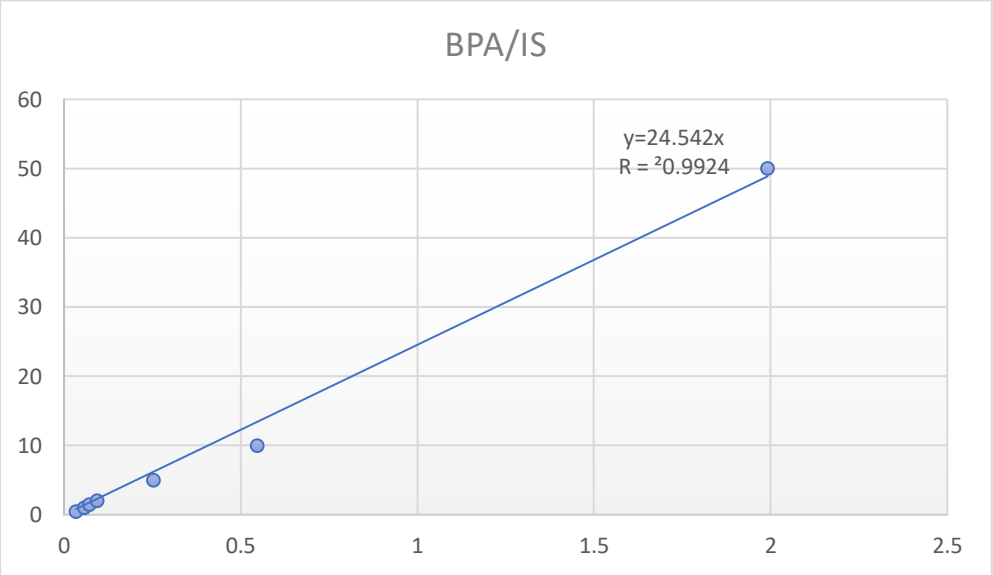

Fig (1): BPA calibration curve**.**

Statistical analysis

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data were summarized using mean, standard deviation, median, minimum, and maximum for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done using unpaired t test when comparing 2 groups and an analysis of variance (ANOVA) with multiple comparisons post hoc test when comparing more than 2 groups [9]. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5 [10]. Correlations between quantitative variables were done using the Pearson correlation coefficient [11]. P-values less than 0.05 were considered as statistically significant.

Results

As expected, females with PCOS had higher BMI than healthy females p value <0.01

We found that 43.3% of cases had hirsutism and 56.7% didn't have hirsutism, while in control group none of the females had hirsutism.
There was a statistically significant difference between both groups regarding presence of hirsutism p value <0.01.

As shown in table (1), a statistically significant difference was found between both groups in LH/FSH ratio, HOMA-IR, FT and DHEA-s.
There was a statistically significant difference in urinary BPA level between cases and controls (2.07 ± 0.64 µg/L) and (1.8 ± 0.56 µg/L) respectively and p value 0.017, also when BPA was adjusted to urinary creatinine level, there was a significant difference between both groups regarding BPA/Creatinine ratio (1.79 ± 0.51µg/g creatinine) for for cases, (1.61 ± 0.41 µg/g creatinine) for controls and p value 0.04 as shown in table (1)
we found significant positive correlation between BPA/creatinine level and HOMA-IR in PCOS patients with p value = 0.005. we also found a statistically significant difference between presence of hirsutism and free testosterone level in PCOS patients, where patients with hirsutism had a higher mean free testosterone level than patients without hirsutism with p value <0.001.

In patients with PCOS, no significant correlation was found between BPA/creatinine and age, BMI, FSH, DHEAs and FT with p values 0.073, 0.343, 0.336, 0.885 and 0.116 respectively, On the other hand, we found significant positive correlation between BPA/creatinine level and LH and HOMA-IR in PCOS patients with p values 0.027 and 0.005 respectively as shown in table (2)

Test	**Group 1** mean ± SD	**Group 2** mean ± SD	**P value**
FSH mIU/mL	5.13 ± 1.8	5.58 ± 2.06	**0.2**
LH mIU/mL	8.34 ± 4.42	5.56 ± 1.93	**<0.001**
LH/FSH	1.68 ± 0.75	1.13 ± 0.55	**<0.001**
Insulin mU/L	13.39 ± 7.99	7.39 ± 2.55	**<0.001**
Glucose mg/dL	92.6 ± 12.03	91.12 ± 10.63	**0.47**
HOMA-IR	3.11 ± 1.93	1.68 ± 0.64	**<0.001**
DHEAs µg/dL	230.18 ± 83	186.93 ± 57.74	**<0.001**
Free testosterone Pg/mL	2.73 ± 1.27	1.71 ± 0.63	**<0.001**
BPA µg/L	2.07 ± 0.64	1.8 ± 0.56	**0.017**
BPA/Creat µg/g	1.79 ± 0.51	1.61 ± 0.41	**0.04**

Table (1) shows the mean, SD, and p-value of studied laboratory tests of both groups.

	BPA/creatinine	
	r	P value
Age	0.233	0.073

BMI	0.125	0.343
FSH mIU/mL	0.126	0.336
LH mIU/mL	0.285	0.027
LH/FSH	0.212	0.104
Insulin mU/L	0.316	0.014
HOMA-IR	0.360	0.005
DHEAs µg/dl	-0.019	0.885
Free testo pg/mL	0.205	0.116

Table (2) shows r and p values of the relation between clinical and laboratory findings and BPA/creatinine in PCOS patients.

Discussion

Polycystic ovary syndrome is a common endocrine disorder, that is characterized by anovulation, hyperandrogenism, and polycystic ovaries. Bisphenol A is a synthetic compound that is widely used in the plastics industry. It is an endocrine disruptor and has been proven to affect steroidogenesis, folliculogenesis, and ovarian morphology [12]. In agreement with our finding, **Chen et al., (2015)** [13] found a similar result between BMI in PCOS patients and non-PCOS patients with p value <0.001. The association between PCOS and obesity has been established by many epidemiological studies that revealed that the majority of women with PCOS are either overweight or obese [14].

Regarding insulin resistance, we found significant difference in HOMA-IR level between both groups. Patients with PCOS had higher HOMA-IR level than control group with p value <0.001. A study by **Zarei et al., (2021)** [15] also found similar results, where they found higher mean HOMA-IR level in PCOS patients than healthy control group with p value 0.04. Regarding serum androgen levels of DHEAs and FT, we found a statistically significant difference between the two groups with p value <0.001. for both parameters. **Emekci ozay et al., (2016)** [16] found higher DHEAs and FT levels in PCOS patients than healthy control group with p values 0.001 and <0.001 respectively.

In our study, we found a statistically significant difference in urinary BPA level between patients with PCOS and control group with p value of 0.017, also when BPA was adjusted to urinary creatinine level, there was a significant difference between both groups regarding BPA/Creatinine ratio with p value of 0.04.

Similar results were found by **Rashidi et al., (2017)** [17] in a study to asses difference in urinary BPA between patients with PCOS and healthy individuals using HPLC, where higher levels were found in PCOS group with p value <0.001. Another study by **Akgül et al., (2019)** [4] Showed similar findings regarding adjusted urinary BPA level (using HPLC), where levels were significantly higher in females with PCOS compared to the control group with p value = .001. These results of association between BPA and PCOS could be explained by the Prescence of direct stimulatory effect of BPA on ovarian theca cells to secrete androgens, BPA also has the ability to decrease estradiol production in the granulosa cells [18]. BPA has also been shown to interact with SHGB, modulating its bioavailability, displacing sex steroids from binding to SHBG and hence, increasing the amount of free testosterone [19]. In sub-group analysis, we found a statistically significant difference between BPA/creatinine level and

insulin resistance in PCOS patients whereas patients with insulin resistance had higher BPA/creatinine with mean value of 1.98µg/g creatinine and patients without insulin resistance had lower BPA/creatinine with mean value of 1.56 µg/g creatinine (p value < 0.001). In agreement with the current study, PCOS women with high urinary BPA had significantly higher HOMA IR (P = 0.0371) as compared to those with low urinary BPA in a study by **Lazúrová et al., (2020).** Many studies have shown that BPA can increase insulin secretion [21], which may be explained by the presence of direct effect on the pancreas, that is considered a receptor for BPA [19]. This could explain the association between higher BPA levels and insulin resistance.

Conclusion

Urinary BPA levels were higher in PCOS patients compared to normal females, which means that there is an association between urinary BPA and PCOS. This needs more studies to confirm our findings.

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