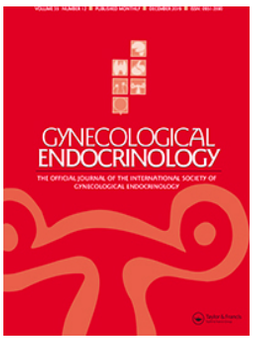

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Bisphenol A and phthalate levels in adolescents with polycystic ovary syndrome

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ABSTRACT

Endocrine disruptors have been proposed in the etiology of polycystic ovary syndrome (PCOS) as they have the potency to interfere with hormone-sensitivity systems. The aim of this study was to evaluate the levels of bisphenol A (BPA) and phtalates in adolescents with PCOS. Sixty-two girls with PCOS and 33 controls, age 12–18 years were enrolled in the study. The diagnosis of PCOS was made using modified Rotterdam criteria. Urinary BPA levels were measured using high-performance liquid chromatography. Di-(2-ethylhexyl)-phthalate (DEHP), the most commonly used phthalate and mono-(2-ethylhexyl)-phthalate (MEHP), its main metabolite were measured by using high-performance liquid chromatography. Adolescents with PCOS had markedly increased BPA levels (15.89 μg/g creatine ± 1.16) when compared with the control group (7.30 μg/g creatine ± 1.38) ($p = .016$). In adolescents with PCOS, BPA was significantly correlated with polycystic morphology on ultrasound but not with obesity androgen levels, or other metabolic parameters. Patients with PCOS (DEHP: 0.40 ppm ± 0.24, MEHP: 0.13 ppm ± 0.23) and controls (DEHP: 0.49 ppm ± 0.27, MEHP: 0.14 ppm ± 0.3) had similar serum phtalate concentrations ($p = .7$ and $p = .3$, respectively). Exposure to specific endocrine disruptors such as BPA could modify neuroendocrine, reproductive, and metabolic regulation favoring PCOS development in adolescents.

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Adolescents; bisphenol A; endocrine disruptor; phthalates; polycystic ovary syndrome

Introduction

The role of the environment in polycystic ovary syndrome (PCOS) pathogenesis has recently received more attention and environmental endocrine disruptors (EEDs) have be proposed in the etiology as they have the potency to interfere with hormone-sensitivity systems. Phthalates and bisphenol A (BPA) are the most abundant EEDs in the environment [1]. These substances are widely used to manufacture polycarbonate plastics, epoxy resins and polystyrene, and are available in a large number of consumer products [2].

BPA is regularly detected in human urine and blood confirming high exposure to these chemicals [43,6]. BPA binds to estrogenic receptors mimicking the action of the estrogen hormone, it can bind to androgen receptors, blocking endogenous androgen action and can also affect steroidogenesis, folliculogenesis, and ovarian morphology [85,10]. Clinical studies have shown that elevated BPA concentrations are observed in both adolescents and adults with PCOS when compared to reproductively healthy women [8].

Di-(2-ethylhexyl)-phthalate (DEHP) is one of the most widespread phthalate plasticizers [11]. Both DEHP and Mono-(2-ethylhexyl)-phthalate (MEHP), the main metabolite of DEHP, has also been detected in high amounts in blood and urine samples from those screened [10]. Phthalates, exert anti-androgenic actions by interfering with steroidogenesis and has been associated with a decrease in infant testosterone concentrations [12], delayed development of pubarchy in girls [13] and potential associations with precocious puberty and premature thelarche [14], so in theory could conceal PCOS findings due to its anti-androgenic effects.

Bearing in mind the high prevalence of PCOS, the existence of an association between exposure to EEDs could have significant impact on public health. Thus, the objective of this case-control study was to determine the levels of bisphenol A and phtalates in adolescents with PCOS. To our knowledge this is the first study to evaluate both the EEDs in adolescents with PCOS.

Materials and methods

Patients and controls

The study took place between March 2016 and March 2018 at Hacettepe University, Division of Adolescent Medicine. Sixty-two patients between the ages of 12 and 18 years diagnosed with PCOS according to the Rotterdam criteria were enrolled into the study [15]. Subjects required any two of the following three criteria to be included in the study: ovulatory dysfunction, clinical and/or biochemical evidence of hyperandrogenism and polycystic ovarian morphology (PCOM) on ultrasound [16].

Ovulatory dysfunction was defined as menstrual cycles more than 45 days in length. All subjects were at least 2 year postmenarcheal. Clinical hyperandrogenism was defined as the presence of hirsutism (Ferriman–Gallwey score >8) or moderate–severe acne. Ferriman–Gallwey scoring was conducted by the same

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physician. Biochemical hyperandrogenism was defined as total testosterone >55 ng/ml. Ultrasonographic findings of PCOM were having 12 or more follicles measuring between 2–9 mm and/or an ovarian volume >10 cm^3 [16].

All patients underwent a detailed physical examination particularly assessing hirsutism using the Ferriman–Gallwey scoring system, presence of acne, acanthosis nigrigans, alopesia, and obesity. For both the PCOS and the healthy control group measurements of weight (kilograms) were obtained using electronic scales (Scale-Seca 220, Hamburg, Germany), and measurements of height (centimeter) were obtained using the Harpenden stadiometer. Obesity was defined as having a Body Mass Index (BMI) at or above the 95th percentile according to the Center for disease control guidelines. Pubertal staging was evaluated according the Marshall–Tanner classification [17]. The control group constituted 33 randomly selected regularly cycling adolescents matched for age with no clinical appearance of hyperandrogenism, obesity or other chronic illness and receiving no medications known to affect hormone, lipid or carbohydrate metabolism.

Bisphenol A and phytalate measurements

Chemicals, kits and equipment

Chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Glucuronidase/aryl sulfatase enzyme (from *Helix pomatia*) from Roche (Mannheim, Germany). High-pressure liquid chromatography (HPLC) equipment was obtained from Agilent (Santa Clara, CA, USA).

Preparation and deplasticization of glassware

In order to remove plastic material residue, glass tubes were put in an oven at 400 °C for 4 h. All the glassware were deplasticized with a deplasticization liquid (n-hexane: tetrahydrofuran (1:1, v/v)) for 4 h and then, put in an incubator to dry.

Preparation of plasma samples

Venous blood samples were drawn by a stainless-steel needle. The sample was allowed to drop directly into heparinized glass test tubes and tube openings were coated by aluminum foil in order to avoid contact with plastic material. Samples were centrifuged at 800×*g* to obtain plasma for 15 min. Plasma was aliquoted and kept at −80 °C until analysis.

BPA was detected by high performance liquid chromatography (HPLC) after a modified extraction according to Yang et al. [18]. Modified extraction and HPLC methods of Paris et al. [19] were used to detect the DEHP and MEHP levels.

Laboratory evaluation

Blood samples for the PCOS group were obtained during the early follicular phase (2–5th day) at 8.30 am in those with spontaneous menstrual cycles or at any time in subjects with amenorrhea. Serum FSH, LH, estradiol, prolactin, thyrotropin, fT3, and fT4 levels were measured by using the 2-step chemiluminescence microparticle immunoassay method. SHBG levels were tested by immunoradiometric assay. Serum dehydroxyepiandrosterone sulfate, 17-hydroxyprogesterone, and total testosterone levels were determined by solid phase chemiluminescence immunoassay. Fasting insulin was studied by using radioimmunoassay method. Insulin resistance was estimated using the homoeostasis model assessment–insulin resistance (HOMA-IR), which was calculated using the following formula: HOMA-IR = Fasting insulin (μU/mL) × Fasting glucose (mg/dL)/405. These parameters were not measured in the control group.

Written informed consent was obtained from both the subject and control group and their parents. The study was approved by the Committee of Ethics of Hacettepe University and funded by Hacettepe University Scientific Reasearch Unit.

Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics 22.0 package program (IBM Corp., Armonk, NY, USA). Data with normal distribution were expressed as mean ± SD. The distribution of BPA, DEHP and MEHP values were examined for skewness by using the Shapiro–Wilk test and were found to have normal distribution. Logarithmic transformation was performed, when appropriate. Differences between groups were tested using *t*-test. Mann–Whitney *U*-test was used for variables without normal distributions. Spearman test were performed to analyze relationship among EEDs and anthropometric, hormonal and biochemical parameters. The value of $p < .05$ accepted as statistical significance.

Results

The study and control groups were similar in terms of age (15.62 ± 1.29 and 16.04 ± 1.59, respectively, $p = .2$). BMI in the study and control group was 25.44 ± 5.2 kg/m^2 and 21.34 ± 3.25 kg/m^2 respectively. This difference was statistically significant ($p = .0002$). The clinical and biochemical data of the study group are represented in Table 1.

Table 1. Clinical and laboratory parameters of the patients.

Parameter	
Age (years) (Mean, SD)	15.62 ± 1.29
Body Mass Index (kg/m^2) (Mean, SD)	25.44 ± 5.2
Ferriman–Gallaway score, (Mean, SD)	10.2 ± 5.9
Ovulatory dysfunction, *n* (%)	58 (93.5)
Biochemical hyperandrogenism, *n* (%)	54 (87)
Clinical hyperandrogenism, *n* (%)	45 (72)
Polycystic ovarian morphology on ultrasound, *n* (%)	49 (79)
FSH (mIU/mL)	5.45 ± 1.81
LH (mIU/mL)	9.04 ± 5.98
Total testosterone (ng/dL)	52.29 ± 26.15
Estradiol (pg/mL)	43.50 ± 18.65
17-OH-progesterone (ng/mL)	0.96 ± 0.59
DHEA-S (ng/mL)	234.00 ± 105.30
SHBG (nmol/L)	35.41 ± 25.90
Prolactin (mIU/L)	12.27 ± 6.80
Triglycerides (mg/dL)	108.23 ± 60.33
HDL-cholesterol (mg/dL)	50.04 ± 12.07
LDL-cholesterol (mg/dL)	109.95 ± 28.11
Total cholesterol (mg/dL)	170.87 ± 36.23
Fasting glucose (mg/dL)	84.77 ± 9.02
HOMA-IR	3.42 ± 3.91
Insulin (μIU/mL)	14.28 ± 14.18
TSH (uIU/ml)	1.92 + 1.25
sT4 (uIU/ml)	11.69 ± 2.21

Bisphenol A measurements

Urine bisphenol A levels were significantly increased in adolescents with PCOS compared to the control group (15.89 ± 1.16 µg/g creatinine, and 7.3 ± 1.38 µg/g creatinine, respectively, $p = .001$).

When we compared the BPA levels in the PCOS group according to the presence of obesity, the BPA levels were higher in the obese group (19.27 ± 3.09 µg/g creatinine) when compared to the non-obese group (13.7 µg/g ± creatinine), but this result was not statistically significant ($p = .269$).

The relation between bisphenol A and clinical and laboratory parameters investigated in the study were analyzed. There was no correlation between BPA and any parameter (Table 2) except for a higher BPA level observed in patients with PCOM ($p = .038$).

DEHP and MEHP measurements

Detectable plasma DEHP and MEHP levels were not significantly different between the study and control group. (PCOS group: DEHP: 0.40 ± 0.24, MEHP: 0.13 ± 0.23) control group (DEHP: 0.49 ± 0.27, MEHP: 0.14 ± 0.3) ($p = .7$, $p = .3$). No relationship was observed between DEHP and MEHP, and clinical and laboratory parameters (Table 2).

Discussion

With widespread global industrialization, reproductive system disorders are increasing rapidly. Substantial evidence points to EDDs as having a role in the initiation of PCOS [20]. In this study, we found that bisphenol A levels were significantly higher in adolescents with PCOS when compared with age-matched healthy controls. On the other hand, we found no relationship between phthalate levels and PCOS.

BPA is one of the most widely produced synthetic chemicals [21]. Many different mechanisms have been proposed to explain its effects on the female reproductive system, which seems to be a predominantly sensitive target of BPA interference, indicated by evidence on ovarian steroidogenesis, folliculogenesis, and ovarian morphology. It is thought that BPA may augment androgen synthesis in ovarian theca cells and may modify their hepatic metabolism by interacting with SHBG and with hydroxylation enzymes [1,22].

A meta-analysis evaluating a total of nine studies involving 493 PCOS patients and 440 controls has demonstrated that high BPA levels were significantly associated with PCOS patients but the authors concluded more evidence using advanced detection methods was necessary to verify the association between BPA and PCOS [13]. In these studies BPA levels were measured from serum examples, to our knowledge the present study is the first to demonstrate the elevation of urinary BPA levels. A study by Vagi et al. [23] also aimed to evaluate urinary concentration of BPA but they found no association between the PCOS and urinary BPA. The only other study evaluating the effect of BPA on adolescent PCOS was conducted by Akın et al. [24] Similar effects were also detected in this study as elevated BPA levels were documented in PCOS adolescents.

Data concerning the effect of obesity and BPA on PCOS is conflicting. In our study although the obese group had a higher BPA level this result was not statistically significant. In the meta-analysis mentioned above high BPA levels showed significant association with high BMI and high HOMA-IR but other studies like ours have not shown this relationship [8]. Kandaraki et al. indicated that bisphenol A levels were higher in PCOS women independently of body weight as both lean and obese PCOS individuals had elevated BPA levels [25]. Similarly, Akin et al. also showed this relationship to be independent of obesity in adolescents with PCOS [24].

Our study showed that BPA levels correlated with PCOM morphology. *In-vivo* studies have demonstrated that BPA could alter ovarian morphology by inducing a large number of cysts [8]. Fernandez et al. [26] described a large number of ovarian cysts in rats that had been exposed to high doses of BPA. Another study found more morphological abnormalities with multioocyte follicles and primordial follicle clusters in rats exposed to BPA when compared with controls [27].

Previous research suggests that phthalates, have anti-androgenic effect [23]. Studies have shown intrauterine DEHP exposure to cause a decrease in testis size and testosterone levels [28]. Phthalate levels have also been associated with decreased testosterone production in men and delayed pubarche in women [29]. As PCOS is characterized by hyperandrogenemia it could be hypothesized that higher levels of phthalates may be protective for PCOS due to their anti-androgenic effects. A previous study evaluating urinary phthalate metabolites found that monobenzyl phthalate was lower in the PCOS group and they concluded that this result was consistent with previous studies in demonstrating the anti-androgenic effects of certain phthalates [23]. We were not able to show any relationship between phthalates and PCOS.

The largest limitation of this study was the number of patients in each group, which may have limited our power to detect smaller differences that could have been of clinical significance, such as correlation between BPA and obesity and androgen levels. We obtained a single spot serum sample for DEPH and MEPH and a urine specimen for BPA, evaluation of both blood and urine for all 3 EEDs may have been more informative. Additionally, phthalates and BPA metabolize quickly and are eliminated from the body within a few hours after exposure, for this reason a single measurement may be insufficient in detecting the association between PCOS and these EDDs. Furthermore,

Table 2. Correlations between BPA, DEHP and MEHP and clinical and hormonal parameters in the study group.

	BPA		DEHP		MEHP	
	r	p	r	p	r	p
Testosterone	0.091	0.543	0.083	0.566	0.042	0.769
DHEASO4	−0.076	0.611	0.030	0.837	0.081	0.573
SHBG	0.267	0.095	−0.180	0.248	0.019	0.900
LH/FSH	0.008	0.959	−0.061	0.675	−0.152	0.286
HO	−0.205	0.163	−0.114	0.432	0.030	0.834
Ferriman–Gallaway score	0.001	0.993	0.147	0.324		
Ovulatory dysfunction		0.858		0.462		1.0
Biochemical hyperandrogenism		0.269		0.559		0.915
Clinical hyperandrogenism		0.776		0.632		0.232
Polycystic ovarian morphology on ultrasound		0.038		0.889		0.303

humans may be exposed to many EDDs at the same time, this study only evaluated 3 EDDs and synergistic or antagonistic effects of other environmental factors that we were not able to control for may have also affected our results.

In conclusion, this study established a significant relationship between urinary BPA concentrations in adolescents with PCOS and found BPA to be significantly correlated with PCOM on ultrasound but not with obesity androgen levels, or other metabolic parameters. As the use of plasticized products have become universal in our environment, more efforts are required to reduce exposure to phthalates and BPA to avoid adverse health effects.

Disclosure statement

The authors report no conflicts of interest.

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