Türk Klinik Biyokimya Derg 2024; 22(3): 113-124 Araştırma Makalesi / *Research Article*
https://doi.org/10.56615/tkbd.2024.13

Investigation Bisphenol A and Phthalate Levels in Idiopathic Hyperandrogenemia

İdiyopatik Hiperandrojenemili Hastalarda Bisfenol A ve Fitalat Düzeylerinin İncelenmesi

Mert Üğe **Saliha Aksun** **Sercan Mehmet Ertürk** **Tuğba Öncel Van** **Leyla Demir** **Barış Önder Pamuk** **Figen Narin**

İzmir Katip Çelebi Üniversitesi Atatürk Eğitim Araştırma Hastanesi, Tıbbi Biyokimya Anabilimdalı, İzmir, Türkiye

Received / Başvuru Tarihi*:* 21 Temmuz 2024 *Accepted* / Kabul Tarihi*:* 10 Kasım 2024

ABSTRACT

Aim: Idiopathic hyperandrogenemia is defined as the disease in women with signs of hirsutism and increased serum androgen levels, a normal menstrual cycle, and normal ovarian morphology. In this study, it was aimed to examine the association of Bisphenol A and mono-ethylhexyl phthalate with this disease.

Material and Methods: A total of 91 individuals between the ages of 18–45 were included in the study. The patient group consisted of 43 women with high androgen levels who applied to the endocrine outpatient clinic with signs of hirsutism, and the control group consisted of 48 healthy women with no signs of hirsutism or any additional disease. Bisphenol A and mono-ethylhexyl phthalate were measured in urine by the liquid chromatography-mass spectrometry method.

Results: Urinary Bisphenol A levels were found to be significantly higher in the patient group compared to the control group (1.6 ng/ml; 0.55 ng/ml, p=0.035). Mono-ethylhexyl phthalate levels were found to be significantly higher in the control group compared to the patient group (1.81 ng/ml, 1.66 ng/ml, p=0.01). In logistic regression analysis, odds ratios were 1.91 (95% CI: 1.13–3.24, p<0.05) for BPA, 0.91 (95% CI: 0.81–1.01, p=0.09) for age, 18.74 (95% CI: 3.86–90.85, p<0.05) for fT3, and 1.06 (95% CI: 0.98–1.14, p=0.09) for prolactin.

Conclusion: Investigation of the effect of endocrine-disrupting chemicals in this group, which is less common in the etiology of hyperandrogenemia, made our study unique. The higher Bisphenol A urinary level in the patient group than in the control group suggests the effect of Bisphenol A in patients with idiopathic hyperandrogenemia. The higher level of mono-ethylhexyl phthalate in the control group suggests that more phthalate metabolites should be examined and more studies should be conducted.

Key Words: Bisphenol A, Phthalate, Idiopathic hyperandrogenemia, Endocrine disruptors

Yazışma adresi: Mert Üğe
İzmir Katip Çelebi Üniversitesi Atatürk Eğitim Araştırma Hastanesi, Tıbbi Biyokimya Anabilimdalı, İzmir, Türkiye
e-posta: mertuge6@gmail.com

Etik onay: İzmir Katip Çelebi Üniversitesi Atatürk Eğitim Araştırma Hastanesi, Etik Kurulu 03.02.2022 tarihi ve 2022/17 sayılı kurul kararı

ÖZET

Amaç: İdiyopatik hiperandrojenemi, kadınlarda hirsutizm belirtileri, artmış serum androjen düzeyleri, normal adet döngüsü ve normal yumurtalık morfolojisi ile seyreden bir hastalık olarak tanımlanır. Bu çalışmada, Bisfenol A ve mono-etilhekzil fitalat'ın bu hastalıkla ilişkisinin incelenmesi amaçlandı.

Materyal ve Metod: Araştırmaya 18-45 yaş arası toplam 91 birey dahil edildi. Hasta grubunu, endokrin polikliniğine hirsutizm bulgusu ile başvuran ve androjen düzeyi yüksek olan 43 kadın; kontrol grubunu ise hirsutizm bulgusu ve herhangi bir ek hastalığı olmayan 48 sağlıklı kadın oluşturdu. Bisfenol A ve mono-etilhekzil fitalat düzeyleri idrarda sıvı kromatografi-kütle spektrometri yöntemiyle ölçüldü.

Bulgular: Hasta grubunda idrar Bisfenol A düzeyleri, kontrol grubuna göre anlamlı olarak yüksek bulundu (1,6 ng/ml; 0,55 ng/ml, p=0,035). Mono-etilhekzil fitalat düzeyleri ise kontrol grubunda, hasta grubuna göre anlamlı olarak yüksek bulundu (1,81 ng/ml; 1,66 ng/ml, p=0,01). Lojistik regresyon analizinde odds oranları, BPA için 1,91 (%95 güven aralığı: 1,13-3,24; p<0,05), yaş için 0,91 (%95 güven aralığı: 0,81-1,01; p=0,09), fT3 için 18,74 (%95 güven aralığı: 3,86-90,85; p<0,05), prolaktin için 1,06 (%95 güven aralığı: 0,98-1,14; p=0,09) olarak bulundu.

Sonuç: Hiperandrojenemi etiyolojisinde daha az sıklıkla yer alan bu grupta, endokrin bozucu kimyasalların etkisinin incelenmesi çalışmamızı özgün kılmaktadır. Hasta grubunda Bisfenol A idrar düzeyinin kontrol grubuna göre yüksek olması, idiyopatik hiperandrojenemililerde Bisfenol A'nın etkisini düşündürmektedir. Kontrol grubunda ise mono-etilhekzil fitalat düzeylerinin daha yüksek bulunması, daha fazla ftalat metabolitinin incelenmesi ve daha fazla çalışma yapılması gerektiğini düşündürmektedir.

Anahtar Kelimeler: Bisfenol A, Fitalat, İdiyopatik hiperandrojenemi, Endokrin bozucular

INTRODUCTION

Hirsutism is a clinical finding that is characterised by the increased growth of terminal hair of a male type in women. Hirsutism may be the only symptom of androgen increase, or it may sometimes be accompanied by acne and androgenic alopecia. Excessive androgen elevation causes an increase in terminal hair (1). The distribution of hair is determined by Modified Ferriman Gallwey (MFG) scoring. Nine different parts of the body are scored between 0 and 4 and the total score is obtained. Cases with an MFG score of 8-15 are considered mild, cases between 16-25 are considered moderate, and cases above 25 are considered severe hirsutism. In Mediterranean coastal countries, an MFG score of 8 and above is considered hirsutism (2).

The most common cause of hirsutism is Polycystic Ovary Syndrome (PCOS). The most important disease group that should be considered in differential diagnosis is PCOS. The clinical presentation may include menstrual disorder, oligo-anovulation, hirsutism, infertility, insulin resistance, dyslipidemia and impaired glucose tolerance. Furthermore, acne, hirsutism, seborrhea, male pattern hair loss and acanthosis nigricans may result from hyperandrogenemia (3). This is followed by late-onset congenital adrenal hyperplasia (LOCAH) and idiopathic hirsutism/ hyperandrogenemia. Rarely, Cushing's syndrome, hyperprolactinemia, thyroid disorders, androgen-secreting tumors, some medications and smoking may increase hair growth (4).

Idiopathic hyperandrogenemia is defined as a group of patients with normal menstrual cycles and normal ovarian morphology, with hirsutism and increased serum androgen levels. In order to diagnose idiopathic hyperandrogenemia, other secondary causes of hirsutism must be excluded.

Endocrine Disrupting Chemicals

Endocrine Disrupting Chemicals (EDCs) are substances extensively used worldwide, particularly in the plastics industry, which has grown considerably in recent years. These chemicals have been recognized for their potential role in endocrine and metabolic

diseases. (5). The US Environmental Protection Agency (EPA) defines EDCs as exogenous substances that interfere with the natural hormones responsible for maintaining homeostasis and developmental processes (6). EDCs can exert both estrogenic and anti-estrogenic effects due to their complex steroidal structures (7). The intracellular signalling network is regarded as comprising both genomic and non-genomic pathways. The genomic pathway transports transcription factors in the target gene directly over nuclear receptors, while the non-genomic pathway transports membrane-bound ER-α and ER-β (estrogen receptors). ER-α receptors are generally found in the breast, uterus, ovarian theca cells, testis, and epididymis; ER-β receptors are found in the prostate, bladder, ovarian granulosa cells, adipose tissue, and colon. EDCs act on hormone-active organs (8,9).

Bisphenol A

Bisphenol A (BPA) is an organic compound consisting of two phenol rings. Bisphenol A is a widely prevalent organic compound found in various consumer products, including plastic food containers, baby bottles, and cans. BPA has been identified in a number of biological samples, including urine, blood, adipose tissue, breast milk and the placenta (10-14).

BPA binds to cell and nuclear receptors or stimulates these receptors through its BPA-mediated effect. There are many studies showing effects on estrogen receptor, androgen receptor, G-protein coupled estrogen receptor, insulin-like growth factor and estrogen-related gamma receptors (15-18). Studies on animals and humans have demonstrated BPA's negative effects on reproductive health (19,20).

After oral ingestion, BPA is metabolized in the liver by CYP2C18 (most commonly), CYP2C19 and CYP2C9. It is conjugated with glucuronic acid in the liver and its major metabolite, Bisphenol A glucuronide (BAPG), is formed. The minor metabolite Bisphenol-sulfate (BPAS) is formed. It has an average half-life of six hours. It is excreted in the urine together with its metabolites within 42 hours (21).

Phthalates

Phthalates are a group of synthetic esters of phthalic acid that differ in the length and branching of their alkyl side chains. Long-chain phthalates are used as plasticizers and short-chain phthalates are used as solvents. Phthalates used as plasticizers are used to increase the durability and flexibility of products. The ingestion of contaminated food, inhalation of phthalates in the air, and skin contact with products containing phthalates are the primary routes of entry for phthalates into the body (22,23).

Di-2-ethylhexyl phthalate (DEHP) is converted to mono-ethylhexyl phthalate (MEHP) metabolite by the catalysis of non-specific lipase enzymes, and then to many metabolites by side chain hydrolysis and cytochrome enzymes. The rate of conversion and excretion of phthalates to their metabolites make exposure assessment difficult. Exposure is usually assessed by measurement of phthalate metabolites in urine. However, analytical detection is possible only for some phthalate metabolites. Not all metabolites can be detected (24,25).

Study Objective

In this study, we aimed to measure BPA and MEHP levels in the urine of women with hirsutism and increased androgen levels and to compare them with the control group.

MATERIAL AND METHOD

Patient and Control group

The study population consisted of 43 female patients with idiopathic hirsutism, aged 18–45 years, who presented to the endocrine outpatient clinic of our hospital with complaints of increased body hair over the past year. At the outpatient clinic, a detailed

patient history, systemic physical examination, laboratory tests, and imaging studies were conducted to rule out potential diseases that could cause hair growth. Individuals were selected for inclusion in the study after thorough evaluation of all examination procedures and laboratory test results. Women with an MFG score of 8 or higher were included in the study. The patient group comprised female patients diagnosed with idiopathic hyperandrogenism.

The control group consisted of 48 healthy, age-matched women who had regular menstrual cycles, normal ovarian morphology, and no risk factors for hirsutism (MFG score below 8). These individuals presented to the same clinic for unrelated reasons. A comprehensive assessment, including laboratory and imaging tests similar to those performed on the patient group, was conducted to exclude any underlying medical conditions associated with increased hair growth. MFG scoring was performed consistently for all participants by the same physician.

Exclusion criteria

Individuals with PCOS, LOCAH, hyperprolactinemia, thyroid dysfunction, anti-androgenic drug therapy (e.g., oral contraceptives, hormone therapy), and smokers were excluded from the study.

Hyperprolactinemia was assessed after ruling out factors such as sleep, exercise, emotional and physical stress, breast or chest wall stimulation, coitus, and high-protein diets. A cut-off value of 25 ng/mL was used to define hyperprolactinemia (26). Similarly, 17-OH progesterone levels were measured to exclude a diagnosis of LOCAH, with a cut-off value of 2 ng/mL (27).

In patients with hirsutism, normal ovarian morphology, and regular menstrual cycles, a total testosterone level above 0.55 ng/mL was considered indicative of hyperandrogenemia (28). The diagnosis of PCOS was made based on the Rotterdam criteria, which require the presence of at least two out of three findings: oligo-anovulation (<6 menstrual periods per year), clinical or laboratory evidence of androgen elevation, or ultrasonographic evidence of polycystic ovary morphology (29).

Laboratory measurements and sample collection

In all cases, biochemical tests were performed on fasting serum samples collected at the same time. All blood samples were taken during the first three days of the menstrual cycle to standardize the hormonal tests. Biochemical tests were carried out using the spectrophotometric method with kits compatible with the Beckman Coulter 5800 (Brea, CA, USA) device. DHEA-S (Dehydroepiandrosterone sulfate), FSH (Follicle-stimulating Hormone), LH (Luteinizing Hormone), estradiol, progesterone, total testosterone, TSH (Thyroid Stimulating Hormone), fT3, fT4, prolactin, and insulin tests were performed with Access brand kits on the Beckman Coulter Unicel DxI 800 (Beckman Coulter, Miami, USA) device. The 17-OH progesterone test was conducted with the Snibe-Maglumi X3 (Shenzhen, China) model device and Snibe (China) brand kit. The androstenedione test was performed using the Immulite 2000 Xpi (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) by immunometric chemiluminescence. The free testosterone test was conducted using the ETI-Max 3000 (Diasorin, Italy) device and DiaMetra (Perugia, Italy) brand kit by the ELISA (Enzyme-Linked ImmunoSorbent Assay) method. The samples collected in gel and clot activator tubes (BD Vacutainer® SST™ II Plus Tubes) were centrifuged for 10 minutes at 2,000 x g within 30 minutes of collection. Urine samples from the cases were immediately transferred from plastic containers to glass tubes and stored at -20°C in glass tubes (BD Vacutainer® Serum Glass Tubes) for BPA and MEHP analysis.

Preparation of calibration standards

A mixed stock solution was prepared from concentrated 50 g BPA (CAS no: 80-05-7, Sigma-Aldrich, Taiwan) and 500 mg MEHP

(CAS no: 4376-20-9, Sigma-Aldrich, Taiwan) standard solution.

1. 50 µL of BPA and MEHP were taken from each bottle and diluted (1/1000) with 99.98% pure methanol (CAS no: 67-56-1, Carlo ERBA, France). A stock solution with a concentration of 10,000 ng/mL was prepared in a volume of 50 mL.
2. It was diluted again with distilled water (1/10).
3. A stock solution with a concentration of 100 ng/mL was prepared by diluting once again with pure methanol (1/10).

By making serial dilutions with methanol; Six-level standard solutions were prepared at concentrations of 100, 25, 10, 5, 1 and 0.25 ng/mL, respectively.

Each prepared standard solution was mixed equally with Acetonitrile precipitator (Cas no: 75-05-8, Carlo ERBA, France) and vortexed for 1 minute. It was centrifuged for 10 minutes at 4000 rpm in a NUVE NF 800 (Ankara, TR) brand centrifuge device, and the supernatants were placed in separate glass vials and made ready for study.

Sample preparation and analysis process

Urine samples stored frozen at -20°C were kept at room temperature until completely thawed. 300 µL urine sample from the patient and control groups and 300 µL acetonitrile were mixed and centrifuged (10 minutes at 4000 rpm). Supernatants were taken into separate glass vials.

Mobile phase solution A contained 0.1% acetic acid and water, and mobile phase solution B contained 0.1% acetic acid and acetonitrile. The column had a size of 100 mm x 2.1 mm, particle width of 2.7 µm (Lot No 220113.1, ReproShel Phenyl-Hexyl, Dr Maisch GmbH, Germany). Column temperature was 40 °C. The flow rate was set to 0.4 mL/min. The total run time for each sample was 12 minutes. The analyzer temperature was kept between 8-10 °C, the room temperature was at 26 °C. The injection volume was optimized to 20 µL.

Analytes were scanned by LC/MS-MS in ESI negative MRM (multiple reaction monitoring) mode on an AB SCIEX QTRAP 4500 (AB Sciex Technologies, Framingham, MA, USA) device.

Statistical evaluation

Descriptive statistics for this study are presented as the number of individuals (n), mean, and standard deviation (SD) from the dataset. The distribution of variables was examined using box plots, and extreme values that did not conform to the distribution were identified. The normality of the data was assessed with the Shapiro-Wilk test. For comparisons of means between two groups, the Independent Sample T-test was applied if the data followed a normal distribution; otherwise, the Mann-Whitney U test was used.

To analyze continuous variables, Spearman correlation was employed when the assumption of normal distribution was not met. The logistic regression model included variables such as age, fT3, and prolactin in both the patient and control groups. This model assessed the effect of BPA on idiopathic hyperandrogenism while controlling for these variables.

In all comparisons, a p-value of <0.05 was considered statistically significant. Data analysis was performed using IBM SPSS Statistics 25 (IBM Corp., Armonk, New York, USA).

RESULTS

Device images of BPA and MEHP results for four patients are shown below. The mass values of the precursor ion, retention times, and scanned areas are presented in Figure 1. Within 12 minutes, the BPA peak was detected at 4 minutes and 56 seconds, while the MEHP peak was detected at 5 minutes and 80 seconds. The scanned area represents the concentration of the measured analyte (Figure 1) and was calculated by a single experienced laboratory technician.

Extreme values of variables not suitable for distribution were identified using box plots. A total of 13 potential outliers were found for BPA and MEHP measurements in the control and patient groups. However, one participant from the patient group (67) was identified as an extreme outlier for BPA measurement. This participant was excluded from the dataset as their inclusion would introduce bias into the analyses (Figure 2).

Four laboratory results indicative of hyperandrogenemia—total testosterone, free testosterone, androstenedione, and DHEA-S—were clinically and statistically significantly higher in the patient group compared to the control group. BPA levels were higher in the patient group than in the control group (95% CI; mean: 1.630 [2.665–0.655], 0.556 [0.803–0.308] ng/mL, $p = 0.035$). Conversely, MEHP levels were higher in the control group than in the patient group (95% CI; mean: 1.817 [1.920–1.715], 1.665 [1.764–1.570] ng/mL, $p = 0.011$) (Table 1).

The table below compares BPA and MEHP levels between the control and patient groups, along with laboratory test results indicative of hyperandrogenemia. A significant negative correlation was observed between MEHP and androstenedione in the patient group ($r = -0.302$, $p < 0.05$) (Table 2).

In logistic regression analysis, the odds ratios were as follows: 1.91 (95% CI: 1.13–3.24, $p < 0.05$) for BPA, 0.91 (95% CI: 0.81–1.01, $p = 0.09$) for age, 18.74 (95% CI: 3.86–90.85, $p < 0.05$) for fT3, and 1.06 (95% CI: 0.98–1.14, $p = 0.09$) for prolactin (Table 3).

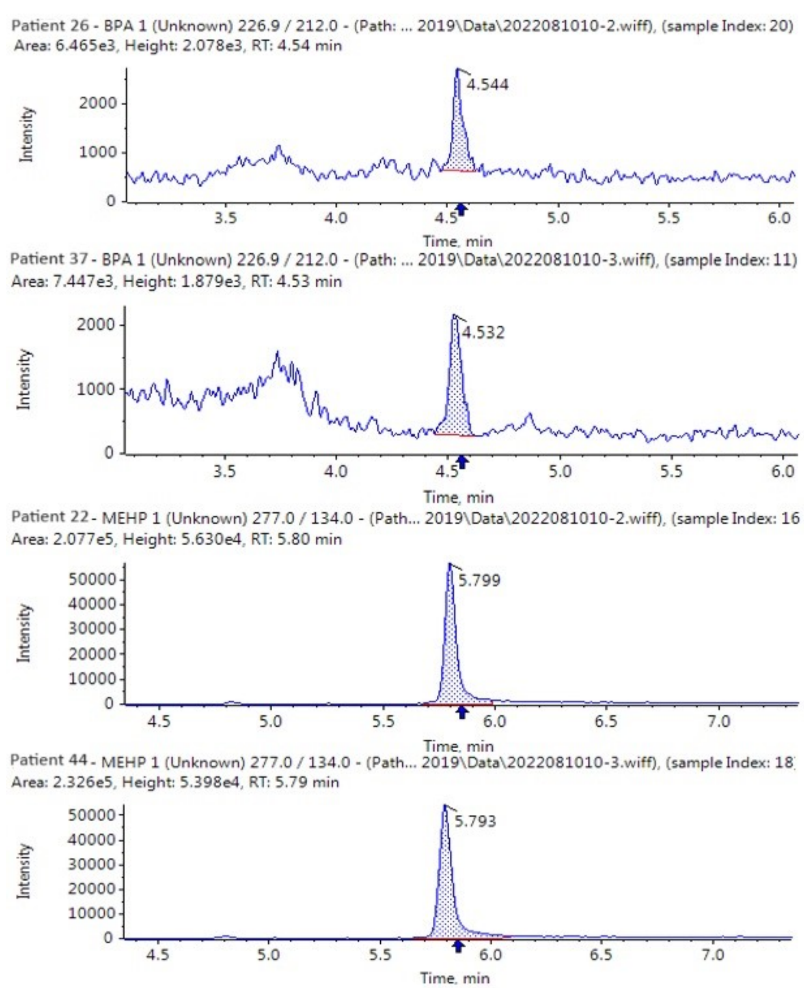

Figure 1. Device images of BPA and MEHP results for 4 patients

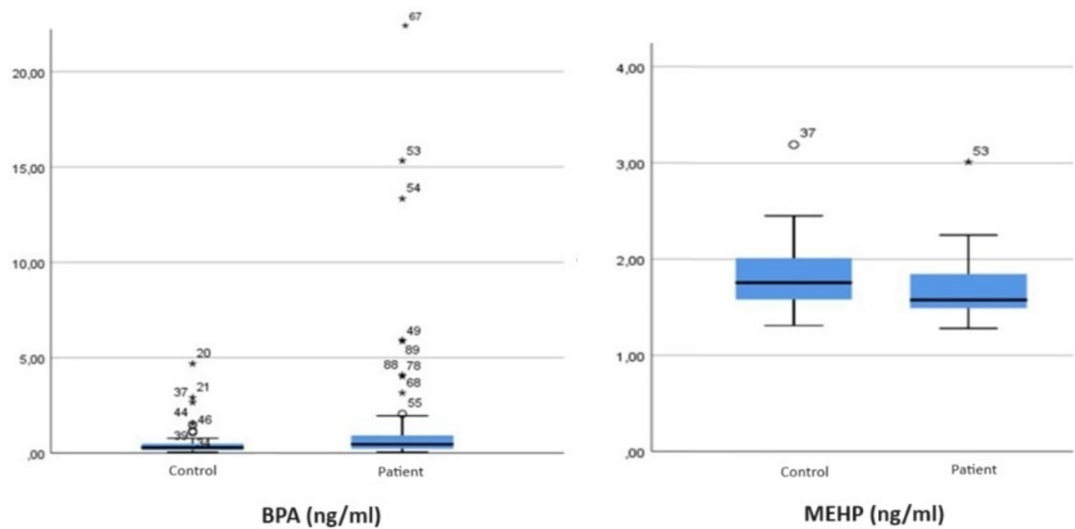

Figure 1. BPA and MEHP measurements in the control and patient groups

Table 1. Comparison of clinical and laboratory findings of patient and control groups
Tablo 1. Hasta ve kontrol gruplarının klinik ve laboratuvar bulgularının karşılaştırılması

		n	Mean	SD	p
Modified Ferriman Gallwey	C	48	3,90	2,136	0,000*
	P	43	17,98	5,930	
Fasting blood glucose (mmol/L)	C	48	4,95	0,49	0,881
	P	43	4,93	0,55	
Total Testosterone (pmol/L)	C	48	1770,9	551,9	0,000*
	P	43	2749,3	719,4	
Free Testosterone (pmol/L)	C	48	2,80	1,62	0,000*
	P	43	8,45	5,20	
Androstenadione (nmol/L)	C	48	5,06	2,86	0,000*
	P	43	12,44	5,75	
DHEA-S (µmol/L)	C	48	5,72	2,57	0,000*
	P	43	8,53	3,84	
TSH (mU/mL)	C	48	1,930	0,919	0,171
	P	43	2,180	0,970	
fT3 (pmol/L)	C	48	5,31	0,57	0,000*
	P	43	5,93	0,67	
fT4 (pmol/L)	C	48	11,32	1,42	0,067
	P	43	10,81	2,06	
Prolactin (µg/L)	C	48	13,341	6,500	0,040*
	P	43	16,769	12,055	
BPA (ng/mL)	C	48	0,556	0,851	0,035*
	P	43	1,630	3,191	
MEHP (ng/mL)	C	48	1,817	0,352	0,011*
	P	43	1,665	0,307	

n: number of participants; SD: standart deviation; *p<0,05; C: Control P: Patient

Table 2. Comparison of BPA and MEHP with laboratory findings indicating hyperandrogenemia

Tablo 2. BPA ve MEHP'nin hiperandrojenemiyi gösteren laboratuvar bulgularıyla karşılaştırılması

		Control		Patient	
		BPA	**MEHP**	**BPA**	**MEHP**
MEHP (ng/ml)	r	0,123	1,000	0,239	1,000
	p	0,404	-	0,122	-
Total Testosterone (pmol/L)	r	0,183	-0,080	0,093	-0,145
	p	0,212	0,591	0,553	0,352
Free Testosterone (pmol/L)	r	0,253	0,080	0,160	-0,198
	p	0,083	0,590	0,306	0,204
Androstenadione (nmol/L)	r	0,234	0,037	0,060	-0,302
	p	0,110	0,803	0,704	0,049*
DHEA-S (μmol/L)	r	0,050	-0,069	0,221	0,061
	p	0,737	0,642	0,154	0,696

*p<0,05 r = Spearman's rank correlation coefficient (Rho)

Table 3. The results of logistic regression analysis

Tablo 3. Lojistik regresyon analizinin sonuçları

	OR	**%95 CI**	***P***
BPA	1.91	1.13-3.24	< 0.05
Age	0.91	0.81-1.01	0.09
fT3	18.74	3.86-90.85	< 0.05
Prolactin	1.06	0.98-1.14	0.09

OD=Odds Ratio, CI=Confidence Interva

DISCUSSION

The diagnosis of idiopathic hyperandrogenism should be made by carefully performing a differential diagnosis using clinical experience, laboratory tests, and imaging studies. Although the etiology of the disease is not fully understood, we believe that environmental factors may play a role. Several studies have indicated that bisphenol and phthalate metabolites may be associated with the development of other endocrine disorders. While BPA and phthalate derivatives have been compared with many diseases in the literature, the lack of a study investigating the relationship between idiopathic hyperandrogenism and these endocrine disruptors makes our study unique.

In chromatographic methods, the pre-analytical steps of sample preparation are crucial. During the pre-analytical phase, storing the samples under appropriate conditions until the day of analysis, avoiding plastic contamination, and ensuring that the tubes used are free of plastic content are important for accurately determining bisphenols and phthalates. Markham et al. added acetonitrile and methanol into standard blood collection tubes and observed BPA exposure at room temperature for 1, 8, and 24 hours. BPA was detected as contamination in the extraction solvents (0.1 to 10 ng/mL) (30). In our study, there was no process of keeping the samples in plastic tubes during the collection phase. However, to evaluate potential plastic contamination from the injection line and pipette tips used during sample preparation, glass vials containing only methanol were included for blind analysis. No signals were detected for the analytes.

Previous studies have noted that liquid-liquid extraction and solid-phase extraction

processes are time-consuming and involve the use of large amounts of organic solvents. Xiao et al. obtained a mobile phase mixture of 70% acetonitrile and 30% water for optimal separation of BPA and interfering substances, using this mixture as an elution phase (31). In our study, we observed that the optimum analyte could be obtained using the acetonitrile precipitation method. Similar to Xiao et al., mobile phase solution A used in our study contained 0.1% acetic acid and water, while mobile phase solution B contained 0.1% acetic acid and acetonitrile. Chen J. et al. demonstrated 13 phthalate metabolites, including MEHP, in urine using the ultra-performance liquid chromatography tandem mass spectrometry (UPLC/MS-MS) method (32). Similarly, in our study, precipitation with acetonitrile was performed after incubation with beta-glucuronidase enzyme, and the eluate was obtained.

Ying Hu et al., in a meta-analysis of nine studies involving 493 PCOS patients and 440 controls, demonstrated elevated serum BPA levels in PCOS patients using HPLC and ELISA methods (standardized mean difference (SMD): 2.437 ng/mL, 95% CI (1.265, 3.609), $p < 0.001$) (33). In our study, BPA levels (95% CI, mean: 1.630 (2.665–0.655), 0.556 (0.803–0.308) ng/mL, $p = 0.035$) were found to be higher in the patient group using the LC/MS-MS method.

Konieczna A. et al. compared serum BPA levels in 106 PCOS patients and 80 controls using the HPLC method. Women with PCOS had significantly higher BPA levels than the control group (geometric mean and (95% CI): 0.202 ng/mL (0.150; 0.255) vs. 0.154 ng/mL (0.106; 0.201), $p = 0.035$). BPA levels were positively correlated with serum total testosterone ($r = 0.285$, $p = 0.004$) and the free androgen index ($r = 0.196$, $p = 0.049$) (34). In our study, we could not evaluate the free androgen index because we did not measure sex hormone-binding globulin. However, similar to Konieczna A. et al., a positive correlation was observed between BPA levels and total testosterone levels ($r = 0.093$, $p = 0.553$).

Takeuchi et al. compared BPA levels using the ELISA method in 19 PCOS patients and 26 women with regular menstruation. They found that BPA levels were positively correlated with total testosterone ($r = 0.39$, $p < 0.001$), androstenedione ($r = 0.68$, $p < 0.001$), DHEA-S ($r = 0.51$, $p < 0.001$), and free testosterone ($r = 0.50$, $p < 0.001$) (35). In our study, positive correlations were observed between BPA levels in the patient group and total testosterone ($r = 0.093$, $p = 0.553$), free testosterone ($r = 0.160$, $p = 0.306$), androstenedione ($r = 0.060$, $p = 0.704$), and DHEA-S ($r = 0.221$, $p = 0.154$).

Chou et al. evaluated urinary concentrations of mono-n-butyl phthalate (MnBP), mono(2-ethylhexyl) phthalate, monobenzyl phthalate, mono(2-ethyl-5-oxo-hexyl) phthalate, and mono(2-ethyl-5-hydroxyhexyl) phthalate in 123 women with endometriosis and 78 controls. They found that only MnBP was associated with endometriosis (95% CI; mean: 1.89 (1.05–3.39) μg/g) (36). Chen J. et al. evaluated urinary phthalate exposure in 220 individuals with thyroid nodules and 220 controls. MEHP levels were found to be higher in the patient group than in the control group (mean: 2.87 vs. 2.66 μg/g, $p = 0.985$) (32). Akın L. et al. compared serum levels of DEHP and MEHP in 63 adolescent PCOS patients and 61 controls, with higher DEHP and MEHP levels observed in the control group (37). Similarly, in our study, MEHP levels were higher in the control group than in the patient group (95% CI, mean: 1.817 (1.920–1.715), 1.665 (1.764–1.570) ng/mL, $p = 0.011$). Since MEHP levels alone do not indicate total phthalate exposure, we believe further studies are needed to include other phthalate derivatives.

Prolactin ($p = 0.040$) and fT3 ($p < 0.001$) levels were statistically significantly higher in the patient group than in the control group. However, since all individuals were within reference ranges for these parameters, they were not considered among the etiological

causes of hirsutism. Logistic regression analysis highlighted BPA as a potential risk factor for idiopathic hyperandrogenism. The findings that prolactin and fT3 levels were statistically significantly higher in the patient group suggest their limited effect on hirsutism.

A limitation of this study is that the level of dihydrotestosterone (DHT), a potent peripherally acting hormone, was not measured. Another limitation is the sample size. Differential diagnosis of idiopathic hyperandrogenism requires considerable time and collaboration with clinicians. Recent experimental studies have shown that BPA disrupts DHT-induced androgen receptor dimerization and negatively affects androgen receptor function. Evidence from laboratory and human studies suggests that BPA has anti-androgenic activity by binding to androgen receptors, adversely affecting cell development and function (38,39).

Our results suggest that environmental factors should be considered in the differential diagnosis of hirsutism and that the possible endocrine-disrupting effects of these chemicals should be further investigated. In conclusion, the association of BPA and MEHP with hirsutism is an important research area that should be supported by further clinical and epidemiological studies. This study provides a foundation for understanding the effects of environmental factors on hormonal imbalances.

Ethic Approval

Approval was obtained from the ethics committee of our institution with the decision numbered 17 and dated February 03, 2022.

Funding

Financial research support was received for this study from University Scientific Research Projects Coordinator.

Conflict of interest

None

KAYNAKLAR

1. Bolu ŞE . Hiperandrojenemili Olguya Yaklas ım; Hirsutizmli Hasta Yönetimi. Adrenal and gonadal diseases guideline. 2019;15:148-150.
2. Goodman NF, Bledsoe MB, Cobin RH, Futterweit W, Goldzieher JW, Petak SM, et al. "American Association of Clinical Endocrinologists Hyperandrogenism Guidelines". Endocrine Practice 2001;7(2):120–134.
3. Azziz R. The evaluation and management of hirsutism. Obstet Gynecol 2003;101:995-1007.
4. Escobar-Morreale HF, Carmina E, Dewailly D, Gambineri A, Kelestimur F, Moghetti P, et al. Epidemiology, diagnosis and management of hirsutism: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome Society. Hum Reprod Update 2013;19(2):207.
5. Jang Y.C, Lee J, Hong S, Choi H. W, Shim W. J, Hong S. Y, et al. Estimating the global inflow and stock of plastic marine debris using material flow analysis: a preliminary approach. Journal of the Korean Society for Marine Environment and Energy. 2015;18(4):2.
6. US Environmental Protection Agency (EPA). Research on Endocrine Disruptors. 2016. Access website: https://www.epa.gov/chemical-research/ research-endocrine-disruptors Update: Nov 30, 2021.
7. Rasier G, Parent AS, Gérard A, Lebrethon MC, Bourguignon JP. Early maturation of gonadotropin-releasing hormone secretion and sexual precocity after exposure of infant female rats to estradiol or dichlorodiphenyltrichloroethane. Biol Reprod. 2007;77(4)734-42.
8. Kiyama R, Wada-Kiyama Y. Estrogenic endocrine disruptors: Molecular mechanisms of action. Environ Int. 2015;83:11-40.
9. Paterni I, Granchi C, Katzenellenbogen JA, Minutolo F, et. al Estrogen receptors alpha (ERα) and beta (ERβ): subtype-selective ligands and clinical potential. Steroids. 2014 Nov; 90:13-29.
10. Pellerin E, Caneparo C, Chabaud S, Bolduc S, Pelletier M. Endocrine-disrupting effects of bisphenols on urological cancers. Environ Res. 2021 Apr;195:110485.
11. Murata M, Kang JH. Bisphenol A (BPA) and cell signaling pathways. Biotechnol Adv. 2018 Jan;36(1):311-327.
12. Li DK, Zhou Z, Miao M, He Y, Wang J, Ferber J. Urine bisphenol-A (BPA) level in relation to semen quality. Fertil Steril. 2011 Feb;95(2):625-30.e1-4.
13. Fernandez MF, Arrebola JP, Taoufiki J, Navalon A, Ballesteros O, Pulgar R. Bisphenol-A and chlorinated derivatives in adipose tissue of women. Reprod Toxicol. 2007 Aug-Sep;24(2):259-64.

14. Vandenberg LN, Chahoud I, Heindel JJ, Padmanabhan V, Paumgartten FJ, Schoenfelder G. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Cien Saude Colet. 2012 Feb; 17(2): 407-34.

15. Dong S, Terasaka S, Kiyama R. Bisphenol A induces a rapid activation of Erk1/2 through GPR30 in human breast cancer cells. Environ Pollut. 2011 Jan;159(1):212-218.

16. Hwang KA, Park MA, Kang NH, Yi BR, Hyun SH, Jeung EB et al. Anticancer effect of genistein on BG-1 ovarian cancer growth induced by 17 β-estradiol or bisphenol A via the suppression of the crosstalk between estrogen receptor α and insulin-like growth factor-1 receptor signaling pathways. Toxicol Appl Pharmacol. 2013 Nov 1;272(3):637-46.

17. Kang NH, Hwang KA, Lee HR, Choi DW, Choi KC. Resveratrol regulates the cell viability promoted by 17β-estradiol or bisphenol A via down-regulation of the cross-talk between estrogen receptor α and insulin growth factor-1 receptor in BG-1 ovarian cancer cells. Food Chem Toxicol. 2013 Sep;59:373-9.

18. Zhang KS, Chen HQ, Chen YS, Qui KF, Zheng XB, Li GC et. al Bisphenol A stimulates human lung cancer cell migration via upregulation of matrix metalloproteinases by GPER/EGFR/ERK1/2 signal pathway. Biomed Pharmacother. 2014 Oct;68(8): 1037-43.

19. Markey CM, Wadia PR, Rubin BS, Sonnenschein C, Soto AM. Long-term effects of fetal exposure to low doses of the xenoestrogen bisphenol-A in the female mouse genital tract. Biol Reprod 2005;72:1344-1351.

20. Souter I, Smith KW, Dimitriadis I, Ehrlich S, Williams PL, Calafat AM et al. The association of bisphenol-A urinary concentrations with antral follicle counts and other measures of ovarian reserve in women undergoing infertility treatments. Reprod Toxicol. 2013;42:224-231.

21. Ye X, Kuklenyik Z, Needham LL, Calafat AM. Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction-high performance liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 2005 Oct;383(4):638-44.

22. Hahladakis JN, Velis CA, Weber R, Iacovidou E, Purnell P. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. J Hazard Mater. 2018 Feb;344:179-199.

23. Katsikantami I, Sifakis S, Tzatzarakis MN, Vakonaki E, Kalantzi OI, Tsatsakis AM, et. al A global assessment of phthalates burden and related links to health effects. Environ Int. 2016 Dec;97:212-236.

24. Frederiksen H, Skakkebaek NE, Andersson AM. Metabolism of phthalates in humans. Mol Nutr Food Res. 2007 Jul;51(7):899-911

25. Choi K, Joo H, Campbell JL Jr, Clewell RA, Andersen ME, Clewell HJ .In vitro metabolism of di(2-ethylhexyl) phthalate (DEHP) by various tissues and cytochrome P450s of human and rat. Toxicol In Vitro. 2012 Mar;26(2):315-22.

26. Melmed S, Casanueva FF, Hoffman AR, Kleinberg DL, Montori VM, Schlechte JA, et al. Diagnosis and treatment of hyperprolactinemia: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011 Feb;96(2):273-88.

27. Wilson RC, Mercado AB, Cheng KC, New MI. Steroid 21-hydroxylase deficiency: genotype may not predict phenotype. The Journal of Clinical Endocrinology and Metabolism 1995; 80(8): 2322-2329.

28. Silfen ME, Denburg MR, Manibo AM, Lobo RA, Jaffe R, Ferin M et al. Early endocrine, metabolic, and sonographic characteristics of polycystic ovary syndrome (PCOS): comparison between nonobese and obese adolescents. J Clin Endocrinol Metab. 2003;Oct 88(10):4682-8.

29. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004; 81:19-25.

30. Markham DA, Waechter JM Jr, Wimber M, Rao N, Connoly P, Chuang JC et. al Development of a method for the determination of bisphenol A at trace concentrations in human blood and urine and elucidation of factors influencing method accuracy and sensitivity. J Anal Toxicol. 2010 Jul-Aug;34(6):293-303.

31. Xiao Q, Li Y, Ouyang H, Xu P, Wu D. High-performance liquid chromatographic analysis of bisphenol A and 4-nonylphenol in serum, liver and testis tissues after oral administration to rats and its application to toxicokinetic study. J Chromatogr B Analyt Technol Biomed Life Sci. 2006 Jan 18;830(2):322-9.

32. Chen J, Chen Y, Liu S, Chen B, Lu Y, Dong R. Urinary Biomarkers of Phthalates Exposure, Blood Lead Levels, and Risks of Thyroid Nodules. Toxics. 2021 Mar 22;9(3):68.

33. Hu Y, Wen S, Yuan D, Peng L, Zeng R, Yang Z et. al The association between the environmental endocrine disruptor bisphenol A and polycystic ovary syndrome: a systematic review and meta-analysis. Gynecol Endocrinol. 2018 May;34(5):370-377.

34. Konieczna A, Rachoń D, Owczarek K, Kubica P, Kowalewska A, Kudłak B et. al Serum bisphenol A concentrations correlate with serum testosterone levels in women with polycystic ovary syndrome. Reprod Toxicol. 2018 Dec ;82:32-37.

35. Takeuchi T, Tsutsumi O, Ikezuki Y, Takai Y, Taketani Y. Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction. Endocr J. 2004; 51:165–169.

36. Chou YC, Chen YC, Chen MJ, Chang CW, Lai GL, Tzeng CR. Exposure to Mono-n-Butyl Phthalate in Women with Endometriosis and Its Association with the Biological Effects on Human Granulosa Cells. Int J Mol Sci. 2020 Mar 5;21(5):1794.

37. Akın L, Kendirci M, Narin F, Kurtoğlu S, Hatipoğlu N, Elmalı F. Endocrine Disruptors and Polycystic Ovary Syndrome: Phthalates. J Clin Res Pediatr Endocrinol. 2020 Nov 25;12(4):393-400.

38. Pathak RK, Jung DW, Shin SH, Ryu BY, Lee HS, Kim JM. Deciphering the mechanisms and interactions of the endocrine disruptor bisphenol A and its analogs with the androgen receptor. J Hazard Mater. 2024 May 5;469:133935.

39. Li X, Wen Z, Wang Y, Mo J, Zhong Y, Ge RS. Bisphenols and Leydig Cell Development and Function. Front Endocrinol (Lausanne). 2020 Jul;11:447.