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RESEARCH ARTICLE

The evaluation of possible role of endocrine disruptors in central and peripheral precocious puberty

Ayşe Derya Buluş[1], Ali Aşci[2], Pinar Erkekoglu[2], Aylin Balci[2], Nesibe Andiran[1,3], and Belma Koçer-Gümüşel[2]

[1]The Ministry of Health, Keçiören Training and Research Hospital, Pediatric Endocrinology Clinics, Ankara, Turkey, [2]Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey, and [3]Department of Pediatrics, Division of Endocrinology, Yıldırım Beyazıt University, Ankara, Turkey

Abstract

Exposure to environmental chemicals can affect genetic and epigenetic molecular pathways and may cause altered growth and development. Among those exposures, endocrine-disrupting chemicals (EDCs) are of particular concern as humans are abundantly exposed to these chemicals by various means in every period of life. Several well-known environmental chemicals, including phthalates and bisphenol A (BPA), are classified as EDCs. These EDCs are suggested to play roles in early onset of puberty in girls. The aim of this study is to determine plasma phthalate (di(2-ethylhexyl)phthalate [DEHP] and its main metabolite mono(2-ethylhexyl)phthalate [MEHP]) and urinary BPA levels in girls with idiopathic central precocious puberty (CPP) and peripheral precocious puberty (PPP). This study was performed on newly diagnosed idiopathic central precocious puberty (CPP) patients ($n = 42$) and peripheral precocious puberty (PPP) ($n = 42$) patients, who were admitted to Keçiören Training and Research Hospital, Clinic of Pediatric Endocrinology between August 2012 and –July 2013. Nonobese healthy girls ($n = 50$) were used as the control group. Urinary BPA levels were not statistically different in control, PPP and CPP groups (medians 10.91, 10.63 and 10.15 μg/g creatinine, respectively; $p > 0.05$). Plasma DEHP levels were significantly higher in PPP group when compared to control. Plasma MEHP levels were not significantly different in control and PPP groups ($p > 0.05$). However, in CPP group, both plasma DEHP and MEHP levels were significantly higher than control and PPP groups. This study showed that phthalates might play a role in the occurence of CPP in girls.

Keywords

Bisphenol A, central precocios puberty, endocrine disrupting chemical, peripheral precocious puberty, phthalate

History

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Introduction

Precocious puberty (PP) is the beginning of secondary sexual characteristics before eight years of age in girls. The most common type is known as idiopathic central precocious puberty (CPP) (Bridges et al., 1994; Lebrethon & Bourguignon, 2000). The process is identical to normal puberty, but happens earlier. Although the real trigger for idiopathic CPP is unkown, it has been proposed that it may be caused by the interactions between genetics, neurotransmitters in central nervous system (CNS) and hormonal factors. Additionally, general health status, nourishment, environmental conditions and socioeconomical status are also possible determinant factors in its occurence (Bridges et al., 1994). Peripheral precocious puberty (PPP) is a rarer and different condition. The triggering factors are estrogen and testosterone; however, hypothalamus and pituitary gland are not involved. It is usually a local problem with the ovaries, testicles, adrenal gland or a severely underactive thyroid gland (Atta et al., 2015). There is growing evidence that girls are developing earlier than in the past with a rising incidence of PP worldwide (De Munich Keizer & Mul, 2001; Mouritsen et al., 2010; Teilmann et al., 2005). Direct or indirect exposure to many widely available chemicals named as ''endocrine disruptoring chemicals (EDCs)'' may also play a role the increased outcome of PP (Mouritsen et al., 2010).

According to U.S. Environmental Protection Agency (US EPA), an EDC was defined as ''an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action or elimination of natural bloodborne hormones that are present in the body and are responsible for homeostasis, reproduction and developmental process'' (Diamanti-Kandarakis et al., 2006) Therefore, EDCs have many mechanisms of action. They may mimic naturally occurring hormones like estrogens, androgens and thyroid hormones in the body or they may potentially cause over-stimulation of hormonal pathways. They may also bind to a receptor within a cell and block the functions of endogenous

Address for correspondence: Belma Koçer-Gümüşel, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Hacettepe University, Ankara, Turkey. Tel: +90 3123052178. E-mail: belmagumusel@yahoo.com

hormones, i.e. acting as antiestrogens and antiandrogens (Caserta et al., 2008).

Endocrine disruptoring chemicals are available in so many environmental agents, such as pesticides, textiles, flame retardants, plastics, fragrances, detergents, lotions, paints and even in foods (Aksglaede et al., 2009; Caserta et al., 2008). The two most common EDCs that humans are widely exposed in everyday urban life are phthalates and bisphenol A (BPA). Phthalates are dialkyl or alkyl aryl esters of phthalic acid. Di(2-ethylhexyl) phthalate (DEHP) is one of the most extensively used phthalate derivative. It is mainly used in construction materials and in numerous polyvinyl chloride (PVC) products, including clothing (footwear, raincoats), food and beverage packaging, children products (toys, grip bumpers) and biomedical equipment (e.g. blood transfusion bags, dialysis bags). Phthalates were shown to exert antiandrogenic effects in laboratory animals (Braun et al., 2013; Hauser & Calafat, 2005). It is possible that these chemicals may particulary affect children in the first decade of life and their effects may be more pronounced during puberty (Massart et al., 2006). On the other hand, BPA is used to harden plastics and manufacture of polycarbonate plastics and epoxy resins. It is considered as estrogen-like EDC (EEDC). There is evidence that BPA functions as a xenoestrogen by binding to different ER receptors (weakly to ERα and ERβ and strongly to ERRγ). Metal food and beverage cans have a thin coating of BPA on the interior surface, which is essential to prevent corrosion of the can and contamination of food. Moreover, fetuses and young infants are commonly exposed to BPA by transplacental transfer of maternal BPA and through ingestion of maternal milk or formula in BPA containing plastic bottles. Perinatal exposure to environmentally relevant BPA doses result in morphological and functional alterations of the male and female genital tract and mammary glands (Braun & Hauser, 2011; Vandenberg et al., 2007).

This study was designed to investigate the possible roles of BPA and phthalates in the pathogenesis of PP in girls. Urinary BPA and plasma phthalate [DEHP and its main metabolite mono(2-ethylhexyl)phthalate (MEHP)] levels were determined in girls with CPP and PPP and their possible role in the etiology of PP was evaluated.

Materials and methods

Chemicals and equipment

All chemicals were obtained from Sigma-Aldrich (St.Louis, MO). All high-performance liquid chromatography (HPLC) equipments were purchased form Agilent (Santa Clara, CA).

Patients

The study was approved by Keçiören Research and Educational Hospital's Ethics Commitee. After an informed consent from the families of the study groups, all girls were participated to the study voluntarily.

Among the patients with complaint of breast development and/or pain accompanied with or without genital and/or axillary hair development applied to Pediatric Endocrinology Clinics of Keçiören Training and Research Hospital of Ministry of Health between August 2012 and July 2013, 42 girls were diagnosed with idiopathic CPP and 42 girls with isosexual PPP. Fifty healthy girls with no secondary sexual characteristics and no history of endocrine disease were recruited into control group.

A standard survey was appplied to all of study groups in order to identify the possible exposure of the subjects to phthalates and BPA's in their daily life. The information collected included their socio-economical and nutritional status, lifestyles, age, birth weight, mother's age, breastfeeding, duration of nipple use in the infancy period, playing with plastic toys, use of creams and lotions.

Hormone measurements

Serum estradiol, total testosterone, dehydroepiandrosterone sulfate (DHEAS) and free T_4 (fT_4) levels were measured by chemiluminescence microparticle immunoassay. Thyroid-stimulating hormone (TSH) levels were determined by electrochemiluminescence immunoassay (ECLIA). Serum luteinizing hormone (LH) levels and follicle-stimulating hormone (FSH) were measured by immunochemiluminometric assay (ICMA). Gonatropin-releasing hormone (GnRH) levels were determined by immunochemiluminometric assay third-generation assay (ICMA).

Diagnosis of precocious puberty

Three of the study groups were examined by the same pediatric endocrinologist. Fasting blood samples (from front arm ordorsum of the hand) were drawn in order to determine serum LH, FSH, estradiol, fT4 and TSH levels at 8:00-8.30 a.m.

GnRH test was only applied to girls who were suspected to have PP. Briefly, 100 μg gonadoreline acetate was applied intravenously (iv) within 15 s. Blood samples were drawn after 20 and 40 min of injection for the measurement of LH.

PPP was diagnosed according to the following criteria: 1. Breast development before eight years old ± pubic and/or axillary hair development; 2. Bone age older than chronological age (at least one year); 3. Body mass index (BMI) of the patients is between 25–85% according to the age and sex; 4. Peak LH response less than 5 mIU/ml in GnRH stimulation test by ICMA; 5. Absence of chronic disease.

CPP was diagnosed according to the following criteria: 1. Breast development before eight years old ± pubic and/or axillary hair development; 2. Bone age older than chronological age (at least one year); 3. BMI between 25–85% according to the age and sex; 4. Peak LH response over 5 mIU/ml in GnRH stimulation test; 5. Normal hypophyseal MR imaging; 6. Absence of chronic disease.

Deplasticization of the glassware

Extreme caution was taken for preventing contact with plastic material throughout the study. All the glassware used for the collection of urine samples were deplasticized with tetrahydrofuran: *n*-hexane (50:50, v/v) for 2 h and later dried in an incubator for 2 h. All the test tubes were deplasticized on a heater at 400 °C for 4 h.

Sampling

For the determination of urinary BPA, morning first urine sample (~30 ml) was taken into the deplasticized beakers and later aliquoted. The urine samples were stored at −70 °C.

For the determination of phthalates, 10 ml of blood sample was drawn from brachial veins of the girls with a sterile stainless steel needle tip (without any plastic structure at backside) into specially prepared heparinized glass tubes by ''dropping method'' in order to prevent contact with plastics. Centrifugation was performed at $800 \times g$. Plasma was separated and all samples were immediately aliquoted into glass vials and stored in a freezer at −70 °C.

After the collection, both urine and plasma samples were transfered to Pharmaceutical Toxicology Laboratory of Faculty of Pharmacy of Hacettepe University on dry ice.

Urinary BPA analysis

Urinary BPA levels were detected by HPLC after its extraction from plasma according to Yang et al. (2003) with some modifications. Briefly, for the analysis of total BPA (conjugated plus free form), 500 µl plasma sample was spiked with 50 µl of 50 ng/ml BPA (5 ng/ml spike in the last volume) and 30 µl of 2.0 M sodium acetate buffer (pH 5.0) was added later. 10 µl β-glucuronidase/aryl sulfatase (from *Helix pomatia*) was added and mixed. The mixture was incubated at water bath at 37 °C for 3 h. After the incubation, 100 µl 2 N HCl was added and the mixture was extracted with 5 ml of ethylacetate and centrifugated at $800 \times g$ for 5 min. 3 ml of supernatant was transfered to a new glass tube and evaporated under nitrogen stream. The extracts were kept at −20 °C until analysis. The residue was dissolved in 300 µl of 60% acetonitrile and 100 µl of this resultant was injected into our HPLC (Hewlett Packard Agilent 1200 Series with Fluorescence Detector, Vienna, Austria). Urinary BPA concentrations were calculated by using the calibration curve of peak height prepared from BPA standards. Limit of detection (LOD) was 1 ppb, and limit of quantitation (LOQ) was 2.5 ppb. In addition, the BPA concentrations were adjusted by urinary creatinine concentrations. Urinary creatinine was analyzed by the method described by Ogata and Taguchi (1988).

Measurement of plasma DEHP and MEHP levels

Plasma DEHP and MEHP levels were detected by HPLC according to Paris et al., (2003). Briefly, 200 µl of plasma was spiked with 20 µl 20 ppm DEHP (1 ppm in the last volume) and 20 µl 20 ppm MEHP (1 ppm in the last volume). Later, 400 µl NaOH (1 N), 100 µl %50 H_3PO_4 and 600 µl asetonitrile were added on the mixture, and the mixture was vortexed for 1 min and later was centrifugated at $1000 \times g$ for 10 min. The extraction was repeated by using 600 µl of acetonitrile and supernatants were collected. 900 µl of the supernatant was evaporated under nitrogen stream and the samples were kept at −20 °C until analysis. The residue was dissolved in 400 µl of 60% acetonitrile and 100 µl of the sample was injected to HPLC. The retention times for DEHP and MEHP were 39.3 min and 4.7 min, respectively. The concentrations of DEHP and MEHP in the samples were calculated by using the calibration curves prepared by using DEHP or MEHP standards. LODs for both DEHP and MEHP were 0.05 ppm, and LOQs for both DEHP and MEHP were 0.1 ppm.

Statistical analysis

For all the statistical analyses, PASW (Predictive Analytics Software version release 18.0.0) packaged software (Quarry Bay, HK, USA) was used. Shapiro–Wilk normality test was used to determine the compliance of the variables to normal distribution. Experimental data were analyzed with one-way analysis of variance (ANOVA) for parametric variables or Kruskal–Wallis variant analysis for nonparametric variables followed by the Student's *t*-test or Mann–Whitney U-test. Bonferroni correction was used after Mann–Whitney U-test. Pearson's or Speerman correlation coefficient was used to determine the correlation between the variables. The p values <0.05 were considered significant.

Results

Anthropometric values and questionnaires

Comparison of anthropometric values of three groups was given in Table 1. The birth weights of all the study groups were not statistically different. Mother's ages and mothers' educational levels were not also different in all of groups. The mean age of control, PPP and CPP groups were 7.4 ± 0.64, 7.4 ± 0.68 and 7.4 ± 0.61 years (y), respectively. There were no significant differences between the ages of the girls in three groups. The means of height were not different in PPP group when compared to control and CPP groups. Height SDS was markedly higher in CPP group (1.58 ± 0.6) compared to control (-0.21 ± 0.5). However, BMI of the CPP, PP and control groups were 17.8 ± 2.8, 17.1 ± 4.0 and 15.9 ± 1.0 kg/m^2, respectively, and there were no statistical differences between the three groups. Body weight standard deviation score (BWSDS) was markedly higher in both PPP and CPP groups versus control. Bone ages of CPP (9.9 ± 1 y) and PPP (9.6 ± 0.7 y) groups were significantly higher than the control group (6.9 ± 0.6 y) ($p < 0.05$, both).

Questionnare data

There were no significant difference between the groups in terms of duration of breastfeeding and duration of nipple use

Table 1. Comparison of anthropometric values of three groups.

	Control ($n = 50$)	PPP ($n = 42$)	CPP ($n = 42$)
Chronological age (year)	7.4 ± 0.64	7.4 ± 0.68	7.4 ± 0.61
Height (cm)	122.7 ± 5.0	131.9 ± 5.3^a	132.6 ± 6.7^a
Height SDS	-0.21 ± 0.5	1.36 ± 0.8	1.58 ± 0.6^a
Body weight (BW) (kg)	24.3 ± 3.2	30.0 ± 5.2^a	31.6 ± 8.0^a
BWSDS	0.7 ± 0.4	1.2 ± 0.7^a	1.1 ± 0.7^a
BMI (kg/m^2)	15.9 ± 1.0	17.1 ± 4.0	17.8 ± 2.8
Bone age	6.9 ± 0.6	9.6 ± 0.7^a	9.9 ± 1.0^a

BMI: body mass index; BWSDS: body weight standard deviation score; CPP: central puberty precocious puberty;height SDS: height standard deviation score; PPP: peripheral precocious puberty.
[a]$p < 0.05$.

in the infancy period. Besides, there were no differences between the groups in means of playing with plastic toys, daily use of creams, lotions and cosmetics or exposure to household products ($p > 0.05$). Past pencil/pen and eraser sucking were not different in CPP and PPP groups (48.10% and 50.0%, respectively, $p > 0.05$). However, the sucking behaviors of PPP and CPP girls were significantly higher than control group (17.4% higher in both PPP and CPP groups, $p = 0.024$ for CPP and control groups, $p = 0.032$ for PPP and control groups). The presence of PVC windows in the house was not different between CPP (92.5%) and PPP (93.3%) ($p > 0.05$); however, both of the groups had presence of PVC windows at their homes when compared to the control group (65.2%, $p < 0.05$ for both).

Ultrasound data and diagnosis

In suprapubic pelvic ultrasonography, the average length of uterus in CPP and PPP groups were 3.92 ± 0.61 (3.70–4.10) cm and 2.54 ± 0.29 (2.4–2.79) cm, respectively. There was a significant difference between two groups ($p < 0.0001$). The mean ovarian volume of CPP group was higher than that PPP group [2.70 ± 0.70 (2.46–2.98) and 2.12 ± 0.27 (2.01–2.23) cm^3, respectively]. However, the difference was not statistically significant ($p = 0.07$).

Hormone results

The hormone levels were shown in Table 2. The mean basal FSH levels were 4.07 ± 2.06, 3.18 ± 1.58 and 1.24 ± 0.43 mIU/mL in CPP, PPP and control groups, respectively, with a significant difference between control and PPP, control and CPP and PPP and CPP ($p < 0.05$, all). In CPP, PPP and control groups, the mean basal LH levels were 0.96 ± 1.05, 0.35 ± 0.24, 0.10 ± 0.01 mIU/and the difference between the CPP, PPP and control groups were statistically significant ($p < 0.05$, all). The mean estradiol levels were found to be 38.56 ± 9.22, 28.88 ± 6.92 and 6.02 ± 3.66 pg/ml in CPP, PPP and control groups, respectively. The differences between control and PPP, control and CPP and PPP and CPP were statistically significant ($p < 0.05$, all). Besides, the mean testosterone levels were also statistically different between control and PPP; control and CPP and PPP and CPP groups.

In GnRH stimulation test, the mean LH levels in CPP at 20th and 40th min were 8.98 ± 6.11 (4.90–32.0) and 10.81 ± 7.56 (5.80–45.0), respectively. These values were both significantly higher than the values of PPP group [2.73 ± 0.87 (0.89–4.17) mIU/mL at 20th min and 3.47 ± 0.89 (1.08–4.14) mIU/mL at 40th min] ($p = 0.001$, both).

Table 2. Comparison of hormone levels of the study groups.

	Control ($n = 50$)	PPP ($n = 42$)	CPP ($n = 42$)
LH	0.10 ± 0.00	0.35 ± 0.24^{a}	$0.96 \pm 1.05^{a,b}$
FSH	1.24 ± 0.43	3.18 ± 1.58^{a}	$4.07 \pm 2.06^{a,b}$
Total testosterone	0.01 ± 0.00	0.24 ± 0.16^{a}	$0.35 \pm 0.27^{a,b}$
Estradiol	6.02 ± 3.66	28.88 ± 6.92^{a}	$38.56 \pm 9.22^{a,b}$
DHEAS	18.26 ± 5.72	44.18 ± 16.68^{a}	$57.31 \pm 28.08^{a,b}$
TSH	2.70 ± 0.73	2.55 ± 0.93	2.41 ± 0.89
fT_4	1.13 ± 0.17	1.14 ± 0.17	1.10 ± 0.18

[a]Significantly different than control group ($p < 0.05$).
[b]Significantly different than PPP group ($p < 0.05$).
CPP: central puberty precocious puberty; DHEAS: dehydroepiandrosterone sulfate; FSH: follicle-stimulating hormone; fT_4: free T_4; LH: luteinizing hormone; PPP: peripheral precocious puberty; TSH: thyroid-stimulating hormone.

Urinary BPA levels

The urinary median BPA levels of the girls were 10.91 (min–max: 2.93–86.87), 10.63 (min–max: 2.46–63.49) and 10.15 (min–max: 2.08–50.22) μg/g creatinine in control, PPP and CPP groups, respectively. The mean urinary BPA levels were not statistically different in the three study groups ($p > 0.05$) (Table 3).

No significant correlations were determined between the urinary BPA levels and basal FSH, LH, estradiol levels, single ovarium volume and average uterus length in PPP and CPP groups. The results are presented in Table 4.

Plasma MEHP and DEHP levels

The mean plasma DEHP levels in CPP, PPP and control groups were 0.141 ± 0.106, 0.109 ± 0.037 and 0.095 ± 0.036 ppm, respectively. The difference between plasma DEHP levels of PPP and control groups were not statistically significant ($p = 0.085$). However, the mean plasma DEHP levels of control and PPP; control and CPP and CPP and PPP groups were statistically significant ($p < 0.05$, all) (Table 5).

The mean MEHP levels of CPP, PPP and control groups were 0.202 ± 0.090; 0.130 ± 0.068 and 0.134 ± 0.085 ppm, respectively. The differences between plasma MEHP levels of PPP and control groups were not statistically significant ($p > 0.05$). However, plasma MEHP levels of CPP group was markedly different when compared to both PPP and control groups ($p < 0.05$, all) (Table 6).

No significant correlations were determined between the plasma mean DEHP and MEHP levels with basal FSH, LH, estradiol, single ovarian volume and average uterus length in PPP and CPP groups. The results are presented in Table 6.

Table 3. Urinary BPA levels of the study groups.

		Control ($n = 50$)	PPP ($n = 42$)	CPP ($n = 42$)
Urinary BPA (μg/g creatinine)	Detectable *(n)*	36	35	38
	Nondetectable *(n)*	6	7	12
	Mean ± SD	20.26 ± 21.8	15.65 ± 45.50	12.21 ± 11.41
	Median	10.91	10.60	10.15
	Min–max	2.93–86.87	2.46–63.49	2.08–50.22

BPA: bisphenol A; CPP: central puberty precocious puberty; PPP: peripheral precocious puberty.

Table 4. Correlations between the urinary BPA levels, hormones and related parameters.

	BPA		
Group	Hormones and related parameters	Pearson's correlation coefficient (r)	Significance (p)
CPP	FSH	0.138	0.252
	LH	−0.086	0.581
	Estradiol	−0.158	0.452
	Average uterus length	0.108	0.636
	Right ovary	0.008	0.979
	Left ovary	−0.100	0.636
	LHRH0	0.590	0.343
	LHRH20	−0.125	0.551
	LHRH40	−0.113	0.590
PPP	FSH	−0.242	0.187
	LH	−0.267	0.226
	Estradiol	−0.126	0.764
	Average uterus length	−0.238	0.129
	Right ovary	−0.174	0.314
	Left ovary	−0.258	0.786
	LHRH0	0.237	0.497
	LHRH20	−0.067	0.630
	LHRH40	0.165	0.825

FSH: follicle-stimulating hormone; LH: luteinizing hormone.

Discussion

Throughout the world, the onset of puberty in girls has been occurring earlier than before, in particular, in the last 20 y (De Munich Keizer & Mul, 2001; Teilmann et al., 2005). Aksglaede et al. showed that during the years 1991–1993, the average age of children displaying secondary sex characterics was 10.88 y. From 2006–2008, the age dropped to 9.86. Therefore, after nearly 15 y, children were showing puberty signs 1.02 y earlier (Aksglaede et al., 2009).

Endocrine-disrupting chemicals are substances that may interfere with the body's endocrine system and may produce adverse developmental, reproductive, neurological and immune effects in both humans and animals. They can mimic natural hormones like estrogens or androgens. These substances may also produce overstimulation of endogenous hormones or they may bind to a cellular receptor and block the functions of endogenous hormones, e.g. acting as anti-estrogens or anti-androgens. They may alter the metabolism of natural hormones. Additionally, the widespread exposure of humans to different EDCs may contribute to the trend of

Table 5. Plasma DEHP and MEHP levels in study groups.

		Control ($n = 50$)	PPP ($n = 42$)	CPP ($n = 42$)
DEHP (ppm)	Detectable	32	31	26
	Nondetectable	18	11	16
	Mean ± SD	0.095±0.036	0.109±0.037[a]	0.141±0.121[a,b]
	Median	0.090	0.103	0.107
	Min–max	0.055–0.166	0.058–0.188	0.052–0.568
MEHP (ppm)	Detectable	36	31	24
	Nondetectable	14	11	18
	Mean±SD	0.134±0.085	0.130±0.068	0.202±0.090[a,b]
	Median	0.106	0.106	0.191
	Min–max	0.050-0.340	0.050–0.325	0.056–0.397

CPP: central puberty precocious puberty; DEHP: di(2-ethylhexyl)phthalate; MEHP: mono(2-ethylhexyl)phthalate; PPP: peripheral precocious puberty.

Table 6. Correlations between the urinary BPA levels, hormones and related parameters.

		DEHP		MEHP	
Group	Hormones and related parameters	Pearson's correlation coefficient (r)	Significance (p)	Pearson's correlation coefficient (r)	Significance (p)
CPP	FSH	0.238	0.252	−0.122	0.560
	LH	−0.086	0.681	−0.434	0.030
	Estradiol	−0.158	0.451	−0.080	0.705
	Average uterus length	−0.123	0.557	−0.340	0.096
	Right ovary	0.006	0.979	−0.465	0.019
	Left ovary	−0.100	0.636	−0.301	0.143
	LHRH0	−0.198	0.343	−0.448	0.025
	LHRH20	−0.125	0.551	−0.148	0.481
	LHRH40	−0.113	0.590	−0.142	0.498
PPP	FSH	−0.252	0.187	0.139	0.463
	LH	−0.290	0.126	0.035	0.854
	Estradiol	−0.026	0.893	0.169	0.371
	Average uterus length	−0.355	0.059	−0.299	0.109
	Right ovary	−0.194	0.314	0.255	0.174
	Left ovary	−0.008	0.967	−0.066	0.729
	LHRH0	0.124	0.520	0.092	0.627
	LHRH20	−0.067	0.730	−0.235	0.210
	LHRH40	0.061	0.755	−0.418	0.022

FSH: follicle-stimulating hormone; LH: luteinizing hormone.

early pubertal onset (Dickerson et al., 2012; Park et al., 2006; Yum et al., 2013).

A wide range of substances, natural or synthetic, are thought to cause endocrine disruption. These substances include pharmaceuticals, polychlorinated biphenyls (PCBs; including dioxin and dioxin-like compounds), dichlorodiphenyltrichloroethane (DDT) and other pesticides, and plasticizers that are substances added to plastics to increase their flexibility, transparency, durability and longevity, such as BPA and phthalates (Caserta et al., 2008).

In the present study, we aimed to investigate the possible exposure of girls to EDCs in neonatal, infancy and childhood periods with a questionnaire. No significant difference was determined in duration of use of nipple, teether and feeding bottle, playing with plastic toys and using plastic dishes between the groups and no correlations were found between urinary BPA levels and these observations. Although the nipple use period was higher in CPP and PPP groups than control group, the difference between the groups was not significant. Between CPP and PPP groups, the presence of PVC windows in the house was not different. However, the presence of PVC windows was significantly higher in both CPP and PPP groups when compared to control. Durmaz et al. (2014) observed that urinary BPA levels were higher in the subjects who used feeding bottle and nipple more than one year during infancy period, compared to those, who never used or used them less than one year. They also did not observe any significant differences between PP and control groups concerning these parameters.

Bisphenol A is a chemical produced in large quantities and is primarily used in the production of polycarbonate plastics and epoxy resins. Polycarbonate plastics are used in food and drink packaging, water and infant bottles, compact discs, impact-resistant safety equipment and medical devices. Epoxy resins are used as lacquers to coat metal products such as food cans, bottle tops and water supply pipes (Dickerson et al., 2012; Mouritsen et al., 2010). BPA is about 10 000 times less potent than 17-β estradiol. BPA was shown to stimulate *in vitro* cell proliferation and cause expression of genes that are responsive to estrogen (Katchy et al., 2014; Pupo et al., 2012; Qin et al., 2012; Sengupta et al., 2013). On the other hand, it was reported that exposure in small quantities of EDCs, particularly to BPA, may cause more significant toxic effects (Johnson & Parry , 2008; Vandenberg et al., 2013).

Urinary concentrations of BPA, especially in spot samples, have usually being used to determine BPA exposure from diet and environment. In ''The National Health and Nutrition Examination Survey (NHANES)'' study performed on 2838 children, median urinary BPA concentration was 2.8 ng/ml (interquartile range, 1.5–5.6). 1047 of the participants (34.1%) were overweight and 590 (17.8%) were obese. Significant associations between urinary BPA concentrations and obesity were found among whites, but not among blacks or Hispanics (Trasande et al., 2012). Kim et al. (2014) found that urinary BPA levels were higher in the females (9.6 ± 6.1 μg/g creatinine) compared to the males (7.8 ± 5.8, 9.6 ± 6.1 μg/g creatinine) in the general population. In literature, there are few studies that evaluated the relationship between BPA exposures, pubertal development or early onset of puberty (Lee et al., 2014; Qiao et al., 2010; Wolff et al., 2010). Wolff et al. (2010) investigated pubertal status, pubertal development, breast and pubic hair development in relation to exposure to hormonally active enviromental compounds among a multiethnic group of 192 healthy nine-year-old girls (mean age 9.5 ± 0.3; age range: 9.002–9.998), who lived in New York City. This cross-sectional study determined that there were not any significant differences between the urinary BPA levels of breast stage 1 and breast stage 2 groups or between pubic hair stage 1 and pubic hair stage 2 groups. Lee et al. (2014) reported that the urinary BPA levels were 8.0 ± 9.9 μg/g creatinine in CPP, 8.7 ± 7.6 μg/g creatinine in PPP and 6.6 ± 7.3 μg/g creatinine in control groups with no significant difference between the three groups. In our study, median urinary BPA levels were 10.15 (2.08–50.22) in CPP group, 10.60 (2.46–63.49) in PPP group and 10.91 (2.93–86.87) μg/g creatinine in control with no significant difference between the study groups. BPA levels in our study were higher than Lee et al.'s study performed in Korea (Kim et al., 2014). This might indicate higher exposure to BPA in our country when compared to Korea. The average bone age in the Korean study was found to be 10.3 ± 1.3 y in CPP; 9.8 ± 1.2 y in PPP and 8.8 ± 0.8 y in control group, which was similar to our study findings. Bone age was higher in CPP and PPP groups compared to the control group, as also observed in the current work. Besides, the researchers found that basal FSH level was 3.7 ± 2.4 IU/l in CPP; 1.9 ± 1.3 IU/l in PPP and 1.6 ± 0.7 IU/l in control group; and basal LH levels were 1.4 ± 2.0 IU/l in CPP; 0.2 ± 0.4 IU/l in PPP and 0.1 ± 0.0 IU/l in control group. They also observed higher LH levels in CPP group compared to the other groups, like in the current study. The peak LH response to GnRH stimulation test in the Korean study was found to be 24.7 ± 18.6 IU/l in CPP. This value was strikingly higher than PPP group (3.9 ± 1.3 IU/l), as also observed in the present study. The researchers also measured testosterone, 17 β-estradiol and pregnenolone levels and observed that the sex hormone levels were higher in subjects with higher BPA levels regardless of the PP type and a relation was determined between BPA levels and estrogen metabolism (Kim et al., 2014).

Durmaz et al. (2014) investigated the relationship between urinary BPA levels of CPP ($n = 26$, mean age: 7.01 ± 0.81) patients. The control group consisted of 21 healthy girls with the mean age of 6.51 ± 0.99 y. They found that urinary BPA levels were significantly higher in CPP group [median 8.34 (0.84–67.35) μg/g creatinine] compared to control group [median 1.62 (0.3–25.79) μg/g creatinine] and suggested that BPA may play a role in etiology of CPP (24). In the present study, urinary BPA levels in all three groups were higher than the findings of Durmaz et al. (2014). We can suggest that as our study was conducted on girls who were living in Ankara and as Ankara is an industrialized city, the girls might be exposed to environmental chemicals more than girls living in Antalya, which is a sea-side city with more agricultural facilities.

Phthalates are used as softeners in plastics. DEHP is a high production volume chemical used in a wide range of common products made of PVC, including some medical devices, food-packaging material and children's toys (Sathyanarayana et al., 2008). Phthalates may have more potential adverse

effects during infancy, pubertal and pregnancy periods. Their most important toxic effects were observed on the reproductive system, as also observed in animal studies (Erkekoglu et al., 2011, 2014). MEHP is its main metabolite in humans (Erkekoglu et al., 2010a, 2010b). As there are no covalent bonds between the phthalates and PVC material, phthalates can easily leach out plastic, causing human exposure by various means. Diet is believed to be the main source of DEHP and other phthalates in the general population. Fatty foods, such as milk, butter, and meats and food, which are packaged by PVC material, are the main sources of phthalate exposure from diet (Clark et al., 2003). Most of the American population was suggested to be exposed to phthalates and they had metabolites of multiple phthalates in their urine as shown by the studies of the Centers for Disease Control and Prevention (Calafat et al., 2011; Kato et al., 2004; Silva et al., 2004).

In general, children's exposure to phthalates is higher than adults. In a 1990s Canadian study that modeled ambient exposures, it was estimated that daily exposure to DEHP was 9 μg/kg bw/day in infants, 19 μg/kg bw/day in toddlers, 14 μg/kg bw/day in children and 6 μg/kg bw/day in adults (Saravanabhavan et al., 2013). Body-care products containing phthalates, like infant lotions, infant powders and infant shampoos, were associated with increased infant urinary concentrations of phthalate metabolites, and this association was strongest in younger infants. These findings suggest that dermal exposures may contribute significantly to phthalate body burden in this sensitive population (Clark et al., 2003; Sathyanarayana et al., 2008; Shelby, 2006). Additionally, infants and toddlers are at the greatest risk of exposure because of their mouthing behavior (Wilkinson & Lamb, 1999).

There are not sufficient number of studies on plasma or urinary levels of phthalates and their metabolites in healthy people in both Turkey and different parts of the world. In the forementioned study of Wolff et al. (2010), the researchers performed recuited 90 girls, from New York City, New York, Cincinnati, Ohio and Northern California, in their study. They reported that the mean phthalate and BPA levels showed wide ranges and were siginificantly different in Asian, Black, Hispanic and White populations. In the United States, in a multicenter cross-sectional study among 28 girls with CPP and 28 age-matched control girls, the researchers did not determine any significant differences in the concentrations of urinary phthalate metabolites [DEHP, dibutylphthalate (DBP) and diethylphthalate (DEP) metabolites] between the study groups (Lomenick et al., 2010). Colon et al. (2000) compared the serum phthalate levels in 41 premature thelarche (PT) and 35 control girls, who were all younger than eight years. The researchers showed that phthalate levels, particularly serum DEHP concentrations in girls with PT were significantly higher than control girls. In our study, plasma MEHP and DEHP levels were found to be significantly higher in girls with CPP (DEHP levels were 0.141 ± 0.121 ppm; MEHP levels were 0.202 ± 0.090 ppm), compared to both PPP (DEHP levels were 0.109 ± 0.037 ppm; MEHP levels were 0.130 ± 0.068 ppm) and control (DEHP levels were 0.095 ± 0.036 ppm; MEHP levels were 0.134 ± 0.085 ppm) groups. Besides, plasma DEHP levels of PPP group were significantly higher than control group.

There are some limitations in the current study. As there were retrospective questions, the answers of the questionnare might not be remembered correctly by the parents or children. Relatively small number of subjects were recruited in the study. Moreover, this is a descriptive study and with our study design, it is not possible to explain the mechanism underlying the causal relationship between urinary BPA and plasma phthalate levels and PP.

In conclusion, we did not observe any significant differences in urinary BPA levels of CPP, PPP and control groups. However, plasma phthalate (DEHP and MEHP) levels were markedly higher in CPP group when compared to both PPP and control girls. These findings might indicate that phthalates may play a more pronounced role in the etiology of CPP than BPA. As girls with CPP have higher phthalate levels, we can suggest that phthalates might have an impact on the central nervous system and may active the cellular molecular pathways that lead to the initiation of puberty. Further studies conducted on larger number of subjects are needed to confirm these findings. Besides, mechanistic studies are needed to enlighten the relationship between phthalate exposure and PP.

Declaration of interest

The authors declare no conflicts of interest.

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