

# Plasma Phthalate Levels in Pubertal Gynecomastia



**WHAT'S KNOWN ON THIS SUBJECT:** Being an androgen antagonist and a possible estrogen agonist, several endocrinologic effects of DEHP have been demonstrated mostly in vitro and in animals. A limited number of studies have linked the endocrinologic effects of DEHP and its metabolite, MEHP, in humans.



**WHAT THIS STUDY ADDS:** To our knowledge, this is the first study to define a possible effect of DEHP and MEHP in pubertal gynecomastia. The etiology of pubertal gynecomastia is attributed either to excessive estrogen, deficient androgen, increased aromatase enzyme activity, or a combination.

## abstract

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**OBJECTIVE:** Several untoward health effects of phthalates, which are a group of industrial chemicals with many commercial uses including personal-care products and plastic materials, have been defined. The most commonly used, di-(2-ethylhexyl)-phthalate (DEHP), is known to have antiandrogenic or estrogenic effects or both. Mono-(2-ethylhexyl)-phthalate (MEHP) is the main metabolite of DEHP. In this study, we aimed to determine the plasma DEHP and MEHP levels in pubertal gynecomastia cases.

**PATIENTS AND METHODS:** The study group comprised 40 newly diagnosed pubertal gynecomastia cases who were admitted to Hacettepe University Ihsan Doğramacı Children's Hospital. The control group comprised 21 age-matched children without gynecomastia or other endocrinologic disorder. Plasma DEHP and MEHP levels were measured by using high-performance liquid chromatography. Serum hormone levels were determined in some pubertal gynecomastia cases according to the physician's evaluation.

**RESULTS:** Plasma DEHP and MEHP levels were found to be statistically significantly higher in the pubertal gynecomastia group compared with the control group ( $P < .001$ ) (DEHP,  $4.66 \pm 1.58$  and  $3.09 \pm 0.90$   $\mu\text{g}/\text{mL}$ , respectively [odds ratio: 2.77 (95% confidence interval: 1.48–5.21)]; MEHP,  $3.19 \pm 1.41$  and  $1.37 \pm 0.36$   $\mu\text{g}/\text{mL}$  [odds ratio: 24.76 (95% confidence interval: 3.5–172.6)]). There was a statistically significant correlation between plasma DEHP and MEHP levels ( $r$ : 0.58;  $P < .001$ ). In the pubertal gynecomastia group, no correlation could be determined between plasma DEHP and MEHP levels and any of the hormone levels.

**CONCLUSIONS:** DEHP, which has antiandrogenic or estrogenic effects, may be an etiologic factor in pubertal gynecomastia. These results may pioneer larger-scale studies on the etiologic role of DEHP in pubertal gynecomastia. *Pediatrics* 2010;125:e122–e129

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### KEY WORDS

phthalate, di-(2-ethylhexyl)-phthalate, mono-(2-ethylhexyl)-phthalate, endocrine disrupter, puberty, gynecomastia

### ABBREVIATIONS

DEHP—di-(2-ethylhexyl)-phthalate

MEHP—mono-(2-ethylhexyl)-phthalate

SPL—stretched penis length

HPLC—high-performance liquid chromatography

LH—luteinizing hormone

FSH—follicle-stimulating hormone

FT3—free triiodothyronine

FT4—free thyroxine

SHBG—sex hormone-binding globulin

DHEA-S—dehydroepiandrosterone sulfate

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Pubertal gynecomastia refers to the benign enlargement of male breast attributable to the proliferation of ductile elements. Pubertal gynecomastia is a common problem occurring in up to 65% of adolescent boys.<sup>1</sup>

Male breast tissue has estrogen and androgen receptors. Estrogens stimulate and androgens inhibit breast tissue proliferation; pubertal gynecomastia is usually caused by an imbalance between these 2 factors, which may be attributable to excessive estrogen activity, deficient androgen activity, increased aromatase enzyme activity, or a combination of these effects on breast tissue.<sup>2</sup>

Phthalates, esters of *a*-phthalic acid, are a group of industrial chemicals with many commercial uses, including personal-care products (eg, perfumes, lotions, cosmetics), paints, building materials, household furnishings, clothing, dentures, children's toys, cleaning materials, insecticides, and most commonly as plasticizers in the food, certain medical devices, and pharmaceuticals.<sup>3–6</sup> Di-(2-ethylhexyl)-phthalate (DEHP) is one of the most widespread phthalate plasticizers. The annual production volume of DEHP alone has been estimated at 2 million tons.<sup>7–9</sup> Consumer products containing phthalates can result in human exposure through direct contact and use, indirectly through leaching into other products, or general environmental contamination. Humans are exposed through ingestion, inhalation, and dermal exposure.<sup>4–6,10</sup> The Agency for Toxic Substances and Disease Registry estimates that the maximum daily exposure to DEHP for the general population is ~2 mg/d.<sup>10</sup> It is also estimated that the total intake of DEHP is higher in all children younger than 19 years old than in adults.<sup>11</sup> DEHP and its metabolites have been detected in blood and urine samples from a high percentage of the people screened

for phthalates. Mono-(2-ethylhexyl)-phthalate (MEHP) is known as the first and the main metabolite of DEHP.<sup>12</sup>

DEHP has been reported to be an androgen antagonist,<sup>13</sup> an initiator of liver and testicular cancer in rats,<sup>14,15</sup> and to interfere with tamoxifen-induced apoptosis in human breast cancer cells because of its estrogenicity.<sup>16</sup> Although DEHP is considered to be an estrogen agonist and a testosterone antagonist, its mechanisms of toxicity are still not well understood, but are thought to be a function of the disruption of endocrine-regulated gene expression by interaction with estrogen receptor  $\alpha$ .<sup>17</sup>

Although there are some *in vivo* studies that show no estrogenic effect,<sup>18</sup> several articles have been recently published in which the authors reported a possible estrogenic effect of DEHP in humans, such as a probable role in premature thelarche,<sup>19</sup> endometriosis,<sup>20</sup> and precocious puberty.<sup>21</sup>

As far as we know there is no report in which the authors investigated the relationship between DEHP/MEHP levels and pubertal gynecomastia, which develops as a result of an imbalance between androgenic and estrogenic activity. In this study, we aimed to determine the plasma DEHP and MEHP levels in patients with pubertal gynecomastia.

## MATERIALS AND METHODS

### Subjects

The study group comprised 40 patients with pubertal gynecomastia, 11 to 15 years old (mean, 13.2  $\pm$  0.9 years), who were admitted to Hacettepe University Ihsan Doğramacı Children's Hospital in Ankara between October and December 2007. Twenty-one healthy male children of comparable age with no history of gynecomastia and any other endocrinologic disorder

comprised the control group (11.5–14.5 years old; mean, 13.2  $\pm$  1.1 years;  $P > .05$ ).

All patients were examined by the same pediatrician. Diagnosis of gynecomastia was made by standard approach<sup>2</sup>; testis volume was estimated by "Prader orchidometry" and stretched penis length (SPL) was measured. Patients who had normal medical history and physical examination findings apart from gynecomastia were classified as pubertal gynecomastia. In both groups, testis volumes were minimum, 8 mm, and the Tanner sexual development stage was 3 and above. The BMI was calculated. Families filled out a questionnaire for possible DEHP exposure.

The study was approved by Hacettepe University's ethical committee. Written informed consent was obtained from the parents and children before participation.

### Blood Sampling

Venous blood samples, for DEHP measurement, were taken by a stainless steel needle from the left arm cubital vein, and the sample was allowed to drop into heparinized glass test tubes directly. The tube openings and all around were covered by clean aluminum foil to protect the sample from contacts with the screw caps and sunlight. Centrifugation was performed at 800*g*, plasma was separated, and all samples were immediately aliquoted into glass vials and stored in a freezer at  $-80^{\circ}\text{C}$  until analysis. All glass equipment was heated in an oven at  $400^{\circ}\text{C}$  for 4 hours after the general cleaning procedure.

### Chemicals

DEHP and MEHP were purchased from Fluka (St Louis, MO) and Cambridge Chemicals Laboratories (Andover, MA), respectively. Acetonitrile (high-performance liquid chromatog-

raphy [HPLC] grade) and all other analytical grade reagents were obtained from Sigma Co (St Louis, MO) and Merck Co (Darmstadt, Germany).

Commercial kits for luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, prolactin, thyrotropin, free triiodothyronine (FT3), and free thyroxine (FT4) were purchased from Abbott Architect (Abbott Park, IL), and the kits for the sex hormone-binding globulin (SHBG) were purchased from Zentech (Angleur, Belgium). Serum dehydroepiandrosterone sulfate (DHEA-S) and total testosterone levels were tested by using the kits from Bio DPC Immulite (Los Angeles, CA).

### Measurement of DEHP and MEHP Levels in Plasma

Determination of DEHP and MEHP concentrations was conducted by HPLC equipped with an auto sampler (Hewlett Packard Agilent 1100 Series, Vienna, Austria) using a UV detector ( $\lambda = 230$  nm). A Spherisorb C18 ODS2 column was used (4.6 mm, inside diameter; length: 25 cm, 5- $\mu$ m particle size; Waters, Milford, MA). The mobile phase was orthophosphoric acid 0.1% (acetonitrile [90:10, vol/vol]), and the flow rate was 1 mL/min.<sup>22</sup>

Two hundred microliters of plasma were extracted by 400  $\mu$ L of NaOH (1N), 100  $\mu$ L of H<sub>3</sub>PO<sub>4</sub> (50%), and 600  $\mu$ L of acetonitrile. The extraction was repeated again by using 600  $\mu$ L of acetonitrile, and supernatants collected after the extractions were evaporated under nitrogen stream. The sample was dissolved in 400  $\mu$ L of mobile phase and injected to HPLC. The injection volume was 100  $\mu$ L. The retention times for DEHP and MEHP were 9.8 minutes and 3.1 minutes, respectively.

Recovery studies were performed on blank samples of plasma spiked with levels of 7.5 ppm of DEHP and 1.25 ppm of MEHP, and the average recoveries were found to be (mean  $\pm$  SD) 89  $\pm$  1.24% for DEHP and 98  $\pm$  1.12% for

MEHP on 20 occasions. Between-run precisions were 7.43  $\pm$  0.14% coefficient of variation (CV) for DEHP and 10.07  $\pm$  1.25% CV for MEHP. Within-day precisions were 9.88  $\pm$  0.51% CV for DEHP and 6.99  $\pm$  0.19% for MEHP. The concentrations of DEHP and MEHP in the samples were calculated by using the calibration curve of peak area prepared for DEHP and MEHP standards (Fig 1). The detection limits were determined as 0.05 ppm for DEHP and as 1 ppm for MEHP.

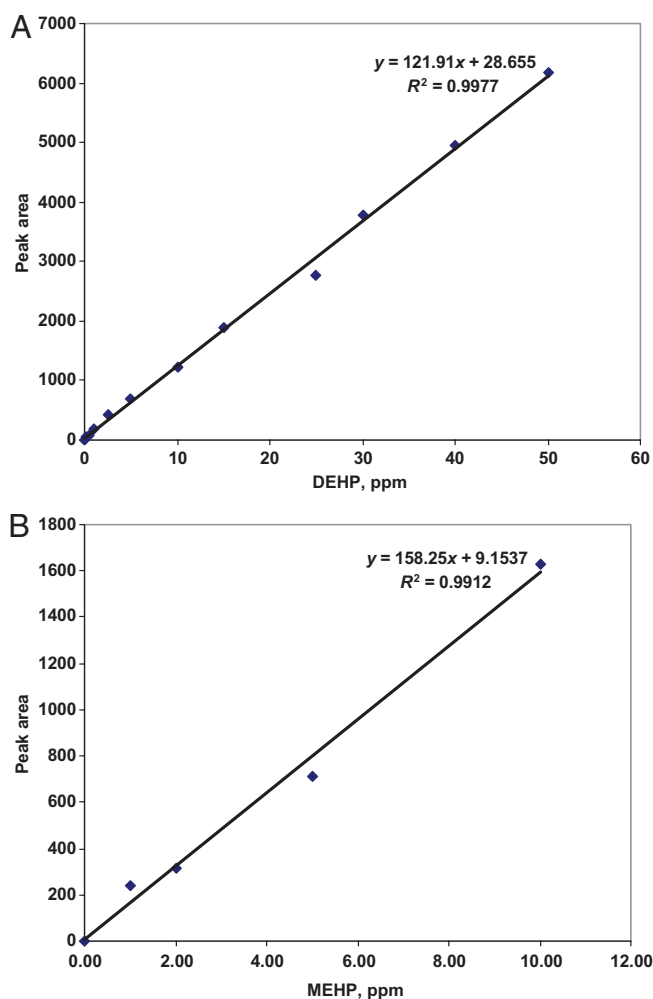
### Determination of Serum Hormone Levels

Serum LH, FSH, 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 DHEA-S and total testosterone levels were also determined by solid-phase chemiluminescence immunoassay. Serum-free testosterone levels were calculated according to the formula<sup>23,24</sup> by using albumin, total testosterone, and SHBG levels.

### Statistical Analysis

Statistical analysis was performed by using SPSS 13.0 (SPSS Inc, Chicago, IL). Data with normal distribution were expressed as mean  $\pm$  SD. The distribution of DEHP and MEHP values were an-



**FIGURE 1** Calibration curves of DEHP (A) and MEHP (B) standards.

alyzed by using the Shapiro-Wilk test and were found to have normal distribution. The comparison between 2 parametric values was made by using Student's *t* test. Comparisons of multiple groups were made by using one-way analysis of variance and Bonferroni correction. The correlation between parametric values was analyzed by using Pearson's correlation. Nonparametric values were compared by using the  $\chi^2$  test. When evaluating DHEA-S, multivariate logistic regression was used for determining the independent variables. A *P* value of  $<.05$  was accepted as significant.

## RESULTS

Forty patients with pubertal gynecomastia and 21 control patients were included in the study. However, 1 patient from the gynecomastia group and 1 patient from the control group were excluded from analysis because the values were at the end points and changed the normal distribution curve.

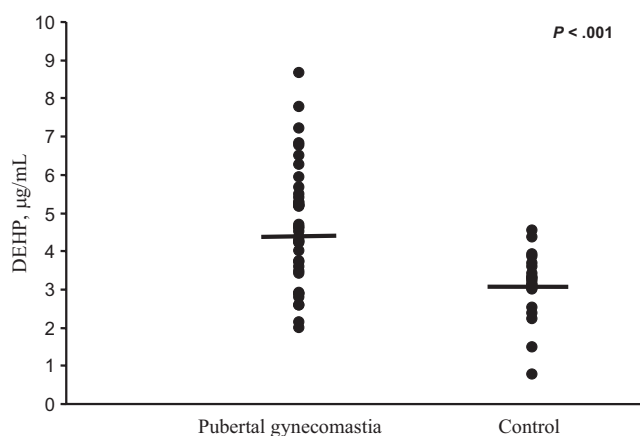
The clinical characteristics of the patients with pubertal gynecomastia and the control patients are displayed in Table 1. There was no statistically significant difference between the groups. Pubertal gynecomastia was bilateral in 30 (76.9%) patients. Severe pain was present in 9 (23%) patients, and 18 patients had been admitted to the hospital with the complaint of gynecomastia.

DEHP levels were detectable in all plasma samples and MEHP levels were detectable in all patients with pubertal gynecomastia and 19 of the 20 control patients. Plasma DEHP and MEHP levels were statistically significantly higher in the pubertal gynecomastia group compared with the control group ( $P < .001$ ) (DEHP,  $4.66 \pm 1.58$  and  $3.09 \pm 0.90$   $\mu\text{g}/\text{mL}$ , respectively [odds ratio: 2.77 (95% confidence interval: 1.48–5.21)]; MEHP,  $3.19 \pm 1.41$  and  $1.37 \pm 0.36$   $\mu\text{g}/\text{mL}$ , respectively [odds ratio:

**TABLE 1** Clinical Characteristics of Patients With Pubertal Gynecomastia and Control Patients

Characteristic	Patients With Pubertal Gynecomastia ( <i>n</i> = 39)	Control Patients ( <i>n</i> = 20)
Age, mo	158.2 $\pm$ 11.7	159.4 $\pm$ 13.1
Height, cm	157.9 $\pm$ 8.4	158.2 $\pm$ 7.1
Weight, kg	50.5 $\pm$ 14.6	51.9 $\pm$ 8.9
BMI	19.9 $\pm$ 3.9	20.6 $\pm$ 3.1
BMI-for-age percentile, <i>n</i> (%)		
1–24	9 (23.1)	2 (10)
25–75	17 (43.6)	10 (50)
76–100	13 (33.3)	8 (40)
Family history of pubertal gynecomastia	12 (30.7)	4 (20)
Left testis, mL	12.8 $\pm$ 4.2	13.1 $\pm$ 4.5
Right testis, mL	12.8 $\pm$ 4.2	12.6 $\pm$ 4.3
Average of testis volumes considering the bigger testis, mL	12.8 $\pm$ 4.1	13.2 $\pm$ 4.4
SPL, cm	9.3 $\pm$ 1.4	9.8 $\pm$ 1.9

Data are presented as mean  $\pm$  SD unless otherwise indicated.  $P > .05$  for all comparisons.



**FIGURE 2**

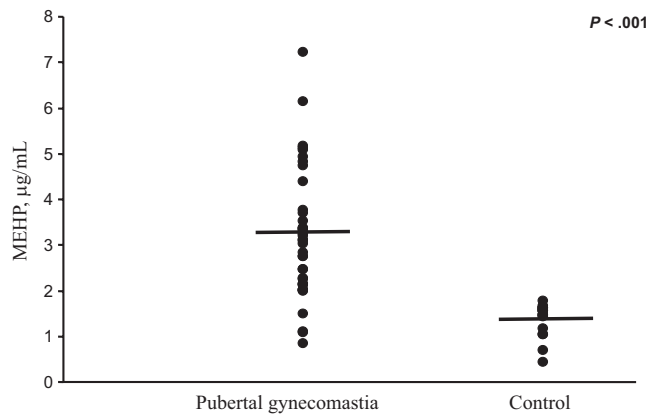
The distribution of DEHP levels in patients with pubertal gynecomastia and control patients.

24.76 (95% confidence interval: 3.5–172.6)]. There was a statistically significant correlation between DEHP and MEHP values in the pubertal gynecomastia group ( $r = 0.44$ ,  $P = .005$ ); however, the correlation was not significant in the control group ( $r = 0.43$ ,  $P = .065$ ). When all of the patients with pubertal gynecomastia and the control patients were evaluated totally again, a statistically significant high correlation was found between DEHP and MEHP values ( $r = 0.58$ ,  $P = .01$ ). The destitution of plasma MEHP and DEHP levels in pubertal gynecomastia and control cases are shown in Figs 2 and 3.

There was no statistically significant relationship between DEHP and

MEHP levels and height, weight, BMI percentile for age, testis volume, and SPL in gynecomastia or control cases. Also, no relationship could be detected between the breast enlargement (disk size) and DEHP and MEHP levels in the pubertal gynecomastia group (Table 2). The only statistically significant difference in DEHP and MEHP levels was found in the gynecomastia group according to the presence of pain on admission (Table 2).

The hormone levels of the patients with pubertal gynecomastia were determined according to the suggested algorithm for the management of gynecomastia.<sup>2</sup> The hormone levels were



**FIGURE 3** The distribution of MEHP levels in patients with pubertal gynecomastia and control patients.

**TABLE 2** Plasma DEHP and MEHP Levels According to Gynecomastia Characteristics and Anthropometric Values

Characteristic	<i>n</i>	DEHP Level, mean $\pm$ SD, $\mu\text{g/mL}$	MEHP Level, mean $\pm$ SD, $\mu\text{g/mL}$
Mammary gland involved			
Unilateral	9	4.30 $\pm$ 1.32	3.47 $\pm$ 1.27
Bilateral	30	4.77 $\pm$ 1.65	3.11 $\pm$ 1.46
1 mammary gland, cm			
<2	7	4.18 $\pm$ 1.08	3.14 $\pm$ 0.90
$\geq 2$	2	4.71 $\pm$ 2.55	4.63 $\pm$ 2.15
Bilateral, cm			
<2	15	5.33 $\pm$ 1.62	3.18 $\pm$ 1.31
$\geq 2$	15	4.22 $\pm$ 1.54	3.03 $\pm$ 1.64
Gynecomastia			
With severe pain	9	5.61 $\pm$ 1.93 <sup>a</sup>	4.14 $\pm$ 1.81 <sup>b</sup>
Without severe pain	30	4.38 $\pm$ 1.38	2.90 $\pm$ 1.16
Admitted to the hospital with the complaint of gynecomastia			
Yes	18	4.63 $\pm$ 1.92	3.32 $\pm$ 1.61
No	21	4.70 $\pm$ 1.27	3.07 $\pm$ 1.25
Tamoxifen treatment			
Given	4	4.24 $\pm$ 2.44	4.10 $\pm$ 2.51
Not given	35	4.71 $\pm$ 1.50	3.08 $\pm$ 1.25
BMI-for-age percentile			
Study group			
1–24	9	5.38 $\pm$ 1.58	3.08 $\pm$ 1.34
25–75	17	4.64 $\pm$ 1.49	3.13 $\pm$ 1.66
76–100	13	4.19 $\pm$ 1.53	3.34 $\pm$ 1.18
Control group			
1–24	2	2.18 $\pm$ 1.99	1.43 ( <i>n</i> = 1)
25–75	10	3.17 $\pm$ 0.68	1.50 $\pm$ 0.30
76–100	8	3.24 $\pm$ 0.86	1.23 $\pm$ 0.40

<sup>a</sup> *P* = .04.

<sup>b</sup> *P* = .018.

all in the normal range (Table 3). There was no correlation between plasma DEHP/MEHP levels and FSH, LH, SHBG, thyrotropin, FT4, FT3, and total and free testosterone levels (Table 4). There was only a statistically significant correlation between DEHP and DHEA-S lev-

els. However, when the effect of SHBG was adjusted, no correlation was found ( $r = -0.268$ ,  $P = .13$ ). All levels for  $\beta$ -human chorionic gonadotropin were found to be <1.2 mIU/mL; therefore, no additional analysis for correlation was made.

**TABLE 3** Hormone Levels in the Pubertal Gynecomastia Group

Hormone	<i>n</i>	Level, mean $\pm$ SD
Thyrotropin, $\mu\text{U/mL}$	38	2.48 $\pm$ 0.96
FT4, pmol/L	38	15.16 $\pm$ 2.02
FT3, pmol/L	31	6.51 $\pm$ 0.86
FSH, mIU/mL	35	2.67 $\pm$ 1.09
LH, mIU/mL	35	1.20 $\pm$ 0.61
Estradiol, pg/mL	35	9.48 $\pm$ 9.55
Total testosterone, ng/dL	32	168.22 $\pm$ 137.35
Free testosterone, ng/dL	29	3.79 $\pm$ 2.81
DHEA-S, $\mu\text{g/dL}$	39	144.87 $\pm$ 68.87
SHBG, nmol/L	33	30.43 $\pm$ 13.77
Prolactin, ng/mL	35	9.77 $\pm$ 4.61

**TABLE 4** The Correlation Between Hormone Levels and DEHP and MEHP Levels in the Gynecomastia Group

Hormone	DEHP, Pearson's Correlation <i>r</i> ( <i>n</i> )	MEHP, Pearson's Correlation <i>r</i> ( <i>n</i> )
Thyrotropin	0.291 (38)	-0.125 (38)
FT4	0.05 (38)	-0.163 (38)
FT3	0.166 (31)	-0.128 (31)
FSH	0.097 (35)	0.166 (35)
LH	0.067 (35)	0.125 (35)
Estradiol	-0.148 (35)	0.11 (33)
Total testosterone	-0.032 (32)	0.126 (35)
Free testosterone	-0.115 (29)	0.065 (29)
DHEA-S	-0.414 (39) <sup>a</sup>	-0.014 (39)
SHBG	0.316 (33)	-0.076 (33)
Prolactin	-0.062 (35)	-0.303 (35)

<sup>a</sup> *P* = .009.

## DISCUSSION

DEHP, the most commonly used plasticizer, is a widespread ubiquitous environmental contaminant. There are many reports showing its long-term toxic effects and tissue accumulation in animals.<sup>15,25–27</sup> The toxic effects depend on dose, the age of the person, and the duration of exposure.<sup>28</sup> It has been shown that the toxic effects are much more during infancy, puberty, and pregnancy periods, and long-term small amounts of exposure can cause evident toxic effects.<sup>18</sup> Experimental animal models showed its effects on developmental stages.<sup>25,29–31</sup> Although DEHP has been shown to have toxic effects for many systems, the most important effects after long-term



exposure are on the reproductive system.<sup>27,28</sup>

In this study, the possible effect of DEHP in the etiology of pubertal gynecomastia was investigated. It was found that for every 1  $\mu\text{g}/\text{mL}$  increase in DEHP levels, the risk of pubertal gynecomastia increased nearly three-fold. Plasma MEHP levels were studied to increase the reliability of the results. A high correlation was found between DEHP and MEHP levels. The increase in the risk of gynecomastia with MEHP levels was found to be much higher ( $\sim 25$ -fold).

This is the first study in which the authors investigated the relationship between pubertal gynecomastia and DEHP levels, making it impossible to compare with other study results. We have tried to minimize the risk for environmental contamination as outlined in the Materials and Methods section. No plastic material was used in any stage of the study. Blood was taken to the laboratory in 30 minutes. Plasma samples were separated and stored at  $-80^{\circ}\text{C}$  immediately. The analyses of the pubertal gynecomastia cases and control blood samples were made simultaneously so if any contamination occurred it would have affected both groups. The diurnal and inter-day repeatability of the DEHP analysis method was found to be very high, the average recovery was 89% and the detection limit was low ( $0.05 \mu\text{g}/\text{mL}$ ). However, the number of the pubertal gynecomastia cases and controls in the study is limited, and the study design does not allow for a direct conclusion of cause-effect.

We could not encounter any study about the DEHP levels in pubertal boys. However, in several studies, the blood DEHP levels were studied in specific risk groups such as women with endometriosis<sup>20</sup> or newborn infants after blood transfusion.<sup>32</sup> Although it is not possible to define a blood range for

DEHP levels, the DEHP levels found in these studies are comparable with our results.

A clue to the cause-effect relationship of DEHP in pubertal gynecomastia was the higher DEHP levels in patients who were admitted to the hospital with pain. These patients mentioned that gynecomastia had started in the previous 3 months and most were bilateral. It is known that pain is experienced in the early florid stage of gynecomastia. Patients who present with symptoms of pain and tenderness generally have gynecomastia of more recent onset, and pathologic findings include hyperplasia of the ductal epithelium, infiltration of the periductal tissue with inflammatory cells, and increased subareolar fat.<sup>33</sup> Still, these results have to be confirmed in larger case series.

Mostly, antiandrogenic properties have been demonstrated in animals. In a study where pregnant rats were given DEHP during their pregnancy, infant rats were found to have decreased anogenital distance, female type areola, and genital malformations.<sup>27</sup> In another study, intrauterine DEHP exposure was found to cause a decrease in testis size and testosterone levels.<sup>28</sup> Recently, similar results were reported for human infants.<sup>34</sup> The antiandrogenic effects of DEHP were related to decreased testosterone levels and it is thought that DEHP does not have a direct effect on androgen receptors.<sup>35–37</sup>

In our study, no difference could be found for testis size among the groups. Also, there was no correlation between DEHP/MEHP levels and testis size. However, there was a reverse but nonsignificant correlation between plasma DEHP levels and free and total testosterone levels. Again, these results need confirmation from larger case series.

One of the etiologic explanations for pubertal gynecomastia is increased estrogenic sensitivity in breast tissue

or increased estrogen levels (disturbed estrogen/androgen ratio, relative elevation of estrogen level), or both. Animal and in vitro studies indicate negligible estrogenic activity for DEHP.<sup>38</sup> However, recent studies in humans indicate a possible estrogenic effect.<sup>17,19,20</sup>

The first study demonstrating a possible estrogenic effect for DEHP was reported by Colon et al<sup>19</sup> in Puerto Rico. They have shown that DEHP levels in 45 girls with premature thelarche were statistically significantly higher compared with the 35 control girls. Unfortunately, these findings were criticized for its statistical methods being vague; the blood samples were stored for 2 to 6 years and the DEHP levels were very high compared with other studies, which may be because of contamination of samples. In our study, all samples were collected and analyzed within 6 months and our results were comparable with the literature. However, being the first study<sup>19</sup> related to estrogenic and/or antiandrogenic effects of DEHP still it is remarkable.

In another study, DEHP/MEHP levels were found to be higher in patients with endometriosis compared with the control group. The etiopathogenesis of endometriosis is not clear, however, it is thought that estrogen receptors in the uterus also play a role. In the study, it was speculated that DEHP may play a role in the development of endometriosis through the estrogen receptor stimulation.<sup>20</sup>

In a recent study conducted in Chinese girls with precocious puberty, DEHP levels were found to be higher compared with the control group. Also, it was noted that girls with high DEHP levels have larger ovarian and uterus size. The authors have concluded that DEHP exposure during the period of rapid development may cause unexplainable estrogenic or antiandrogenic effects.<sup>21</sup>

All these studies point to a possible estrogenic effect of DEHP. However, this is not simply via increasing the estrogen levels but probably through receptors or gene regulation, or both.

It is known that estrogen or estrogen-like substances increase the cellular proliferation in breast cancer cell culture (MCF-7). Bloom et al<sup>39</sup> have demonstrated a dose-dependent relationship between DEHP and cellular proliferation in these cell cultures. The authors of a recent study have demonstrated that DEHP inhibits tamoxifen-induced apoptosis in breast cancer cell cultures.<sup>16</sup> Tamoxifen is an estrogen receptor antagonist used in breast cancer treatment. Tamoxifen decreases the *BCL-2* gene expression, which is an antiapoptotic oncogene and increases the expression of *BAX* gene, which increases apoptosis. However, adding DEHP to the culture reverses all these effects. The antiapoptotic *BCL-2* gene expression increases and apoptotic *BAX* gene expression decreases. In this study, it was concluded that DEHP may alleviate

tamoxifen-induced apoptosis through estrogen receptor interaction, and it was recommended that women using tamoxifen use less cosmetic material (because of its DEHP content).<sup>16</sup> Tamoxifen is also a drug of choice in pubertal gynecomastia. It is effective in decreasing breast size and pain.<sup>40,41</sup>

In this study, plasma DEHP and MEHP levels were found significantly higher in patients with pubertal gynecomastia. Although in this study it is not possible to show a cause-effect relationship, it may be speculated that DEHP may lead to pubertal gynecomastia via receptor or intracellular gene regulation, rather than effecting hormone levels, at a period of rapid growth or increased sensitivity.

The hormonal evaluation was not one of the objectives of the study. Therefore, plasma hormone level determination was made only from some of the pubertal gynecomastia cases according to the algorithms.<sup>2,35</sup> All hormone levels were within the normal range and no relationship between DEHP or MEHP levels and thyrotropin, LH, FSH, total

testosterone, free testosterone, FT3, FT4, and SHBG were found. A negative significant relationship between plasma DEHP levels and DHEA-S was found. However, after correction with SHBG levels, no relationship persisted.

## CONCLUSIONS

Plasma DEHP and MEHP levels were found to be significantly higher in patients with pubertal gynecomastia compared with control patients. Several studies that included humans as the subjects have demonstrated a possible estrogenic effect of DEHP besides antiandrogenic effects. Also, the effects of DEHP in cell cultures support an estrogenic effect at the receptor level. Being a common problem, this subject deserves additional studies in larger case series to confirm our results and explain the cause-effect relationship.

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