Plasma Phthalate Levels In Children With Speech Delay

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Abstract

Speech delay is one of the most common developmental problems. One of the risk factors may be the exposure to environmental chemicals. There is increased environmental exposure to phthalates, an endocrine-disrupting chemical. In this study, we aimed to determine the relationship of phthalates with speech delay. We included 50 children with isolated speech delay and 40 healthy children of similar age. Children were surveyed for risk factors for speech delay and phthalate exposure. Plasma di-(2-ethylhexyl) phthalate (DEHP), mono-(2-ethylhexyl) phthalate (MEHP) and dibutyl phthalate (DBP) levels were measured by high pressure liquid chromatography. The DEHP, MEHP and DBP levels in study and control groups were 0.377 [0.003 - 1.224] µg/ml, 0.212 [0.007 - 1.112] µg/ml ($p = 0.033$), 0.523 [0.031 - 2.477] µg/ml, 0.152 [0.239 - 2.129] µg/ml ($p < 0.001$), and 0.395 [0.062 - 1.996] µg/ml, 0.270 [0.006 - 0.528] µg/ml ($p = 0.004$), respectively. Multiple linear regression analysis was used to adjust the association between the phthalate levels and factors differing between the two groups in terms of delayed speech risk factors. While there was no significant difference between the study and control groups in terms of DEHP level ($p=0.233$), the MEHP and DBP levels were found significantly higher in the study group ($p<0,001$).

Conclusion: The statistically significant higher phthalate levels in those with speech delay indicate that these children are more exposed to phthalates and more epidemiological studies are needed to evaluate the association between phthalates and speech delay.

What Is Known

The adverse effects of phthalates, one of the environmental pollutants, on the neurological development of infants have been shown in many studies. However, studies on language development and therefore speech delay are limited.

What is New

MEHP and DBP, which are commonly used phthalates in daily life, were significantly higher in children with speech delay after adjusted analyzes. This result indicates that children with speech delay are more exposed to phthalates in any way.

1. Introduction

Speech delay, one of the most common developmental problems, is considered as child's inability in attaining to the language development they should reach at certain ages and to lag behind their peers [1]. Delay in language acquisition without any identified cause is observed at a rate of 2.3-19% in pre-school children aged between 2-5 years [2]. Children with a speech delay can have a higher risk of suffering psychological and behavioral adjustment problems both in preschool and later life [3, 4].

Speech delay has been associated with multiple risk factors, such as maternal and pregnancy related problems in prenatal period, prematurity [5], hypoxic birth [6], hearing loss [7], and environmental factors

such as low socioeconomic level, caregiver education level and lack of stimuli [8].

Another risk factor for speech delay may be the mother's or baby's exposure to environmental chemicals during prenatal or postnatal periods. Exposure to certain chemicals may negatively impact on the baby's neurological development [9] which in turn may affect language skills. One of the environmental chemicals are phthalates.

Phthalates are semi-volatile synthetic chemicals that are used to soften plastics. Many products used in daily life, such as food packages, cosmetics/personal care products, medical materials and toys can contain certain amounts of different phthalate derivatives [10, 11]. Since phthalates are not covalently bound to the plastic matrix, they can easily contaminate the environment. Moreover, humans are exposed to these chemicals by different routes (oral, dermal, inhalation and intravenous) [12, 13]. In many studies, phthalates were detected in different body fluids such as blood, urine, saliva and breast milk [14, 15]. Phthalates were also determined in amniotic fluid and this finding shows that they can cross the placental barrier and humans are exposed to these plasticizers even during the fetal period [16].

Phthalates, such as di-(2-ethylhexyl) phthalate (DEHP) and dibutyl phthalate (DBP), are the most abundant chemicals in the environment. These chemicals affect many systems, particularly the reproductive system. They are suggested to have anti-androgenic properties [17, 18]. Due to their effects on androgens, they may disrupt testosterone-dependent brain development during prenatal period [19]. Although there are some studies that evaluated the association of phthalate metabolites with neurodevelopmental status in the neonatal, infancy and childhood periods [20, 21], there is limited research concerning their effects on language development. Published studies showed contradictory and inconclusive results on the relationship between phthalates and speech delay [22, 23].

Due to the limited number of studies exploring the effects of phthalate exposure on language development, we aimed to examine the relationship between phthalate levels and speech delay in children aged between 2-6 years in the present study.

2. Materials And Methods

Study Participants

This study is a case-control study. Ethical approval was obtained from the Ethical Review Board of Hacettepe University Non-Interventional Clinical Research in September 2019 (Approval number: GO 19/748).

The study groups applied to Hacettepe University İhsan Doğramacı Children's Hospital were as follows:

1. Study group (n=50): Children between 24-72 months admitted to the Developmental Pediatrics Outpatient Clinic with isolated speech delay were recruited as the study group.

2. Control group (n=40): Healthy children between 24-72 months admitted with acute complaints to the General Pediatrics Outpatient Clinic were recruited as the control group.

Patients who were previously diagnosed with a neurodevelopmental, genetic, or metabolic disorder or living in a stimuli poor environment which was related to cause speech delay were excluded from the study.

During the patients' evaluation, families were informed and written consent was obtained. A questionnaire to evaluate the possible routes of phthalate exposure, demographic information, and risk factors of speech delay was given after obtaining consent. This questionnaire mainly investigated whether mothers' were in contact with products containing phthalate derivatives during pregnancy and the postpartum period and whether children's dietary habits and environment might cause significant phthalate exposure.

Data collection

Data were collected between October 2019 and February 2020. Language and other developmental domains such as gross motor, fine motor, problem-solving and personal-social development of the children in the study and control groups were assessed with the Ages and Stages Questionnaire (ASQ). ASQ is a screening tool widely used in large-scale screening programs and research, and can be filled by parents/other caregivers directly or in the company of a trained professional [24]. We used Turkish version of the Ages and Stages Questionnaires (ASQ-TR). The sensitivity and specificity of ASQ-TR are 0.94 and 0.85, respectively [25]. Children who were below the cutoff scores in the language domain and within the normal range in other domains were included in the isolated speech delay and were followed up. Children whose total scores were within the normal range in all developmental domain evaluations were included in the control group.

From the patient's file, information on the demographic data [child's age, gender, birth order, mother's and father's age and education level, family's socioeconomic status, the place where they lived (urban/suburban)], and breastfeeding status/duration were obtained. The missing data were recorded on the form by questioning the family. The socioeconomic status of the children was determined by the Hollingshead-Redlich Scale [26].

Laboratory Analyses of Serum Phthalates

Chemicals and reagents

DEHP, DBP, acetonitrile, n-hexane, tetrahydrofuran, NaOH, HNO₃ and H₃PO₄ were purchased from Sigma-Aldrich (Mannheim, Germany). MEHP was from Cambridge Isotope Laboratories (Tewksbury, MA).

Deplasticization of the glassware

All glassware were washed and were kept in 10% nitric acid for 24 h. Later, glassware were rinsed 4 times and then cleaned with n-hexane:tetrahydrofuran (50:50) for 2 h. They were dried at 37°C. High pressure liquid chromatography (HPLC) vials were kept at 400°C for 4 hours to avoid plastic contamination. Aluminum foil was used in order to prevent contact with the plastic material on the lids of all glass materials.

Biological samples

Venous blood samples were taken into heparinized tubes, prepared in 5 ml drip form with a sterile needle tip without plastic structure at the rear end. Samples were centrifuged (3500 rpm for 10 min). Plasma samples were kept at -80°C and stored until analysis.

Extraction of DBP, DEHP, and MEHP from plasma

For the analyses of plasma DEHP, DBP and MEHP levels, the method of Paris et al. [27] was used with some modifications [28]. Briefly, after spiking plasma (200 µL) with phthalates (1 ppm in the last volume), NaOH (1N, 400 µL), H₃PO₄ (50%, 100 µL) and ACN (800 µL) were added and mixed. The mixture was vortexed and centrifuged. Supernatant (600 µL) was taken into another tube and evaporated until dryness under nitrogen stream. Residues were stored at -20°C.

Chromatographic analysis

Residues were dissolved in 60% ACN (300 µl). Standards (0.2, 0.5, 1, 2 and 5 ppm for DEHP; 0.2, 0.5, 1, 2 and 5 ppm for DBP; 0.2, 0.5, 1, 2.5 and 5 ppm for MEHP) and samples (100 µl) were injected into HPLC (Agilent 1100 series, Santa Clara, CA). HPLC columns were Spherisorb C18 ODS2 column (25 cm x 5 µm x 4.6 mm i.d.) (Waters, Milford, MA), and ODS C18 precolumn (4 cm) (Waters, Milford, MA). Mobile phase was 0.1% H₃PO₄ and ACN [pH 3.0, 80:20 (v/v)]. Flow rate was 1 ml/min. Retention times for DBP, DEHP, and MEHP were 4.1 min, 32.5 min and 4.5 min, respectively. Due to close retention times of DBP and MEHP, their analyses were performed separately.

Plasma concentrations of DBP, DEHP, and MEHP were calculated from standards and peak areas were used for quantification. Limit of detections (LODs) were 0.38 µg/ml for DBP, 0.09 µg/ml for DEHP, and 1.4 µg/ml for MEHP. Limit of quantifications (LOQs) were 1.15 µg/ml for DBP, 0.27 µg/ml for DEHP, and 4.26 µg/ml for MEHP.

Recovery studies were performed on blank samples of plasma spiked with levels of 9.1 µg/ml of DBP, 9.8 µg/ml of DEHP, and 10.1 µg/ml of MEHP. Within-day precisions were DBP 0.71±0.40% CV, DEHP 3.09±1.29% CV, and 3.27±1.05% CV for MEHP. Between-run precisions were 1.06±0.56% CV for DBP, 9.21±1.19 CV for DEHP and 7.92±2.11% CV for MEHP.

Statistical Analysis

Statistical analysis and documentation were performed using IBM SPSS 21.0 (Chicago, IL). For descriptive statistics, mean and standard deviation or median and smallest-largest values were given in numerical variables, while number and percentage values were given for categorical variables. Kolmogorov-Smirnov test was used for the assumption of normality. In the comparison of the groups, if the assumption of normality was provided, the significance test of the difference between the two means was used. If the assumptions of normality were not provided, the Mann-Whitney U Test was used. Categorical variables were compared by using chi-square test. Multiple regression analysis was used to explain the total change in the dependent variable studied with independent variables differing between groups. p values <0.05 were considered as statistically significant.

3. Results

Sociodemographic characteristics and risk factors for speech delay in both groups are shown in Tables 1 and 2. The number of boys ($p = 0.036$) and history of speech delay ($p < 0.001$) was higher in the study group. The father's education level ($p = 0.049$) and smoking during pregnancy ($p = 0.008$) was higher in the control group. There was no significant statistical difference between the two groups in terms of other characteristics and factors.

Table 1
Sociodemographic Characteristics of the Study and Control Groups

Characteristics	Study Group (n=50)	Control Group (n=40)	p
Gender, n (%)	12 (24.0)	18 (45.0)	0,036
Female	38 (76.0)	22 (55.0)	
Male			
Age (month)**	39 [24 - 70]	43.5 [24 - 72]	0.199
Mother's age (year)*	33.6 ± 5.28	34.73 ± 5.39	0.322
Father's age (year)*	36.86 ± 4.93	37.85 ± 6.25	0.403
BMI Z scores, n (%)	3 (6)	1 (2.5)	0.16
<-2 SD	6 (12)	4 (10)	
≥-2 SD- <-1 SD	27 (54)	31 (77.5)	
≥-1 SD- <1 SD	12 (24)	4 (10)	
≥1 SD- 2 SD	2 (4)	0 (0.0)	
≥2SD			
Mother's educational level, n (%)	10 (20.0)	10 (25.0)	0.724
8 years	16 (32.0)	10 (25.0)	
9-12 years	24 (48.0)	20 (50.0)	
>12 years			
Father's educational level, n (%)	8 (16.0)	1 (2.5)	0.049
8 years ^a	17 (34.0)	12 (30.0)	
9-12 years	25 (50.0)	27 (67.5)	
>12 years			

* Mean ± SD ** median [min – max]

Abbreviations: SD, standard deviation

^aDifference in education level due to group with 8 years or less education.

Characteristics	Study Group (n=50)	Control Group (n=40)	p
Total breastfeeding duration (month), n (%)	8 (16.0)	9 (22.5)	0.434
< 6 months	42 (84.0)	31 (77.5)	
≥6 months			
Birth order, n (%)	18 (36.0)	20 (50.0)	0.522
1	21 (42.0)	13 (32.5)	
2	8 (16.0)	4 (10.0)	
3	3 (6.0)	3 (7.5)	
4+			
Place of residence, n (%)	45 (90.0)	40 (100.0)	0.063
Urban	5 (10.0)	0 (0.0)	
Suburban			
Monthly income (x10³ Turkish Lira)**	5 [2 - 18]	6 [1 - 16]	0.139
Social-economic level, n (%)	25 (50.0)	24 (60.0)	0.162
Higher education, profession or higher administrative positions	17 (34.0)	13 (32.5)	
	4 (8.0)	3 (7.5)	
Smaller business owners, government officials or skilled laborers, high school graduates	4 (8.0)	0 (0.0)	
Semi-skilled laborers; educational level below high school			
Semi-skilled laborers; primary school graduates or not educated			
* Mean ± SD ** median [min – max]			
Abbreviations: SD, standard deviation			
^a Difference in education level due to group with 8 years or less education.			

Table 2
Comparison of Risk Factors for Speech Delay in the Study and Control Groups

	Study Group n(%)	Control Group n(%)	p
Prenatal period	5 (10.0)	13 (32.5)	0.008
Smoking	0 (0.0)	0 (0.0)	NA
Alcohol consumption	10 (20.0)	8 (20.0)	1.000
Infection	6 (12.0)	9 (22.5)	0.184
Hypothyroidism	2 (4.0)	0 (0.0)	0.501
Radiation			
Natal period	24 (48.0)	16 (40.0)	0.448
Delivery type	26 (52.0)	24 (60.0)	0.429
<i>NSVD</i>	3.33 ± 0.49	3.25 ± 0.43	0.058
<i>C-section</i>	39.12 ± 1.45	38.57 ± 1.17	1.000
Birth weight (kg)*	3 (6.0)	2 (5.0)	0.444
Birth week*	0 (0.0)	1 (2.5)	
Hypoxia history			
Congenital anomaly			
Postnatal period	6 (12.0)	5 (12.5)	1.000
Disease/Disability	0 (0.0)	1 (20.0)	0.322
<i>Hearing</i>	4 (66.7)	2 (40.0)	<0.001
<i>Visual</i>	2 (33.3)	2 (40.0)	
<i>Other</i>	8 (16.0)	11 (27.5)	
Screen exposure	21 (42.0)	12 (30.0)	
0-1 hour	21 (42.0)	17 (42.5)	
1-2 hour	33 (66.0)	7 (17.5)	
> 2 hours			
Speech delay in the family			
* Mean ± SD.			
Abbreviations: NSVD, Normal spontaneous vaginal delivery; C-section, Cesarean section			

The median values of detectable DEHP, MEHP and DBP levels were significantly higher in the study group compared to the control group (Mann-Whitney U test, $p = 0.033$, $p < 0.001$ and $p = 0.004$, respectively) (Table 3).

Table 3

Plasma di-(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP) and mono-(2-ethylhexyl) phthalate (MEHP) levels of the children in the study and control groups.

Phthalate	Study Group	Control Group	P
DEHP ($\mu\text{g/ml}$)*	0.377 [0.003 – 1.224] (n=47)	0.212 [0.007 – 1.112] (n=35)	0.03
MEHP ($\mu\text{g/ml}$)*	0.523 [0.031 – 2.477] (n=47)	0.152 [0.239 – 2.129] (n=33)	<0.001
DBP ($\mu\text{g/ml}$)*	0.395 [0.062 – 1.996] (n=42)	0.270 [0.006 – 0.528] (n=30)	0.004
*Median [min – max]			
Abbreviations: DEHP: di-(2-ethylhexyl) phthalate, DBP: dibutyl phthalate, MEHP: mono-(2-ethylhexyl) phthalate			

Multiple linear regression analysis for risk factors including gender, smoking during pregnancy, father's education level, and family history of speech delay between the study and control groups showed no significant difference in terms of DEHP levels ($p=0.233$). In contrast, MEHP and DBP levels were found to be significantly higher in the study group ($p<0.001$) (Table 4). Since the data were skewed in multiple linear regression models, square root transformation for DEHP and DBP and logarithmic transformation for MEHP were performed.

Table 4
a. Multiple linear regression analysis of DEHP levels in the study and control groups.

Model		Unstandardized Coefficients		t	Sig.	95,0% Confidence Interval for B	
		B	Std. Error			Lower Bound	Upper Bound
1	(Constant)	,985	,227	4,338	,000	,533	1,438
	Group	-,081	,067	-1,203	,233	-,215	,053
	Gender	,026	,061	,421	,675	-,096	,148
	Father's education	-,036	,043	-,836	,406	-,121	,049
	Smoking during pregnancy	-,063	,076	-,824	,412	-,214	,089
	Speech delay history in family	-,074	,067	-1,101	,274	-,208	,060
a. Dependent Variable: square root_ di-(2-ethylhexyl) phthalate (DEHP)							

Table 4
b. Multiple linear regression analysis of MEHP levels in the study and control groups.

Model		Unstandardized Coefficients		t	Sig.	95,0% Confidence Interval for B	
		B	Std. Error			Lower Bound	Upper Bound
1	(Constant)	-,470	,822	-,572	,569	-2,108	1,168
	Group	-1,150	,243	-4,731	,000	-1,635	-,666
	Gender	-,045	,220	-,204	,839	-,482	,393
	Father's education	,162	,156	1,043	,300	-,148	,473
	Smoking during pregnancy	,024	,275	,087	,931	-,525	,573
	Speech delay history in family	,419	,245	1,707	,092	-,070	,907
a. Dependent Variable: logarithm_ mono-(2-ethylhexyl) phthalate (MEHP)							

Table 4
c. Multiple linear regression analysis of MEHP levels in the study and control groups.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95,0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	-,470	,822		-,572	,569	-2,108	1,168
	Group	-1,150	,243	-,573	-4,731	,000	-1,635	-,666
	Gender	-,045	,220	-,021	-,204	,839	-,482	,393
	Father's education	,162	,156	,110	1,043	,300	-,148	,473
	Smoking during pregnancy	,024	,275	,009	,087	,931	-,525	,573
	Speech delay history in family	,419	,245	,211	1,707	,092	-,070	,907
a. Dependent Variable: logarithm_ mono-(2-ethylhexyl) phthalate (MEHP)								

The questionnaire results applied to evaluate the possible phthalate exposure routes of children and their mothers in the study and control groups are shown in Tables 5 and 6.

Table 5
Possible exposure routes of the mothers to phthalates in the study and control groups

	Mother's exposure at any time			Mother's exposure during pregnancy		
	Study (n=50,%)	Control (n=40,%)	p	Study (n=50, %)	Control (n=40,%)	p
Shampoo	49 (98.0)	40 (100.0)	1.000	50 (100.0)	40 (100.0)	NA
Hair conditioner	17 (34.0)	24 (60.0)	0.014	19 (38.0)	24 (60.0)	0.038
Hair spray	4 (8.0)	7 (17.5)	0.206	3 (6.0)	4 (10.0)	0.695
Hair dye	24 (48.0)	25 (62.5)	0.170	4 (8.0)	4 (10.0)	1.000
Make up	26 (52.0)	27 (67.5)	0.138	21 (42.0)	23 (57.5)	0.144
Nail polish	7 (14.0)	9 (22.5)	0.295	2 (4.0)	5 (12.5)	0.235
Shower gel	19 (38)	19 (47.5)	0.365	13 (26.0)	18 (45.0)	0.059
Perfume/ deodorant	38 (76.0)	36 (90.0)	0.084	23 (46.0)	30 (75.0)	0.005
Detergents	50 (100.0)	40 (100.0)	NA	50 (100.0)	40 (100.0)	NA
Fabric softeners	37 (74.0)	24 (60.0)	0.158	37 (74.0)	26 (65.0)	0.355
Use of dishwashing gloves	6 (12.0)	5 (12.5)	1.000	6 (12.0)	6 (15.0)	0.677
Use of medicine	11 (22.0)	11 (27.5)	0.546	10 (20.0)	13 (32.5)	0.177

Table 6

Possible exposure routes to phthalates in the newborn and early childhood periods of children in the study and control groups

	Study (n=50, %)	Control (n=40, %)	p
Baby bottle usage	34 (68.0)	29 (72.5)	0.643
Pacifier use	13 (26.0)	18 (45.0)	0.059
Plastic toy	47 (94.0)	40 (100.0)	0.251
Baby shampoo	50 (100.0)	40 (100.0)	NA
Hospitalization in intensive care	6 (12.0)	5 (12.5)	1.000
Mechanic ventilation	4 (8.0)	1 (2.5)	0.377
Dialysis by peritoneum or hemodialysis	1 (2.0)	0 (0.0)	1.000
Blood transfusion	2 (4.0)	0 (0.0)	0.501
Frequent infections/use of antibiotics (≥ 6/year)	3 (6.0)	7 (17.5)	0.102
Plastic plate/spoon	9 (18.0)	9 (22.5)	0.596
Plastic storage container	34 (68.0)	25 (62.5)	0.585
Plastic bottle/carboy	44 (88.0)	37 (92.5)	0.726
Frozen food consumption	6 (12.0)	10 (25.0)	0.109
Canned food consumption	3 (6.0)	5 (12.5)	0.458
Packaged food consumption	47 (94.0)	37 (92.5)	1.000
PVC products at home	40 (80.0)	29 (72.5)	0.403
PVC products in the nursery	25 (50.0)	20 (50.0)	NA
Smoking mother	11 (22.0)	16 (40.0)	0.064
Smoking at home	19 (38.0)	23 (57.5)	0.065
Abbreviation: PVC, Polyvinyl chloride; NA, Not applicable			

DEHP, DBP and MEHP levels of children in the study group were analyzed according to possible phthalate exposure routes of mothers and children. We observed that plasma DEHP levels were higher in those whose mothers used hair dyes ($0,574 \pm 0,305 \mu\text{g/ml}$) and conditioners ($0,486 [0,211 - 1,224] \mu\text{g/ml}$) at any time and those who used perfume/deodorant ($0,535 \pm 0,340 \mu\text{g/ml}$) during pregnancy compared to those who did not ($0,313 \pm 0,173 \mu\text{g/ml}$, $0,312 [0,003 - 1,096] \mu\text{g/ml}$, and $0,364 \pm 0,188 \mu\text{g/ml}$, respectively) ($p=0.001$, $p=0.041$, and $p=0.048$, respectively). For DBP and MEHP, no statistically significant difference was found for any of the exposure routes.

When phthalate levels of children in the control group were examined according to phthalate exposure routes, no statistically significant difference was found in terms of DEHP and DBP levels according to the mothers' phthalate exposure pathways (at any time and during pregnancy). In childhood, plasma DBP levels were found to be higher in those who used plastic storage containers for food (0,284 [0,076 – 0,468] µg/ml) than those who did not (0,062 [0,006 – 0,528] µg/ml) ($p = 0.049$). While plasma MEHP levels of children in the control group were higher than those whose mothers used fabric softeners at any time (0,313 [0,063 – 2,129] µg/ml) and during pregnancy (0,267 [0,063 – 2,129] µg/ml) compared to those who did not (0,137 [0,024 – 0,348] µg/ml and 0,124 [0,024 – 0,348] µg/ml, respectively) ($p = 0.010$, $p = 0.011$, respectively). There was no difference in MEHP results in terms of phthalate exposure routes in childhood.

4. Discussion

In studies on speech delay, male gender [29, 30], education level of parents [8, 30] and family history of speech delay [8] are some of the well-known risk factors. In our study, the males were diagnosed with isolated speech delay three times more. Moreover, the family history of speech delay was higher and the father's education level was lower in the study group compared to the control group.

In numerous studies, it is reported that exposure to smoking in prenatal and postnatal periods have adverse effects on children's neurological development process and thus on language development [31, 32]. However, contrary to expectations, smoking rate during pregnancy was reported to be higher in the control group. However, there was no difference between the two groups in terms of maternal smoking in the postnatal period and smoking in the home.

Many epigenetic factors may play a role in the etiology of speech delay [33]. Among these factors, genetics, lifestyle, and environmental pollutants are suggested to be the main factors [34]. The development of the central nervous system in children is sensitive to the environment during the intrauterine period and first years of life [35]. Considering that language development is one of the primary markers of the neurodevelopmental process, studies investigating the role of phthalates, one of the environmental pollutants, in the etiology of speech delay attract more attention day by day. Phthalates have been reported to impair testosterone-induced brain development. Exposure to these chemicals, in prenatal period may have substantial impact on speech. Moreover, they may also interfere with the sex-related neurodevelopmental stages and males are suggested to be more susceptible to such effects [36].

In our study, median plasma levels of DEHP, its metabolite MEHP, and DBP were significantly higher in the study group compared to control group. After correcting for speech delay risk factors, which differed between the two groups with direct regression analysis, there was no significant difference between the two groups in terms of DEHP levels, while MEHP and DBP levels were found to be significantly higher in the study group.

In a prospective study conducted in Denmark, the researchers examined the relationship between prenatal phthalate exposure and language development of children aged 20-36 months. In the study, high urinary diethyl phthalate (DEP), butyl benzyl phthalate (BBP), and DEHP metabolite levels in the third trimester of mothers were associated with low language scores (word count and complex language use) in boys, but this relationship was not observed in girls [22].

In a two-centered cohort study from Sweden and the United States, which examined the relationship between language development and urinary phthalate metabolites, in corrected analyses, doubling the prenatal DBP and BBP metabolite exposure significantly increased the estimated relative risk (odds ratio, OD) for language delay in the Swedish group by approximately 25–40%. However, no association with any DEHP metabolite with language delay was observed in either cohort [23]. Unlikely, we found an inverse relationship between MEHP and speech delay.

In a study published in Singapore, a large number of metabolites of different environmental chemicals were measured by gas chromatography-mass spectrometry in hair samples taken from mothers at 26-28 weeks of gestation and their relationship with the developmental areas of 24-month-old children were examined. The researchers found that high phthalic acid levels were associated with low expressive language scores (univariate p value=0.022) [37].

In our study, in which possible exposure routes from the intrauterine period to early childhood were questioned, the plasma DEHP levels were found to be higher in the study group whose mothers reported using hair dyes and conditioners at any time and those who used deodorant during pregnancy. Plasma MEHP levels of children in the control group whose mothers used softeners (at any time and during pregnancy) were higher. Although it is stated that oral and respiratory exposure is more prominent for phthalates [14], these results also show the importance of dermal exposure. In the control group, plasma DBP levels were higher in those whose mothers used plastic storage containers for food. DBP is a phthalate predominantly found in personal care and cosmetic products, and enteric-coated drug tablets [14], and no difference was found in both the study and control groups in the inquiries made in this regard. Therefore, more research should be done with DBP-related exposure sources and their possible undesired outcomes.

Our study has some limitations and strengths. Firstly, although the number of participants in the study and control groups was determined using the G-power 3.0.10 program with 0.80 effect size, 80% power, and 5% margin of error based on previous studies, repeating a similar study with larger groups will increase the strength of the study. There was no gender match between the groups, but this was adjusted when evaluating the results, along with other confounding risk factors that differed between the two groups. Although a single blood sample was taken in our study and a cross-sectional evaluation was made, it should be kept in mind that exposure routes mostly continue in the same environment and through similar routes in pregnancy. However, prospective follow-up studies should be conducted in terms of half-lives and long-term effects of endocrine disruptors. Although questioning about possible intrauterine and childhood exposure routes in our study is one of the advantages of this study, it should

be considered that the results may be affected by the possibility of incomplete or incorrect recall in the retrospective responses of the questionnaire studies. In our study, in order to prevent possible contamination during the collection of blood samples and experimental procedures, the use of plastic materials was avoided, and other materials used were pre-treated as described in the method section. In addition, although the early development inventory we used in our study was a parent-centered and easy-to-use screening questionnaire, each child in our study was evaluated by the same clinician, taking into account the parents' views. Thus the results achieved provided a more objective evaluation.

5. Conclusion

Plasma DEHP, MEHP, and DBP levels were found to be significantly higher in children with isolated speech delay compared to healthy controls. After the adjusted analysis of the factors differing between the two groups in terms of delayed speech risk factors, there was no significant difference in terms of DEHP levels, while MEHP and DBP levels were markedly higher in the study group. The statistically significant higher phthalate levels in those with speech delay indicate that these children are more exposed to phthalates in whatever form in different ways.

In conclusion, endocrine-disrupting agents in the etiology of speech delay and their mechanism of action are still not fully explained. Our study contributes to the limited number of studies in this area, but more epidemiological and pathophysiological studies with higher sample sizes are needed to confirm this cause-effect relationship.

Abbreviations

ASQ-TR, Turkish version of the Ages and Stages Questionnaires; DEHP, di-(2-ethylhexyl) phthalat; DBP, dibutyl phthalate; HPLC, high pressure liquid chromatography; MEHP, mono-(2-ethylhexyl) phthalate

Statements & Declarations

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Code availability: N/A (software application or custom code)

Author's Contribution:

Dr Yaman conceptualized and designed the study and the data collection instruments, collected data, drafted the initial manuscript, and reviewed and revised the manuscript.

Prof Erkekoğlu planned and supervised the plasma phthalate measurement experiments, and critically reviewed and revised the manuscript.

Dr Özkemahlı and Msci Yirun performed the plasma phthalate measurement experiments, worked out the technical details and performed the numerical calculations for the suggested experiments.

Drs Akkus and Bahadur, and child development specialist Özdemir took part in developmental assessment and data collection.

Prof Özmert conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed and revised the manuscript for important intellectual content.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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