Urinary bisphenol-A levels in children with type 1 diabetes mellitus

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Abstract

Background: Bisphenol-A (BPA) is one of the most abundantly produced chemicals globally. Concerns have been raised about BPA's possible role in the pathogenesis of type 1 diabetes mellitus (T1DM). The main aim of the current study was to evaluate the possible association between BPA exposure and T1DM. The second aim was to investigate children's possible BPA exposure routes in Turkey.

Methods: A total of 100 children aged between 5 and 18 years including 50 children with T1DM and 50 healthy children were included. Urinary BPA levels of all children were measured using high-performance liquid chromatography. Mothers of children enrolled in the study were also requested to complete a survey that included questions on the sociodemographic characteristics, medical history and possible BPA exposure routes of their children.

Results: In the T1DM group, urinary BPA levels were slightly higher compared to the control group, but this difference was not significant ($p = 0.510$). However, there was an inverse relationship between current urinary BPA levels and birth weight. It was found that the use of plastic kettles and the consumption of dairy products in plastic boxes significantly increased the urinary BPA concentrations in all subjects.

Conclusions: Although there was no significant association between urinary BPA levels and T1DM, we found an inverse relationship between current urinary BPA levels and birth weight. This finding might be important for prenatal exposure, and further prospective research must be conducted. Also, the use of plastic kettles, which has not been mentioned much in the literature before, was found to be an important exposure route for BPA.

Keywords: bisphenol-A; children; endocrine disrupting chemicals; type 1 diabetes mellitus.

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Introduction

Modernization and technology, although increasing convenience, increase the exposure risk to man-made chemicals. The potential toxic effects of many of these chemicals are largely unknown. Some of these chemicals are suggested to interrupt the endocrine system and are named as "endocrine disrupting chemicals (EDCs)". Bisphenol-A (BPA) is suggested to be an EDC and is used extensively in the manufacturing of polycarbonate plastics and epoxy resins. The main utilization of BPA is in the production of various common consumer products such as water containers, baby bottles, the resin linings of food and beverage cans, food packaging, reusable water bottles, plastic tableware, food storage containers, children's toys and sealants in dentistry [1, 2]. Due to its many applications it is one of the most abundantly produced man-made chemicals globally [3]. Human BPA exposure is continuous and ubiquitous as BPA metabolites are measured in more than 90% of children and adults throughout the globe [4].

Diabetes mellitus is the most commonly encountered endocrine-metabolic disorder of childhood. It is an epidemic worldwide and might be affecting more than 300 million people. Although type 2 diabetes (T2DM) is dominating this epidemic, type 1 diabetes (T1DM) should not be overlooked because it represents 10–15% of diabetes mellitus cases [5]. Among children under the age of 15 years, the risk of developing T1DM has been rapidly

increasing over the past 30 years [6]. This rapid increase in the incidence of T1DM cannot be explained only by genetic predisposition. Environmental agents which were seldom referred to in the pathogenesis of diabetes have become suspected factors in disease development. In recent years, EDCs, including BPA, have come under suspicion as factors in diabetes development [4]. Recent studies have found some evidence that BPA exposure could contribute to the development of insulin resistance, obesity and T2DM [7–10]. Recent studies have found that BPA exposure can affect β-cells of the pancreas and promote autoimmunity, which accelerates insulitis and diabetes development in an animal model of T1DM [4, 11, 12].

Though an association between BPA and T2DM has been suggested by different studies in the literature, the relationship between T1DM and BPA has not been evaluated previously [2, 7–10]. Therefore, the main aim of the current study was to evaluate the possible relationship between BPA exposure and T1DM by comparing the urinary BPA levels of children with T1DM to those of children of a control group. In addition, a survey was taken to find out the children's possible BPA exposure routes in Turkey.

Materials and methods

This case-control study was conducted between May and November 2016 in Ankara, Turkey. A total of 100 children (aged between 5 and 18 years) participated in the study. The study group consisted of 50 children who had been diagnosed with T1DM and followed in the Hacettepe University Faculty of Medicine, Department of Pediatric Endocrinology, Ankara, Turkey. The control group comprised 50 healthy age- and sex-matched children (aged between 5 and 18 years) with no history of T1DM. Control cases were chosen from the Hacettepe University Faculty of Medicine Department of Social Pediatrics outpatient clinic; children with any endocrine disorder, obesity or chronic disease were excluded.

All participants were examined by the same pediatrician and their weights and heights were measured. The body mass index (BMI) was calculated by weight (kg)/height $(m)^2$ formula. BMI z-scores were calculated by using the World Health Organization (WHO) "Anthro" program [13]. Mothers of children who were enrolled in the study were asked to complete a survey, which included questions on the sociodemographic characteristics, medical history and possible BPA exposure routes of their children. Possible BPA exposure routes were determined according to the literature [1–3, 8]. The questionnaire was completed using face-to-face interview technique.

Spot urine samples from both the study and control groups were collected in deplasticized glass beakers in the Social Pediatrics outpatient clinic. The samples were aliquoted and kept at –20 °C. The urine samples of all subjects were sent on dry ice to the Hacettepe University, Faculty of Pharmacy, Department of Toxicology. All samples were kept at –20 °C until BPA extraction.

All subjects participated in the study voluntarily and written consent was obtained from the parents of the children in the study. This study was approved by the Scientific and Ethical Committee of the Hacettepe University Faculty of Medicine (Number: GO 15/147-08).

Deplasticization of the glassware

In order to prevent contact with plastic material, extreme caution was taken throughout the study. All the glasswares were deplasticized with tetrahydrofuran: n-hexane (50:50, v/v) for 2 h and later were kept in an incubator for 2 h. All test tubes were deplasticized on a heater at 400 °C for 4 h and later cooled, sealed with aluminum foil and kept in glass collectors until use.

Chemicals

All the chemicals, including BPA, were obtained from Sigma-Aldrich (St. Louis, MO, USA). Glucuronidase/arylsulfatase enzyme (from *Helix pomatia*) was purchased from Roche (Mannheim, Germany). All high-performance liquid chromatography (HPLC) equipment were obtained from Agilent (Santa Clara, CA, USA).

Extraction of BPA from urine

For the analysis of urinary BPA, the method of Yang et al. [14] was used with some modifications. Briefly, after spiking urine (500 µL) with BPA (5 ng/mL), sodium acetate buffer (200 M, pH 5) and glucuronidase/arylsulfatase were added, mixed and the mixture was incubated at 37 °C for 3 h in order to free the conjugated BPA (glucuronidated plus sulfonated). After incubation, HCl (2 N, 100 µL) and ethyl acetate (5 mL) were added, vortexed and centrifuged. The pellet was discarded and the supernatant (2.5 mL) was evaporated; the glass tubes with residues were kept at –20 °C until analysis.

Chromatographic analysis

The residue was dissolved in acetonitrile (60% [v/v], 400 µL) and 100 µL of the sample was injected into an HPLC (Hewlett-Packard Agilent 1100 series, Santa Clara, CA, USA). The HPLC parameters were as follows: BPA standards used were 1.25, 2.5, 5, 10, 25, 50 and 100 ng/mL; C18 column (25 cm × 5 µm × 4.6 mm i.d.); column temperature: 25 °C; fluorescence detector ($\lambda_{exitation} = 230$ nm, $\lambda_{emission} = 315$ nm). The mobile phase was acetonitrile: tetrahydrofuran (2.5%, v/v) (gradient elution was applied as 60:40–5:95), flow rate was 0.4 mL/min; retention time was 18.3–19.2 min and analysis duration were 40 min. The limit of detection (LOD) according to the method of the U.S. Environmental Protection Agency (EPA) for BPA was 0.5 ng/mL and the limit of quantitation (LOQ) was 1.25 ng/mL [15]. The urinary BPA levels were calculated by the peak heights obtained from the chromatogram. Recovery studies were performed on urine samples spiked with 5 ng/mL of BPA. The average recoveries were found to be (mean ± standard deviation [SD]) 97.37 ± 1.23% on 10 occasions. The Between-run precision was 2.76 ± 0.24% coefficient of variation (CV) and within-day precision was 2.63 ± 1.23% CV.

Urinary creatinine concentrations were analyzed simultaneously using an HPLC according to Jen et al. [16] with slight modifications. The urinary BPA concentrations were adjusted by urinary creatinine concentrations in order to eliminate the differences in urinary dilution.

Statistical analysis

Statistical analysis was conducted using SPSS version 21.0 for Windows software (SPSS Inc., Chicago, IL, USA). The distribution of BPA values was analyzed using the Shapiro-Wilk test. Descriptive statistics were performed to determine means and standard deviations for continuous variables and frequencies for categorical variables. The comparison between two parametric values was performed using Student's t-test or the Mann-Whitney U-test where appropriate. While investigating the associations between normally distributed variables, the correlation coefficients were calculated using Pearson's test. In ordinal variables and skewed data, Spearman's test was used to calculate the correlation coefficients.

Multiple logistic regression was used to detect the association between urinary BPA levels and T1DM after adjusting age, gender, height z-score, BMI z-score, mothers' education (≤8 years/>8 years), fathers' education (≤8 years/>8 years), type of delivery (cesarean section/vaginal birth) birth weight.

Two-way analysis of variance (ANOVA) was used to examine the effect of possible exposure routes (significant in univariate analysis) and group (case vs. control) on urinary BPA levels.

Multiple linear regression analysis (STEPWISE) was used to identify independent exposure routes of urinary BPA after controlling for age, sex, height z-score, BMI z-score, mother's educational background, mother's working status, birth weight, duration of exclusively breastfeeding.

A p-value of <0.05 was considered statistically significant.

Results

The study included 50 children with a T1DM diagnosis and 50 non-diabetic, healthy controls. The characteristics of the study population are shown in Table 1. Except z-scores for height-for-age, there was no statistically significant difference between the T1DM and control groups.

There were no significant associations between urinary BPA concentrations and some sociodemographic characteristics of cases between or within the groups (Table 2). When the correlations between urinary BPA concentrations and some characteristics of the participants were examined, an inverse correlation was found between urinary BPA concentrations and z-scores for height-for-age and birth weight (only in the case group) (Table 3). Though the difference was not statistically significant, mean urinary BPA concentrations appeared higher in the T1DM group than in the control group (27.71±15.53 [min: 5.28, max: 81.11] μg/g creatinine and 25.37±17.89 [min: 3.86,

Table 1: Some sociodemographic and clinical characteristics of the subjects in the study and control groups.

Characteristics	T1DM group n (%)	Control group n (%)	p-Value
Age, months	155.2±44.2	145.5±46.8	0.274
Sex			
Boys	27 (54)	27 (54)	1.000
Girls	23 (46)	23 (46)	
Height z-score	0.18±0.81	−0.29±0.85	0.005[a]
BMI z-score	−0.10±1.08	0.11±1.01	0.314
Mother's educational level			
≤8 years	16 (32)	12 (24)	0.504
>8 years	34 (68)	38 (76)	
Father's educational level			
≤8 years	8 (16)	10 (20)	0.795
>8 years	42 (84)	40 (80)	
Working mother	23 (46)	28 (56)	0.424
Gestational week	38.1±1.2	38.4±1.0	0.286
Birth weight, g	3333.8±278.3	3242.0±308.2	0.121
Urinary creatinine, mg/dL	99.0±68.1	94.7±71.7	0.760
Urinary BPA, ng/mL	24.13±19.68	22.82±22.08	0.756
Urinary BPA/creatinine, μg/g creatinine	27.71±17.53	25.37±17.89	0.510

[a]<0.05. BMI, body mass index; BPA, bisphenol-A; T1DM, type 1 diabetes mellitus.

Table 2: Some sociodemographic characteristics and urinary BPA (μg/g creatinine) concentrations.

	n	T1DM group Mean±SD	n	Control group Mean±SD	p-Value
Age, years					
≤11	14	28.82±14.33	21	25.18±19.75	0.558
>11	36	27.29±18.79	29	25.50±16.78	0.692
p-Value		0.785		0.950	
Sex					
Boys	27	28.24±14.72	27	23.07±16.93	0.237
Girls	23	27.10±20.67	23	28.06±18.98	0.870
p-Value		0.821		0.331	
Mother's educational level					
≤8 years	16	24.94±20.72	12	20.49±20.95	0.581
>8 years	34	29.02±15.98	38	26.91±16.83	0.589
p-Value		0.449		0.283	
Father's educational level					
≤8 years	8	30.04±25.54	10	20.05±23.41	0.400
>8 years	42	27.27±15.95	40	26.70±16.33	0.873
p-Value		0.687		0.298	
Mother's working status					
Working	23	30.72±19.70	28	28.90±20.74	0.750
Housewife	27	25.15±15.35	22	20.88±12.50	0.299
p-Value		0.267		0.117	

SD, standard deviation; T1DM, type 1 diabetes mellitus.

Table 3: The correlations between urinary BPA levels and some characteristics of the participants.

	T1DM group		Control group	
	Pearson correlation coefficient	p-Value	Pearson correlation coefficient	p-Value
Age, months	−0.162	0.261	0.028	0.847
HbA_{1c}, %	0.213	0.180	–	–
Height z-score	−0.286	**0.044**[a]	0.001	0.993
BMI z-score	−0.106	0.466	0.140	0.331
Mothers' education	0.164	0.256	0.250	0.079
Fathers' education	0.125	0.388	0.111	0.441
Birth weight, g	−0.331	**0.019**[a]	0.081	0.575
Gestational week	−0.219	0.127	0.226	0.115

[a]<0.05. BMI, body mass index; NICU, neonatal intensive care unit; T1DM, type 1 diabetes mellitus.

max: 88.41] µg/g creatinine, respectively, p = 0.510) (Figure 1). Potential confounders were identified from our results and the literature (age, gender, height z-score, BMI z-score, mothers' education [≤8 years/>8 years], fathers' education [≤8 years/>8 years], type of delivery [cesarean section/vaginal birth], birth weight). There was no statistically significant association between urinary BPA levels and T1DM when logistic regression analysis was performed after these confounders were adjusted (odds ratio [OR] = 1.02 [95% confidence interval (CI): 0.99–1.05], p = 0.142).

Children were investigated for possible environmental BPA exposure pathways by a questionnaire. As BPA is used for the inner lining of tins and carton containers, milk is a possible route of exposure. The frequency of ultra-high temperature-pasteurized (UHT) milk consumption in children with T1DM was significantly higher than in the controls (92% and 62%, respectively, p = 0.001). In addition, the frequency of use of laminated parquet in the houses of children with T1DM was significantly higher than in those of controls (88% and 68%, respectively, p = 0.03). There were no other significant differences in the frequency of possible exposure routes between the groups. The relationships between possible BPA exposure pathways and urinary BPA concentrations are shown in Table 4. The urinary BPA concentrations of children who used a pacifier during infancy were significantly higher in the T1DM group than in the control group (34.42 ± 17.23 and 22.54 ± 12.58, respectively, p = 0.007). There were no other differences between urinary BPA concentrations concerning the possible exposure routes among the study groups. When the effects of possible exposure routes on the urinary BPA concentrations in children in the T1DM group were examined, it was found that the children who used pacifiers in infancy, plastic food container and plastic kettle had significantly higher urinary BPA concentrations than those who did not use. In addition, urinary BPA levels were also higher in children who kept hot foods in plastic storage containers, washed these containers in the dishwasher, consumed dairy products in plastic boxes or packaged ready-to-eat foods (Table 4).

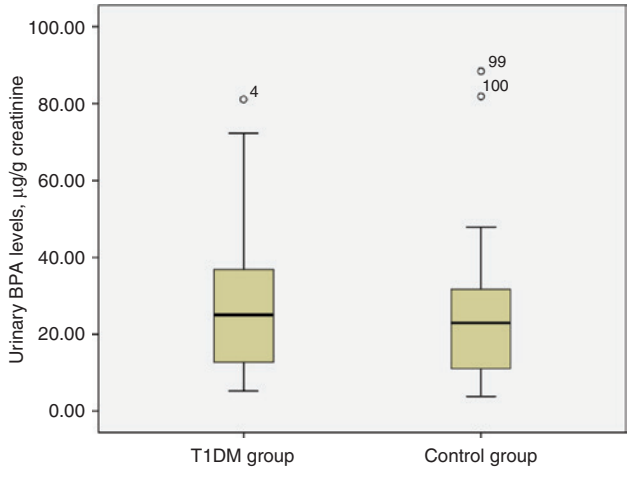

Figure 1: The distribution of urinary bisphenol-A (BPA) levels in T1DM and control groups.
T1DM, type 1 diabetes mellitus.

Exposure routes which were found to be important in the initial statistical analyses (pacifier use in infancy, use of plastic food containers at home, keeping hot foods in plastic containers, and washing them in the dishwasher, using plastic kettle, consumption of dairy products in the plastic boxes, consumption of packaged ready-to-eat products) were later analyzed by two-way ANOVA. It was found that the use of plastic kettles and the consumption of dairy products in plastic boxes causes an increase in the urinary BPA concentrations in all children regardless of their study group (p = 0.012 and p = 0.009, respectively).

Multiple linear regression analysis (STEPWISE) was used to evaluate the association between urinary BPA

Table 4: The relationship between the environmental risk factors surveyed by the questionnaire and urine BPA levels (μg/g creatinine).

	n	T1DM group Mean ± SD	n	Control group Mean ± SD	p-Value
NICU history					
Yes	2	32.20 ± 16.13	6	22.05 ± 12.20	0.374
No	48	27.53 ± 17.72	44	25.82 ± 18.59	0.654
p-Value		0.716		0.632	
Exclusively breastfeeding					
≤4 months	16	33.54 ± 23.50	12	24.55 ± 20.37	0.313
>4 months	34	24.98 ± 13.45	38	25.75 ± 17.63	0.836
p-Value		0.190		0.848	
Formula feeding					
Yes	30	28.50 ± 18.48	32	27.17 ± 15.86	0.762
No	20	26.54 ± 16.38	18	22.17 ± 21.14	0.479
p-Value		0.702		0.348	
Pacifier use					
Yes	23	34.42 ± 17.23	28	22.54 ± 12.58	**0.007**
No	27	22.01 ± 15.94	22	28.97 ± 22.78	0.215
p-Value		**0.011**		0.244	
Baby bottle usage					
Yes	39	28.68 ± 19.15	38	28.07 ± 18.55	0.887
No	11	24.28 ± 9.75	12	16.82 ± 12.80	0.134
p-Value		0.307		0.057	
Window frame material					
PVC	34	28.11 ± 17.93	30	26.73 ± 18.38	0.764
Others	16	26.87 ± 17.16	20	23.32 ± 17.39	0.544
p-Value		0.819		0.514	
Laminate parquet usage					
Yes	44	27.89 ± 16.13	34	27.57 ± 17.55	0.933
No	6	26.43 ± 27.75	16	20.69 ± 18.28	0.576
p-Value		0.850		0.208	
Smoking at home					
Yes	17	26.08 ± 17.58	24	22.41 ± 12.69	0.442
No	33	28.56 ± 17.71	26	28.10 ± 21.52	0.929
p-Value		0.640		0.266	
Using plastic food containers					
Yes	35	31.44 ± 18.87	27	24.81 ± 18.35	0.170
No	15	19.03 ± 9.74	23	26.03 ± 17.72	0.172
p-Value		**0.004**		0.813	
Storing hot foods in the plastic food container					
Yes	3	48.37 ± 13.11	4	22.68 ± 17.17	0.085
No	47	26.39 ± 17.03	46	25.60 ± 18.12	0.828
p-Value		**0.034**		0.758	
Washing the plastic food containers in a dishwasher					
Yes	20	34.74 ± 18.18	16	25.61 ± 15.91	0.123
No	30	23.03 ± 15.68	34	25.26 ± 18.98	0.614
p-Value		**0.019**		0.948	
Using plastic kettle					
Yes	28	32.48 ± 20.03	23	29.15 ± 16.65	0.528
No	22	21.66 ± 11.51	27	22.15 ± 18.58	0.913
p-Value		**0.021**		0.171	
Canned food consumption					
Yes	18	27.52 ± 10.14	11	27.63 ± 12.66	0.981
No	32	27.82 ± 20.72	39	24.73 ± 19.20	0.517
p-Value		0.946		0.641	
Consumption of carbonated beverages in tin cans					
Yes	34	28.51 ± 17.28	29	22.95 ± 13.69	0.168
No	16	26.03 ± 18.50	21	28.71 ± 22.39	0.700
p-Value		0.647		0.265	
UHT milk consumption					
Yes	46	28.03 ± 17.80	31	25.96 ± 17.54	0.617
No	4	24.11 ± 15.63	19	24.40 ± 18.90	0.977
p-Value		0.672		0.768	
Consumption of dairy products in plastic boxes					
Yes	42	30.26 ± 17.85	36	27.31 ± 19.11	0.484
No	8	14.35 ± 6.35	14	20.38 ± 13.64	0.256
p-Value		**<0.001**		0.222	
Consumption of packaged ready-to-eat foods					
Yes	16	35.62 ± 21.83	10	25.68 ± 22.27	0.274
No	34	23.99 ± 13.96	40	25.29 ± 16.96	0.724
p-Value		**0.027**		0.952	

BPA, bisphenol-A; NICU, neonatal intensive care unit; PVC, polyvinyl chloride; SD, standard deviation; T1DM, type 1 diabetes mellitus; UHT, ultra-high temperature.

concentrations in children and are important possible exposure routes found in single analyses (age, sex, height z-score, BMI z-score, mother's educational background, mother's working status, birth weight, duration of exclusively breastfeeding, using plastic food containers in the home, keeping hot foods in these containers, and washing them in the dishwasher, using plastic kettles, consuming canned foods in tin can, consuming UHT milk, dairy products in plastic boxes and packaged ready-to-eat foods). This analysis revealed that urinary BPA concentrations were significantly increased only with the use of plastic kettles in all groups (B = 8.23 [%95 CI: 0.24–16, 21], p = 0.044, F = 4.47, adjusted R^2 = 0.11).

Discussion

BPA is produced in large quantities globally and mainly used as an inner protective lining in canned foods, drinks and bottle caps [17]. Urinary BPA, particularly in spot samples, has often been used to evaluate the environmental BPA exposure [18, 19]. Based on the high rate of presence of BPA in human urine, it can be concluded that virtually everyone is exposed and the exposure is continuous in modern life [20, 21]. According to the literature, urinary BPA concentrations are age-dependent. Children are highly exposed to EDCs, particularly to phthalates and BPA in their food intake is higher than adults on

weight-food intake basis. Therefore, exposure to BPA containing materials (e.g. canned foods, plastic baby bottles, food containers) can be considered as the main sources of BPA exposure in childhood [1, 8]. The restriction of BPA use in baby bottles in the European Union (EU) countries and the increase in public awareness of BPA's potential adverse health effects have resulted in a decline in BPA levels in biological matrices. The mean urinary BPA level was found to be approximately 2 μg/L (0.7–4.2 μg/L) in EU countries [22]. In a study conducted by the National Health and Nutrition Examination Survey (NHANES), the geometric mean of urinary BPA concentrations for children ranged from 1.81 μg/L to 3.74 μg/L between 2003 and 2010 [23]. Biomonitoring studies for BPA in Turkey are very few, especially for children. Ellialtı [24] found that the urinary BPA concentrations in children (2–11 years old) were between 6.8 and 32 μg/g creatinine in Ankara. Another study from Durmaz et al. [25] found that the urinary BPA concentrations were between 0.3 and 67.35 μg/g creatinine in girls with idiopathic central precocious puberty. The mean concentration of urinary BPA in children aged between 3 and 18 years was found to be 56.6 μg/g creatinine in Mersin city [26]. In our study, we found that the mean urinary BPA level was 26.54 ± 17.66 μg/g creatinine in all study groups. It is not possible to give a normal range of urine BPA levels in humans. Nevertheless, we think that the high urinary BPA levels measured in Turkey as well as in other countries reveal a continuous and serious BPA exposure throughout the globe.

Concerns have been raised about BPA's possible role in the pathogenesis of some chronic diseases and auto immunity such as T1DM [7–12, 27–29]. Cetkovic-Cvrlje et al. [30] showed that sub-chronic daily exposure of a chemically induced experimental mouse model to low as well as high BPA doses increased the incidence of T1DM. Recently in another study, BPA was found to be more potent than the phthalate metabolites in affecting insulin secretion of the pancreatic β-cells [31]. To our knowledge, an epidemiological study evaluating the relation between BPA exposure and T1DM development has not been performed yet. In the current work, we evaluated whether there is any association between urinary BPA levels and T1DM in children. The T1DM group was found to have a higher mean BPA level (27.71 ± 15.53 μg/g creatinine) when compared to controls (25.37 ± 17.89 μg/g creatinine) in our study. However, we could not find any significant difference between urinary BPA levels of children with T1DM and controls ($p = 0.510$). After adjusting for potential confounders, we again did not find any significant difference between urinary BPA concentrations in children with T1DM and controls. To the best of our knowledge, this is the first study to investigate the relationship between urinary BPA levels and T1DM in children. We think that the limited number of subjects in the current work did not enable us to show a possible relationship. As there is no similar work done in children in the literature, it is impossible for us to make comparisons with other studies.

Although we could not find a relationship between urinary BPA levels and BMI among children in our study, there was a statistically significant, inverse correlation between birth weight and urinary BPA levels in children. BPA is able to cross the placenta, and although some studies provided conflicting results, prenatal exposure to BPA has been reported to reduce birth weight [32–37]. Prenatal exposure to BPA seems to cause adverse effects on fetal growth. Growth is primarily affected by insulin and insulin-like growth factor in this period. As we found a negative correlation between BPA levels and birth weight only in T1DM patients, we speculate that prenatal exposure of BPA might start to affect insulin metabolism in T1DM patients. Due to our study design, it is not possible to say that children in the T1DM group were prenatally exposed to BPA, but further studies about this topic should be conducted.

Although oral exposure from canned foods is the primary source of BPA exposure in childhood, other potential routes for exposure (such as plastic food containers or polycarbonate food packages, plastic kettles, thermal papers) may be equally important from the standpoint of biological monitoring [1, 8]. In our study, we could not find any association between canned food consumption and urinary BPA concentrations. However, we found higher urinary BPA concentrations in children whose families kept hot foods in plastic storage containers and washed these containers in the dishwasher. Besides, children who consumed dairy products in plastic boxes or packaged ready-to-eat foods had higher urinary BPA levels in our study. It is known that considerable amounts of BPA leach from plastic food containers and heating can increase this leaching [37–39]. For example, increased migration of BPA was found in polycarbonate baby bottles after boiling and dishwashing, repeated washing with detergents, rubbing and sterilization [40, 41]. In one study from Turkey, urinary BPA levels were found to be higher in users of plastic food containers [26]. It may be for these reasons that have observed high levels of urinary BPA in these children. However, it is impossible to explain why we observed this relationship only in the T1DM group, but not in the control group. Additionally, we found that urinary BPA levels of children who used a pacifier during infancy are higher in the study group than in the control group. However, it is difficult to explain an association

between infancy and childhood in terms of BPA levels. Therefore, studies on the metabolism and kinetics of BPA should be performed in children with T1DM.

Data retrieved by the European Food Safety Authority (EFSA) reported BPA migration from plastic kettles was below the limits [42, 43]. Some studies showed that BPA was not detected after boiling water in plastic containers (at the LOD of 1 μg/L) [44] However, urinary BPA concentrations in our study were significantly correlated with the use of plastic kettles in all children. When polymers of BPA in plastic kettles are exposed to high temperatures, the ester bond linking monomers of BPA can be hydrolyzed. This phenomenon causes the migration of free BPA monomers into the boiling water [21]. Considering the high usage rates, we think that plastic kettles are one of the important sources of BPA in children.

Our study had several limitations. First, with our study design, it is not possible to explain the mechanism underlying the relationship between urinary BPA levels and T1DM. Second, we have relatively small sample size. Third, in the T1DM group, the age at diagnosis and the time after the diagnosis of cases were different from each other. If newly diagnosed children with T1DM were recruited, a better study design would have been achieved. Last, the possible BPA exposure routes were assessed using face-to-face questionnaire and this may be affected by social desirability bias. However, the face-to-face questionnaire method remains the most practical method for assessing these parameters.

In conclusion, we can state that there was no statistically significant difference between urinary BPA concentrations in children with T1DM and in controls. However, we found an inverse relationship between current urinary BPA levels and birth weight. This finding might be important for prenatal exposure and further research must be conducted. Our study showed that children who consume dairy products in plastic boxes or use plastic kettles at home had higher levels of urinary BPA. We think that the use of plastic kettles, which has not been mentioned much in the literature before, is an important risk factor for BPA exposure.

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