European Journal of Pediatrics (2019) 178:541–550
https://doi.org/10.1007/s00431-019-03329-4

ORIGINAL ARTICLE

Preliminary study on bisphenol A levels and possible exposure history of mother and exclusively breastfed infant pairs

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Received: 31 August 2018 / Revised: 18 January 2019 / Accepted: 21 January 2019 / Published online: 1 February 2019

Abstract

The aims of this study were to determine bisphenol A (BPA) levels in breast milk and urine specimens of healthy mother and exclusively breastfed infant pairs having no known BPA exposure, and also to examine the relationship between BPA levels and possible BPA exposure history. Forty mothers and their 1–2-month-old exclusively breastfeed infant were included in the study. The questionnaires about sociodemographic characteristics and possible BPA exposure history were filled out. Breast milk and urine samples were taken. BPA analyses of these samples were conducted using high-performance liquid chromatography coupled with mass spectrometry. All mother-infant pairs showed detectable BPA concentrations. The geometric means of BPA levels in breast milk, maternal urine, and infant urine were determined as 0.12 μg/L (0.03–0.59), 0.12 μg/L (0.03–0.73), and 0.13 μg/L (0.02–0.44), respectively. Infants whose mothers were consuming yoghurt in plastic containers had relatively higher urinary BPA levels ($p = 0.00$). Mothers consuming hot beverages in plastic glass showed higher breast milk BPA levels ($p = 0.033$). There were no statistical associations between BPA levels and the use of plastic materials and tools ($p > 0.05$).

Conclusion: The measurable BPA concentrations in all breast milk specimens of healthy mothers may reflect possible exposure from dietary or non-dietary sources. Exclusively, breastfed healthy infants without any known BPA exposure may be exposed to BPA from their mothers through breastfeeding.

What is Known:

• *Fetuses, neonates and infants are exposed to BPA from their mothers through placental transfer and breastfeeding.*
• *Breast milk is considered a continuous low-level exposure to BPA.*

What is New:

• *BPA was detected in 100% of maternal urine, infant urine, and breast milk in healthy mother-infant pairs having no known BPA exposure.*
• *The measurable amount of BPA in breast milk and infant urine may reflect possible BPA exposure of mother-infant pairs.*

Keywords Bisphenol A · Breast milk · Maternal urine · Infant urine · Exclusively breastfeeding · Exposure

Communicated by Mario Bianchetti

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Abbreviations

BMI	Body mass index
BPA	Bisphenol A
HPLC	High-performance liquid chromatography
LC-MS/MS	High-performance liquid chromatography coupled with mass spectrometry
PVC	Polyvinylchloride
UHT	Ultra high temperature

Introduction

Bisphenol A (BPA) is a high-production synthetic chemical used in the manufacture of polycarbonate plastics and epoxy resins that are found in a variety of consumer products, such as food and beverage storage containers, baby bottles, food can linings, medical equipment, dental sealants, thermal papers, and children's toys [11, 29]. Whereas diet is considered the primary source of exposure as a result of migration of BPA from the packaging, exposure from non-dietary sources may also occur [11, 12, 19]. The widespread exposure due to the ubiquitous presence of BPA is of concern because BPA has the ability to interfere with the endocrine system by mimicking, blocking, and triggering actions of hormones [24, 26]. In humans, BPA is rapidly metabolized and excreted in the urine, mainly as a glucuronide conjugate with a half-life of 6 h [27, 29].

Early life is particularly vulnerable to the endocrine disrupting effects of BPA, because exposures that occur during specific periods of development can have a lifelong impact on health [9, 21, 22]. Previous biomonitoring studies conducted on pregnant women and lactating mothers have reported the presence of BPA in the serum, urine, amniotic fluid, placenta, and cord serum, as well as in the breast milk [2, 5, 8, 14, 16]. It is suggested that fetuses and neonates are exposed to BPA from their mothers through placental transfer and breastfeeding [4, 19]. Besides many chemical and environmental factors, various maternal factors including age, health status, dietary habits, parity, lactation period, and smoking may affect the transfer of environmental pollutants to breast milk [19, 25].

Biomonitoring studies evaluating BPA levels in mothers and infants on a pairwise basis highlight the concerns about potential exposure for the infants. Previous mother-infant pair studies assessing postnatal BPA levels have been conducted on various heterogeneous groups, such as newborns or infants, breastfed or formula fed infants, preterm or term infants, and hospitalized infants using medical devices [3, 10, 17, 18]. Since human breast milk is considered a continuous low-level exposure to endocrine-active compounds such as BPA [19, 26], breast milk may seem the only BPA exposure route for exclusively breastfed healthy infants having no known BPA exposure. However, to the best of our knowledge, there is no study to examine the BPA levels and possible BPA exposure history in healthy lactating mothers and their healthy, exclusively breastfed infants having no known BPA exposure. Therefore, the aim of our study was to determine the BPA levels in breast milk and urine specimen of pairs of healthy mothers and healthy, exclusively breastfed infants having no known BPA exposure, and also to examine the relationship between the BPA levels and any possible BPA exposure history.

Materials and methods

The study was conducted at the Ankara University Faculty of Medicine, Department of Pediatrics, Division of Social Pediatrics. Ethics approval was obtained from the Ethics Committee of the Faculty of Medicine.

Totally 40 1–2-month-old infants, brought for routine well-child visits, along with their mothers were included in this study. Inclusion criteria for the infants were to be followed up by the Division of Social Pediatrics as a healthy infant, being exclusively breastfed, no bottle-fed, not having an acute or chronic disease, no histories of premature birth or delivery complication or medical device use, not hospitalized, and not having a BPA exposure history. Inclusion criteria for the mothers were to be healthy, not having an acute or chronic disease or a nutritional disorder, no history of medical device usage, and not having known BPA exposure history. A written informed consent was obtained from the mothers who were admitted to the study.

All infants were examined by the same pediatrician in their well-child visit. Weight, height, and head circumference measurements of infants were taken and daily weight gains were calculated. Also, weight and height measurements of mothers were measured and the body mass index (BMI) was calculated by the formula weight (kg)/[height (m)]^2.

Following the routine well-child visit, the surveys containing the questions on the participants' sociodemographic characteristics and possible BPA exposure routes of the mother-infant pairs were filled out during face-to-face interviews. Possible BPA exposure survey included the questions about living near industrial zones, mothers' occupational BPA exposure status, mothers' detailed food and beverage consumption in plastic containers and other plastic tool and material usage, the construction material usage which causes possible BPA exposure in their home environment, and the infants' pacifier, baby bottle, toy and baby teething ring usage.

After the interview, breast milk and urine specimens were taken under proper conditions. Infant urine and maternal breast milk were collected on the same day from mother-infant pairs. The breast milk samples were collected at the beginning of a single breastfeeding. The mothers were instructed to clean their hands and nipple using purified water and gauze provided to them by the study personnel. Maternal milk samples of 2 ml were obtained from each breast by means of an electric breast pump (Dual Electric Breast Pump, Ameda, USA). All parts of this pump that came in contact with expressed milk are of BPA-free polypropylene plastic. Milk samples in polypropylene bottles of the pump were taken into BPA-free polypropylene cryovials and stored at − 20 °C until analyzed. The mothers were instructed to urinate into glass beakers. Then, 5-ml maternal urine samples in the glass beakers were taken into BPA-free polypropylene cryovials and stored at − 20 °C until analyzed. Urine samples were collected from each infant by means of a BPA-free baby urine collector (Amsino, AMSure®, NY, USA). Five microliters of infant urine samples in urine collectors was quickly taken into BPA-free polypropylene cryovials and stored at − 20 °C until analyzed.

Laboratory analysis

BPA analyses of urine and breast milk samples were conducted by high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) using an AB/Sciex3200 QTrap® LC/MS-MS system (Foster City, CA, USA).

For the analysis of urinary free BPA, the experimental procedures described previously [15, 30] were used with some modifications. Briefly, urine samples (500 μL) were thawed at room temperature and vortex-mixed for 3–5 s. Thereafter, samples were fortified with 10 μL$^{13}C_{12}$-BPA (Cambridge isotope laboratory, USA) as internal standard and 100 μL of ammonium acetate buffer (Sigma-Aldrich, USA) (0.1 M, pH 6.5). The resulting mixture was incubated at 37 °C for 2 h. Three hundred microliters of incubated sample was mixed with 500 μL acetonitrile and centrifuged at 3000 rpm for 10 min. Urine-free BPA analysis was performed by LC-MS/MS using an AB/Sciex 3200 QTrap® LC/MS-MS system (Foster City, CA, USA). The mobile phase composition used in chromatographic separation was optimized by binary mixtures of 0.1% ammonium acetate in water (solvent A) and 0.1% acetonitrile in water (solvent B). The flow rate of the mobile phase was 300 μL/min. A volume of 5 μL of each sample was injected into the HPLC (high-performance liquid chromatography) system. BPA levels were calculated by the peak heights obtained from the chromatogram. All detectable BPA concentrations in the samples were calculated. Free BPA concentrations of urine specimens were given as μg/L.

For the analysis of BPA in breast milk, the experimental procedures described previously [6, 31] were used with some modifications. Briefly, breast milk (1 mL) samples were thawed at room temperature and vortex-mixed prior to sample processing. The samples were spiked with 10 μL of 1 μg/mL$^{13}C_{12}$-BPA (Cambridge isotope laboratory, USA) as internal standard, and 500 μL of 1 M ammonium acetate was added. Thereafter, the samples were incubated at 37 °C for 2 h. The incubated samples were diluted with 2 mL of 0.1 M formic acid and were eluted with 3 mL of acetonitrile. Breast milk-free BPA analysis was quantified by LC-MS/MS using the AB/Sciex 3200 QTrap® LC/MS-MS system (Foster City, CA, USA). The mobile phase composition used in the chromatographic separation was optimized by binary mixtures of 0.1% ammonium acetate in water (solvent A) and 0.1% acetonitrile in water (solvent B). The flow rate of the mobile phase was 0.2 mL/min. A volume of 10 μL of each sample was injected into the HPLC system. BPA levels were calculated by the peak heights obtained from the chromatogram. All detectable BPA concentrations in the samples were calculated. Free BPA concentrations of breast milk specimens were given as μg/L.

Quality-control materials were analyzed with the analytical standards, reagent blanks, field blanks, and study samples. The coefficients of variation of quality-control materials prepared with spiked pooled human urine or milk were less than 10%. In order to avoid any possibility of contamination, laboratory supplies such as containers, tubes, and vials were pre-washed with Milli-water and acetonitrile, and it was monitored to confirm them prior to each assay. The accuracy of the validated method ranged from 93.5 to 107% for urine, and 83.3 to 105% for breast milk. Urinary BPA concentrations were adjusted for dilution by using specific gravity.

Statistical analysis

Sociodemographic, anthropometric, and possible BPA exposure data were evaluated using descriptive statistics. These descriptive statistics were performed to determine means and standard deviations for continuous variables and frequencies for categorical variables. The BPA levels were analyzed using descriptive statistics and the results were presented as geometric mean and median (min-max). The distribution of BPA values was analyzed using the Shapiro-Wilk test. Since the results were not normally distributed, statistical differences between BPA levels and sociodemographic, anthropometric, and possible BPA exposure data were tested using the Mann-Whitney U test. The correlations among BPA levels were tested using Spearman's rho test.

A p value of < 0.05 was considered statistically significant. Statistical analysis was conducted using SPSS version 24.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Sociodemographic and anthropometric characteristics

Totally, 40 mother-infant pairs were included in the study. Sociodemographic characteristics of the study population are given in Table 1. The mean age of the mothers was 29.7 ± 5.4 years (21–40 years). The majority had high school education and was not employed. The mean BMI of the mothers was calculated as 24.7 ± 2.9 (18.3–29.8). The mean age of the infants was 41.1 ± 14 days (25–65 days). The mean infant weight, height, and head circumference were found as 4704 ± 641.5 g (3500–5970 g), 55.5 ± 2.6 cm (51–65 cm), and 37.4 ± 1.5 cm (35–41 cm), respectively. The mean daily weight gain of infants was found as 38.8 ± 14.7 g/day (20–72 g/day).

Possible BPA exposure history

According to the statements of mothers, none of them lived close to industrial zones and none had an occupational BPA exposure history. Additionally, none of the mothers were smoking. Table 2 shows the findings about possible environmental or individual exposure of the mother-infant pairs. The

Table 1 Sociodemographic and anthropometric characteristics of the study group

	Number (n)	Percent (%)
Maternal age (years)		
20–30	20	50
31–40	20	50
Maternal education status		
Primary school	9	13.5
High school and university	31	87.5
Maternal occupation		
Housewife	23	57.5
Employed	17	42.5
Maternal BMI		
BMI < 30	40	100
BMI ≥ 30	0	0
Number of family members at home		
3	14	35
4 or above	26	65
Infant gender		
Female	26	65
Male	14	35
Infant age		
< 32 days	21	52.5
≥ 32 days	19	47.5
Infant weight gain		
< 35 g/day	19	47.5
≥ 35 g/day	21	52.5

frequencies of water consumption in plastic bottles and of ultra high temperature (UHT)-treated milk were both found as 75%, and these frequencies seemed the highest dietary exposure frequencies in our study.

The frequency of non-dietary BPA exposure due to plastic tools and materials was relatively less compared to that of dietary exposure (Table 2). While none of the mothers reported the use of plastic toys, baby bottles, and baby teething ring for their infants, 11 (27.5%) mothers reported the use of BPA-free pacifiers by their infants.

Urinary and breast milk BPA levels

All mother-infant pairs in the analysis population had detectable BPA concentrations. Descriptive analysis of BPA levels of the study population is shown in Table 3. The geometric mean of BPA levels in breast milk, maternal urine, and infant urine were determined as 0.12 μg/L (0.03–0.59), 0.12 μg/L (0.03–0.73), and 0.13 μg/L (0.02–0.44), respectively. Figure 1 shows the distribution of the BPA levels. No significant correlations were observed between breast milk and maternal urine BPA levels, between breast milk and infant urine BPA levels, and between maternal urine and infant urine BPA levels ($p = 0.759$, $r = -0.050$; $p = 0.381$, $r = -0.142$, and $p = 0.326$, $r = 0.160$; respectively).

BPA levels and sociodemographic and anthropometric characteristics

There were no significant associations between BPA levels and various sociodemographic and anthropometric characteristics of the study population ($p > 0.05$) (Table 4). Although there was not any statistically significant association ($p > 0.05$), BPA levels in the breast milk and in the maternal urine of high educated mothers were found lower than in those of less educated mothers. Further, infants aged ≥ 32 days had higher urinary BPA levels than others; however, there was no significant relationship ($p > 0.05$).

BPA levels and possible BPA exposure history data

No significant associations between the consumption of various foods and beverages in plastic containers/bottles/boxes and BPA levels were found ($p > 0.05$) (Table 5).

Infants whose mothers were consuming yoghurt in plastic containers had significantly higher urinary BPA levels ($p = 0.00$) (Fig. 2). Urinary BPA levels of those mothers consuming yoghurt in plastic containers were found higher, but it was not statistically significant ($p > 0.05$).

The mothers consuming hot beverages in plastic glasses had significantly higher breast milk BPA levels ($p = 0.033$) (Fig. 3). Although mothers consuming hot beverages in plastic glasses showed higher urinary BPA levels, it was not statistically significant ($p > 0.05$).

Further, there were no statistical associations between BPA levels and the use of plastic materials and tools ($p > 0.05$). The infants whose mothers use plastic home furniture and plastic kettles had higher urinary BPA levels, but it was not statistically significant ($p > 0.05$).

Discussion

The present study provides the BPA levels and any possible BPA exposure history of pairs of mothers and exclusively breastfed infants with no known exposure. All pairs of mothers-exclusively breastfed infants showed detectable BPA concentrations. Healthy, exclusively breastfed infants without any known BPA exposure are more likely to be exposed to BPA from their mothers through breastfeeding.

Fetuses, neonates, and infants are exposed to BPA due to their mothers through placental transfer and breastfeeding [4, 22]. BPA was detected in 100% of maternal and infant urine samples and breast milk in our mother-infant pair study. The geometric means of BPA levels in breast milk, maternal urine,

Table 2 The frequency of consumption of foods and beverages in plastic containers and the use of plastic tools and materials

	Yes *N* (%)	No *N* (%)
UHT (ultra high temperature)-treated milk consumption	30 (75)	10 (25)
Canned food consumption	11 (27.5)	29 (72.5)
Yoghurt consumption in plastic container	16 (40)	24 (60)
Oil consumption in plastic bottle	14 (35)	26 (65)
Vinegar consumption in plastic bottle	26 (65)	14 (35)
Milk consumption in plastic box	8 (20)	32 (80)
Ayran consumption in plastic box	6 (15)	34 (85)
Water consumption in plastic bottle	30 (75)	10 (25)
Beverage consumption in plastic bottle	17 (42.5)	23 (57.5)
Cheese consumption in plastic container	22 (55)	18 (45)
Hot beverage consumption in plastic glass	12 (30)	28 (70)
Gravy consumption in plastic container	14 (35)	26 (65)
Pickle consumption in plastic container	16 (40)	24 (60)
Plastic plate, fork, spoon, knife usage	1 (2.5)	39 (97.5)
Plastic kettle usage	15 (37.5)	25 (62.5)
Plastic glove usage	6 (15)	34 (85)
Plastic furniture usage	2 (5)	38 (95)
Plastic floor usage	2 (5)	38 (95)
Polyvinylchloride (PVC) usage in doors and windows	33 (82.5)	7 (17.5)
Presence of dental prosthesis	8 (20)	32 (80)
Presence of domestic plastic sanitary pipes	16 (40)	24 (60)
BPA-free baby pacifier usage	11 (27.5)	29 (72.5)
Baby bottle usage	0 (0)	40 (100)
Plastic baby toy usage	0 (0)	40 (100)
Plastic baby teething ring usage	0 (0)	40 (100)

and infant urine were determined as 0.12 μg/L (0.03–0.59), 0.12 μg/L (0.03–0.73), and 0.13 μg/L (0.02–0.44), respectively. There is limited number of postnatal BPA biomonitoring studies conducted on mother-infant pairs. A study involving pairs of mothers and infants showed that the total BPA was detected in 93% of urine samples in a healthy infant population aged 3–15 months with different feeding procedures and in 75% of breast milk samples [18]. Arbuckle et al. show that the median total BPA concentrations were 1.21 and 0.24 μg/L in maternal and infant urines, respectively [3]. In a recent birth panel of mother-neonate pairs study using LC-MS-MS system, the median BPA levels in neonatal urine and maternal urine were found as 4.75 μg/L and 2.86 μg/L, respectively [17]. In comparison to previous studies, we observed that the BPA levels in both maternal and infant urine were found lower in this healthy Turkish population having no known BPA exposure. Differences in dietary or non-dietary exposures, dietary habits, and sampling protocols may explain the variances in BPA levels among study populations. In agreement with this, there are various studies reporting newborn/infant BPA exposure due to medical device usage, exclusive formula feeding, or undergoing intensive therapeutic medical interventions [3, 7, 10, 28]. In a previous study, infants who were exclusively fed with formula had significantly higher total BPA in their urine, compared to those who were exclusively breastfed [3]. As the strength of the present study, the mother and infant population reflects a homogenous pattern according to sociodemographic and anthropometric characteristics. Therefore, urine BPA levels found in this study may

Table 3 Free BPA levels in breast milk, maternal urine, and infant urine

	Breast milk	Maternal urine	Infant urine
N	40	40	40
Mean	0.15	0.16	0.17
Median	0.11	0.12	0.14
Minimum	0.03	0.03	0.02
Maximum	0.59	0.73	0.44
Geometric mean	0.12	0.12	0.13

Data was presented as μg/L

Fig. 1 The distribution of free BPA levels

reflect the standard concentrations of pairs of healthy mothers-exclusively breastfed infants with no known exposure to BPA. Previous data show that the estrogenic activity of BPA at low levels is of increasing concern [13, 24]. Thus, we suggest that even lower BPA levels in infant urine may pose a risk to infant health.

Since human breast milk is considered a continuous low level exposure to endocrine-active compounds such as BPA, it is taken into account as a biomarker of maternal BPA exposure [19, 29]. Previous studies observed varied breast milk BPA concentrations because of mothers' different medical and lifestyle status, the stage of lactation, environmental factors, and source of exposure [8, 14, 23, 32, 33]. In previous mother-infant pair studies, Mendonca et al. reported that total BPA was detected in 75% of breast milk samples [18], and Lee et al. reported that BPA was detected in the breast milk at a level of 0.74 μg/L [17]. The differences of breast milk BPA levels among the studies may be explained based on individual exposure routes or lactation characteristics [19, 25]. Even though breast milk BPA levels seem relatively lower compared to previous

Table 4 Free BPA levels and sociodemographic and anthropometric characteristics

	Number (%)	Breast milk*	Maternal urine*	Infant urine*
Maternal age				
20–30	20 (50)	0.11	0.10	0.13
31–40	20 (50)	0.13	0.13	0.13
p value		*0.482*	*0.344*	*0.914*
Maternal education				
Primary school	9(13.5)	0.15	0.13	0.12
High school and university	31(87.5)	0.11	0.11	0.14
p value		*0.128*	*0.517*	*0.871*
Maternal occupation				
Housewife	23(57.5)	0.13	0.13	0.12
Employed	17(42.5)	0.11	0.10	0.14
p value		*0.547*	*0.198*	*0.381*
Number of family members at home				
3	14 (35)	0.10	0.13	0.17
4 or above	26 (65)	0.13	0.11	0.11
p value		*0.178*	*0.372*	*0.074*
Infant gender				
Female	26 (65)	0.12	0.11	0.14
Male	14 (35)	0.12	0.12	0.13
p value		*0.843*	*0.514*	*0.691*
Infant age				
< 32 days	21(52.5)	0.11	0.11	0.12
≥ 32 days	19(47.5)	0.14	0.13	0.16
p value		*0.294*	*0.395*	*0.294*
Infant weight gain				
< 35 g/day	19(47.5)	0.14	0.12	0.14
≥ 35 g/day	21(52.5)	0.11	0.12	0.13
p value		*0.218*	*0.978*	*0.432*

*Data was presented as geometric mean and μg/L

Table 5 Free BPA levels and possible BPA exposure routes of the study population

	Number	Breast milk BPA	*p*	Maternal urine BPA	*p*	Infant urine BPA	*p*
UHT (ultra high temperature)-treated milk consumption							
Yes	30	0.13	0.164	0.11	0.731	0.12	0.274
No	10	0.10		0.12		0.16	
Canned food consumption							
Yes	11	0.12	0.990	0.11	0.916	0.13	0.762
No	29	0.12		0.12		0.13	
Yoghurt consumption in plastic container							
Yes	16	0.13	0.934	0.15	0.129	0.23	0.000
No	24	0.12		0.10		0.09	
Oil consumption in plastic bottle							
Yes	14	0.11	0.630	0.11	0.640	0.11	0.183
No	26	0.13		0.12		0.15	
Vinegar consumption in plastic bottle							
Yes	26	0.12	0.702	0.12	0.887	0.11	0.100
No	14	0.13		0.11		0.17	
Milk consumption in plastic box							
Yes	8	0.11	0.995	0.16	0.310	0.10	0.132
No	32	0.12		0.11		0,14	
Ayran consumption in plastic box							
Yes	6	0.11	0.850	0.17	0.096	0.11	0.507
No	34	0.12		0.11		0.14	
Water consumption in plastic bottle							
Yes	30	0.12	0.628	0.11	0.719	0.13	0.662
No	10	0.13		0.13		0.13	
Beverage consumption in plastic bottle							
Yes	17	0.10	0,101	0.12	0.712	0.14	0.784
No	23	0.14		0.11		0.13	
Cheese consumption in plastic container							
Yes	22	0.11	0.881	0.12	0.817	0.11	0.157
No	18	0.13		0.11		0.16	
Hot beverage consumption in plastic glass							
Yes	12	0.17	0.024	0.15	0.065	0.14	0.883
No	28	0.10		0.10		0.13	
Gravy consumption in plastic container							
Yes	14	0.11	0.234	0.14	0.281	0.10	0.217
No	26	0.13		0.10		0.15	
Pickle consumption in plastic container							
Yes	16	0.11	0.355	0.11	0.989	0.12	0.610
No	24	0.13		0.12		0.14	
Plastic plate, fork, spoon, knife usage							
Yes	1	0.10	0.931	0.15	0.573	0.13	0.634
No	39	0.12		0.12		0.13	
Plastic kettle usage							
Yes	15	0.14	0.342	0.13	0.665	0.16	0.276
No	25	0.11		0.11		0.12	
Plastic glove usage							
Yes	6	0.10	0.353	0.13	0.570	0.15	0.483
No	34	0.12		0.11		0.13	
Plastic furniture usage							
Yes	2	0.09	0.136	0.12	0.951	0.21	0.121
No	38	0.12		0.12		0.13	
Plastic floor usage							

Table 5 (continued)

	Number	Breast milk BPA	*p*	Maternal urine BPA	*p*	Infant urine BPA	*p*
Yes	2	0.10	0.598	0.07	0.214	0.08	0.642
No	38	0.12		0.12		0.14	
Polyvinylchloride (PVC) usage in doors and windows							
Yes	33	0.13	0.068	0.12	0.873	0.14	0.346
No	7	0.16		0.12		0.11	
Presence of dental prosthesis							
Yes	8	0.13	0.588	0.09	0.532	0.12	0.624
No	32	0.12		0.12		0.14	
Presence of domestic plastic sanitary pipes							
Yes	16	0.11	0.730	0.13	0.912	0.13	0.235
No	24	0.12		0.11		0.14	
BPA-free baby pacifier usage							
Yes	11	0.15	0.250	0.10	0.413	0.15	0.694
No	29	0.11		0.12		0.13	

data, measurable BPA concentrations in all breast milk specimens of our healthy mothers may reflect BPA exposure from dietary or non-dietary sources. Since BPA content in maternal milk reflects the recent exposure of the mother [20], we suggest that there may be a high potential for exposure of exclusively breastfed infants to BPA via breast milk.

In comparison to previous mother-infant pair studies, an important strength of our study is the determination of factors that influence the inter-individual variability in BPA exposure based on a detailed survey. Currently, diet is considered the primary source of BPA exposure as compared to non-dietary sources [12, 29]. Similarly, the frequency of dietary BPA exposure was relatively higher than that of non-dietary exposure routes in our study. The migration of BPA, as an additive of plastic polymeric structures, can determine a further contamination of dietary milk and dairy products such as yoghurt [11, 12]. Similarly, in our small study group, we found that infants whose mothers consumed yoghurt in plastic containers had higher urinary BPA levels and the mothers who consumed hot beverages in plastic glasses showed higher breast milk BPA levels. These findings may be significant due to the risk of breast milk and infant exposure. However, other sources of BPA exposure such as non-dietary causes should also be considered. Although long-term BPA exposure in infants may occur through saliva exposure due to pacifiers and toys [1, 12], we have not observed any potential saliva exposure risk in these healthy infants. This finding can be explained by the small number of infants using these devices in our study.

The present study provided BPA levels and possible BPA exposure history of pairs of mothers-exclusively breastfed infants with no known exposure. Having said this, we faced several limitations during the study. First, the sample size was small. Despite this fact, we suggest that our preliminary data may provide interesting insights for future studies.

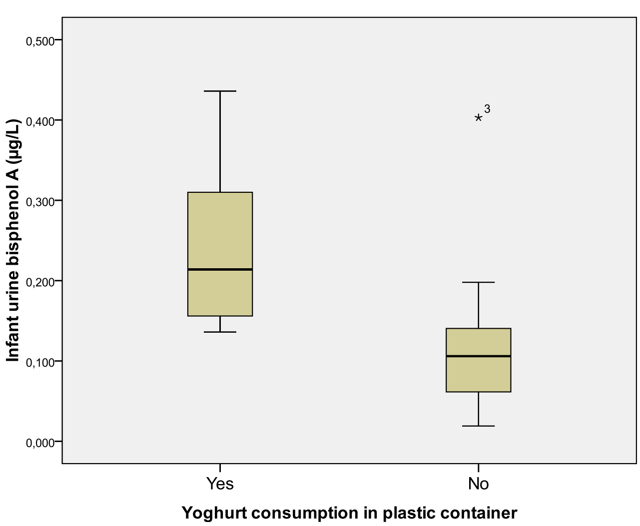

Fig. 2 The association between free BPA levels in infant urine and mothers' yoghurt consumption in plastic container ($p = 0.00$)

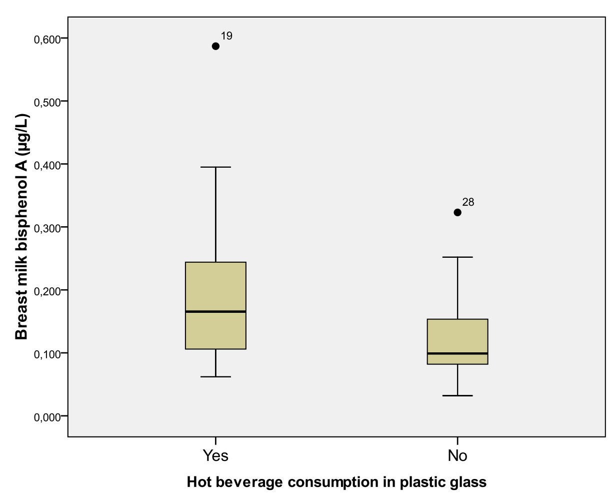

Fig. 3 The association between free BPA levels in breast milk and mothers' hot beverage consumption in plastic glasses ($p = 0.033$)

Another limitation of our study is that the BPA concentrations measured at one spot time may not reflect the BPA exposure accurately. Moreover, the timing of sample collection should be standardized to account for the variability in BPA levels. Further, in statistics, multiple testing may refer to the potential increase in type 1 error. Therefore, we should indicate that multiple comparisons run on our small sample may cause the type I error which may have led to the statistically significant associations observed in our study.

Conclusions

To our knowledge, this study is the first to identify BPA levels and possible BPA exposure history of pairs of mothers-exclusively breastfed infants with no known exposure. Whereas BPA was detected in 100% of maternal urine, infant urine, and breast milk, BPA concentrations of the specimens were found relatively lower as compared to previous studies. Urine-free BPA levels of healthy mothers and healthy, exclusively breastfed infants not having BPA exposure may be used as a standard concentration for this population. We suggest that the measurable BPA concentrations in all breast milk specimens of healthy mothers may reflect BPA exposure from dietary or non-dietary sources. Moreover, the measurable amount of BPA in infant urine may reflect possible BPA exposure via breast milk in exclusively breastfed infants without any known BPA exposure. In our study, we were able to determine the factors that influenced inter-individual variability in BPA exposure. Although, dietary exposure, such as the consumption of yoghurt and hot beverages in plastic containers may be observed as the sources of exposure, non-dietary exposure should also be considered due to the ubiquitous presence of BPA. Considering the vulnerability of infants, it is essential to determine their exposure to BPA to decide which efforts of risk reduction should receive the highest priority. Further studies conducted on larger healthy mother-infant pair populations are needed to obtain standard BPA concentrations and to establish the exposure sources to BPA.

Authors' contributions Dr. Sayıcı prepared the application report for funding source, designed the data collection instruments, collected data, and performed the statistical analysis.

Prof. Orhon conceptualized and designed the study, designed the data collection instruments, revised the application report for funding source, performed the statistical analysis, interpreted the data, and prepared and edited the manuscript.

Assoc. Prof. Topcu collected data, and interpreted the statistical data.

Prof. Ulukol supervised the data collection process.

Prof. Baskan supervised the data collection process.

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

Funding The study is supported by Ankara University Research Fund (Project code 16 L0230005).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research ethics committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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