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RESEARCH ARTICLE

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Estimated exposure to bisphenol A in breastfed and breastfed plus formula-fed infants in Turkey: a comparison study

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ABSTRACT

This study aimed to estimate and compare dietary exposure to bisphenol A (BPA) in exclusively breastfed (EBF) and breastfed plus formula-fed (BF + FF) infants. A total of 70 mothers and their 0–6 month-old infants (40 in the EBF group and 30 in BF + FF group) were included in the study. After the questionnaire form was applied to the mothers, maternal breast milk, infant formula, and infant urine were collected from mother-infant dyads. Total BPA levels in breast milk, infant formula, and infant urine samples were analyzed by the high-pressure liquid chromatography (HPLC). While BPA was detected in 92.5% of the breast milk samples in the EBF group (mean ± SD = 0.59 ± 0.29 ng/mL), BPA was detected in all of the breast milk samples in the BF + FF group (mean ± SD= 0.72 ± 0.37 ng/mL) ($p < 0.05$). Similarly, 100% of the infant formula samples in the BF + FF group had detectable levels of BPA (mean ± SD = 7.54 ± 1.77 ng/g formula). The mean urinary BPA levels in the EBF infants (4.33 ± 1.89 μg/g creatinine) were not statistically different from the BF + FF infants (5.81 ± 0.11 μg/g creatinine) ($p > 0.05$). The average daily BPA intake in EBF infants (0.18 ± 0.13 μg/kg body weight (bw)/day) was found to be significantly higher than in BF + FF infants (0.12 ± 0.09 μg/kg bw/day) ($p < 0.05$). The estimated dietary intakes of BPA for infants in both groups were below the temporary tolerable daily intake (t-TDI) (4 μg/kg bw/day). Consequently, BPA intake of EBF and BF + FF infants were within safe daily limits during the first six months of life.

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Bisphenol A; urine; exposure; exclusive breastfeeding; formula-fed

1. Introduction

Bisphenol A (BPA), which is widely used in the inner surface coating of plastic bottles, baby feeding bottles, plastic kitchenware, dental sealants, electronic materials, thermal papers, medical supplies, and food and beverage cans, has been defined as an 'endocrine disrupting chemical' and declared as a social, environmental, and global problem by the United States Environmental Protection Agency (EPA) and the World Wide Fund for Nature (WWF) (Kuruto-Niwa *et al.* 2007, Vandenberg *et al.* 2010, Shafei *et al.* 2018). BPA can affect the synthesis, secretion, and transport of hormones (Ma *et al.* 2019). As a result, the reproductive system, nervous system, metabolic functions, immunity, growth, and development may be adversely affected (FDA 2010). Considering the potential effects of BPA on human health, legal regulations have been made by various international authorities as a result of risk assessments (Almeida *et al.* 2018). According to European Union (EU) Regulation 2018/213 on plastic materials and articles intended to come into contact with food, the use of BPA is authorized under the consideration of the specific migration limit (SML) of 0.05 mg/kg food. EU has banned BPA in food contact varnished or coated materials and articles for infants and toddlers (European Commission 2018). In January 2015, the European Food Safety Authority (EFSA) set a temporary tolerable daily intake (t-TDI) of 4 μg/kg body weight (bw)/day for BPA (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids 2015). EFSA (2021) published a reevaluation of BPA exposure in November 2021 and recommended reducing the t-TDI from 4 μg/kg bw/day to 0.04 ng/kg bw/day. However, it should be noted that this is still a draft opinion. The SML for BPA is defined as 0.05 mg/kg food in 2019/44 numbered Turkish Food Codex Food Contact Plastic Substances and Materials Communique. In addition, it is prohibited the use of BPA in the production of polycarbonate substances and materials used for infants and toddlers in 2019/14 numbered Turkish Food Codex on Infant Formula and Follow-on Formula Communique (Turkish Food Codex 2019).

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Infants are more vulnerable to BPA due to their physiological characteristics. Due to their rapid cellular differentiation and immature metabolism, they may not be able to eliminate this chemical effectively (Kim *et al.* 2020). BPA detoxification is insufficient in infants because their protective mechanisms such as detoxification enzymes, liver biotransformation capacities, mechanisms of DNA repair, and immune systems are not sufficiently developed during the first year after birth (Kadawathagedara *et al.* 2018, Kawa *et al.* 2021). In addition, infants are more exposed to BPA than the general population because they consume more food per unit of body weight (Bergkvist *et al.* 2008). The main sources of dietary exposure to BPA in infants not yet introduced to complementary foods are breast milk and infant formula (Yesildemir and Akdevelioglu 2021).

Although many studies have been carried out to detect BPA levels in breast milk and infant formula, there are limited studies that determine and compare dietary BPA exposures of infants according to feeding type. This study was conducted to estimate and compare dietary exposure to BPA in exclusively breastfed (EBF) and breastfed plus formula-fed (BF + FF) infants. The secondary aims of the study were to determine the BPA levels in breast milk and infant urine in the EBF and BF + FF groups.

2. Materials and methods

2.1. Study population

This case-control study was carried out at the Gazi University Medical Faculty Social Pediatrics Well Child Care Clinics, between January 2021 and July 2021. It was conducted on 0–6-month-old infants, brought for routine well-child care visits, and their mothers. We recruited 70 infants, who were assigned into two different groups fed breast milk exclusively (40 babies) and breast milk plus formula (30 babies), and their mothers.

The ethics approval was obtained from the meeting on 07.12.2020 (decision number: 828) from Gazi University Clinical Research Ethics Committee. The mothers were notified about the study with an informed consent form. They also stated that they were volunteers for this study.

All infants in this study had the following inclusion criteria: born at term (gestational age: 38–42 weeks), singleton, normal growth and development, EBF or BF + FF, not introduced complementary foods. The mothers were aged between 18 and 35 years and healthy.

Exclusion criteria for the infants were: the presence of congenital malformation or other diseases, hospitalization in the neonatal intensive care unit, performing an interventional procedure (catheter or tube insertion, etc.), histories of premature birth or delivery complication, having a chronic feeding problem, and having known BPA exposure history. Exclusion criteria for the mothers were: unhealthy, having a chronic disease or a nutritional disorder, exposed to an interventional procedure, and having a known BPA exposure history. Any mother who might have had occupational exposure to BPA was also excluded.

2.2. Study design

Participating infants were divided into two groups: 40 infants for the EBF group and 30 infants for the BF + FF group. The questionnaire containing the questions on the general information of the mother-infant dyads were filled out during face-to-face interviews. The height, body weight and head circumference of infants at birth were obtained from hospital records. The height, body weight, and head circumference of infants were measured at the hospital by outpatient nurses. The World Health Organization (WHO) growth standards for 0–5 years (2006–2007) and Neyzi Growth Charts were used to calculate weight, height, and head circumference Z-scores. The measurements were classified according to the Z-score junctions (Neyzi *et al.* 2008, WHO 2008). The height and body weight of mothers were measured by the dietitian, and body mass index (BMI) was calculated by dividing weight in kg by height in meters squared (m^2). BMI was categorized according to WHO classification: underweight ($<18.50\ kg/m^2$), normal (18.50–24.99 kg/m^2), overweight (25.00–29.99 kg/m^2), and obese ($\geq 30.00\ kg/m^2$) (WHO 2010a).

After the interview, maternal breast milk and infant urine samples were collected from mother-infant dyads in EBF group. Also, maternal breast milk, infant formula used by the babies during the time they were included in the study, and infant urine samples were collected from mother-infant dyads in the BF + FF group. Breast milk samples were collected in the middle of the day.

After the mothers were ensured to breastfeed their babies for about 10 min, they were instructed to clean their hands and nipple with purified water and gauze provided to them by the researcher. Mature milk samples of 10–20 ml were obtained from a single breast using a hand. Breast milk specimens were taken into BPA-free glass tubes. Powdered infant formula samples of 10–20 g were taken into BPA-free glass tubes. Spot urine samples were collected from infants by means of a BPA-free pediatric urine bags. About 2–3 mL of urine samples in urine bags were quickly taken into BPA-free glass vials. Maternal breast milk and infant urine samples were stored at low temperature (-80 °C) until analyses. Formula samples were kept at room temperature (23–25 °C) and in the dark until the day of analysis.

2.3. Bpa measurement

2.3.1. Purification of glass materials from plastic

As also applied by Ayar et al. (2021), HPLC vials and glass tubes used throughout the study were deplasticized for 4 hours on a sand bath at 400 °C. In addtion, to remove all the plastic material, all flasks, beakers and funnels were put in n-hexane:tetrahydrofuran (1:1, v/v) for 4 hours. Then, they were dried at 37°C.

2.3.2. Determination of total BPA levels in breast milk and infant formula by HPLC

2.3.2.1. Solid-phase extraction for infant formula. 5 mL acetonitrile was added to 5 g of reconstituted (in 5 mL) infant formula with added BPA (BPA spike: 100 ng/mL, 100 µL). The

samples were vortexed for 30 seconds. They were centrifuged at 4000 $\times g$ for 20 min. Following that, the supernatant was transferred to another centrifuge tube with maximum care not to collect the precipitate. The supernatant was diluted with 50 mL of deionized water before being taken into the SPE Cartidge, CHROMABond C18 45 μm, 6 mL/500 mg (Macherey Nagel, Düren, Germany). SPE columns were conditioned with 10 mL chloroform, 10 mL acetonitrile, and 10 mL methanol. Then, 10 mL of deionized water was passed through the column and dried. The cartridges were washed with 10 mL of n-hexane and dried again. The samples were eluted from the cartridge with 15 mL of chloroform. Chloroform extracts were evaporated under nitrogen gas. The residue was then stored at −80 °C until analysis.

2.3.2.2. Liquid-liquid extraction for breast milk. Frozen milk samples were thawed by the sonification for 60 min. 100 μL of milk sample was taken into a glass tube and 200 μL of deionized water was added to it. Subsequently, 10 μL glucuronidase/arylsulfatase (*Helix pomatia*) was added to the mixture. The new mixture was incubated in a water bath for 3 hours at 37^0 C. 5 mL acetonitrile was added to breast milk with added BPA (BPA spike: 100 ng/mL, 100 μL). The samples were vortexed for 30 seconds. After adding 1 mL hexane to the samples, the mixture was vortexed for 1 minute. Following this, the mixture was centrifuged at 1300 $\times g$ for 10 min. The aqueous layer (280 μL) was collected (carefully handled to avoid transfer of the n-hexane layer). 100 μL 0.2 M hydrochloric acid was added to the it. Then, 1 mL chloroform was added to the mixture and mixed for 1 minute. Finally, after centrifugation at 1300 $\times g$ for 10 min, 900 μL organic fraction was evaporated under nitrogen gas.

2.3.2.3. Derivatization. The eluate was dissolved in 100 μL acetonitrile containing 5 mM 4-(4,5-diphenyl-1H-imidazol-2-yl) benzoyl chloride (DIB-Cl) and 5 μL 1.5 M triethylamine. Afterwards, it was kept in a hot dry bath at 35 °C for 20 min. The reaction was stopped by adding 10 μL 5% acetic acid. The mixture was then passed through a microfilter (0.45 μm, HLC-DISK 3). The mixture obtained from both solid-solid extraction and liquid-liquid extraction was given to high-pressure liquid chromatography (HPLC) as 5 μL. All detectable BPA levels in the samples were calculated from the peaks of the standards.

2.3.2.4. Chromatographic system. The BPA levels in infant formulas and breast milk were measured by HPLC (Hewlett Packard Agilent 1200 Series, Vienna, Austria). We used a Spherisorb C18 ODS2 column (particle size 5 μm, 25 cm, 4.6 mm i.d.) (ACE) throughout the experiments. The detections were made by a fluorescence detector at an excitation λ of 230 nm and emission λ of 315 nm. The samples and standards were injected to the instrument with a volume of 5 μl. The column temperature was set at 25 °C.

2.3.2.5. Application of the method. Acetonitrile (A) and tetrahydrofuran 2.5% (v/v, in water) (B) were used as the mobile phase. The flow rate was set at 0.4 mL/min (with gradient elution as 60:40 to 5:95, for 40 min). In this period, the volumes of A and B mobile phases changed gradually (Durmaz *et al.* 2018, Ayar *et al.* 2021). BPA levels in infant formulas and breast milk were measured for least for two times.

2.3.3. Determination of urinary total BPA levels by HPLC

2.3.3.1. Extraction. For the determination of total BPA (conjugated plus free), the urine samples (500 μl) were spiked with 50 μl 100 ng/mL (ppb) BPA. Later, 2.0 M sodium acetate buffer (30 μl, pH 5.0) was added. To free the conjugated BPA, glucuronidase/arylsulfatase (10 μl, from *Helix pomatia*) was added to the mixture and vortexed. For 3 hours, this mixture was incubated in a water bath at 37 °C. Afterwards, the BPA in the urine samples was extracted by using ethyl acetate (5 mL). After centrifugation in 3500 $\times g$ for 5 min, the supernatants (3 mL) were transferred to deplasticized glass tubes. Nitrogen stream was used to evaporate the solvent to dryness. All the residues were kept at −20 °C until analyses were performed (Durmaz *et al.* 2018, Ayar *et al.* 2021).

2.3.3.2. Chromatographic system. The residues were dissolved in the mobile phase and determined by HPLC. As also used for infant formulas and breast milk, a Spherisorb C18 ODS2 column and fluorescence detector ($\lambda_{excitation}$ =230 nm, $\lambda_{emission}$ =315 nm) were used. The column temperature was set at 25 °C (Durmaz *et al.* 2018, Ayar *et al.* 2021).

2.3.3.3. Application of the method. The urine samples were dissolved in 300 μL 60% acetonitrile. The injection volumes for both standards and samples were 100 μL. Acetonitrile (A) and 2.5% (v/v, in water) tetrahydrofuran (B) were used as the mobile phase with a flow rate set at 0.4 mL/min and gradient elution was applied as 60:40 to 5:95 in 40 min. In this period, the volumes of A and B mobile phases changed gradually (Durmaz *et al.* 2018, Ayar *et al.* 2021). BPA measurements for each urine sample were performed for at least for two times.

2.3.4. Determination of the urinary creatinine levels

The urinary creatinine levels were determined by HPLC in order to normalize the results. The mobile phase was 15 mM potassium dihydrogen phosphate buffer (with 2.5% methanol, pH:7). C18 column was used and the detections were made by a UV detector at $\lambda = 235$ nm (Durmaz *et al.* 2018, Ayar *et al.* 2021).

2.3.5. Limit of detection, limit of quantification, and coefficient of variation

The limits of detection (LOD) were 0.89 ng/mL, 0.84 ng/mL, and 0.79 ng/mL, and the limits of quantification (LOQ) were 1.87 ng/mL, 1.72 ng/mL, and 1.65 ng/mL for infant formulas, breast milk, and infant urine, respectively. The within-day coefficient of variation (CV) was determined to be 6.75 ± 1.18%, and the interday CV was determined to be 11.57 ± 2.86% for infant formulas. The within-day CV was 5.89 ± 0.57% and 2.93 ± 0.38%, and the interday CV was 10.72 ± 1.17% and 7.61 ± 0.87% for breast milk and infant

urine, respectively. The recovery was found to be 89.72%, 91.42%, and 96.87% for infant formula, breast milk, and infant urine, respectively. The levels below the LOQ were also included in the data since all samples were spiked.

2.4. Exposure assessment

Dietary BPA exposures of infants were calculated using the BPA level in breast milk/infant formula, the current body weight of the infant, and the total amount of breast milk/formula consumed per day by the infant. Precise measurement of infants' daily breast milk consumption is very challenging. Therefore, a practical method was used to estimate daily breast milk intake. The frequency and duration of breastfeeding were determined using the questionnaire. Accordingly, if breastfeeding lasted 10 min or longer for 0–6 month-old babies, the amount of breast milk for each breastfeeding was accepted as 125 mL. In addition, it was calculated as 12.5 mL/min if the duration of breastfeeding was shorter. This method has been used in many studies conducted by the Avon Longitudinal Study of Parents and Children (ALSPAC) working group with infants aged 0–6 months (Kent *et al.* 2006, Noble and Emmett 2006, EFSA Panel on Dietetic Products, Nutrition and Allergies 2017).

The estimated daily intake (EDI) for exclusively breastfed infants of BPA from breast milk was estimated by the following equation:

$$\text{EDI (ng/kg bw/day)} = \frac{\text{CBM (ng/mL)} \times \text{VBM (mL/day)}}{\text{BW (kg)}}$$

where CBM is the individual BPA concentration in breast milk (ng/mL), VBM is the daily breast milk consumption for the infant (mL/day), and BW is the body weight of the infant (kg).

The EDI for breastfed plus formula-fed infants of BPA from breast milk and formula was estimated by the following equation:

$$\text{EDI (ng/kg bw/day)} = \frac{[\text{CBM (ng/mL)} \times \text{VBM (mL/day)}] + [\text{CIF (ng/g)} \times \text{VIF (g/day)}]}{\text{BW (kg)}}$$

where CBM is the individual BPA concentration in breast milk (ng/mL), VBM is the daily breast milk consumption for the infant (mL/day), CIF is the BPA concentration in infant formula (ng/g), VIF is the daily powdered formula consumption for the infant (g/day), and BW is the body weight of the infant (kg). The calculated value for the EDI (ng/kg bw/day) was divided by 1000 and the unit of the estimated daily intake was given as μg/kg bw/day.

For BPA, the hazard quotient (HQ) was calculated as the ratio between the EDI and the t-TDI. A HQ above 1 was accepted as a BPA exposure risk (Sanchis *et al.* 2020).

2.5. Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences Version 23 (IBM Inc., Armonk, NY, USA). Numerical variables were expressed as arithmetic mean (mean) and standard deviation (SD). Categorical variables were expressed as numbers (n) and percentages (%). The Kolmogorov– Smirnov test was used to determine if the variables were normally distributed. The differences between categorical variables were evaluated using the chi-squared test. The independent sample t-test was used with parametric data, while the Mann-Whitney U test was used for non-parametric data. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Subjects

The study included 40 breastfed (boys = 47.5%; girls = 52.5%) and 30 breastfed plus formula-fed infants (boys = 46.7%; girls = 53.3%). The average age of the infants in EBF and BF + FF were 4.40 ± 1.65 months and 3.93 ± 1.74 months, respectively. More than half of the infants (61.4%) were born by cesarean section. There were no statistically significant differences in gender, age, and birth type of infants from the EBF and BF + FF groups ($p > 0.05$). Also, there were no significant differences in the body weight, height, and head circumference of infants between the groups ($p > 0.05$) (Table 1).

The mean age was 29.20 ± 3.91 years for mothers in EBF and 30.60 ± 3.52 years for mothers in BF + FF ($p > 0.05$). The difference between the groups concerning level of education, occupation, working status, and income status was not statistically significant ($p > 0.05$). The mean mothers' height in the EBF and BF + FF were 162.7 ± 6.67 cm and 165.2 ± 5.44 cm, respectively ($p > 0.05$). The mean body weight was higher in the BF + FF mothers (73.7 ± 13.36 kg) than in the EBF mothers (66.8 ± 13.27 kg) ($p < 0.05$). There was no statistically significant difference in the BMI classification of the mothers ($p > 0.05$) (Table 1).

3.2. BPA levels in breast milk and infant formula

BPA was detected in 92.5% and 100% of the breast milk samples in EBF and BF + FF groups, respectively. The mean BPA levels in breast milk appeared higher in the BF + FF group than in the EBF group (0.72 ± 0.37 ng/mL and 0.58 ± 0.29 ng/mL) ($p < 0.05$). In addition, BPA was detected in all formula samples, with mean levels of 7.54 ± 1.77 ng/g (Table 2).

BPA levels of all breast milk ($n = 70$) and infant formula ($n = 30$) samples were also compared in the study. The mean value of BPA levels in breast milk and infant formula were determined as 0.64 ± 0.33 ng/mL (<LOD-2.29) and 7.54 ± 1.77 ng/g formula (4.98–11.54), respectively. Since the density of breast milk is approximately 1.03 g/mL, a milliliter of milk was accepted as equivalent to a gram of milk. The average BPA levels in infant formula were significantly higher than in breast milk ($p < 0.05$).

It was found that the BPA levels of the infant formulas of four different brands included in our study were not statistically different from each other ($p > 0.05$). The levels of total BPA in canned formula samples (mean ± SD = 7.74 ± 1.73 ng/g formula) were higher than those in formula samples in paper containers

Table 1. General characteristics of infants and their mothers.

Infants	EBF ($n = 40$)	BF + FF ($n = 30$)	
Gender, *n* (%)			
Boy	19 (47.5)	14 (46.7)	$p = 0.945$
Girl	21 (52.5)	16 (53.3)	
Age (month), *n* (%)			
0-1	3 (7.5)	2 (6.7)	$p = 0.281$
1-2	3 (7.5)	8 (26.7)	
2-3	6 (15.0)	2 (6.7)	
3-4	6 (15.0)	3 (10.0)	
4-5	8 (20.0)	8 (26.7)	
5-6	14 (35.0)	7 (23.2)	
Birth type, *n* (%)			
Normal	19 (47.5)	8 (26.7)	$p = 0.076$
Cesarean	21 (52.5)	22 (73.3)	
Anthropometric measurements at birth, mean ± SD			
Height (cm)	49.8 ± 2.60	49.8 ± 2.83	$p = 0.838$
Body weight (g)	3191.1 ± 361.91	3271.8 ± 355.43	$p = 0.355$
Head circumference (cm)	35.0 ± 1.66	34.9 ± 1.89	$p = 0.832$
Current anthropometric measurements, mean ± SD			
Height (cm)	62.2 ± 5.17	60.8 ± 5.14	$p = 0.292$
Body weight (g)	6429.0 ± 1366.73	5886.7 ± 1227.10	$p = 0.091$
Head circumference (cm)	40.3 ± 2.74	39.3 ± 0.53	$p = 0.130$
Height-for-age z-score, mean ± SD	0.3 ± 1.12	0.3 ± 1.19	$p = 0.811$
Weight-for-age z-score, mean ± SD	0.2 ± 0.63	−0.1 ± 0.73	$p = 0.083$
Weight-for-height z-score, mean ± SD	0.1 ± 1.02	−0.3 ± 1.06	$p = 0.158$
Head circumference-for-age z-score, mean ± SD	0.1 ± 1.20	−0.3 ± 1.66	$p = 0.233$
Mothers			
Age, mean ± SD	29.20 ± 3.91	30.60 ± 3.52	$p = 0.225$
Level of education, *n* (%)			
Primary school	6 (15.0)	6 (20.0)	$p = 0.298$
High school	8 (20.0)	11 (36.7)	
University	23 (57.5)	11 (36.7)	
Post-graduate	3 (7.5)	2 (6.7)	
Occupation, *n* (%)			
Housewife	12 (30.0)	4 (13.3)	$p = 0.203$
Officer	17 (42.5)	15 (50.0)	
Self-employment	9 (22.5)	6 (20.0)	
Worker	2 (5.0)	5 (16.7)	
Working status, *n* (%)			
Working	17 (42.5)	10 (33.3)	$p = 0.436$
Not working	23 (57.5)	20 (66.7)	
Income status, *n* (%)			
Low	4 (10.0)	4 (13.3)	$p = 0.894$
Middle	34 (85.0)	24 (80.0)	
High	2 (5.0)	2 (6.7)	
Anthropometric measurements, mean ± SD			
Height (cm)	162.7 ± 6.67	165.2 ± 5.44	$p = 0.095$
Body weight (g)	66.8 ± 13.27	73.7 ± 13.36	$p = 0.034$*
BMI (kg/m^2)	25.1 ± 5.25	27.1 ± 5.01	p = 0.062
BMI classification, *n* (%)			
Underweight	1 (2.5)	–	$p = 0.189$
Normal	24 (60.0)	12 (40.0)	
Overweight	6 (15.0)	9 (30.0)	
Obese	9 (22.5)	9 (30.0)	

Chi-squared test, EBF: Exclusively breastfed; BF + FF: Breastfed plus formula-fed; SD: Standard deviation; BMI: Body mass index.
*$p < 0.05$.

Table 2. BPA levels in breast milk, infant formula, and infant urine and infants BPA exposure.

	EBF ($n = 40$)			BF + FF ($n = 30$)			
	n>LOD (%)	mean ± SD	Min–Max	*n*>LOD (%)	mean ± SD	Min–Max	
BPA levels							
Breast milk (ng/mL)	92.5	0.58 ± 0.29	<LOD–1.13	100	0.72 ± 0.37	0.36–2.29	$p = 0.037$*
Infant formula (ng/g)[a]	–	–	–	100	7.54 ± 1.77	4.98–11.54	
Urine (μg/g creatinine)	100	4.33 ± 1.89	2.02–11.02	100	5.81 ± 3.11	2.57–15.87	$p = 0.091$
BPA exposure (μg/kg bw/day)	92.5	0.12 ± 0.09	0.00–0.36	100	0.18 ± 0.13	0.04–0.69	$p = 0.040$*

Independent sample *t*-test; BPA: Bisphenol A; EBF: Exclusively breastfed; BF + FF: Breastfed plus formula-fed; LOD: LOD: Limit of detection; SD: Standard deviation; Min: Minimum; Max: Maximum; bw: Body weight.
[a]BPA concentration in infant formula refers to powder (as sold).
*$p < 0.05$.

(mean ± SD = 7.19 ± 1.86 ng/g formula), but it was not statistically significant ($p > 0.05$).

3.3. BPA levels in infant urine

The mean urinary BPA levels in EBF and BF + FF groups were found as 4.33 ± 1.89 μg/g creatinine and 5.81 ± 3.11 μg/g creatinine, respectively. The urinary BPA levels in EBF group were in the range of 2.02–11.02 μg/g creatinine, those in BF + FF group were in the range of 2.57–15.87 μg/g creatinine. No significant differences were shown in creatinine-corrected levels of BPA between the groups ($p > 0.05$) (Table 2).

3.4. Infant BPA exposure

The mean dietary exposure to BPA in EBF and BF + FF infants were 0.12 ± 0.09 μg/kg bw/day (0.00–0.36) and 0.18 ± 0.13 μg/kg bw/day (0.04–0.69), respectively. It was determined that the dietary intake of BPA in BF + FF infants was higher than those in EBF infants ($p < 0.05$) (Table 2). While the highest exposure was 11 times below the t-TDI in the EBF group, it was 6 times below the t-TDI in the BF + FF group. The BPA levels in breast milk, infant urine, and infant BPA exposure are shown in Figure 1.

The percentile values of HQs, the ratio between the EDI and the t-TDI, are given in Figure 2. The percentiles of HQs for BPA are below one in all EBF and BF + FF infants.

4. Discussion

We compared daily BPA intake between EBF and BF + FF infants in this study. Although the estimated daily intake of BPA for BF + FF infants was higher than for EBF infants, those for all infants are far below the t-TDI (4 μg/kg bw/day).

Breast milk, because of its fat content, can be contaminated with many environmental chemicals, mainly those with lipophilic properties. Thus these chemicals can be passed from mother to infant through the contaminated breast milk (Mercogliano and Santonicola 2018, Iribarne-Durán *et al.* 2022). In breast milk, determining the levels of BPA, which is among these environmental chemicals, is necessary to estimate dietary intake of BPA for infants aged 0–6 months (Kuruto-Niwa *et al.* 2007). In our study, almost all breast milk samples showed detectable BPA levels (Table 2). In other studies, this rate varies between 20–100% (Ye *et al.* 2006, Ye *et al.* 2008, Mendonca *et al.* 2014). It was reported that BPA was detected in 58.4% of 1525 breast milk samples in a recent systematic review. Moreover, it was notified that BPA was present in more than 70% of the samples in all but three studies (Iribarne-Durán *et al.* 2022).

Many studies have been carried out in different countries such as Japan (Sun *et al.* 2004, Kuruto-Niwa *et al.* 2007), USA (Ye *et al.* 2008, Mendonca *et al.* 2014), South Korea (Yi *et al.* 2010, Lee *et al.* 2018), France (Migeot *et al.* 2013, Deceuninck *et al.* 2015), Spain (Rodríguez-Gómez *et al.* 2014, Dualde *et al.* 2019), Canada (Cao *et al.* 2015), and China (Niu *et al.* 2017, Jin *et al.* 2020) to determine the levels of BPA in breast milk. The current results are generally consistent with previous studies (Sun *et al.* 2004, Mendonca *et al.* 2014). However, these results were found to be lower (mean ± SD = 3.41 ± 0.13 ng/mL and 2.1 ± 4.9 ng/mL) (Kuruto-Niwa *et al.* 2007, Mendonca *et al.* 2014) or higher (median = 0.10 ng/mL ve 0.11 ng/mL) (Cao *et al.* 2015, Deceuninck *et al.* 2015) than published studies. There are differences between total BPA levels in breast milk in studies conducted in different countries. It can be explained by the age, health status, lifestyle, nutritional habits, use of plastic materials, and dietary exposure risk of mothers, apart from the difference in the applied analysis method in each study (Mercogliano and Santonicola 2018). In this study, BPA levels in breast milk samples are generally low, which suggests that Turkish mothers have relatively low BPA exposure. The possible explanation for the variability in results could be that dietary habits change from country to country since the primary source of BPA exposure is diet. Moreover, it is known that BPA migration from kitchenware and packing materials increases with temperature in summer (Vilarinho *et al.* 2019). In our study, the total BPA

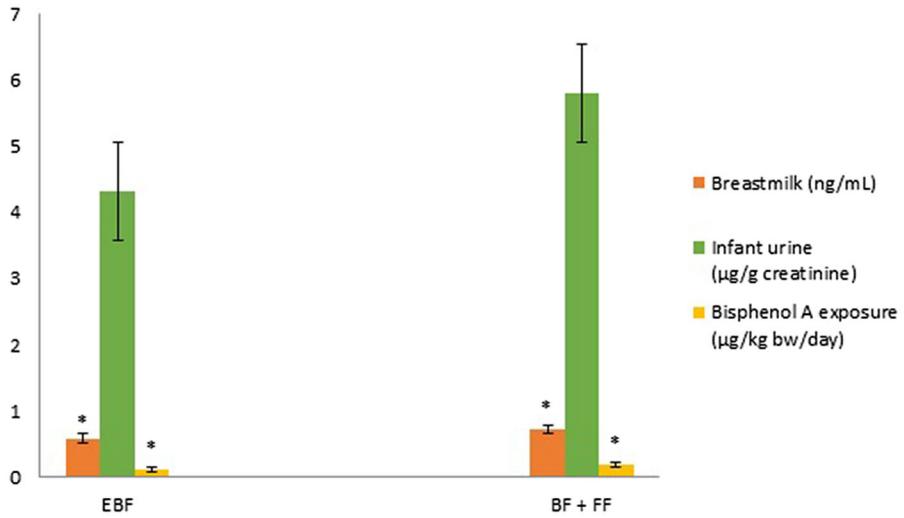

Figure 1. The comparison of BPA levels in breast milk and infant urine, and infants' BPA exposure between both groups. EBF: Exclusively breastfed, BF + FF: Breastfed plus formula-fed. *$p < 0.05$. Total BPA levels of breast milk samples in the BF + FF group were significantly higher than those in the EBF group. Infants in the BF + FF group had significantly higher dietary exposure to BPA than those in the EBF group.

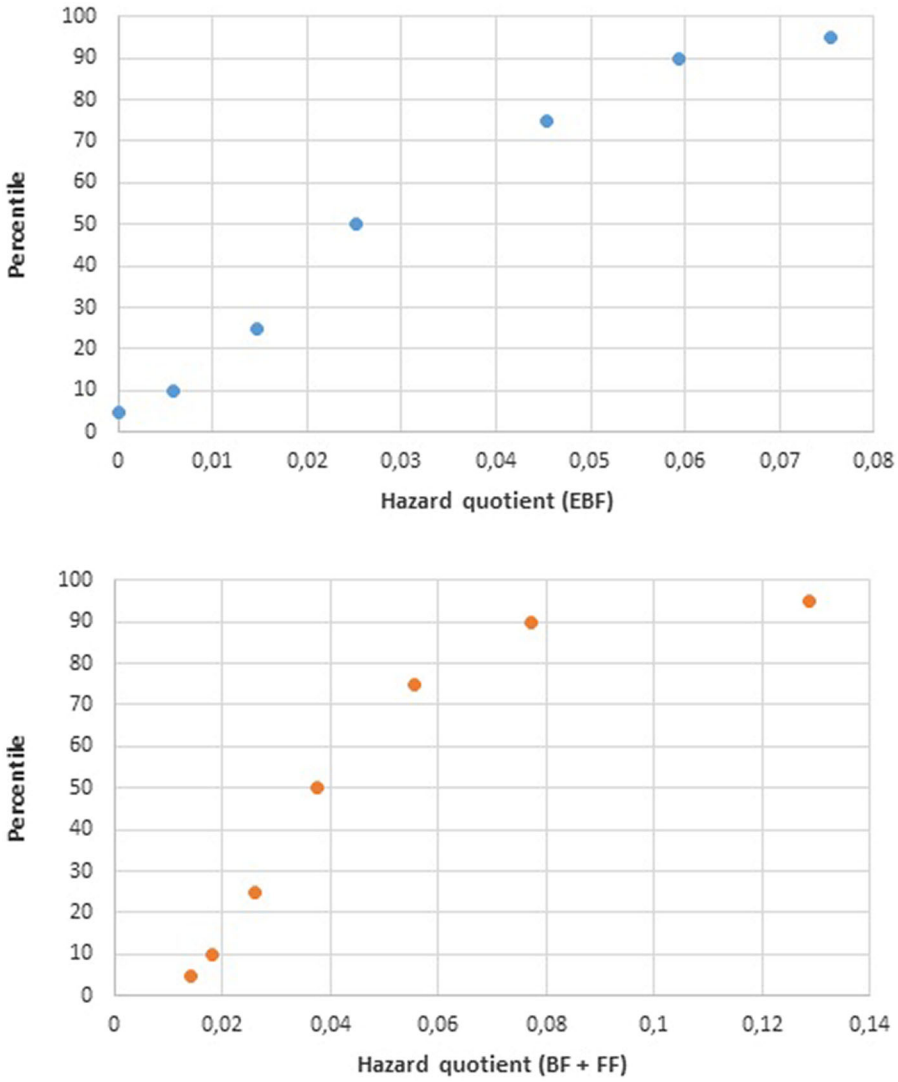

Figure 2. The percentile values of hazard quotient for BPA.

level in breast milk may be lower than in other countries since most of the breast milk samples were collected during months of winter. We think that BPA migration from kitchen utensils to foods may decrease with the lower ambient temperature and thus BPA exposure of mothers may reduce in winter.

In this study, BPA was detected in all infant formulas (Table 2). In a recent study examining 68 infant formulas in India, 76.5% of the breast milk samples had detectable levels of total BPA (mean = 5.46 ng/g formula) (Karsauliya *et al.* 2021). In a study conducted in China, BPA was not detected in any of 76 powdered infant formulas (Sun *et al.* 2017). Similarly, another study showed that total BPA was detected in only one of fourteen powdered infant formulas in the USA (Ackerman *et al.* 2010). In studies carried out in Italy (Ferrer *et al.* 2011), Canada (Cao *et al.* 2008), the USA (Schecter *et al.* 2010), and India (Karsauliya *et al.* 2021), BPA was detected in infant formulas at different levels. It was shown that the BPA levels in infant formula in Turkey are generally higher than the BPA levels in those in other countries. Despite using different analysis methods in each study, we think this high difference may be due to contaminations occurring at formula production. However, our results suggest that even the highest BPA level in infant formulas was below the SML (0.05 mg/kg or 50 ng/g of food) for BPA. Hereby, it can be suggested that the total BPA in widely consumed infant formula in Turkey was within acceptable limits.

Although BPA use has been banned in polycarbonate food-contact materials intended for babies and toddlers in Turkey, there is no regulation for epoxy resins used in the internal coating of cans (Turkish Food Codex 2019). However, it has been claimed that BPA-based epoxy resins are not used as coatings in baby food packages (Cao *et al.* 2008). In this study, we observed that the majority of formula-fed infants were given a canned formula (63.3%). The canned formulas had significantly higher BPA levels than formulas in paper containers in our study. The previous studies have reported values of total BPA in canned formulas ranging from undetectable to 1290 ng/g formula (Ackerman *et al.* 2010, Ferrer *et al.* 2011, Zheng *et al.* 2021). It is reported that migration of BPA from epoxy-coated can surfaces to infant formula, with the range of 0.1–11.6 ng/g formula (Biles *et al.* 1997). This suggests that can surfaces in infant formulas on the market in Turkey may be epoxy-coated.

BPA levels were significantly higher in infant formulas compared to breast milk samples in our study. In another study, free BPA (mean = 0.26 μg/L) and total BPA (mean = 1.30 μg/L) were detected in 38% and 76% of breast milk samples, respectively. Additionally, the mean level of free BPA in formula samples was 0.88 μg/L, while the mean level of total BPA in formula samples was 3.85 μg/L. The content of free BPA and total BPA was higher in infant formula samples than in breast milk samples (Martínez *et al.* 2019). Although BPA migration from packaging is expected to decrease in recent years, due to the increasing use of BPA-free packages in infant formulas, infant formulas still contain more BPA than breast milk samples. This situation reveals again that breast milk is the best source of nutrition for babies during the first six months. Even though EFSA declares that BPA levels in breast milk and infant formula are not harmful to infants, infancy is a critical period for mental and physical development. Therefore, it is suggested that healthcare professionals and parents should pay full attention to the exposure to chemicals such as BPA during infancy (Ma *et al.* 2019).

Numerous studies have been conducted to determine urinary BPA levels in adults and children (Ye *et al.* 2015, Gao *et al.* 2016, van Woerden *et al.* 2019, Cai *et al.* 2020). However, there is limited number of studies on the determination of the urinary BPA levels in babies, and the majority of these studies were carried out in newborns (Calafat *et al.* 2009, Lee *et al.* 2018). This may reflect prenatal exposure of infants rather than dietary exposure. It has been observed that infants had low levels of urinary BPA in most of the studies. Völkel *et al.* (2011) stated that the maximum level of urinary BPA in infants aged 1–5 months was 17.85 μg/L. In a study conducted with 152 newborns in South Korea, BPA was detected in 82.1% of neonatal urine samples (median = 10.1 μg/g creatinine) (Lee *et al.* 2018). On the other hand, Mendonca *et al.* (2014) detected total BPA in 93% of the urine samples of 29 infants aged 3–15 months (mean ± SD = 6.0 ± 16.2 μg/L). A study involving mother-infant dyads showed that the urinary BPA levels in babies were between 0.02 and 0.44 μg/L (Sayıcı et al. 2019). In the current study, BPA was detected in 100% of infant urine samples (Table 2). The current results are consistent with previous research conducted on newborns and older babies. Another study from Calafat *et al.* (2009) found that the mean BPA levels in low birth weight infants in a neonatal intensive care unit were found as 30.3 ± 5.2 μg/L. This value is higher than those observed in our and in other studies. A possible explanation for this difference could be related to the exposure of premature infants to BPA caused by some medical instruments used during intensive care treatments.

In a study comparing urinary BPA levels of infants by feeding pattern, urinary total BPA levels in EBF and BF + FF infants were in the range of 0.52–7.3 μg/L and 1.7–4.4 μg/L, respectively ($p > 0.05$) (Mendonca *et al.* 2014). In another cohort study, urinary total BPA levels were shown to be significantly higher in formula-fed (geometric mean = 1.04 μg/L) compared to EBF infants (geometric mean = 0.09 μg/L). Besides, the geometric mean urinary total BPA level was 0.35 μg/L in BF + FF infants (Arbuckle *et al.* 2015). Generally, it can be said that feeding with infant formula increases the urinary BPA level. In the current study, BF + FF infants had higher urinary BPA levels than EBF infants, but it was no statistically significant difference (Table 2). As a result of this study, it has been observed that 0–6 months infants without any known BPA exposure have low urinary BPA levels. Some infants had high urinary BPA levels, which may be due to differences in dietary and non-dietary exposures to BPA in our study. The BPA levels in the spot urine samples show the infants' BPA exposure in the last 24 hours. Whether the infants were exposed to BPA on the day the samples were taken or not may be another reason for this difference.

International organizations have indicated that babies and children have the highest estimated daily intake of BPA (Mendonca *et al.* 2014). However, there are only limited number of published studies that report estimate BPA exposure in infants. These studies have shown the estimated daily intake in infants ranged from 0.007 to 0.475 μg/kg bw/day. This difference may depend on the age and feeding type of babies (Huang *et al.* 2017). In our study, the BPA exposure of BF + FF infants was much higher than the BPA exposure of EBF infants. WHO Expert Committee determined the estimated exposure of EBF infants aged 0–6 months to BPA as 0.3 μg/kg bw/day (WHO 2010 b). EFSA in 2015 reported that the estimated dietary exposures of breastfed infants aged 1–5 days, 6 days-3 months, and 4–6 months to BPA were 0.225, 0.165, and 0.145 μg/kg bw/day, respectively (EFSA 2015). Dualde *et al.* (2019) stated that the mean BPA exposure of Spanish breastfed infants was 0.20 μg/kg bw/day (0.001–5.0 μg/kg bw/day). In addition, WHO stated in 2010 that the highest estimated exposure occurred in infants fed with liquid formula in polycarbonate plastic packages and polycarbonate-free packages were 2.4 μg/kg bw/day and 0.5 μg/kg bw/day, respectively (WHO 2010 b). Consistent with our study, Martínez *et al.* (2019) estimated that the dietary exposure of BPA for formula-fed and EBF infants aged 1–6 months were 0.51 and 0.04 μg/kg bw/day, respectively. It can be stated that BPA exposure is higher in infants who are formula-fed for the first six months.

In our study, estimated BPA exposure of EBF and BF + FF infants were below the t-TDI limit of 4 μg/kg bw/day. Likewise, it was shown that HQ values were below 1 in all infants (Figure 2). It is suggested that breast milk and infant formula may not pose considerable health risks to infants, concerning BPA exposure (Karsauliya *et al.* 2021). However, it should be noted that babies can also encounter exposure to BPA from non-dietary sources. The health effects of BPA are still a public concern, even though BPA exposure in infants is below the accepted limits in almost all of the research. Due to rapid mental and physical changes during the prenatal and neonatal period, infants may be more sensitive to adverse effects of BPA exposure (Mercogliano and Santonicola 2018). It has been reported that exposure to BPA, even at low levels (4% of total daily intake), can cause neurotoxicity and abnormal behavior in mouse pups (Jin *et al.* 2020). In the light of recent studies, EFSA has proposed lowering the TDI to 0.04 ng/kg bw/day in November 2021 (EFSA 2021). Compared to the newly proposed TDI value the EFSA draft opinion, the daily intake of BPA would be 3000 times higher in the EBF infants and 4000 times higher in the

BF + FF infants of our study. This is also the case with similar studies in the literature. However, we also underline that EFSA's draft opinion of 2021 opinion received numerous critical comments during the public consultation phase (EFSA 2022).

5. Strengths and limitations

Several limitations of this study should be noted:

1. There were difficulties in collecting breast milk samples from mothers of BF + FF infants because of the high amount of breast milk required for BPA analysis and using a hand expressed.
2. Breast milk samples were taken from the mothers at a one-time point. A single sample collection may not fully reflect the BPA exposure of an infant. A possible more appropriate approach for it would be to collect breast milk samples several times on different days.
3. We did not consider recording whether the breast milk samples were foremilk or hindmilk. It isn't known whether BPA levels differ in hindmilk, which usually has a higher fat content.
4. We also had problems taking urine samples from babies, especially girls. A urine sample could not be collected from a baby using a single urine bag, and the insertion of the urine bag was repeated many times. For this reason, some mothers left the research study.
5. Our study measured total BPA levels in breast milk, infant formula, and urine samples. Since free BPA has endocrine disrupting properties, it would be better if we measured both free and total BPA levels in the samples. However, we measured total BPA levels in all samples due to available opportunities and lack of budget.

6. Conclusion

Exposure to BPA is a concern because of its possible health effects on infants. This study extends our knowledge of BPA exposure among Turkish infants under 6 months of age by their feeding type. Our results indicate that BF + FF infants aged 0–6 months have significantly higher dietary BPA intake than EBF infants.

This result supports the importance of exclusive breastfeeding in the first six months of life. We observed that EBF and BF + FF infants aged 0–6 months in Turkey encounter dietary BPA exposures which are lower than the tTDI value derived by EFSA in 2015, yet exceed the now proposed far lower limits. However, long-term exposure to bisphenol A, even in low doses, can cause serious health problems in infants. For this reason, it is important to prevent BPA exposure to protect and maintain the health of babies whose nutrition sources are breast milk and/or infant formula during the first six months of life. We recommend that pregnant and lactating women should not use plastic kitchenware, reduce the consumption of plastic packed and canned foods, keep plastic utensils away from piping hot meals, and reduce the consumption of ready-made meals, canned foods, and fast food. Although BPA levels in infant formulas were in the safe range, indicating that exposure to BPA from infant formulas does not represent health risk in Turkey, government regulatory sectors should continue to strengthen the supervision to ensure the quality and safety of infant formulas. In addition, it may be advisable for parents should pay attention to the shelf life, special storage conditions, and usage instructions to choose the safest infant formula for their babies. It should also be considered that infants may also expose to BPA from dermal absorption and inhalation. Thus, the use of BPA should be limited in all products intended for babies and lactating women and these products should be strictly controlled by the government regulations.

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Author contributions

OY, YA, and ADC contributed to the conception or design of the work. OY, ADC, and BCC contributed to the acquisition of data. SBEK, DAC, AY, ABO, SS, and PE performed laboratory analysis. OY, YA, ADC, AY, ABO, SS, and PE contributed to the statistical analysis and interpretation of data for the work. OY prepared the draft of the manuscript. YA, ADC, and PE reviewed and corrected the final manuscript. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Disclosure statement

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