

Bisphenol A Exposure in Exclusively Breastfed Infants and Lactating Women: An Observational Cross-sectional Study

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What is already known on this topic?

Bisphenol A (BPA) is a known endocrine disruptor. Exposure to BPA throughout breastfeeding may impair health. There is no data concerning the exposure of Turkish exclusively breastfed infants to BPA.

What this study adds?

This is the first study to estimate exclusively breastfed infants' daily BPA exposure and BPA risk index in a Turkish population. BPA was measured in human milk samples and the median concentration of free BPA in milk was 0.63 µg/L. Exclusively breastfed infants' estimated exposure was below the temporary tolerable daily intake limit of 4 µg/kg body weight/day. There was a weak negative correlation between exclusively breastfed infants' BPA exposure and their current body weight.

Abstract

Objective: Bisphenol A (BPA) is a known endocrine disruptor and free BPA will interact with estrogen. BPA is also fat soluble and will therefore contaminate breast milk. The European Food Safety Authority has set a limit for temporary tolerable daily intake of 4 µg/kg body weight/day in breastfeeding infants. The aim of this study was to measure human milk BPA concentrations in Turkish women and thus exclusively breastfed infants' exposure to BPA.

Methods: Healthy, postnatal, exclusively breastfeeding women were recruited and breast milk samples were collected. Free BPA concentration was analyzed in the milk samples using competitive enzyme-linked immunosorbent assay. Participants' demographic characteristics and nutritional habits were investigated through face-to-face interviews using a detailed questionnaire.

Results: Eighty women participated. Median milk free BPA level was 0.63 µg/L. There was no statistically significant association between maternal body mass index, birth type, parity, infant birth week, infant birth weight, and human milk BPA concentration. Nevertheless, there was a significant association between human milk BPA level and consumption of fast-food and carbonated drinks ($p = 0.022$ and $p = 0.018$, respectively). Exclusively breastfed infants' mean BPA exposure was 0.0099 ± 0.0079 µg/kg bw/day. There was a moderate negative significant correlation between infant BPA exposure and infant current body weight ($r = 0.327$, $p = 0.003$).

Conclusion: BPA exposure in exclusively breastfed infants was within accepted limits and the current dietary exposure level of infants in this cohort was safe.

Keywords: Bisphenol A, breastfeeding, exposure, lactation, maternal exposure

Introduction

Bisphenol A (BPA) is a human-made chemical compound used in the production of polycarbonate (PC) plastics, such as food packaging materials, and epoxy resins which are

used to coat the inside of food cans and water storage tanks (1). The primary route for BPA contamination of food is direct contact with packages, and BPA penetrates foods rapidly (2). Almost all dietary free BPA, which is absorbed



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through the gastrointestinal system, is metabolized in the liver, and then conjugated with glucuronide. In a healthy population, conjugated BPA (BPA-glucuronide) is mostly excreted through the urine (3,4).

However, free (unconjugated) BPA is biologically active and a known endocrine disruptor; free BPA has been detected in human samples, especially in lipid-rich biofluids such as breast milk (5,6). When evaluating exposure to endocrine disrupting chemicals, exposure to free BPA should be considered (7). Free BPA is rapidly metabolized, has a short half-life (<6 hours), and excretion can be measured as it will normally be completely excreted within 24 hours of ingestion, although BPA can also accumulate in the human body. BPA exposure may be of particular concern for fetal and neonatal development. This is because fetal/neonatal and childhood liver has inadequate metabolic enzymic capacity to inactivate BPA via conjugation. This inability to safely metabolize ingested BPA will lead to relatively high free BPA concentrations in urine and plasma in toddlers compared to adults (8,9). Fetuses and neonates are sensitive to perturbation by hormone-like chemicals and early-life exposure to low-dose BPA can alter the epigenetic mechanism (10). These epigenetic changes may increase the risk of developing adult-onset diseases.

BPA has been measured in a range of human biological fluids, such as serum, urine, saliva (11,12), human milk, and colostrum (5). The European Food Safety Authority (EFSA) has published an expert opinion on the safety of BPA and has assigned a new threshold value of 4 µg/kg of body weight/day for temporary tolerable daily intake (t-TDI) (12).

Human milk is the optimal food for human infants and babies and contains a considerable amount of essential nutrients. Accordingly, exclusive breastfeeding is recommended by the World Health Organization up to six months and breastfeeding in conjunction with appropriate complementary foods up to two years of age or beyond (13). Human milk contains proteins, lipids, and carbohydrates. In addition, chemical contaminants such as persistent organic pollutants, polychlorinated biphenyls, polybrominated diphenyl ethers, dichlorodiphenyltrichloroethane, and dichlorodiphenyl-dichloroethylene isomers may also be present. Furthermore, human milk composition will vary depending on maternal diet, genetics, lactation stage, breastfeeding practice, maternal and infant health status, and environmental exposure (14). Diet is considered the primary route of BPA exposure and accounts for the majority of estimated DI of BPA per body weight among the general population, including infants and children (12). Exclusively breastfed infants BPA exposure can be assessed by measuring maternal human milk BPA concentration (15).

In vitro studies have suggested that postnatal BPA exposure impacted sperm production and reproductive success (16), and caused genomic damage, and significant alterations in liver enzymes and lipid profile (17). The aim of this study was to measure maternal human milk BPA levels in a cohort of Turkish women and thus, the exposure of their exclusively breastfed infants to BPA and the BPA exposure risk index. Furthermore, the association between breast milk BPA concentration and participants' baseline characteristics, such as nutritional habits, preferred cuisine and food packaging types would be investigated.

Methods

Study Setting and Participants

This study was conducted at Hacettepe University İhsan Doğramacı Child Hospital, Social Pediatrics Department in Ankara province, between August 2018-December 2018. The study protocols were approved by the Ethics Board and Commission of Hacettepe University. Exclusively breastfeeding women, aged between 19-40 years, and their healthy 1-3 month-old infants attending for routine health checks were approached to participate. All participants gave informed consent before being included in the study. Exclusion criteria were: smokers; multiple pregnancies; gestational diabetes; diabetes mellitus; other chronic disease; and those who regularly used medicines or took vitamin/mineral supplements or used alcohol. Any woman who might have had occupational exposure to BPA was also excluded.

The questionnaire was completed by face-to-face interview. The first section of the questionnaire recorded demographic and anthropometric data about the mother and her infant. Mothers' weight and height were measured, and body mass index (BMI) was calculated by dividing weight in kg by height in meters squared (m²) (18). Infants' weight and height were measured at the hospital by trained nurses. The second section of the questionnaire investigated daily culinary behaviour, nutritional habits, and packaging for purchased food. All participants provided 5 mL mature human milk (hindmilk) samples directly from the nipple into a BPA-free, sterile tube. Samples were stored at low temperature (-80 °C) until the day of analysis.

Investigation of human milk free-BPA levels was performed using a commercially available enzyme-linked immunosorbent assay (ELISA) (EuroProxima, Holland) according to manufacturers instructions. The detection limit of the ELISA kit was between 0.2 and 10 ng/mL. The EuroProxima BPA ELISA is validated for water and milk

samples using sample preparation procedures developed and validated in cooperation with the RIKILT (Wageningen, the Netherlands) (19).

Estimation of Infant Daily Milk Consumption

Exclusively breastfed infants BPA exposure was calculated by estimating the mean ingestion of human milk based on each infant's age in months and combining this with the concentration of BPA measured in breast milk. Precise measurement of exclusively breastfed infants' daily human milk consumption is very challenging. Therefore, a human milk intake average constant value was used. Maternal milk production of an exclusively breastfeeding woman between 1- and 6-months averages to approximately 750 to 800 mL/day (20). Thus, it was assumed that infants daily ingestion would be approximately 800 mL of human milk (21). Both the daily infant intake of BPA in breastmilk and the risk index (RI) was determined for human milk samples, as previously described (15):

$$DI \text{ (ng/kg bw/day)} = \frac{\text{Milk Consumption (mL/day)} \times \text{BPA concentration (ng/mL)}}{\text{Infant body weight (kg)}}$$

$$RI = \frac{DI \text{ (ng/kg bw/day)}}{\text{Tolerable Daily BPA Intake (}\mu\text{g/kg bw/day)}}$$

Statistical Analysis

Two critical assumptions were made when manipulating measurements below the detection limit. Firstly, because the number of data points below the detection limit value was less than 15% of all data, human milk BPA concentrations below the limit of detection (LOD) were assigned a value equal to the LOD divided by the two. In the case of the ELISA kit used, the LOD was 0.2 ng/mL. Samples were analyzed according to standards specified by the U.S. Environmental Protection Agency. Analytical data were very carefully reviewed to ensure quality control (22). Human milk BPA data in this cohort were normally distributed. Secondly, different countries have used different approaches for assigning values to measurements below the LOD. However, the Canadian Health Measures Survey and the Korean National Environmental Health Survey both used a value of LOD/2 for measures below the LOD. Thus, we believe adopting this approach in this study was justified (23). Statistical analysis was performed to compare distribution among groups. Data sets were assessed for normality of distribution and appropriate comparative tests were used depending on data set distribution. The independent sample t-test was used with parametric data while the Mann-Whitney U and Kruskal-Wallis tests were used for non-parametric data. Correlation analysis was performed using

the Pearson coefficient test for non-parametric data and the Spearman coefficient test for parametric data. Statistically, significance was set at $p < 0.05$. Data management and analysis were performed using Statistical Package for the Social Sciences version 23 (IBM Inc., Armonk, NY, USA).

Results

Subjects

In total 80 women who were exclusively breastfeeding their babies were recruited to the study. The baseline characteristics of the participants are presented in Table 1. The mean \pm standard deviation of maternal age and BMI was 28.88 ± 5.17 years and 26.41 ± 4.28 kg/m², respectively. All participants reported being healthy and not using any medicines regularly. All infants were singleton, born at full-term with normal neonatal outcome, and they were healthy

Table 1. Characteristics of mothers and their infants

Features	n (%)	Human milk BPA level (µg/L)	p
Mothers			
Age (years)			
19-28	40 (50 %)	0.5792 (0.3432-0.7878)	0.554*
29-40	40 (50 %)	0.6457 (0.3127-0.8033)	
BMI (kg/m²)			
18.5-24.9	35 (43.75 %)	0.6464 (0.4136-0.8388)	0.521***
25.0-29.9	30 (37.5 %)	0.5686 (0.2779-0.7674)	
30.0-34.9	9 (11.25 %)	0.6790 (0.4524-1.0234)	
35.0-39.9	6 (7.5 %)	0.4684 (0.2153-0.7204)	
Birth type			
Normal spontaneous	40 (50 %)	0.6667 (0.3607-0.8180)	0.225*
Vaginal delivery			
Caesarean	40 (50 %)	0.5711 (0.3044-0.7464)	
Parity			
0	38 (47.5 %)	0.6857 (0.4891-0.8224)	0.095**
≥1	42 (52.5 %)	0.5328 (0.2711-0.7181)	
Infants			
Birth week (week)			
Boy	40 (50 %)	0.6518 (0.3430-0.8114)	0.513**
Girl	40 (50 %)	0.5738 (0.3044-0.7559)	
Total	n = 80 (100 %)		

P25: 25th percentile (P25) and P75: 75th percentile (P75).
BPA: bisphenol A, BMI: body mass index

according to parental report and had no complications during pregnancy or delivery.

Bisphenol A Exposure in Exclusively Breastfeeding Infants

The median value of free-BPA concentrations in breast milk was 0.63 ng/mL, and the mean ± SD was 0.49 ± 0.37 ng/mL. Therefore, the estimated mean exposure of exclusively breastfed infants to free-BPA was 0.0099 ± 0.0079 µg/kg bw/day, and the geometric mean was 0.0073. In this study, estimated free-BPA exposure levels were in the range of 0.0008-0.489 (Table 2). Furthermore, exclusively breastfed infants mean RI was calculated as 0.002 ± 0.0019 and the geometric mean was 0.0018 (Table 2).

There was no statistically significant correlation between human milk free-BPA concentration and infant birth week, birth weight, body weight (see Table 3). However, there was a weak negative correlation between infant BPA exposure and infant body weight ($r = -0.327$, $p = 0.003$). However, there was again no correlation between infant BPA exposure and infant birth week and infant birth weight.

The exposure risk index for these infants based on the estimated human milk-free BPA concentration was calculated (Figure 1). We identified a direct high positive correlation between exclusively breastfed infants' BPA exposure and BPA risk index ($r = 0.990$; $p < 0.05$).

Nutritional Habits and Human Milk Bisphenol A Levels

To identify sources of human milk BPA contamination, a nutritional survey of participating women was undertaken.

Table 2. Infants BPA exposure level and risk index due to human milk BPA concentration

	Ingested human milk free-BPA level (µg/kg bw/day)					
	\bar{x} (SD)	GM	S_x	Median	Min	Max
Exclusively breastfed infants BPA exposure	0.0099 (0.0079)	0.0073	0.0008	0.008	0.0008	0.489
RI	0.002 (0.0019)	0.0018	0.0002	0.002	0.0002	0.0122

\bar{x} : mean, SD: standard deviation, GM: geometric mean, S_x : standard error, Min: minimum, Max: maximum, RI: risk index, BPA: bisphenol A

Table 3. Association between human milk BPA concentration, infant BPA exposure and birth week, birth weight, and infant current body weight

BPA	Infant birth week		Infant birth weight		Infant current body weight	
	r	p	r	p	r	p
Human milk free-BPA concentration (µg/L)	-0.010 ^b	0.932 ^b	0.071 ^a	0.530 ^a	0.000 ^a	0.997 ^a
Infant BPA exposure (µg/kg bw/day)	-0.024 ^b	0.836 ^b	0.035 ^b	0.759 ^b	-0.327 ^b	0.003^{b*}

^aPearson correlation analysis ($p < 0.05$); ^bSperman correlation analysis ($p < 0.05$); ^{b*}Sperman correlation analysis ($p < 0.01$).

BPA: bisphenol A

The findings are summarized in Table 4, below. Culinary equipment such as kettles, drinking water storage bottles, and baking moulds play are used on a daily basis in most homes. Analysis did not reveal any significant association between human milk BPA level and culinary equipment. In terms of nutritional habits, the only significant association to emerge was that between breast milk BPA concentration and fast-food consumption. Within the fast-food consumption group, women who consumed fast food once a month had higher BPA levels than participants who did not consume fast food ($p = 0.02$) but the BPA concentration was also higher than in women who consumed fast food twice a month. There was no correlation between human milk BPA level and the packaging type used for drinking water or vegetable oil. However, a significant association emerged between human milk BPA level and packaged carbonated drinks ($p = 0.018$). When pairwise comparison was made participants who did not consume carbonated beverages had higher human milk BPA level than those who did consume carbonated drinks either from glass or polyethylene terephthalate (PET) bottles ($p = 0.042$ and $p = 0.020$, respectively).

Table 5 shows the distribution of parameters regarding human milk free-BPA and comparison with other studies.

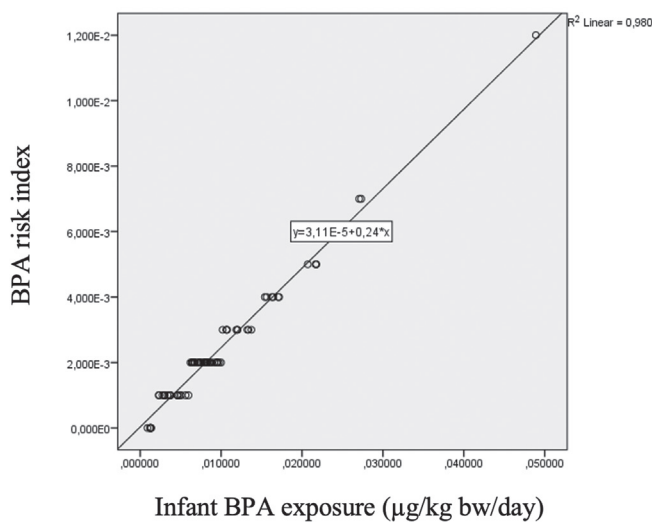


Figure 1. Association between infants BPA exposure and BPA risk index

BPA: bisphenol A

The median concentration value of human milk free-BPA found in this study was with that reported in studies from Japan and the USA. Concentrations reported from Spain

and Canada were around a sixth of the value found in this Turkish population while the value reported from a South Korean population was much higher than that found in any other study shown here.

Table 4. Association between the human milk BPA level (µg/L) and culinary materials, nutritional habits, and packaged information

	Human milk BPA level (µg/L)		
	N	Median (P25-P75)	P
Culinary equipment			
Water heater			
Steel teapot	50	0.6482 (0.3180-0.7951)	0.989
Steel kettle	18	0.6122 (0.2904-0.8392)	
Plastic kettle	12	0.5919 (0.4385-0.7038)	
Water bottle			
Plastic	25	0.6450 (0.4136-0.7858)	0.451
Glass	55	0.5172 (0.2766-0.8351)	
Baking moulds			
Teflon	42	0.5973 (0.3359-0.7354)	0.937
Glass (heat resistance)	14	0.5993 (0.3352-1.0234)	
Silicon	9	0.7078 (0.2554-0.8557)	
Granit	15	0.6412 (0.2538-0.7858)	
Nutritional habits			
Main meal consumption in a day			
2 times a day	25	0.615 (0.29-0.79)	0.976
3 times a day	55	0.641 (0.34-0.79)	
Fast-food consumption			
Once a month	22	0.7536 (0.6035-0.9238)	0.022
Twice a month	9	0.5152 (0.2716-0.8123)	
Not consumed	49	0.5124 (0.2603-0.7101)	
Instant- packaged meal consumption			
No	58	0.6301 (0.2956-0.7726)	0.714
Yes	22	0.6103 (0.4255-0.8392)	
Canned food consumptions (tuna fish, soup, corn, peas)			
No	60	0.6431 (0.3374-0.8180)	0.764
Yes	20	0.5713 (0.3044-0.7512)	
Canned beverage consumptions			
No	65	0.6152 (0.3195-0.7632)	0.315
Yes	15	0.6501 (0.4544-1.1269)	
Packaged information			
Drinking-water			
PET plastics	30	0.6631 (0.3950-0.7994)	0.678
Carboy (recycling code = 7)	26	0.6518 (0.3359-0.7588)	
Tap	24	0.5327 (0.2683-0.9536)	
Carbonated drinks			
PET plastics	62	0.5696 (0.2975-0.7607)	0.018
Canned	9	0.6450 (0.5541-0.8297)	
Glass	4	0.4414 (0.1509-0.6723)	
Not consumed	5	1.0818 (0.8723-1.3696)	
Vegetable oil			
PET plastics	37	0.5632 (0.3185-0.7820)	0.788
Canned	36	0.6438 (0.3484-0.8224)	
Glass	6	0.7409 (0.2544-0.7990)	
Not consumed [‡]	1	-	

[‡]Because the “no consumed” sample size was 1, it was not included in the statistical assessment.

PET: polyethylene terephthalate, BPA: bisphenol A

Discussion

In this study free-BPA level in human milk samples were measured in Turkish lactating women using an ELISA method. Total BPA is calculated from the sum of free BPA and BPA glucuronide (27). Moreover, the exposure of exclusively breastfed infants to BPA was estimated and the BPA exposure risk index was calculated. The implications of these findings are discussed below.

Participants were healthy mothers with 1-3 month-old, exclusively breastfed, healthy infants. Exclusively breastfed infants estimated breast milk consumption was assumed to be 750-800 mL/day (20). BPA is reported to be incorporated into human milk (28) and is subsequently naturally transferred to the infant via breastfeeding (5). Thus exposure of exclusively breastfed infants to BPA is inevitable. The adverse outcome of BPA exposure varies according to the exposure dose and term (29).

BPA is metabolized in humans through the activity of uridine diphosphate glucuronosyltransferase (UGT) enzymes, which gradually rises from the age of around 3-6 months to 10 years and then reaches normal adult levels. Infants between 1-3 months of age do not have sufficient UGT activity to metabolize BPA effectively (30). A recently developed ELISA method kit for the measurement of BPA has high sensitivity and comparatively low cost compared to other methods for measuring BPA in biological samples (28,31).

As conjugated BPA is not an active biologic form, both conjugated and free BPA concentration should be determined together (25). Studies have measured both free and total BPA levels in human milk (6,28,32). The primary BPA exposure source is dietary (12,33), but dietary habits vary considerably from society to society so countries should determine local BPA exposure risks in their own populations. The study presented here is one of the first investigations to assess the impact of BPA exposure and risk index for exclusively breastfed Turkish infants. The mean BPA exposure value in our cohort was 0.0099 µg/kg bw/day. The EFSA suggested that the limit of BPA exposure during the first three months for exclusively breastfed infants should be 0.2 µg/kg bw/day (12). Furthermore, we found a BPA exposure risk index value of 0.002 for exclusively breastfed infants based on the EFSA's BPA TDI value. The RI value is less than one so that there is no BPA exposure risk among exclusively breastfed infants (15). The correlation between human milk

Table 5. Distribution of the parameters and comparison with other studies for free BPA concentration of human milk

Country	(n)	Method	n > LOD*	Free-BPA (ng/mL)			Reference
				X (S)	Median	Min-Max	
Turkey	80	ELISA	71 (88.75%)	0.49 (0.37)	0.63	< LOD*-1.9	This study
Japan	23	HPLC	23 (100%)	-	0.61	0.28-0.97	Sun et al (24)
USA	21	UHPLC-MS/MS	13 (62%)	3.13	0.68	< 0.22-10.8	Zimmers et al (8)
Spain	120	HPLC-MS/MS	92 (77.4%)	0.15 (4.8)	0.10	< LOD-41	Dualde et al (6)
Canada	278	GC-MS/MS	46 (16.5%)	0.11	0.10	< 0.036-2.3	Cao et al (25)
Korea	100	LC/MS/MS	100	-	6.60	0.65-29.9	Yi et al (26)

BPA: bisphenol A, LOD: Limit of detection (0.2-10 ng/mL), ELISA: enzyme-linked immunosorbent assay, HPLC: high-performance liquid chromatography, MS: mass spectrometry, Min: minimum, Max: maximum

BPA level and infant birth week, weight, and current body weight were not statistically significant (Table 3). Casas et al (34) did not find a statistically significant association between BPA exposure during pregnancy and fetal growth parameters. Intriguingly, we found a moderate negative correlation between BPA exposure in the infants studied and infant current body weight (Table 3). This suggests that an increase in BPA exposure may have a negative effect on weight gain. Normally after delivery, when lactation begins, estrogen levels start to decrease (35), but elevated levels of BPA may inhibit lactation. Kasper et al (36) demonstrated an association between maternal BPA exposure and decreased breastfeeding at one month postpartum. As presented in Figure 1, a strong positive linear relationship was found between estimated exposure of the infants to BPA and their BPA risk index. This is because the only source of BPA for exclusively breastfed infants is contamination of their mother's milk. Research has shown that the primary source of BPA exposure is diet (37,38). Thus, pregnant and lactating women should carefully consider their diet.

The effect of different types of culinary equipment, nutritional habits and food packaging was investigated in the current study. The EFSA has reported that estimated BPA exposure caused by PC plastic water kettles ranged from 2.0 to 3.2 ng/kg bw/day (12). Moreover, plastic kettle use has been identified as a critical exposure route for BPA (39). It should be noted that the highest values are observed in adults, due to their higher consumption of coffee and tea. However, in the present study there was no significant difference in BPA exposure associated with metal or PC kettles. This association may be explained by lower coffee and tea consumption among lactating women than in other adults.

Interestingly, mothers who consumed fast food once a month had significantly higher BPA breast milk concentrations than women who reported never eating fast food. Somewhat confusingly, mothers who ate fast food twice a month also had similar breastmilk BPA concentrations to those who never ate fast food but the sample sizes for the twice-a-

month consumers was small. In a cross-sectional study among the U.S population, Zota et al (40) showed that fast-food consumption did not appear to be a BPA exposure source (40). In addition, the scientific opinion of the EFSA was that PC plastic food packaging did not pose a health risk to consumers of any age group, including unborn children, infants, and adolescents (12). Differences in exposure due to fast food consumption could be due not only to frequency of consumption but also preferred diet for fast food stuffs and the local regulations concerning the packaging of fast foods. For example, it has been reported that the concentration of BPA in a hamburger was 10.9 ng/g (41).

PC plastics, which contain BPA are used in reusable water carboys (42). Considerable amounts of BPA (approximately 0.15 µg/L) have been reported to leach from PC bottles within the first 24 hours of storage (43). If PC carboys are stored at or below room temperature, BPA water levels are expected to be normal. PC carboys are widely used for water storage because of the properties of PC, that is clear and rigid plastic (44). In our study, human milk BPA level did not change based on the mothers' preferred storage material for drinking water. According to the Food and Drug Administration, scientific evidence has supported the safety of BPA for the currently confirmed utilization in food containers and packaging (45).

In this cohort, women who reported carbonated drinks from glass bottles had significantly higher human milk BPA levels than either those who did not consume carbonated beverages or those who drank carbonated drinks from PET bottles ($p = 0.042$ and $p = 0.020$, respectively). This rather intriguing result may be due to the specific ingested carbonated drinks and would also be a function of consumption quantity and frequency, which was not investigated. Previous studies have reported BPA concentrations in canned carbonated drinks and plastic bottled water. The BPA concentrations in canned carbonated drinks were between 83-340 ng/L. And two canned carbonated beverages BPA concentrations was detected below the limit of quantitation (46).

Previous studies investigating human milk free-BPA levels are summarized in Table 5. The current results are consistent with previous researches (8,47) although there are discrepant results reported by other studies (6,25,26), they are consistent with some other published studies (8,24). Firstly, a possible explanation for these differences could be related to the applied analysis method in each study, as suggested by Yi et al (26). There are methodological differences between studies so that detection limits vary between studies. Studies utilizing high-performance liquid chromatography (HPLC) would have a wider detection range than those using LC-tandem mass spectrometry (LC-MS/MS) method. However, studies using HPLC with a fluorescence detector (HPLC/FLD) analysis would have a lower sensitivity than LC-MS/MS analysis. Secondly, the primary BPA exposure source is nutritional habits (12), and these will vary from country to country (33). Another possible explanation for variability in results could be that dietary habits change over time and from region to region within countries.

Study Limitations

Several limitations of this study should be noted. Firstly, human milk samples were only collected from each woman at one time point. Unfortunately, a single day sample collection is likely to introduce bias because a single sample collection may not reflect BPA exposure precisely. Possible solutions for this would be to collect two or more non-consecutive samples to estimate the normal mean human milk BPA level (48). A further solution would be to have a very large cohort. Secondly, used an ELISA method for measuring BPA concentrations in samples whereas most other studies have used chromatographic methods instead of ELISA for determining the BPA level of samples of human milk. However, ELISA can be used for screening purposes, and it is an inexpensive method (49). Furthermore, we did not consider the seasonal difference while collecting human milk samples. We collected the first sample in August and the last in November. BPA may leach into foods and beverages from packaging or storage containers, especially when heated to high temperatures as may happen in the summer months (50).

Conclusion

Exposure to BPA is a concern because of the possible health effects, and it plays a role in the pathogenesis of several endocrine disorders, including obesity, and asthma and neurobehavioural disturbances. The present study extends our knowledge of BPA exposure among breastfeeding Turkish women and their infants. The BPA exposure of exclusively breastfed infants positively correlates with

human milk BPA concentrations. These results suggest that BPA exposure of exclusively breastfed Turkish infants was far below the EFSA tolerable BPA level of 4 ($\mu\text{g}/\text{kg}$ bw/day) and we suggest that exclusively breastfed Turkish infants have negligible BPA exposure risk. It is thought that current regulatory restrictions on BPA use have reduced exposure levels. However, the possibility of low dose BPA exposure cannot be ruled out. Further, larger, well-designed national studies are warranted to confirm and extend these findings.

Ethics

Ethics Committee Approval: This research study was ethically approved on July 24, 2018, by the Hacettepe University Clinical Research Ethical Board with project number GO 18/715 and decision number GO 15/715-33.

Informed Consent: All procedures performed in this study involving human participants followed the institutional and national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept - Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: Seda Çiftçi, Sıdıka Songül Yalçın, Gülhan Samur.

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