Quantifying bisphenol A in maternal and cord whole blood using isotope dilution liquid chromatography/tandem mass spectrometry and maternal characteristics associated with bisphenol A

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Abstract

Bisphenol A (BPA) is endocrine disrupting chemical that have been detected among general population. Exposure levels among pregnant women and their fetus are yet largely unknown among Japanese. We have developed a new method of measuring total BPA in whole blood samples by using isotopic dilution liquid chromatography-tandem mass spectrometry (ID-LC/MS/MS). For eliminating possible contaminations, we have used glass cartridge instead of polypropylene cartridge and successfully reduced background levels. Additionally gap retention technique was applied to improve sensitivity. We also confirmed no external contamination by measuring free BPA in the samples. The limit of quantification (LOQ) was 0.040 ng/ml. With this developed method, we determined total BPA concentrations of 59 maternal blood at delivery and 285 cord blood samples in prospective birth cohort study and investigated factors possibly related to total BPA levels. Total BPA levels ranged from below LOQ to 0.419 ng/ml and for maternal blood and from below LOQ to 0.217 ng/ml for cord blood, respectively. The geometric mean was 0.051 ng/ml for maternal blood and 0.046 ng/ml for cord blood, respectively. Although no correlation was observed between maternal and fetal blood levels of total BPA, our result suggested fetal exposure to BPA. We have found that younger mothers, frequent beef and pork consumption during pregnancy were positively associated with maternal total BPA levels. We confirmed in utero exposure to BPA, which highlights the importance of further studies of investing the effects of fetus BPA exposure on health outcomes.

1. Introduction

Bisphenol A (BPA) is widely used in the manufacture of polycarbonate and epoxy resins. The wide spread of BPA usage in many consumer products such as toys, plastic containers for food and drinks, dental material, metal can linings, and medical equipment have been reported (Vandenberg et al., 2007). The major sources of BPA exposure of human were considered to be diet (Hengstler et al., 2011; Willhite et al., 2008; Geens et al., 2012). Several studies already have reported the presence of BPA in human blood, placental tissue and umbilical cord blood (Lee et al., 2008; Wan et al. 2010; Chou et al., 2011). BPA is an estrogen receptor agonist and an androgen receptor antagonist and is endocrine disrupting chemical (Rubin, 2011; Teng et al., 2013). Exposure to BPA has been associated with various adverse health effects (Melzer et al., 2010; Clayton et al., 2011; Rochester, 2013; Bhandari et al., 2013). The fetus is considered to be highly susceptible to adverse effects of environmental chemicals. Prenatal exposure to BPA and its adverse health effects such as reducing birth weight (Snijder et al., 2013), child behavior problems (Perera et al., 2012; Harley et al., 2013; Braun et al., 2011) have been reported.

It has been reported that certain lifestyle, demographic and dietary factors such as

smoking habit, social class and consumption of canned food were associated with BPA levels in general population (Covaci et al., 2014; LaKind et al., 2015). However, no current studies of assessing maternal characteristics in association with BPA exposure in pregnant women and no studies to reveal fetus BPA exposure levels in Japan.

In most of the epidemiological studies, urine samples were used to evaluate BPA exposures. Previously we have reported the exposure levels of persistent organic pollutants (POPs) and mercury and demographical characteristics among pregnant women in Japan by using prospective birth cohort study (Miyashita et al., 2015). Despite the rich data of demographic, behavioral, dietary, and socioeconomic characteristics obtained from our cohort study, we were only able to collect maternal and cord blood samples but not urine samples in our cohort study. Consequently, we had to develop reliable analytical methods to detect low concentration of BPA in human blood samples. Thus the objectives of this study were, 1) to establish reliable analytical methods to determine BPA exposure levels in human blood by using isotope dilution liquid chromatography tandem mass spectrometry (ID-LC/MS/MS) with minimized background levels and 2) to evaluate BPA exposure levels of pregnant women and their fetus and correlation between maternal and fetal levels in

general population and to evaluate the association between demographic and lifestyle characteristics and BPA levels of mothers and fetus.

2. Material and methods

2.1. Sample collection

Maternal and cord whole blood samples at delivery were collected from study subjects who enrolled in a hospital based prospective birth cohort entitled “The Hokkaido Study on Environment and Children’s Health”. Details of the study population and data collection have been published (Kishi et al., 2011; Kishi et al., 2013). In brief, Japanese women participated with this study at 23-35 weeks of gestation and delivered between 2002 and 2005. They completed a self-administered questionnaire at the time of enrollment regarding lifestyle and demographic characteristics including educational level, annual household income, smoking habit and alcohol intake, food consumption during pregnancy and medical history. Their medical records including maternal age, maternal anthropometric measures before and during pregnancy, pregnancy complications, parity were obtained. Whole blood samples of mothers and fetus were obtained at birth and stored at -80^{o}C until analysis. Sample storage glassware was heated at 400 ^{o}C for 4

hours to avoid potential environmental contamination of BPA. This study was conducted with the written informed consent from all subjects. The Institutional Ethical Board for Epidemiological study at Hokkaido University Graduate School of Medicine and Hokkaido University Center for Environmental and Health Sciences approved the study protocol.

2.2. Chemicals and reagents

Environment analysis grade BPA standard, acetone, acetic acid, sodium acetate and biochemistry grade β-glucuronidase solution from Helix Pomatia were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). HPLC grade acetonitrile was purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). BPA-d_{16} as surrogate and BPA-d_4 as recovery standard were purchased from CDN isotopes (Quebec, Canada). Purified water was obtained from MiliQ Gradient.

2.3. Sample preparation

To each 1.0 ml of whole blood sample, BPA-d_{16} β-glucuronidase spiking solution was added and shaken for 10 seconds. Then 50 µl of β-glucuronidase from Helix pomatia source and 0.2 M acetate buffer solution (pH 5.0) were added and samples were

held in an incubator at 37 ℃ for 1.5 hrs.

The solid phase extraction procedure was adjusted as follows. Isolute multimode (500 mg/6 ml) cartridges were prepared by filling packing material to the acetone prewashed glass cartridges (Sigma-Aldrich Japan, Tokyo, Japan). Conditioning was conducted by using 20 ml of acetone, 20 ml of acetonitrile and 10 ml of purified water. Then samples were loaded onto column without vacuum then solid phase column and washed by 3.0 ml of water and 5.0 ml of 20% acetonitrile solution. Then the column was rinsed with 2.5 ml of 100% acetonitrile solution to elute BPA. The sample was collected into 2ml vial. The 1.25 ml of eluent from solid phase extraction was evaporated to the volume of 0.125 ml under gentle stream of nitrogen, 40 ℃ using Heat Block (Nippon Genetics Co. Ltd., Tokyo, Japan). Reconstituted with purified water to the volume of 0.25 ml and then 2.5 ng of BPA-d_4 was added to each sample and for derivatization prior to LC/MS/MS analysis.

2.4. Instrumental analysis

The chromatographic separation was performed on an Agilent Eclipse XDB-C8 capillary column (2.1mm × 150mm × 5μm) from Agilent (Agilent technology, Tokyo, Japan) using an Agilent 1100 liquid chromatograph equipped with autosampler. A 20

μl sample was injected at 40 ℃. A flow rate of 0.3 ml/min was used with the mobile phase initially consisting of 20 % acetonitrile and 80 % water held for 1 min, then increased linearly to 60 % of acetonitrile from 1 min to 7 min then to 99% and held there approximately for 6 min and finally reversed to 20% of acetonitrile and held there approximately for 6 min, with 19 min hold between injections. Mass spectrometric experiments were performed using API 4000 Q Trap mass spectrometer (AB SCIEX, Tokyo, Japan). BPA was detected with MS operating in the ESI negative selected reaction monitoring (SRM) mode. The following SRM transitions (m/z) were monitored: BPA (m/z 227.0→132.9), BPA-d16 (m/z 241.0 →142.0), BPA-d4 (m/z 231.0→134.9).

2.5. Prevention of contamination

All necessary precautions were taken to avoid contamination with BPA during sample preparation. All glassware used in extraction procedure was washed with acetone. Potential contamination of samples with BPA from external source was evaluated with additional control blank experiments. The experiment was conducted by collecting distilled water using the same equipment (syringe, needle and glass tube) instead of actual blood samples. Afterwards, the extraction and instrumental

analysis were performed following the same procedures as blood samples. We have eliminated all the possible external sources of BPA contamination by using only baked glassware for storage of samples, using glass cartridge instead of polypropylene one, which contained BPA. BPA is considered to exist mostly as a form of conjugate in human blood (Teeguarden et al., 2015) and thus presence of free BPA in blood samples is considered as an indication of possible contamination. It was reported that conversion of BPA to metabolite was very efficient, with less than 1% of the dose reaching the bloodstream as free BPA (Teeguarden et al., 2015). We analyzed subset of each maternal whole blood and cord blood samples (n=10) of for free BPA to assure no external BPA contamination. These each 10 samples of maternal and cord blood were selected among the highest concentration of BPA as highest might be due to suspected external contamination. Free BPA, if detected at all represented less than 10% of total BPA in these samples indicating that external contamination was unlikely (Koch et al., 2012; Volkel et al., 2011). No free BPA was detected from 10 of each maternal and cord blood samples. Thus, no external BPA contamination was confirmed and we confirmed total BPA in whole blood samples were valid biomarker of BPA exposure.

2.6. Data analysis

To examine the correlation between maternal and fetal exposure to BPA, we calculated Spearman correlation coefficients between total BPA levels in maternal and cord blood as they were not normally distributed. BPA levels were $\log_{10}$ transformed to prevent high concentration values to have an undue high influence on the regression model. Regression coefficients (β) of maternal characteristics related to maternal BPA levels were obtained from linear regression models. All data analyses were conducted using the Statistics Package for Social Sciences (SPSS, Inc., USA) software for Windows version 22.0J. The limit of quantification (LOQ) was calculated according to the US EPA method (http://dnr.wi.gov/regulations/labcert/documents/guidance/-LODguide.pdf). The LOQ was mathematically defined as equal to 10 times the standard deviation (SD) of the results for a series of 7 replicates used to determine Method Detection Limit (MDL). For statistical analyses BPA concentrations below the LOQ were assigned values equal to the half the value of LOQ.

3. Results and Discussion

3.1. Method development and quality control

In this study, we analyzed total (conjugated plus free) BPA levels using maternal blood and cord blood samples. Analyzing total BPA concentration using whole blood samples made it possible to measure total BPA concentration with small volume of samples. Additionally, no need of separation of serum from whole blood sample enabled simple procedure in sample preparation. It is noted that reports of high levels of unconjugated serum BPA in human biomonitoring studies have been rejected based on the prediction that findings from these studies are due to assay contamination (vom Saal and Welshons, 2014). However, serum BPA can be accurately assayed without contamination according to NIH round robin study (Vandenberg et al., 2014). In fact, review article found that assay contamination is well controlled in most labs (vom Saal and Welshons, 2014). Thus using blood samples as BPA exposure measurement considered to be reasonable, especially for those studies without available urine samples for reasons. Authors of NIH round robin study made it clear that it was essential to confirm that equipment used in sample collection process should be determined not to contain any potential contamination of BPA prior to sample collection. In our study, evaluation of potential contamination from external source including equipment used during sample collection was conducted, and the experiment found no detectable BPA, thus the

method considered to be valid. It is necessary to minimize background contributions during sample extraction, storage and analysis as BPA is a ubiquitous chemical. A number of trials were carried out to minimize the background levels. Early in method developmental stage, we encountered several problems. First, BPA peak was detected at the same retention time without sample injection. The solvent and instrument used for measurement is suspected as the cause. To solve this problem, the retention gap technique was used by installing retention gap columns to protect the analytical column from contamination from solvent and instrument. Second, background levels of BPA were high due to polypropylene solid phase extraction cartridges which contained higher levels of BPA. After changing to glass cartridges, background BPA levels were lowered to one third of original method (mean ± SD, 0.19 ± 0.28 ng/ml, 0.065 ± 0.051ng/ml, respectively). Adequate control of contamination is required for accurate measurement of BPA analysis of human biomonitoring (Markham et al., 2010) and a good example of showing well control of contamination is a report from CDC which could identify and eliminate contamination during the development of LC-MS/MS assays for BPA (Ye et al., 2013). Our method was able to control contamination by applying retention gap technique and changing cartridge thus achieved accurate measurement of total BPA in blood

samples. Procedural blank was processed with each batch of five samples. The calibration standard was prepared from the mixture of equal concentrations of the native and BPA-d_{16} standards. The overall native BPA/BPA-d_{16} relative response factor (RRF) was 1.03. This was determined by calculating the ratio of the slope of the native BPA calibration curve to the slope of the BPA-d_{16} calibration curve. The calibration curve was linear over a concentration ranging from 1 to 100 ng/ml with a coefficient of correlation (r^2) greater than 0.995. The MDL of BPA was calculated as follows according to the procedure of the manual of Analyses of Chemicals by the Ministry of Environment of Japan (https://www.env.go.jp/chemi/anzen/chosa/tebiki-h20.pdf). The SD associated with replicate analyses of BPA was multiplied by t-value (1.943) and then was multiplied by 2. The data was adjusted for presence of BPA blank samples. The median was 0.015 ng/ml and the SD was 0.00423 ng/ml and 7 blanks were used to determine MDL. The MDL was determined to be 0.017 ng/ml. The LOQ was calculated according to the US EPA method (http://dnr.wi.gov/regulations/labcert/documents/guidance/-LODguide.pdf). The LOQ was mathematically defined as equal to 10 times the SD (0.00423ng/ml) of the results for a series of replicates used to determine MDL and the LOQ for total BPA

was determined 0.040 ng/ml. All reported results were corrected for their respective blank samples. For quality control purpose, replicate measurements of randomly selected eight samples were conducted. The discrepancy in levels of BPA in duplicate measurements varied 5.3 to 20 %, which were within the described standard range in the Guidelines establishing test procedures for the analysis of pollutants by the Ministry of Environment of Japan.

3.2. BPA levels in maternal and fetal blood and correlation

We have measured total BPA levels in 59 maternal blood samples and 285 cord blood samples. The distribution of maternal and cord blood BPA levels were shown in Table 1 and Figure S1. The detection rate of maternal and cord blood samples were 68.8 % and 76.3 %, respectively (Table 1). The detected levels of BPA might be from exposure to hospital equipment such as IV tubes during delivery. Especially higher detection rate in cord blood samples may be explained by medical equipment used during delivery. In this study, BPA levels in maternal blood at delivery ranged from not detected to 0.419 ng/ml (geometric mean (GM) = 0.051 ng/ml), in cord blood ranged from not detected to 0.217 ng/ml (GM= 0.046 ng/ml). Placenta may not reduce or protect the transfer of BPA from mother to fetus as we observed the GM of

BPA in maternal blood was similar to that of cord blood. This also may indicate that maternal exposure to BPA directly influence on fetus. Among 59 pairs with both maternal and cord blood BPA levels measured, 27 pairs had higher maternal levels than fetal levels, 30 pairs had higher fetal levels than maternal levels and 2 pairs had below LOQ levels in both maternal and fetal. We analyzed the correlation between maternal and cord blood levels of BPA. There was no significant correlations between maternal and fetal BPA levels (Figure 2). This could be due to time difference between cord blood sample collection and maternal blood sample collection. Maternal blood samples were obtained right after delivery to within 24 hours of delivery, time between two collections of blood samples was larger than a few hours, thus it was reasonable that no correlation was observed due to short half-life of BPA. In this study, the detection frequency of BPA was higher compared to the previous study of maternal and cord blood serum samples (Kosarac et al., 2012). In their study, the median levels of BPA in maternal and cord blood serum was 1.46 ng/ml and 1.82 ng/ml, respectively with the LOQ of 0.087 ng/ml. Previous study of 300 pregnant women from Korea examined maternal and fetal exposure to BPA (Lee et al., 2008). In their study, 84 % of maternal serum and 40 % of cord blood serum samples had detectable levels of BPA and the median concentration of

total BPA in maternal serum and umbilical cords was 2.73 μg/l and < 0.625 μg/l, respectively with the LOD of 0.625 μg/l. Another small sample size study conducted in Korea (Wan et al., 2010), the mean concentration of BPA in maternal serum (n=26) and in fetal serum (n=25) was 0.7 ± 0.1 ng/ml and less than LOD (0.6 ng/ml), respectively. A study from China found that the detection rate of BPA in maternal blood samples were higher than in cord blood and BPA levels in maternal blood were higher than in fetal cord blood (Zhang et al., 2013). In contrast to Korean and Chinese studies, we found higher detection rate in cord blood than in maternal blood. Most recently, a study from Canada (Aris, 2014) investigated maternal and fetal serum BPA levels. In their study BPA was detected over 95% of both maternal and fetal serum samples (n=61, respectively). The ranges were from non-detected (nd) to 4.46 ng/ml for maternal serum and nd to 4.60 ng/ml for fetal serum with the LOD of 0.01 ng/ml. Relatively higher detection rate of their study could be due to lower LOD compared to the other studies. Our developed method of measuring BPA levels in blood samples with relatively lower LOQ made it possible to evaluate lower levels of total BPA observed in our study. This study showed lower levels of detected BPA in both maternal and cord blood samples compared to the values reported from previous studies. The differences in LOD/LOQ among studies can possibly explain

inconsistent results of detection rate of maternal and cord blood samples.

Previous studies suggested strong correlation between maternal and fetal blood BPA levels (Aris, 2014; Lee et al., 2008), however, we did not find correlation (Spearman's rho = 0.051). Both of these previous studies showed much higher levels of BPA exposure among both mothers and their fetus compared to our study. Maternal and fetal correlations may only be seen in higher exposure population. The present study recruited pregnant women from one hospital in Sapporo and sample size was relatively small, thus our findings may not represent accurately nationwide results. Nevertheless, this was the first study of presenting both maternal and fetal total BPA levels in Japan by using whole blood samples.

3.3. BPA levels and maternal characteristics

Potential lifestyle, demographic and dietary factors were investigated in association with total BPA levels of maternal and cord blood (Table 2). Maternal age, oral contraceptive (OC) use were associated with maternal BPA levels with borderline significance ($p<0.10$). Maternal frequent consumption (≧ 1/wk) of beef and pork were significantly associated with maternal BPA levels ($p=0.020$ and 0.036, respectively). However, none of these factors were associated with fetal BPA levels.

Maternal BMI was weakly associated with fetal BPA levels (p=0.077). Linear regression analyses were conducted for further investigation. Table 3 showed regression coefficients of maternal characteristics found to be related to maternal BPA levels. Maternal age was negatively associated with $\log_{10}$ transformed BPA levels (< 25 years vs. ≧ 35 years, p= 0.011). OC use was negatively associated with $\log_{10}$ transformed BPA levels (p=0.039). Frequent consumption of beef and pork (≧1/wk) were positively associated with $\log_{10}$ transformed BPA levels (p=0.013 and p=0.074, respectively, <1/wk vs. ≧1/wk). We also investigated the association between cord blood BPA levels and the same characteristics, and found no significant associations (Data not shown). Cord blood samples were obtained at delivery and maternal blood samples were collected right after to within 24 hours of delivery. The associations between maternal BPA levels and frequent consumption of beef or pork maybe a random association due to small sample size. Several cohort studies have identified major predictors of BPA concentration of pregnant women. Our results found that higher BPA levels in younger mothers, which agreed with two of the previous reports from Spain and Canada (Arbuckle et al., 2015; Casas et al., 2013). On the other hand, other studies from United States and Puerto Rico suggested no significant associations between BPA levels and maternal age (Meeker

et al., 2013; Braun et al., 2011; Quiros-Alcala et al., 2013; Robledo et al., 2013). Smoking were also significant predictor of higher BPA levels in studies from Canada and Spain (Arbuckle et al., 2015; Casas et al., 2013). In our study, we did not find significant associations between current smoking and BPA levels. Maternal education was considered to be a great predictor of BPA levels in the study in Ohio, USA (Braun et al., 2011), Spain (Casas et al., 2013) and Canada (Arbuckle et al., 2015) where mothers with lower education showed higher BPA levels, contrary higher BPA levels were observed in higher education mothers in Korean study (Lee et al., 2014). Our study did not show any significant differences in BPA levels between lower and higher education groups, which agreed to the observation from the studies in New Your City (Hoepner et al., 2013) and California (Quiros-Alcala et al., 2013). We observed mothers with lower annual income showed higher BPA levels without statistical significance, contrary, the opposite trend was observed in Korean study (Lee et al., 2014). Findings from these studies had some similarities as well as discrepancies. This could be due to differences in study methods among studies. For example timing of obtaining information varied (early pregnancy, late pregnancy), and method of obtaining information differed (by self-answering questionnaires, telephone interview and so on). Additionally we should note that our

findings were based on simple linear regression but not multiplicity. Thus, result may change with consideration of multiple factors. We observed that frequent consumption of beef and pork were positively associated with BPA levels. According to the European Food Safety Authority (EFSA), canned food and non-canned meat and meat-product were the two main dietary contributors of BPA in majority of countries and age classes (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)). Our findings were supported by the report of EFSA although our finding may possibly be a random association. CEF stated that BPA could also migrate into meat from any food contact materials or from articles used in the processing of the product. They also reported that fish and other seafood also contained highest levels of BPA, however, we did not find any association between BPA levels and maternal fish and seafood consumption in this study. Though we used questionnaire regarding frequency of food consumption, data of canned food and drink consumption, which considered to be one of the major sources (Schecter et al., 2010), was not obtained from our questionnaire. This limited our further investigation. Additionally we should note that BPA concentrations can vary with time, elevated levels were observed immediately after consuming BPA rich diet (Teeguarden et al., 2011), thus possibility of

misclassification of BPA levels cannot be excluded.

4. Conclusions

We have measured maternal and cord blood total BPA levels in whole blood samples by ID-LC/MS/MS method. The levels of total BPA in cord blood were similar to those of maternal blood and the percentage of detection was higher in cord blood than in maternal blood. Our result confirmed that there was BPA exposure to pregnant women and their fetus in the similar levels. We also provided information on associations between BPA levels and demographic and lifestyle characteristics of mothers. Effects of in utero BPA exposure need more human epidemiological evidences. We are following children up to school age and puberty and going to investigate associations between prenatal or postnatal exposure to BPA and adverse developmental effects.

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Table 1 Percentile and summary statistics (ng/ml) of bisphenol A in maternal (N=59) and cord blood (N=285).

	Detection rate (%)	**Percentile**					
		LOQ	**25th**	**50th**	**75th**	**Mean**	**GM**
Maternal blood (N=59)	68.8	0.040	0.040	0.057	0.072	0.063	0.051
Cord blood (N=285)	76.3	0.040	< LOQ	0.051	0.076	0.057	0.046

Abbreviations: LOQ: Limit of quantification, GM: Geometric Mean.

Table 2 Characteristics of participants with maternal (N=59) and cord blood (N=285) BPA levels.

Characteristics		Maternal BPA (ng/ml)	Number (%)	Fetal BPA (ng/ml)	Number (%)
Age (years)	**< 25**	0.078**	8 (13.6)	0.051	38 (13.3)
	25-29	0.048	16 (27.1)	0.047	80 (28.1)
	30-34	0.053	24 (40.7)	0.046	108 (37.9)
	≧ 35	< LOQ	11 (18.6)	0.045	59 (20.7)
BMI (kg/m^2)	**< 18.5**	0.052	12 (20.3)	0.049**	46 (16.1)
	18.5-25.0	0.050	46 (78.0)	0.045	218 (76.5)
	≧ 25.0	0.066	1 (1.7)	0.061	20 (7.0)
Education (years)	**12 ≧**	0.051	28 (47.5)	0.047	130 (45.6)
	13 ≦	0.050	31 (52.5)	0.046	155 (54.4)
Annual income (million yen)	**< 5**	0.054	40 (67.8)	0.047	202 (71.4)
	5 ≦	0.044	19 (32.2)	0.046	81 (28.6)
Parity	**0**	0.054	28 (47.5)	0.047	147 (51.6)
	≧ 1	0.048	31 (52.5)	0.046	138 (48.4)
Smoking habit	**Never**	0.050	34 (57.6)	0.046	120 (42.1)
	Former	0.059	17 (28.8)	0.047	119 (41.8)
	Current	0.039	8 (13.6)	0.046	46 (16.1)
Alcohol intake during pregnancy	**No**	0.048	38 (64.4)	0.046	187 (65.6)
	Yes	0.056	21 (35.6)	0.047	98 (34.4)

OC use	**No**	0.052**	57 (96.6)	0.047	264 (92.6)
	Yes	< LOQ	2 (3.4)	0.043	21 (7.4)
Frequency of food consumption during pregnancy					
Beef	**< 1/wk**	0.045*	44 (74.6)	0.046	209 (73.3)
	≧ 1/wk	0.072	15 (25.4)	0.046	76 (26.7)
Pork	**< 1/wk**	< LOQ*	4 (6.8)	0.052	21 (7.4)
	≧ 1/wk	0.052	55 (93.2)	0.057	264 (92.6)
Chicken	**< 1/wk**	0.054	11 (18.6)	0.052	43 (15.1)
	≧ 1/wk	0.050	48 (81.4)	0.057	242 (84.9)
Fish	**< 1/wk**	0.058	24 (40.7)	0.053	102 (35.8)
	≧ 1/wk	0.046	35 (59.3)	0.059	183 (64.2)

Values shown are geometric means.

Abbreviations: LOQ: Limit of quantification, BMI: Body Mass Index, OC: Oral Contraceptives.

*P<0.05 by Mann-Whitney U test or Kruskal-Wallis test.

**P<0.10 by Mann-Whitney U test or Kruskal-Wallis test.

Table 3 Regression coefficients of maternal characteristics related to maternal BPA levels.

Characteristics		**β (95%CI)**	**p value**
Age (years)	**< 25**	Ref.	
	25-29	-0.22 (-0.45, 0.20)	0.071
	29-34	-0.17 (-0.39, 0.05)	0.134
	≧ 35	-0.33 (-0.59, -0.08)	0.011
OC use	**No**	Ref.	
	Yes	-0.42 (-0.81, -0.02)	0.039
Beef consumption	**< 1/wk**	Ref.	
	≧ 1/wk	0.21 (-0.04, 0.37)	0.013
Pork Consumption	**< 1/wk**	Ref.	
	≧ 1/wk	0.26 (-0.03, 0.55)	0.074

(a) (b)

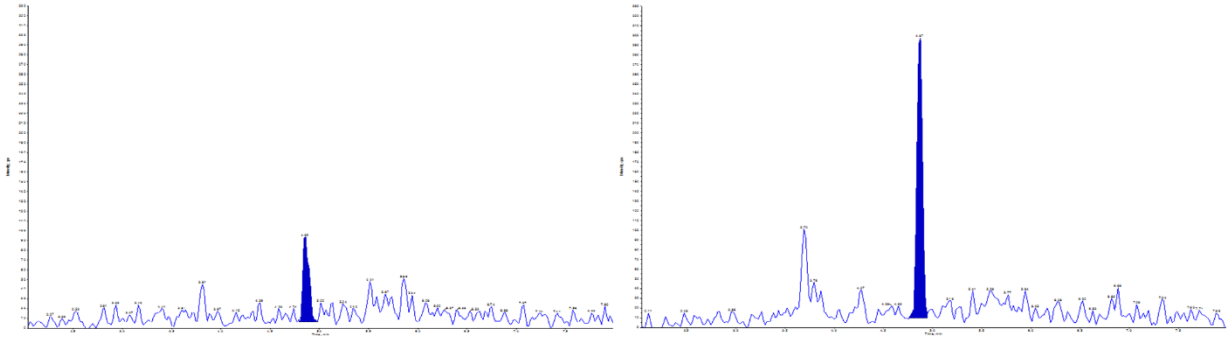

Figure 1. SRM chromatograms of procedural (a) blank and (b) maternal blood sample of median concentration (0.057 ng/ml).

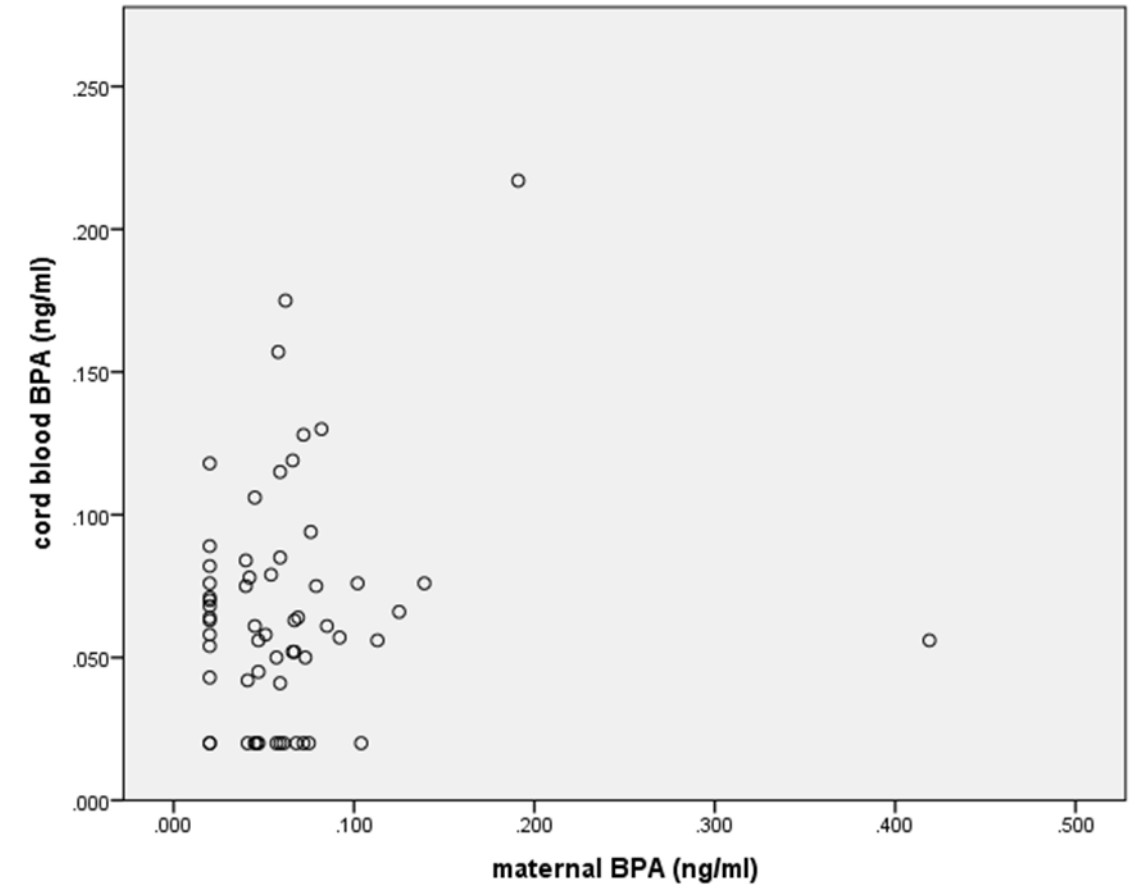

Figure 2. Scatter plot of maternal and cord blood BPA levels.