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Assessment of Bisphenol a in Umbilical Cord Blood and Maternal Blood

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Abstract
Bisphenol A (BPA) is an industrial material used to make polycarbonate plastics and epoxy resins. BPA is found to leach from food and beverage containers. However, BPA is an endocrine disruptor that may interfere with the body's endocrine system and affect development in infants and children. The purpose of this study was to determine the distribution of bisphenol A (BPA) levels in umbilical cord blood and maternal blood. Thirty maternal blood and 39 umbilical cord blood samples during pregnancy and delivery were obtained from the hospitals in this study, respectively. Results demonstrated the detection rates of plasma BPA in pregnant woman and neonates were 26.7% and 51.0%. The maternal BPA concentrations were 0.896 ± 6.80 (mean ± SD) μg/L, with a range from <0.48 to 71.7 μg/L. The neonatal BPA concentrations were 1.86 ± 3.24 μg/L, with a range from <0.48 to 26.8 μg/L. The present study showed the fetus was exposed to BPA through umbilical cord blood. Future research needs to focus on the effects of BPA on fetus growth and development.
Keywords: Bisphenol A, Pregnant woman, Neonates
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1. Introduction

Bisphenol A (2,2-bis(4-hydroxyphenyl)propane; BPA) is an industrial material used to make plastic products and epoxy resins [1]. Many food and liquid containers, are made of polycarbonate, or have a lining that contains BPA [2-3]. Dental sealants, thermal printing paper and polyvinyl chloride plastics can also contain low levels of bisphenol A [4-6]. Due to its extensive use, humans are frequently exposed to BPA in daily life, which result in its wide distribution in human blood, amniotic fluid, placenta, breast milk, and urine [7-10]. In addition, BPA has also been detected in a variety of environmental substances, including water, wastewater, river and sediment [11-13].

BPA has been reported for endocrine-disruption potentials in a number of in vitro and in vivo studies. In general, BPA is considered to have weak estrogenic effect [14]. Several studies also indicated that BPA altered the postnatal growth rate and reproductive function in female mice [15-18]. Low dose of BPA possibly altered development of the fetal prostate and mammary gland, and decreased efficiency of sperm production in mice [19-22].

BPA is also suspected to affect development in infants and children. Philippat et al. [23] indicated that maternal BPA concentrations in urine were positively associated with head circumference of male newborns. In addition, prenatal exposure to BPA may affect children behavior and increase the risk of wheezing [24-26]. In Taiwan, the use of plastic products is more common than other developed countries. It is necessary to investigate the distribution of BPA levels in maternal blood and neonatal cord blood.

2. Materials and Methods

2.1 Chemicals and Reagents

Bisphenol A (BPA) standard (99%) andβ-glucuronidase were purchased from Sigma-Aldrich (St. Louis, USA). HPLC grade methanol and analytical grade ammonium acetate, hydrochloric acid, glacial acetic acid, perchloric acid were provided by Merck (Darmstadt, Germany). Internal standard of bisphenaol B [2,2-bis(hydroxyphenol)butane] (99.5%) was obtained from Dr. Ehrenstorfer GmbH (Augsburg, German). Bisphenol

A sulfate sodium salt (95%) and bisphenol A β-Glucuronide (95%) were obtained from Toronto Research Chemicals (Ontario, Canada).

2.2 Sample collection

Thirty maternal blood samples during pregnancy were obtained from a northern Taiwan hospital. Thirty-nine umbilical cord blood samples were obtained at delivery from a central Taiwan hospital. All pregnant women consented to participate in this study, and the bio-sampling process was approved by the institutional review board of the National Yang-Ming university.

2.3 Sample preparation

The pretreated method of plasma samples was referred to the procedures as reported by Chou et al. [27]. The plasma (500 µL) was added 500µl water, 200 µl of 0.01 M ammonium acetate buffer (pH 4.5) and 4 mL mixture of n-hexane and diethyl ether. The samples were sonicated for 1 minute, incubated for 10 minute in a shaker bath, and then 9 µL of 9.187 M perchloric acid was added. After centrifugation at 3,500 rpm for 5 minutes, the organic layer was evaporated to dryness, and reconstituted with 200 µl of mobile phase (methanol:water 80:20 v/v) for HPLC system.

2.4 Instrumental analysis

The analytes in blood samples were determined by using a reversed-phase HPLC fluorescent detector. The reverse-phase column was a Chromolith® RP-18e (100 × 4.6 mm) i.d., 5-µm particle size (Merck, Germany) column. The isocratic mobile phase was a mixture of acetonitrile:water (30:70, v/v), and the flow rate was 1.5 mL/min. The fluorescent detector was operated with an excitation wavelength of 275 nm and an emission wavelength of 300 nm. The samples were injected in quantities of 20 µL. The limit of detection (LOD) of the analyte was calculated based on the standard deviation (SD) of seven replicates of the spiked sample. The LOD was defined as 3 times of the SD. The LOD was determined at 0.48 ng/mL, which was similar to those reported at 0.6-0.625 ng/mL in some studies [28, 29], but substantially higher than those used in other studies at 0.01 ng/L-0.1 ng/mL [30-36]. Recoveries for BPA in blood samples were 96.9±11.8% (mean±SD).

2.5 Statistical analysis

All statistical analyses were conducted using the SPSS statistical package (SPSS version 18.0, Chicago, IL). Values below the LODs were replaced with half of the values of LODs. Arithmetic mean and ranges were used to demonstrate the distribution and disparity of BPA levels in maternal blood urine and neonatal cord blood, respectively.

3. Results and Discussion

The distributions of BPA levels respectively in the 30 maternal blood samples and 39 fetal umbilical cord blood samples were shown in Table 1 and Table 2. The BPA detection rate in maternal blood was 27%, which was similar to that reported at 26.9% in a study [28], but substantially lower than those used in other studies at 67.0-100% [29-34]. The BPA detection rate in fetal umbilical cord blood was 51%, which was similar to those used in other studies at 40-47% [29, 35], but substantially higher than those used in other studies at 8-27% [28, 31] and lower than those used in other studies at 76.3-100% [30, 32-34, 36].

The maternal BPA concentrations were 0.896 ± 6.80 (mean ± SD) µg/L, with a range from <0.48 to 71.7 µg/L, which were similar to those reported for pregnant women from Canada, Korea, the United States, China and Germany (Table 1). Lee et al. [29] found a mean ± SD serum concentration of 9.04 ± 14.03 ng/mL (range: <0.625 to 66.48) in 300 Korean pregnant women. Aris [30] found a mean ± SD serum concentration of 1.36 ± 1.18 ng/mL (range: <0.01 to 4.46) in 61 Canadian pregnant women. Zhang et al. [31] found a mean ± SD serum concentration of 3.58 ± 4.27 ng/mL (range: <0.10 to 29.0) in 30 Chinese pregnant women. Schönfelder et al. [33] found a mean ± SD serum concentration of 4.4 ± 3.9 ng/mL (range: 0.30 to 18.9) in 37 German pregnant women. In the present study, the maternal BPA concentrations were higher than those for 26 Korean pregnant women (mean ± SD: 0.7 ± 0.1 ng/mL; Range: <0.6-5.4 ng/mL) in the study of Wan et al. [28], 9 Japsnese pregnant women (mean ± SD: 0.46 ± 0.20 ng/mL; Range: 0.21-0.79 ng/mL) in the study of Kuroda et al. [32] and 59 Japanese pregnant women (mean: 0.063 ng/mL; Range: <0.04-0.419 ng/mL) in the study of Yamamoto et al. [34].

The neonatal BPA concentrations were 1.86 ± 3.24 (mean ± SD) µg/L, with a range from <0.48 to 26.8 µg/L, which were similar to those reported for neonates from Canada, Korea, Germany, the United States, and France (Table 2). Aris [30] found a mean ± SD serum concentration of 1.23 ± 1.04 ng/mL (range: <0.01 to 4.60) in 58 Canadian fetal cord samples. Lee et al. [29] found a mean ± SD serum concentration of 1.13 ± 1.04 (range: <0.625 to 8.86) in 300 Korean fetal cord samples. Gerona et al. [35] found a mean ± SD serum concentration of 2.18 ± 8.10 ng/mL (range: <0.05 to 52.26) in 85 U.S. fetal cord samples. Fenichel et al. [36] found a mean ± SD

serum concentration of 1.12 ± 0.86 ng/mL (range: 0.14 to 4.76) in 106 French fetal cord samples of control newborns. On the other hand, our fetal cord BPA concentrations were higher than those for 25 Japanese neonates (mean: <0.6 ng/mL; range: <0.6-0.7) in the study of Wan et al. [28], 30 Chinese neonates (mean ± SD: 0.13 ± 0.12 ng/mL; range: <0.10-0.79 ng/mL) in the study of Zhang et al. [31], 9 Japsnese neonates (mean ± SD: 0.62 ± 0.13 ng/mL; Range: 0.45-0.70 ng/mL) in the study of Kuroda et al. [32] and 285 Japsnese neonates (mean: 0.057 µg/L; Range: <0.04-0.217 ng/mL) in the study of Yamamoto et al. [34].

4. Conclusions

The present study showed that BPA was found in the maternal blood and fetal umbilical cord blood. The maternal and neonatal plasma BPA concentrations were similar to those reported from Canada, Korea and Germany, respectively. In order to protect the health of the fetus, it is necessary to limit the use of consumer products that contain BPA. Future research needs to focus on the effects of BPA on fetus growth and development.

References

1. Kang, J. H., Kondo, F., & Katayama, Y. (2006). Human exposure to bisphenol A. Toxicology, 226 (2-3), 79-89.
2. Howe, S.R., Borodinsky, L., Lyon, R.S. Potential exposure to bisphenol A from food-contact use of epoxy coated cans. *J. Coat. Technol.* 1998, 70 (877), 69-74.
3. Sajiki, J., Yonekubo, J. Leaching of bisphenol A (BPA) to seawater from polycarbonate plastic and its degradation by reactive oxygen species. *Chemosphere* 2003, 51 (1), 55-62.
4. Arenholt-Bindslev, D., Breinholt, V., Preiss, A., Schmalz, G. Time-related bisphenol-A content and estrogenic activity in saliva samples collected in relation to placement of fissure sealants. *Clin. Oral invest.* 1999, 3 (3), 120-125.
5. Biedermann, S., Tschudin, P., Grob, K. Transfer of bisphenol A from thermal printer paper to the skin. *Anal. Bioanal. Chem.* 2010, 398 (1), 571-576.
6. Yamamoto, T., Yasuhara, A. Quantities of bisphenol A leached from plastic waste samples. *Chemosphere* 1999, 38 (11), 2569-2576.
7. Schönfelder, G., Wittfoht, W., Hopp, H., Talsness, C. E., Paul, M., & Chahoud, I. (2002). Parent bisphenol A accumulation in the human maternal-fetal-placental unit. *Environmental health perspectives*, *110*(11), A703-A707.
8. Ikezuki, Y., Tsutsumi, O., Takai, Y., Kamei, Y., & Taketani, Y. (2002). Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. *Human reproduction*, *17*(11), 2839-2841.
9. Sun, Y., Irie, M., Kishikawa, N., Wada, M., Kuroda, N., Nakashima, K. Determination of bisphenol A in human breast milk by HPLC with column-switching and fluorescence detection. *Biomed. Chromatogr.* 2004, 18, 501–507.
10. Calafat, A.M., Kuklenyik, Z., Reidy, J.A., Caudill, S.P., Ekong, J., Needham, L.L. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. *Environ. Health Perspect.* 2005, 113 (4), 391-395.
11. Rudel, R.A., Melly, S.J., Geno, P.W., Sun, G., Brody, J.G. Identification of alkylphenols and other estrogenic phenolic compounds in wastewater, septage, and groundwater on Cape Cod, Massachusetts. *Environ. Sci. Technol.* 1998, 32, 861–9.
12. Ding, W.H., Wu, C.Y. Determination of estrogenic nonylphenol and bisphenol A in river water by solid-phase extraction and gas chromatography–mass spectrometry. *J. Chin. Chem. Soc-Taip.* 2000, 47, 1155–60.
13. Bolz, U., Hagenmaier, H., Komer, W. Phenolic xenoestrogens in surface water, sediments and sewage sludge from Baden-Wurttemberg, South-west Germany. *Environ. Pollut.* 2001, 115, 291–301.
14. Matthews, J.B., Twomey, K., Zacharewski, T.R. In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors alpha and beta. *Chem. Res. Toxicol.* 2001, 14 (2), 149-157.
15. Howdeshell, K.L., Hotchkiss, A.K., Thayer, K.A., Vandenbergh, J.G., vom Saal, F.S. Environmental toxins: exposure to bisphenol A advances puberty. *Nature* 1999, 401, 763–764.
16. Caserta, D., Segni, N.D., Mallozzi, M., Giovanale, V., Mantovani, A., Marci, R., Moscarini, M. Bisphenol A and the female reproductive tract: an overview of recent laboratory evidence and epidemiological studies. *Repro. Biol. and Endocrin.* 2014, 12 (1), 37.
17. Mallozzi, M., Leone, C., Manurita, F., Bellati, F., Caserta, D. Endocrine Disrupting Chemicals and Endometrial Cancer: An Overview of Recent Laboratory Evidence and Epidemiological Studies. *Int. J. Environ. Res. Public health* 2017, 14 (3), 334.
18. Mallozzi, M., Bordi, G., Garo, C., & Caserta, D. The effect of maternal exposure to endocrine disrupting

chemicals on fetal and neonatal development: A review on the major concerns. *Birth Defects Res. C Embryo Today* 2016, 108, 224–242.
19. Timms, B.G., Howdeshell, K.L., Barton, L., Bradley, S., Richter, C.A., vom Saal, F.S. Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra. *Proc. Natl. Acad. Sci. USA* 2005, 102, 7014–7019.
20. Markey, C.M., Luque, E.H., de Toro, M.M., Sonnenschein, C., Soto, A.M. In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. *Biol. Reprod.* 2001, 65, 1215–1223.
21. vom Saal, F.S., Cooke, P.S., Buchanan, D.L., Palanza, P., Thayer, K.A., Nagel, S.C., Parmigiani S., Welshons W.V. A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. *Toxicol. Ind. Health* 1998, 14, 239–260.
22. Richter, C.A., Birnbaum, L.S., Farabollini, F., Newbold, R.R., Rubin, B.S., Talsness, C.E., Vandenbergh J.G., Walser-Kuntz D.R., vom Saal F.S. In vivo effects of bisphenol A in laboratory rodent studies. *Reprod. Toxicol.* 2007, 24, 199–224.
23. Philippat, C., Mortamais, M., Chevrier, C., Petit, C., Calafat, A. M., Ye, X., ... & Cordier, S. Exposure to phthalates and phenols during pregnancy and offspring size at birth. Environmental health perspectives 2011, 120(3), 464-470.
24. Perera, F., Vishnevetsky, J., Herbstman, J. B., Calafat, A. M., Xiong, W., Rauh, V., & Wang, S. Prenatal bisphenol A exposure and child behavior in an inner-city cohort. Environmental health perspectives 2012, 120(8), 1190-1194.
25. Casas, M., Forns, J., Martínez, D., Avella-García, C., Valvi, D., Ballesteros-Gómez, A., ... & Vrijheid, M. Exposure to bisphenol A during pregnancy and child neuropsychological development in the INMA-Sabadell cohort. Environmental research 2015, 142, 671-679.
26. Spanier, A. J., Kahn, R. S., Kunselman, A. R., Hornung, R., Xu, Y., Calafat, A. M., & Lanphear, B. P. Prenatal exposure to bisphenol A and child wheeze from birth to 3 years of age. Environmental health perspectives 2012, 120(6), 916-920.
27. Chou, W. C., Chen, J. L., Lin, C. F., Chen, Y. C., Shih, F. C., & Chuang, C. Y. (2011). Biomonitoring of bisphenol A concentrations in maternal and umbilical cord blood in regard to birth outcomes and adipokine expression: a birth cohort study in Taiwan. Environmental health, 10(1), 1-10.
28. Wan, Y., Choi, K., Kim, S., Ji, K., Chang, H., Wiseman, S., ... & Lam, M. H. (2010). Hydroxylated polybrominated diphenyl ethers and bisphenol A in pregnant women and their matching fetuses: placental transfer and potential risks. *Environmental science & technology*, *44*(13), 5233-5239.
29. Lee, Y. J., Ryu, H. Y., Kim, H. K., Min, C. S., Lee, J. H., Kim, E., ... & Park, E. Y. (2008). Maternal and fetal exposure to bisphenol A in Korea. *Reproductive toxicology*, *25*(4), 413-419.
30. Aris, A. (2014). Estimation of bisphenol A (BPA) concentrations in pregnant women, fetuses and nonpregnant women in Eastern Townships of Canada. *Reproductive toxicology*, *45*, 8-13.
31. Zhang, T., Sun, H., & Kannan, K. (2013). Blood and urinary bisphenol A concentrations in children, adults, and pregnant women from china: partitioning between blood and urine and maternal and fetal cord blood. *Environmental science & technology*, *47*(9), 4686-4694.
32. Kuroda, N., Kinoshita, Y., Sun, Y., Wada, M., Kishikawa, N., Nakashima, K., ... & Nakazawa, H. (2003). Measurement of bisphenol A levels in human blood serum and ascitic fluid by HPLC using a fluorescent labeling reagent. *Journal of pharmaceutical and biomedical analysis*, *30*(6), 1743-1749.
33. Schönfelder, G., Wittfoht, W., Hopp, H., Talsness, C. E., Paul, M., & Chahoud, I. (2002). Parent bisphenol A accumulation in the human maternal-fetal-placental unit. *Environmental health perspectives*, *110*(11), A703-A707.
34. Yamamoto, J., Minatoya, M., Sasaki, S., Araki, A., Miyashita, C., Matsumura, T., & Kishi, R. (2016). Quantifying bisphenol A in maternal and cord whole blood using isotope dilution liquid chromatography/tandem mass spectrometry and maternal characteristics associated with bisphenol A. *Chemosphere*, *164*, 25-31.
35. Gerona, R. R., Woodruff, T. J., Dickenson, C. A., Pan, J., Schwartz, J. M., Sen, S., ... & Hunt, P. A. (2013). Bisphenol-A (BPA), BPA glucuronide, and BPA sulfate in midgestation umbilical cord serum in a northern and central California population. *Environmental science & technology*, *47*(21), 12477-12485.
36. Fenichel, P., Dechaux, H., Harthe, C., Gal, J., Ferrari, P., Pacini, P., ... & Brucker-Davis, F. (2012). Unconjugated bisphenol A cord blood levels in boys with descended or undescended testes. *Human reproduction*, *27*(4), 983-990.

Table 1. BPA concentrations in the blood samples of pregnant women from different countries

BPA concentration (ng/mL)	Country	N	Mean±S.D.	Range	Detection rate
Tsai et al. (the present study)	Taiwan	30	0.896±6.80	<0.48-71.7	27%
Aris (2014)	Canada	61	1.36 ± 1.18	<0.01-4.46	97%
Lee et al. (2008)	Korea	300	9.04±14.03	<0.625-66.48	84%
Wan et al. (2010)	Korea	26	0.7 ± 0.1	<0.6-5.4	26.9%
Zhang et al. (2013)	China	30	3.58 ± 4.27	<0.10−29.0	67%
Kuroda et al. (2003)	Japan	9	0.46± 0.20	0.21-0.79	100%
Schönfelder et al. (2002)	German	37	4.4±3.9	0.3-18.9	100%
Yamamoto et al. (2016)	Japan	59	0.063	<0.04-0.419	68.8%

Table 2. BPA concentrations in the fetal umbilical cord blood from different countries

BPA concentration (ng/mL)	Country	N	Mean±S.D.	Range	Detection rate
Tsai et al. (the present study)	Taiwan	39	1.86±3.24	<0.48--26.8	51%
Aris (2014)	Canada	58	1.23 ± 1.04	<0.01-4.60	95%
Lee et al. (2008)	Korea	300	1.13±1.43	<0.625-8.86	40%
Wan et al. (2010)	Korea	25	<0.6	<0.6-0.7	8%
Zhang et al. (2013)	China	30	0.13 ± 0.12	<0.10−0.79	27%
Kuroda et al. (2003)	Japan	9	0.62±0.13	0.45 - 0.7	100%
Schönfelder et al. (2002)	German	37	2.9±2.5	0.2-9.2	100%
Yamamoto et al. (2016)	Japan	285	0.057	<0.04-0.217	76.3%
Gerona et al. (2013)	US	85	2.18±8.10	<0.05-52.26	47%
Fenichel et al. (2012)	France	106	1.12 ± 0.86	0.14–4.76	100%