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Research Article

# Cord Blood Bisphenol-A Level in Relation to Gestational Age and Neonatal Anthropometric Measurements in A Sample of Egyptian New Borns

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**ABSTRACT**

Background: Wide spread human exposure to bisphenol-A and evidence of developmental toxicity in experimental animals has raised significant public health concerns. Objective: To estimate levels of Bisphenol-A in cord blood samples of Egyptian newborns, correlating these levels with gestational age and neonatal anthropometric measurements. Subjects and methods: Eighty neonates were recruited randomly from public and private Gynecology and Obstetrics Hospitals. Their gestational age ranged from 31 to 39 weeks. Assessment of gestational age was performed in the delivery room. Neonatal anthropometric measurements were carried out within the first 24 hours and cord blood Bisphenol-A level was assayed using High Performance Liquid Chromatography (HPLC). Results: BPA was detected in all cord blood samples with levels ranged from 0.87 to 15.11 ng/ml and median level was 5.06 ng/ml. Neonates with BPA level above the median level had lower gestational age, lower birth weight, length and head circumference when compared to neonates with BPA level below the median; the differences were statistically highly significant ($p < 0.001$). BPA level showed highly significant negative correlations with gestational age and anthropometric measures in neonates with BPA level above the median. Conclusion: All neonates in this study were subjected to prenatal BPA exposure with varying grades. Adverse effects of BPA on fetal growth are dose-dependent and to some extent sex-dependent. High cord blood levels of BPA are negatively associated with gestational length and birth size.

**Keywords:** Cord blood, bisphenol-A, gestational age, anthropometry

## INTRODUCTION

Bisphenol-A (BPA) is an estrogenic monomer used to produce polycarbonate plastics and resins. Polycarbonate plastics are used in some food and drinking containers as water and baby bottles; the resins are used to coat metal products such as beverage and food cans, bottle tops and water supply pipes. It can also be used in the processing of some medical equipment, children's toys, carbonless paper, cigarette filters and in some polymers used in tooth coatings[1]. Humans are frequently exposed to Bisphenol-A which has been shown to leach from a variety of resin-based and plastic products[2]**.** BPA is known to exert estrogenic activity and is considered one of the most potent endocrine-disrupting chemical (EDC)[3]. Concern about EDCs stems from their potential effects via diverse mechanisms, including estrogenic/anti-androgenic properties, inhibition of cell cycles and effects on cell differentiation[4,5]. Some animal studies have shown that exposure to EDCs that mimic estrogen affected fetal growth and organ differentiation[6]. These studies support the hypothesis that increased estrogen signaling during inappropriate times of fetal development can lead to intrauterine growth retardation (IUGR) or preterm birth. Different animal studies on BPA dosages have presented inconsistent results with both a reduction and a gain in body weight[6,7]. Widespread exposure to BPA has created a great deal of concern regarding its potential adverse effects on human health. The developing fetus and neonates are especially susceptible to BPA exposure resulting in adaptations and organizational changes that appear to predispose them to later dysfunctions[8]. Study of the human health impact of bisphenol-A has been hampered by the high cost of laboratory analyses of the compound. There are just a few human epidemiological studies that shed light on the effect of bisphenol-A on pregnancy outcomes[9-11]. Observational data have proposed that increased incidence of IUGR and low birth weight among certain ethnic groups is caused, at least partially, by increased exposure to endocrine disrupting chemicals such as BPA[12]. The health and social consequences of low birth weight/IUGR have higher perinatal morbidity and mortality, greater risk of cognitive impairment[13] and increased risk of disabling adult onset diseases, as cardiovascular disease,

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Table 1: Comparison between group A and group B regarding the mean cord blood BPA concentration.

| Groups | BPA level(ng/ml) | | Independent t-test | |
|---|---|---|---|---|
| | Mean ± SD | Min-Max | t | p-value |
| Group A< 5.06 ng/dl (n=40) | 3.22± 1.33 | 0.87 – 5.06 | -12.457 | <0.001 |
| Group B ≥ 5.06ng/dl (n=40) | 9.52 ± 2.90 | 6.19– 15.11 | | |

P <0.001 is highly significant

Table 2: Comparison between group A and group B regarding sex and gestational age.

| Parameter | | Group A | | Group B | | $X^2$ | P-value |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | | |
| Sex of neonate | Female | 22 | 55.0% | 25 | 62.5% | 0.000 | 1.000 |
| | Male | 18 | 45.0% | 15 | 37.5% | | |
| Gestational age | Mean±SD | 37.47±2.20 | | 33.58±2.01 | | 7.663* | <0.001 |

P <0.001 is highly significant * t: Independent sample t-test

Table 3: Comparison of neonatal anthropometric measures in group A and group B.

| Parameter | Group A | Group B | P |
|---|---|---|---|
| Weight (gm) Median [Percentiles ($25^{th}$-$75^{th}$)] | 3225 (3025-3500) | 1900 (1662.5-2237.5) | <0.001‡ |
| Length (cm) (Mean±SD) | 49.31±2.95 | 44.61±3.48 | <0.001* |
| Head circumference (cm) (Mean±SD) | 34.77±2.18 | 31.13±2.30 | <0.001* |

P <0.001 is highly significant * t: Independent sample t-test; ‡ z- Mann-Whitney test

hypertension, type II diabetes and obesity[14]. The main source of BPA exposure is through food. After ingestion, unconjugated BPA-the biologically active form of BPA-has been thought to be rapidly conjugated in the liver and then excreted through bile or urine[15]. Scientific belief holds that BPA cannot be a biologically important pollutant since it is metabolized and excreted relatively quickly. Detectable urinary BPA concentrations have been found in various populations, including pregnant women[16-20]. It has been detected in the serum of pregnant women and follicular fluid[21-23,2]. Bisphenol-A can pass through placental barrier and it has been measured in placental tissue and umbilical cord blood at birth[24]. Many studies have found bioactive BPA in blood of pregnant women, in newborns and in amniotic fluid. Ikezuki et al., (2002)[22] reported accumulation of BPA in early fetuses with no significant correlation between maternal and fetal serum concentrations, suggesting that BPA may be partly metabolized in the fetus. Two recent studies - one human and one animal - show that the active form of BPA in the fetus remains active while the inactive form can be converted to the active form which may carry a bigger risk to the developing fetus than previously thought[25,26]. A number of human studies examined BPA exposure in relation to gestational or birth outcomes[27-30,2]. No clear conclusions can be drawn at present due to a lack of consistent evidence. Therefore, this issue still warrants further investigation[31]. We hypothesized that variability in cord blood BPA concentrations at birth reflected diverse prenatal exposure and would be associated with variable gestational length and birth sizes. The aim of this study was to estimate cord blood levels of Bisphenol-A at birth and to correlate these levels with gestational age and neonatal anthropometric measurements in a sample of Egyptian newborns.

## SUBJECTS AND METHODS

Eighty healthy pregnant women and their neonates were included in this study. Forty mothers and their offspring were recruited from the delivery room and neonatal care unit of Gynecology and Obstetrics Hospital of Ain Shams University and the rest of pregnant women were recruited from a private hospital to allow for diversity in socioeconomic status. Written informed consent was obtained from each participating pregnant woman, and the study protocol was approved by the National Research Centre Ethical Committee. Any pregnant woman came to the defined hospitals in the period of the study was invited to participate if her age was ≥ 18 years and ≤ 35 years and she had apparent fair physical and mental health. Mothers who experienced complicated labor or suffered from any problem which might affect fetal growth (e.g. preeclampsia, diabetes mellitus, chronic renal or hepatic disease) were excluded. Exclusion criteria for the newborns were: encephalopathy, malformations and unstable vitals.

*Methods*

All neonates included in this study were subjected to the following

*Assessment of gestational age*

using new Ballard score (NBS). The NBS is a valid and accurate gestational age assessment tool until 96 hours postnatal[32]. It is accurate for all newborns and was expanded to include extremely premature infants. The agreement between NBS and prenatal ultrasonography and the last menstrual period (US/LMP) is good, but differences of more than 2 weeks in GA were frequent[33].

*Anthropometric Measurements*

All measurements were carried out within 24 hours of birth, while the newborn is naked and lying down. Birth weight in grams (g), Length in centimeters (cm) and Head

Table 4: Comparison of studied parameters between male and female neonates of group B.

| Parameter | Male | | Female | | t-test | |
|---|---|---|---|---|---|---|
| | Mean | ±SD | Mean | ±SD | t | p-value |
| Gestational age (wks.) | 33.4 | 1.3 | 32.9 | 1.5 | 1.12 | 0.271 |
| BPA level(ng/ml) | 8.2 | 2.6 | 10.2 | 3.3 | -2.06 | 0.047* |
| Weight (gm) | 2000 | 300 | 1700 | 300 | 2.67 | 0.011* |
| Length (cm) | 44.1 | 2.0 | 43.1 | 2.3 | 1.47 | 0.149 |
| Head circumference (cm) | 31.1 | 1.3 | 30.1 | 1.4 | 2.34 | 0.024* |

P <0.05 is significant

circumference in centimeters (cm) were assessed according to anthropometry report of WHO, (1995)[34].

*Assessment of BPA level in plasma*

The BPA concentrations in cord blood were determined using a high performance liquid chromatography HPLC (Agilent 1100 series) according to the technique described by Chou et al., (2011)[27].

*Sample preparation*

The umbilical cord blood samples were collected in glass heparin tubes. Plastics were excluded to avoid BPA contamination. Whole blood was centrifuged at 12,000 rpm for 10 minutes to separate the plasma to be stored at 80˚C until analysis. To 500 µl plasma was added 100 µl of 0.01 M ammonium acetate buffer (ph 4.5) and 4 ml mixture of n-hexane (HPLC grade) and diethyl ether (70:30 v/v). The samples were mixed for 5 seconds, vortexed for 10 minutes, immobilized for one minute and then 8.71 µl of 9.187 M perchloric acid (purity 60-62%, Sigma- Aldrich, St. Louis, Mo) was added. After centrifugation at 3,000 rpm for 5 minutes, the organic layer was evaporated to dryness, and reconstituted with 100 µl of mobile phase (methanol: water 80:20 v/v) for BPA determination by reverse – phase high performance liquid chromatography (HPLC).

*Standard preparation*

The eluted peak of BPA (bis-(4-hydroxy phenyl)-propane, purity > 99%, Sigma- Aldrich, St. Louis, Mo) was detected at 226 nm. Both the initial standard stock solution as well as the serial dilutions from the stock solution in methanol were 0.5 mg/ml. linear calibration curve obtained for BPA ranged from 5-220 ng/ml, and the coefficient of determination ($r^2$) were $\geq$ 0.995. A linear standard curve was constructed by plotting peak areas vs the corresponding concentrations.

*HPLC condition*

Twenty µl of the filtrate were injected on to a C18 reversed phase column (25cm×10.00 mm, 5 µm particle size) and isocratically eluted with a mobile phase consisting of methanol: water (80:20 v/v) and was delivered at a flow rate of 0.7 ml/min for run time 20-minute. UV detection was performed at 226 nm. The concentrations in samples were obtained from the standard curve.

*Statistical analysis*

Data were analyzed using Statistical Program for Social Science (SPSS) version 18.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage. Independent-samples t-test of significance was used in parametric data when comparing two means. Mann Whitney U test: for two-group comparisons in non-parametric data. Chi-square (X2) test of significance was used in order to compare proportions between two qualitative parameters. Spearman's rank correlation coefficient (r) was used to assess the degree of association between two sets of variables if one or both of them was skewed. P-value was considered significant when $p<0.05$ and was considered highly significant when $p<0.001$.

## RESULTS

Eighty neonates with gestational age ranged from 31 to 39 weeks were recruited randomly in the period of the study. They were 46 females and 34 males. BPA was detected in all cord blood samples of the included neonates with concentrations ranged from 0.87 to 15.11 ng/ml and the median level was 5.06 ng/ml. Each neonate in this study was located in either of two groups according to the median level of BPA: group A whose BPA levels were below the median (<5.06ng/ml) and group B whose BPA levels were above the median (≥5.06ng/ml). Mean of BPA cord blood concentration in group B was statistically significantly higher than that of group A (9.52 ± 2.90 ng/ml vs. 3.22± 1.33 ng/ ml, $p< 0.001$) (table 1). The distribution of male and female neonates was not significantly different in either of the two groups. Neonates with BPA levels above the median (group B) had statistically significant lower mean gestational age when compared with neonates in group A as shown in table (2). Lengths and head circumferences of all neonates were normally distributed while their weights were not. Comparison of these measurements revealed that neonates in group B had statistically significant lower mean of lengths and head circumferences and lower median of weights than their comparable group (table 3). It was found that female neonates in group B had statistically significant higher concentrations of cord blood BPA than male neonates in the same group. These female neonates appeared to have statistically significant lower weight and smaller head circumference than males in the same group. They had shorter gestational age and lower length but the difference was not statistically significant (table 4). These gender-specific differences were not apparent in group A (not shown). BPA cord blood concentrations showed highly significant strong negative associations with gestational age and anthropometric measurements in group B neonates ($r = 0.6$ to $0.7$, $p< 0.01$) (table 5). These associations were not manifested in group A (table 6).

Table 5: Association of cord blood BPA level with gestational age and anthropometric measurements in group B neonates.

| Parameter | BPA level(ng/ml) | |
|---|---|---|
| | r | p-value |
| Gestational age (wks.) | -0.671 | <0.001 |
| Weight (gm) | -0.717 | <0.001 |
| Length (cm) | -0.640 | <0.001 |
| Head circumference (cm) | -0.716 | <0.001 |

P <0.001 is highly significant

Table 6: Association of cord blood BPA level with gestational age, and anthropometric measurements in group A neonates

| Parameter | BPA level(ng/ml) | |
|---|---|---|
| | r | p-value |
| Gestational age (wks.) | -0.205 | 0.182 |
| Weight (gm) | -0.228 | 0.137 |
| Length (cm) | 0.023 | 0.885 |
| Head circumference (cm) | -0.285 | 0.061 |

P <0.05 is significant

## DISCUSSION

As BPA has been recently shown to cross the placental barrier, in humans and animals, the potential effects of maternal BPA exposure on prenatal development has become a more focal area of research[35]. Current epidemiological evidence for the association of BPA exposure with adverse birth outcomes, are inconsistent[9,11,29,30]. This study is the first to show the correlation between cord blood BPA concentration and birth outcomes in Egypt. BPA was detected in all cord blood samples of the recruited neonates with levels ranged from 0.87 to 15.11 ng/ml (median 5.06 ng/ml). Results reported in different studies are characterized by wide variability and inconsistency. In Taiwan, the levels of BPA in cord serum ranged from 0.3 to 18.5 ng/ml[27]. In Berlin, levels of BPA ranged from 0.2 to 9.2 ng/ $mL^{24}$; in Korea the levels ranged from non-detectable to 8.86 ng/ $mL^{2}$. Some studies reported much lower levels as the Canadian study[36], where levels ranged from non-detectable to 4.60 ng/ml in Eastern Townships of Canada. Genetic differences in metabolism of BPA between different ethnic groups may be a partial explanation for the varying findings. Different levels of maternal exposure to BPA in different nationalities are another explanation. In the present study, the mean gestational age of neonates in group B (cord blood BPA level> 5.06 ng/ml) was statistically significantly lower than that of group A (33.58±2.01 vs37.47±2.20, p< 0.001). A highly significant negative correlation between BPA level in this group and gestational age was obvious. Evidence for decreased gestational duration in relation to BPA has also been reported by other studies[9-11]. Other studies link maternal BPA exposures to an increase in premature births, as well as small for gestational age babies[28]. Other small-scale human studies of BPA exposure during pregnancy have reported increases in the risk of spontaneous abortion[37]. BPA has been shown to stimulate the production of pro-inflammatory cytokines[38,39]. It can induce Th-1 type cytokines while simultaneously suppressing Th-2 cytokines[40]. Additionally, it has been shown in human populations that BPA concentrations are associated with increased serum C-reactive protein levels[41]. These effects together are thought to initiate an inappropriate inflammatory cascade resulting in preterm birth[42]. However, other studies found no association between prenatal BPA exposure and gestational age. In the Children's Environmental Health study in New York City, no association was found between BPA exposure during the third trimester and gestational length among 404 pregnant women[30]. A small scale study (N = 40) in South eastern Michigan measured BPA in blood of women at the time of delivery and found no differences in gestational length between women with plasma BPA concentrations > 5 and ≤ 5 ng/mL[29]. Within nested case–control study of mothers giving birth in the Boston area, Cantonwine et al., (2015)[43] found no significant associations between averaged or cross-sectional urinary BPA levels and preterm birth (PTB). Reasons for the conflicting evidence between BPA exposure and either risk of PTB or short gestational length may include differences in study size and design, differences in populations, use of differing biological media for exposure assessment, or other factors[42]. Multiple animal and human studies have reported evidence of sex-specific adverse health effects resulting from BPA exposure[44-46]. In this study, significant higher BPA levels in female neonates were detected compared to male neonates. Female neonates had lower gestational age than male neonates but the difference was not statistically significant. Cantonwine et al., (2015)[42] suggested that female infants may be more sensitive to being delivered preterm in relation to gestational BPA exposure than males. Past research has demonstrated that female fetuses are more sensitive to the changes in inflammatory stressors[47]. The present study found that high levels of cord blood BPA (above the median) were inversely associated with birth weight, length and head circumference in both male and female neonates, whereas low levels (below the median) were not significantly correlated with growth indicators. These findings indicated that adverse effects of BPA on fetal growth were dose-dependent and somewhat sex-dependent as female neonates with high levels of cord blood BPA are more affected than male neonates. The risks of fetal low birth weight, small for gestational age based on maternal BPA exposure were documented in previous studies[2,27,48]. Miao et al., (2011)[28] reported that mothers with greater occupational exposure to BPA during pregnancy had offspring with lower birth weight. Some animal studies supported our findings. Kim et al., (2001)[6] reported that administration of a high BPA level (300 mg/kg) during the entire gestational period in Sprague-Dawley rats reduced the weight of the fetuses. Maternal exposure in sheep to BPA levels of 30 - 50 ng/ml during days 30 to 90 of gestation resulted in low birth weight in offspring[49]. The magnitude of BPA effect on fetal growth may be

influenced by subtle changes of hormones in utero[27]. BPA may harm fetal growth and promote early parturition through various mechanisms, as it has been shown to disrupt a variety of biologic functions including steroid hormone synthesis and metabolism[50], peroxisome proliferation[51], cytokine networks[52], genotoxicity[53], and oxidative stress[54,55]. Low doses of BPA also induced apoptosis and increased output of matrix metalloproteinase-9, an enzyme associated with preterm birth, in ovarian granulosa cells in dose-dependent patterns[56,57]. It has been shown that human primary cytotrophoblast cells undergo a dose-dependent increase in TNF-α production and apoptosis with increasing environmentally relevant (0.0002 to 0.2 ug/mL) levels of BPA[51]. Morck et al., (2010)[34] also demonstrated that levels of BPA exposure can induce cell death in a human choriocarcinoma cell line and increase secretion of β-HCG and caspase-3 cleavage in first human chorionic villous explant cultures. However, certain studies provided conflicting results, reporting an increased weight in offspring whose mothers were exposed to BPA during gestation[58]. Philippat et al., (2012)[59] reported positive associations between maternal urinary BPA concentrations and birth weight and head circumference. Some animal studies suggested that effect of BPA on birth weight may be dose-dependent. Offspring of rats exposed to BPA exhibited an increase in body weight[7]. Other studies showed no correlation between prenatal bisphenol-A and birth weight[10,29,30]. These ambiguous findings may reflect the need for well-designed and adequately powered studies of the influence of BPA on fetal growth.

Assay of BPA in many studies (whether in blood or urine) is usually hindered by high cost per sample. Consequently, this study has several limitations. First; small sized sample, so results can't be generalized. Second; lack of estimation of maternal BPA. Third, samples were analyzed for total BPA, instead of unconjugated BPA and conjugated BPA separately. Fourth, the cross-sectional design of the study which may affect the precision of results, as BPA exposure is variable overtime. However, results of this study threw the light on this point of research and indicated the importance of gathering efforts to explore the influence of BPA on fetal growth and development. To our knowledge, this is the first study in Egypt to estimate BPA concentrations in cord blood. We concluded that all neonates in this study were subjected to prenatal BPA exposure with varying grades. Adverse effects of BPA on fetal growth are dose-dependent and to some extent sex-dependent. High cord blood levels of BPA are negatively associated with gestational length and birth size.

## REFERENCES


1. Chapin RE, Adams J, Boekelheide K, Gray LE, Hayward SW, Lees PSJ et al. NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A. Birth Defects Res B Dev Reprod Toxicol 2008; 83(3):157-395.
2. Lee YJ, Ryu HY, Kim HK, Min CS, Lee JH, Kim E et al. Maternal and fetal exposure to bisphenol A in Korea.Reprod. Toxicol 2008; 25(4): 413–419.
3. Alonso-Magdalena P, Ropero AB, Soriano S, García-Arévalo M, Ripoll C, Fuentes E, et al. Bisphenol-A acts as a potent estrogen via non-classical estrogen triggered pathways. Mol Cell Endocrinol 2012; 355:201–207.
4. Hotchkiss AK, Rider CV, Blystone CR, Wilson VS, Hartig PC, Ankley GT et al. Fifteen years after "wingspread" environmental endocrine disrupters and human and wildlife health: where we are today and where we need to go. Toxicol. Sci .2008; 105(2): 235-259.
5. McLachlan JA, Simpson E, Martin M. Endocrine disrupters and female reproductive health. Best Pract Res Clin Endocrinol Metab 2006; 20:63–75.
6. Kim JC, Shin HC, Cha SW, Koh WS, Chung MK, Han SS. Evaluation of developmental toxicity in rats exposed to the environmental estrogen bisphenol A during pregnancy McLachlan. Life Sci (2001); 69:2611–2625.
7. Rubin BS, Murray MK, Damassa DA, King JC, Soto AM. Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. Environmental Health Perspectives (2001); 109(7): 675-680.
8. Ranjit N, Siefert K, Padmanabhan V. Bisphenol-A and disparities in birth outcomes: a review and directions for future research. J Perinatol 2010; 30(1):2-9.
9. Cantonwine D, Meeker JD, Hu H, Sanchez BN, Lamadrid-Figueroa H, Mercado-Garcia A et al. Bisphenol A exposure in Mexico City and risk of prematurity: a pilot nested case control study. Environmental Health 2010; 18(9):59-62.
10. Tang R, Chen MJ, Ding GD, Chen XJ, Han XM, Zhou K, et al. Associations of prenatal exposure to phenols with birth outcomes. Environ Pollut 2013; 178:115–120.
11. Weinberger B, Anna M, Vetrano, Faith E, Archer, Stephen W et al. Effects of maternal exposure to phthalates and bisphenol A during pregnancy on gestational age. J Matern Fetal Neonatal Med. March, 2014; 27(4): 323–327.
12. Emanuel I, Leisenring W, Williams MA, Kimpo C, Estee S, O'Brien W, et al. The Washington State Intergenerational Study of Birth Outcomes: methodology and some comparisons of maternal birthweight and infant birthweight and gestation in four ethnic groups. Paediatric and Perinatal Epidemiology 1999; 13:352–369.
13. Behrman R, Butler A. Committee on Understanding Premature Birth and Assuring Health Outcomes, Institute of Medicine of the National Academies 2006. National Academies Press; Washington D.C. Preterm Birth: Causes Consequences and Prevention.
14. Nathanielsz PW, Padmanabhan V. Editorial: Developmental origin of health and disease. J Physiology (Lond) 2006; 572:3–4

15. Genuis SJ, Beesoon S, Birkholz D, Lobo RA. Human Excretion of Bisphenol A: Blood, Urine, and Sweat (BUS) Study. J Environ Public Health 2012; 27:185731: 1-10.
16. Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003–2004. Environ.Health Perspect 2008; 116(7):39–44.
17. He Y, Miao M, Herrinton L J, Wu C, Yuan W, Zhou Z, et al. Bisphenol A levels in blood and urine in a Chinese popZulation and the personal factors affecting the levels. Environ Res 2009; 109: 629–633
18. Meeker JD, Cantonwine DE, Rivera-González LO, Ferguson KK, Mukherjee B, Calafat AM, et al. Distribution, variability, and predictors of urinary concentrations of phenols and parabens among pregnant women in Puerto Rico. Environ SciTechnol 2013; 47:3439–3447.
19. Vandenberg LN, Chahoud I, Heindel JJ, Padmanabhan V, Paumgartten FJ, Schoenfelder G. Urinary, Circulating, and Tissue Biomonitoring Studies Indicate Widespread Exposure to Bisphenol A. Environ Health Perspect 2010; 118(8): 1055–1070.
20. Ye X, Pierik FH, Hauser R, Duty S, Angerer J, Park MM et al. Urinary metabolite concentrations of organophosphorous pesticides, bisphenol A, and phthalates among pregnant women in Rotterdam, the Netherlands: the Generation R study. Environ Res .2008; 108(2):260–267.
21. Edlow AG, Chen M, Smith NA, Lu C, McElrath TF. Fetal bisphenol A exposure: concentration of conjugated and unconjugated bisphenol A in amniotic fluid in the second and third trimesters. Reprod Toxicol 2012; 34:1–7.
22. Ikezuki Y, Tsutsumi O, Takai Y, Kamei Y, Taketani Y. Determination of bisphenol A concentration in human biological fluids reveals significant early prenatal exposure. Human Reproduction 2002; 17(11):2839-2841.
23. Yamada H, Furuta I, Kato EH, Kataoka S, Usuki Y, Kobashi G, et al. Maternal serum and amniotic fluid bisphenol A concentrations in the early second trimester. Reprod Toxicol 2002; 16:735–739.
24. Schonfelder G, Wittfoht W, Hopp H, Talsness CE, Paul M, Chahoud I. Parent bisphenol A accumulation in the human maternal-fetal-placental unit. Environ Health Perspect. 2002;110: A703–A707.
25. Balakrishnan B, Henare K, Thorstensen EB, et al. Transfer of bisphenol A across the human placenta. Am J Obstet Gynecol 2010; 202:393. e1-7.
26. Nishikawa M, Iwano H, Yanagisawa R, Koike N, Inoue H and Yokota H. Placental transfer of conjugated bisphenol A and subsequent reactivation in the rat fetus. Environ Health Perspect 2010; 118:1196-1203
27. Chou WC, Chen JL, Lin CF, Chen YC, Shih FC, Chuang CY. 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. Environ Health 2011; 10(3):10 94.
28. Miao M, Yuan W, Zhu G, He X, Li DK. In utero exposure to bisphenol-A and its effect on birth weight of offspring. Reprod Toxicol 2011; 32, 64-68.
29. Padmanabhan V, Siefert K, Ransom S, Johnson T, Pinkerton J, Anderson L et al., Maternal bisphenol-A levels at delivery: a looming problem?J. Perinatol. 2008; 28, 258–263.
30. Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C et al. Prenatal phenol and phthalate exposures and birth outcomes. Environ Health Perspect 2008;116(8): 1092–1097.
31. EFSA. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. Part II – Toxicological assessment and risk characterization. EFSA Panel on Food Contact Materials, Enzymes, Flavorings and Processing Aids (CEF), European Food Safety Authority (EFSA), Parma, Italy. EFSA Journal 2015; 13, 3978. http://www.efsa.europa.eu/en/efsajournal/doc/3978part2.pdf CDC.
32. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R. New Ballard Score, expanded to include extremely premature infants. J Pediatrics 1991; 119(3): 417-423
33. Lee KG. Identifying the high risk newborn and evaluating gestational age and small for gestational age infant. In: Coloherty JP, Eichenwald EC, Stark AR (eds), Manual of Neonatal Care 2008. 6th edition. Lippincott Williams & Wilkins. Philadelphia; 5: 41-58.
34. WHO. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser 1995; 854: 1–452.
35. Morck TJ, Sorda G, Bechi N, Rasmussen BS, Nielsen JB, Ietta F et al. Placental transport and in vitro effects of Bisphenol A. Reprod Toxicol 2010; 30(1):131-137.
36. Aris A. Estimation of bisphenol A (BPA) concentrations in pregnant women, fetuses and nonpregnant women in Eastern Townships of Canada. Reprod Toxicol 2014; 45:8-13
37. Sugiura-Ogasawara M, Ozaki Y, Sonta S, Makino T, Suzumori K Exposure to bisphenol A is associated with recurrent miscarriage. Human Reproduction (Oxford, England) 2005; 20(8):2325–2329.
38. O'Brien E, Dolinoy DC, Mancuso P. Perinatal bisphenol A exposures increase production of pro-inflammatory mediators in bone marrow-derived mast cells of adult mice. J Immunotoxicol 2014; 11(3):205-12.
39. Tian X, Takamoto M, Sugane K. Bisphenol A promotes IL-4 production by Th2 cells. Int. Arch. Allergy Immunol .2003;132:240-247
40. Youn J-Y, Park H-Y, Lee J-W, Jung I-O, Choi K-H, Kim K, et al.. Evaluation of the immune response following exposure of mice to bisphenol A: induction of Th1 cytokine and prolactin by BPA exposure in the mouse spleen cells. Arch Pharm Res 2002; 25:946-953.

41. Lang IA, Galloway TS, Scarlett A, Henley WE, Depledge M, Wallace RB, et al. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. JAMA 2008; 300:1303-1310.
42. Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss JF, Petraglia F. Inflammation and pregnancy. Reprod Sci. 2009; 16:206-215.
43. Cantonwine DE, Ferguson KK, Mukherjee B, McElrath TF, and Meeker JD (2015). Urinary Bisphenol A Levels during Pregnancy and Risk of Preterm Birth. Environ Health Perspect. Sep; 123(9): 895–901.
44. Braun JM, Kalkbrenner AE, Calafat AM, Yolton K, Ye X, Dietrich KN, et al. Impact of early-life bisphenol A exposure on behavior and executive function in children. Pediatrics 2011; 128:873-882.
45. Perera F, Vishnevetsky J, Herbstman JB, Calafat AM, Xiong W, Rauh V, et al. Prenatal bisphenol Aexposure and child behavior in an inner-city cohort. Environ Health Perspect 2012; 120:1190-1194.
46. Rubin BS, Lenkowski JR, Schaeberle CM, Vandenberg LN, Ronsheim PM, Soto AM. Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. Endocrinology 2006; 147:3681-3691.
47. Clifton VL, Murphy VE. Maternal asthma as a model for examining fetal sex-specific effects on maternal physiology and placental mechanisms that regulate human fetal growth. Placenta. 2004; 25: S45-S52.
48. Smarr MM, Grantz KL, Sundaram R, Maisog JM, Kannan K, Louis GM. Parental urinary biomarkers of preconception exposure to bisphenol A and phthalates in relation to birth outcomes. Environ Health. Sep 2015;11; 14:73
49. Savabieasfahani M, Kannan K, Astapova O, Evans NP, Padmanabhan V. Developmental programming: differential effects of prenatal exposure to bisphenol-A or methoxychlor on reproductive function. Endocrinology 2006; 147(12): 5956-5966.
50. Padmanabhan V, Sarma HN, Savabieasfahani M, Steckler TL, Veiga-Lopez A. Developmental reprogramming of reproductive and metabolic dysfunction in sheep: native steroids vs. environmental steroid receptor modulators. Int J Androl 2010; 33:394-404.
51. Rashid H, Ahmad F, Rahman S, Ansari RA, Bhatia K, Kaur M, Islam F, RaisuddinS. Iron deficiency augments bisphenol A-induced oxidative stress in rats. Toxicology 2009; 256:7–12.
52. Benachour N, Aris A. Toxic effects of low doses of Bisphenol-A on human placental cells. Toxicol Appl Pharmacol 2009; 241(3):322-328.
53. Tayama S, Nakagawa Y, Tayama K. Genotoxic effects of environmental estrogen-like compounds in CHO-K1 cells. Mutat Res 2008; 649(1-2):114-125.
54. Kabuto H, Amakawa M, ShishiboriT. Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice. Life Sci, 2004; 74:2931–2940
55. Chitra KC, Latchoumycandane C, Mathur PP. Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. Toxicology 2003; 185(1-2):119-127.
56. Dominguez MA, Petre MA, Neal MS, Foster WG. Bisphenol A concentration-dependently increases human granulosa-lutein cell matrix metalloproteinase-9 (MMP-9) enzyme output. Reprod Toxicol. 2008; 25:420–425.
57. Xu J, Osuga Y, Yano T, Morita Y, Tang X, Fujiwara T, Takai Y, Matsumi H, Koga K, Taketani Y, et al. Bisphenol A induces apoptosis and G2-to-M arrest of ovarian granulosa cells. Biochem Biophys Res Commun. 2002; 292:456–462
58. Newbold RR, Jefferson WN, PadillaBanks E. Long-term adverse effects of neonatal exposure to Bisphenol-A on the murine female reproductive tract. Reproductive Toxicology 2007; 24 (2): 253–258.
59. Philippat C, Mortamais M, Chevrier C, et al. Exposure to phthalates and phenols during pregnancy and offspring size at birth. Environ Health Perspect. 2012; 120:464–470.