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. Humans are frequently exposed to Bisphenol-A which has been shown to leach from a variety of resin-based and plastic products. BPA is known to exert estrogenic activity and is considered one of the most potent endocrine-disrupting chemical (EDC). 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. Some animal studies have shown that exposure to EDCs that mimic estrogen affected fetal growth and organ differentiation. 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. 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. 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. 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. The health and social consequences of low birth weight/IUGR have higher perinatal morbidity and mortality, greater risk of cognitive impairment and increased risk of disabling adult on set diseases, as cardiovascular disease.

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hypertension, type II diabetes and obesity. 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. 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. It has been detected in the serum of pregnant women and follicular fluid. BPA can pass through the placental barrier and it has been measured in placental tissue and umbilical cord blood at birth. Many studies have found bioactive BPA in blood of pregnant women, newborns and in amniotic fluid. Ikezuki et al., (2002) 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 fetuses 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. A number of human studies examined BPA exposure in relation to gestational or birth outcomes. No clear conclusions can be drawn at present due to a lack of consistent evidence. Therefore, this issue still warrants further investigation. 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 hospital 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 newborns 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. 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. **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...
circumference in centimeters (cm) were assessed according to anthropometry report of WHO, (1995)\textsuperscript{34}.

**Assessment of BPA concentration 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)\textsuperscript{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 (r²) 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 ($<$5.06ng/ml) and group B whose BPA levels were above the median ($\geq$5.06ng/ml). Mean of BPA cord blood concentration in group B was statistically significantly higher than that of group A (9.52 ± 2.90 ng/ml vs. 3.22± 1.33 ng/ ml, p<0.001) (table 1). The distribution of male and female neonates was not significantly different in either of the two groups. Neonates with BPA levels above the median (group B) had statistically significant lower mean gestational age when compared with neonates in group A as shown in table (2). Lengths and head circumferences of all neonates were normally distributed while their weights not. Comparison of these measurements revealed that neonates in group B had statistically significant lower mean of lengths and head circumferences and lower median of weights than their comparable group (table 3). It was found that female neonates in group B had statistically significant higher concentrations of cord blood BPA than male neonates in the same group. These female neonates appeared to have statistically significant lower weight and smaller head circumference than males in the same group. They had shorter gestational age and lower length but the difference was not statistically significant (table 4). These gender-specific differences were not apparent in group A (not shown). BPA cord blood concentrations showed highly significant strong negative associations with gestational age and anthropometric measurements in group B neonates ($r = 0.6$ to $0.7$, p<0.01) (table 5). These associations were not manifested in group A (table 6).

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

P <0.05 is significant
Table 5: Association of cord blood BPA level with gestational age and anthropometric measurements in group B neonates.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BPA level(ng/ml)</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wks.)</td>
<td>-0.671</td>
<td>0.061</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (gm)</td>
<td>-0.717</td>
<td>0.061</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>-0.640</td>
<td>0.061</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>-0.716</td>
<td>0.061</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

P <0.001 is highly significant

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BPA level(ng/ml)</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wks.)</td>
<td>-0.205</td>
<td>0.182</td>
<td>0.137</td>
</tr>
<tr>
<td>Weight (gm)</td>
<td>-0.228</td>
<td>0.182</td>
<td>0.885</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>0.023</td>
<td>&lt;0.001</td>
<td>0.061</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>-0.285</td>
<td>&lt;0.001</td>
<td>0.061</td>
</tr>
</tbody>
</table>

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. Current epidemiological evidence for the association of BPA exposure with adverse birth outcomes, are inconsistent. 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. In Berlin, levels of BPA ranged from 0.2 to 9.2 ng/mL. In Korea the levels ranged from non-detectable to 8.86 ng/mL. Some studies reported much lower levels as the Canadian study, 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 vs 37.47±2.20, p < 0.001). A highly significant negative correlation between BPA level in this group and gestational age was observed. Evidence for decreased gestational duration in relation to BPA has also been reported by other studies. Other studies link maternal BPA exposures to an increase in premature births, as well as small for gestational age babies. Other small-scale human studies of BPA exposure during pregnancy have reported increases in the risk of spontaneous abortion. BPA has been shown to stimulate the production of pro-inflammatory cytokines. Additionally, it has been shown in human populations that BPA concentrations are associated with increased serum C-reactive protein levels. These effects together are thought to initiate an inappropriate inflammatory cascade resulting in preterm birth. 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. 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. Within nested case-control study of mothers giving birth in the Boston area, Cantonwine et al., (2015) 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. Multiple animal and human studies have reported evidence of sex-specific adverse health effects resulting from BPA exposure. 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) 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. 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. Miao et al., (2011) 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) 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. The magnitude of BPA effect on fetal growth may be
influenced by subtle changes of hormones in utero. 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, peroxisome proliferation, cytokine networks, genotoxicity, and oxidative stress. 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. 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 μg/mL) levels of BPA. Morck et al., (2010) 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 choriocarcinoma villous explant cultures. However, certain studies provided conflicting results, reporting an increased weight in offspring whose mothers were exposed to BPA during gestation. Philippat et al., (2012) 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 increased in body weight. Other studies showed no correlation between prenatal bisphenol-A and birth weight. 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.

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