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High fetal bisphenol A exposure enhances IL22 secretion: A proinflammatory cytokine

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Abstract

Background: Bisphenol A (BPA) is an industrial product, widely used in human consumed types of equipment that can be transmitted orally, by inhalation or through dermal absorption and is detectable in many body fluids including cord blood. A correlation between BPA concentration in maternal serum and cord blood has been demonstrated previously, suggesting a possible transfer of BPA via the transplacental path.

Objective: Our objective is to determine the impact of cord blood BPA level on cytokine responses.

Methods: In this cross-sectional study, healthy pregnant women who delivered healthy newborns followed by the Obstetrics and Gynecology Department between September 2016 to June 2017 were enrolled. Cord blood samples were obtained and BPA and IL4, IL5, IL10, IL17, IL22, IFN gama and TGF beta levels were studied by ELISA.

Results: Among 197 deliveries, 176 of them were included in the study. Due to lack of cut-off value, BPA levels were stratified as percentiles. No statistically significant difference was detected in comparison of cytokine levels based on BPA concentrations below and above the 25th and 50th percentiles. Significantly higher IL22 levels (p = 0.007) and increased ratio of IL22/TGFβ (p = 0.04) were detected in those with BPA level above 75th percentile (> 19.16 ng/ml) compared to the below group.

Conclusions: This in vivo real-life study demonstrated that very high BPA levels in cord blood of expectant mothers enhances IL22 secretion in cord blood which is a proinflammatory cytokine. Studies evaluating long term immunological effects on those highly exposed newborns are necessitated.

Key words: Cord blood, cytokines, Interleukin 22, maternal exposure, newborn

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Introduction

Bisphenol A (BPA) is widely used in the manufacturing of many human consumer products such as beverage/food cans, plastic bottles, cosmetics, toys and medical types of equipment, etc.[1,2]

BPA is readily passed into those products, when exposed to high temperatures, and can be transmitted to human orally, by inhalation or through dermal absorption.[3]

Bio-monitoring studies have reported that BPA is detectable in many body fluids such as blood serum, urine, amnionic fluid, breast milk, and cord blood.[4] Previous studies demonstrated that there is a correlation between BPA concentration in maternal serum and cord blood, which raises the possibility of the transfer of BPA via the transplacental path.[5,6]

BPA is accepted as an endocrine-disrupting chemical (EDC) in humans, and the accumulation of the compound has a significant impact on the endocrine system.[3,7,8,9]

Besides causing an endocrine system disruption, fetal BPA exposure was associated with various autoimmune and inflammatory diseases, including allergy and asthma later in life.[1,6,10] Studies have shown that the immune system is sensitive to BPA exposure, and the fetal time is a critical period for the development of the immune system. This susceptibility may result from the immaturity of fetal organs and undeveloped detoxification systems.[11]

Previously, it has been hypothesized that BPA may have immune dysregulating effects such as susceptibilty to allergic immune responses.[10,12] Several studies on experimental animal models have shown that the effect of prenatal BPA exposure may have a modifying impact on the synthesis of cytokines.[1,9,12-14] Studies are indicating that BPA exposure may be linked to immunological outcomes in humans, however, as the human studies have limitations, the link between BPA exposure during pregnancy and immune effects in humans remains uncertain.[15]

Our objective in this study was to determine the impact of different cord blood BPA levels on cytokine responses including IFN-γ, TGF-β, IL10, IL4, IL5, IL17A, IL22. The shifts in the balance of those cytokines may have impact on the skewing of innate and specific immune responses. Based on those preliminary results, hereby, we aimed to evaluate whether the transplacental BPA exposure has an impact on the cord blood cytokine milieu.

Materials and methods

Study Population and Anthropometric Data

The Institutional Ethics Committee approval was obtained before the study (YDU/2015/32-215), and informed parental consent was obtained for each participant.

In this cross-sectional study, All expentant mothers with a healthy pregnancy process who had no known chronic diseases and chronic drug addiction followed by the Obstetrics and Gynecology Department of the University hospital between September 2016 and June 2017 were invited to participate in the study. Pregnant mothers who accepted to participate and delivered healthy newborns were included in the study. Exclusion criteria were: Delivery of newborns with congenital anomalies, perinatal asphyxia and major surgical operation during labour. Cord blood samples were collected during labour for BPA and cytokine measurements.

Blood Sampling and BPA Level Measurement

Cord blood samples from the umbilical vein were obtained at birth and collected into BPA-free polystyrene tubes (BD Diagnostics Preanalytical Systems, BE). Each blood sample was left to coagulate for 30 minutes. Then samples were centrifuged at 2000 g for 10 minutes at room temperature to obtain serum, which was stored in aliquots in BPA-free Eppendorf (Eppendorf AG, GE) vials at -80°C until analysis. On the day of analysis, the aliquots were brought to room temperature and thoroughly vortexed before the analysis.

Biological markers were analyzed by sandwich enzyme-linked immunosorbent assays (ELISAs) kits for BPA (General Bisphenol A ELISA, MyBioSource, Inc., San Diego, California, USA), TGF-β (ELISA, e-bioscience, Vienna, Austria), IL10 (DIASource Immunoassays SA, Louvain-LA-Neuve, Belgium), IL4 (ELISA, e-bioscience, Vienna, Austria), IL5 (ELISA, e-bioscience, Vienna, Austria), IL17a (ELISA, e-bioscience, Vienna, Austria), IL22 (ELISA, e-bioscience, Vienna, Austria), IFN-α (ELISA, e-bioscience, Vienna, Austria). The methods of measurements were carried out according to the manufacturer's instructions.

ELISAs were read with a Spectramax M5 Series Multi-Mode Microplate Reader (Molecular Devices, Sunnyvale, California, USA). The standard curves were created with a software program called Softmax Pro 5.2 that is cable of generating four parametric logistics (4PL) curve-fit.

Statistical Analysis

Statistical analysis was performed using SPSS version 22 for Macintosh (SPSS Inc., Chicago, Illinois, USA). The results were expressed as mean and standard deviation of the mean. Cord blood cytokines were compared with below and above the 25th, 50th, and 75th percentile of cord blood BPA levels. To determine the relationship between principal variables and the other continuous variables, the Pearson correlation test was used. The Mann-Whitney U test was used to determine the relationship between grouped variables. A p-value of less than 0.05 was considered statistically significant.

Results

Among 197 deliveries, 21 of them were excluded based on inclusion and exclusion criteria, leaving 176 healthy term labors in this study. Seventy-seven (43.7%) of newborns were male, and 99 (56.3%) were female. The mean gestational week was 38.20 ± 1.8 weeks. Demographic characteristics of delivery and newborns are presented in **Table 1**. Mean values of the cord blood BPA and cytokine measurements are shown in **Table 2**.

Table 1. Demographic characteristics of study group.

Characteristics (n:176)	
Male (n/%)	77/43.70
Female (n/%)	99/56.30
Caeserean section (n/%)	167/94.80
Normal delivery (n/%)	9/5.20
Birth weight (gr) (Mean ± SD)	3156.76 ± 493.45
Birth height (cm) (Mean ± SD)	48.28 ± 2.04
Head circumference (cm) (Mean ± SD)	34.14 ± 1.74

Table 2. Cord blood BPA and cytokine levels.

Parameter	Concentration (ng/ml) Mean ± SD
BPA	14.5 ± 6.20
IFN-γ	2.52 ± 4.17
TGF-β	40.54 ± 22.70
IL-10	81.18 ± 256.62
IL-4	8.85 ± 10.30
IL-5	1.86 ± 4.96
IL-17A	0.96 ± 0.81
IL-22	2.19 ± 10.65

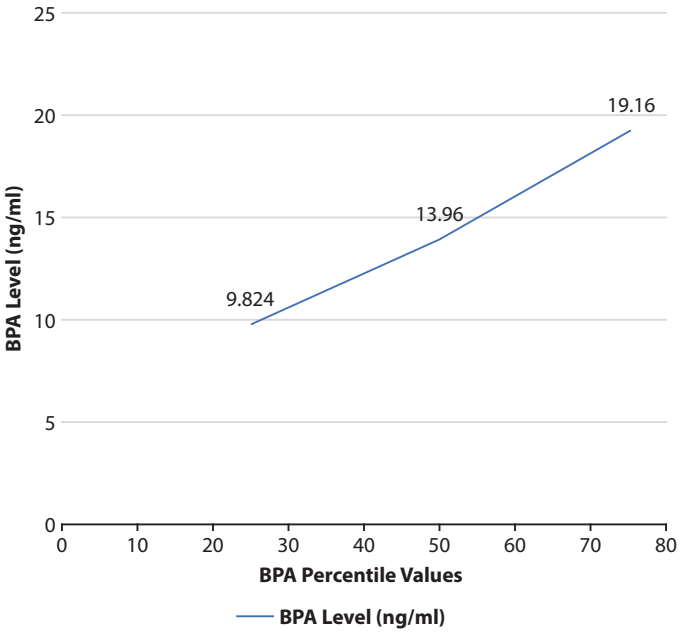

Figure 1. The measured BPA levels were subgrouped as percentiles. 25th, 50th, 75th percentile values of measured cord blood BPA values were calculated (9.84 ng/ml, 13.96 ng/ml, 19.16 ng/ml respectively). Below versus above percentile comparisons were made for all cytokine measurements.

For further comparisons based on the concentration of BPA levels, the measured BPA levels were stratified as percentiles of 25th, 50th, 75th to evaluate the effect of different levels of exposure to BPA on cytokine synthesis. The 25th, 50th, and 75th percentiles of cord blood BPA levels of the whole group were found to be 9.84 ng/ml, 13.96 ng/ml, 19.16 ng/ml, respectively (**Figure 1**).

Below versus above percentile comparisons were made for all cytokine measurements.

There was no statistically significant difference in cytokine levels in the comparisons of BPA concentrations below and above the 25th and 50th percentile groups (**Table 3**). Whereas, the comparison between BPA below versus above 75th percentile groups revealed statistically significant higher IL22 levels (p = 0.007) in the more exposed group (above 75th percentile group) (**Table 3**).

To evaluate whether there is an association between higher BPA level and ratio of cytokine levels in cord blood, multiple comparisons were performed for below versus above 25th, 50th, 75th percentiles for the following ratios: IFNγ/TGFβ, IL4/TGFβ, IL5/TGFβ, IL17/TGFβ, IL22/TGFβ and IFNγ/IL10, IL4/IL10, IL5/IL10, IL17/IL10, IL22/IL10.

There was a statistically significant increase in IL22/TGFβ (p = 0.04) and a decrease in IL5/TGFβ (p = 0.05) ratios in those with BPA level above 75th percentile compared to the below group (**Table 4**). No statistically significant difference was detected in comparisons of the ratio to IL10 of the studied cytokines (data not shown).

Table 4. Comparison of each cytokine level relative to TGF-beta level based on ≤ and > 75th percentiles.

Percentile*	BPA ≤ 75th Mean ± SD	BPA > 75th Mean ± SD	*P* Value
IFNγ/TGFβ	0.15 ± 0.40	0.09 ± 0.16	0.12
IL4/TGFβ	0.58 ± 1.50	0.29 ± 0.59	0.09
IL5/TGFβ	0.13 ± 0.59	0.03 ± 0.07	0.05
IL17A/TGFβ	0.07 ± 0.16	0.04 ± 0.07	0.12
IL22/TGFβ	0.01 ± 0.10	0.16 ± 0.88	0.04

*75th percentile BPA value is 19.16 ng/ml

Table 3. Comparison of cord blood cytokine levels according to 25th, 50th, and 75th percentiles.

Cytokine levels (ng/ml)	≤ 25th Mean ± SD	> 25th Mean ± SD	*P*	≤ 50th Mean ± SD	> 50th Mean ± SD	P	≤ 75th Mean ± SD	> 75th Mean ± SD	*P*
IFN-γ	2.59 ± 4.50	2.3 ± 2.97	0.7	2.05 ± 2.51	2.99 ± 5.30	0.1	2.4 ± 3.70	2.8 ± 5.30	0.7
TGF-β	40.19 ± 52.60	41.58 ± 23.36	0.7	42.9 ± 23.10	38.19 ± 22.20	0.2	41.5 ± 23.50	37.7 ± 10.20	0.3
IL-10	86.2 ± 289.00	85.96 ± 115.90	0.5	57.3 ± 90.50	105.3 ± 350	0.2	61.6 ± 113.90	139.99 ± 473.00	0.3
IL-4	8.9 ± 10.50	8.62 ± 9.50	0.9	8.7 ± 11.20	9.04 ± 9.40	0.8	9.2 ± 11.40	7.6 ± 6.10	0.2
IL-5	1.85 ± 4.70	1.89 ± 5.60	0.9	1.78 ± 0.92	5.1 ± 1.94	0.8	2.01 ± 5.20	1.2 ± 3.90	0.3
IL-17A	0.97 ± 0.80	0.95 ± 0.81	0.9	0.87 ± 0.80	1.05 ± 0.81	0.1	0.97 ± 0.80	0.96 ± 0.80	0.9
IL-22	2.03 ± 10.20	2.5 ± 12.03	0.8	2.5 ± 10.10	1.8 ± 10.00	0.7	0.1 ± 2.10	2.91 ± 12.20	0.007

*25th, 50th, 75th percentile values are 9.82 ng/ml (n = 44), 13.96 ng/ml (n = 45), 19.16 ng/ml (n = 87) respectively

Discussion

Recent studies have revealed that the potential role of endocrine-disrupting chemicals such as BPA may also have an impact on the modification of cytokine responses.[15] Previously conducted in vitro human studies, as well as, murine studies revealed that BPA could pass through the placental barrier, and there is a correlation between maternal and placental BPA levels.[16] Moreover, the correlation between maternal and cord blood BPA concentrations has been demonstrated previously.[5,6] Therefore, in this study, we considered to measure only cord blood BPA levels based on those previous results.

BPA concentrations have been adjusted to supraphysiologic levels in in-vitro and murine studies for the evaluation of their effect on the immune responses. However, those studies do not represent real-life exposures. Hereby, we aimed to measure the impact of real-life BPA exposure during pregnancy by means of measuring cord blood BPA and evaluate its impact on cytokine responses in cord blood.

Cytokines are substances that enable the activity, stimulation, and intra-communication of immune cells, which in turn affects the development of the innate and T cell immune responses. Several murine studies have demonstrated that environmental in utero costimulatory signals have an impact on T helper cell differentiation at the early fetal immune system development.[17] Limited studies are showing the effect of prenatal BPA exposure on the differentiation of newborn T helper cells. Meanwhile, ILCs represent innate immunity which mediate both pro-inflammatory and anti-inflammatory responses not requiring recombination or expansion from memory cells, but responding to antigens rapidly through cytokine secretion. These cells are classified as 3 subgroups - ILC1, ILC2, ILC3- based on their secreted cytokines and transcription factor expressed. ILCs are programmed at the embryogenic stage suggesting their role in the neonatal innate immune system with a higher number and activity level during the neonatal period, unlike the other innate immune cells including neutrophils and monocytes.[17]

In previous studies, it has been demonstrated that there is a dose-related impact of BPA on fetal growth, adiposity, and neonatal complications. There were no significant differences in birth weight, length, and head circumference based on different cord BPA levels.[2] To our knowledge, this is the first in-vivo study in humans that investigated the cord blood BPA exposure level on the cytokine secretion in cord blood. Cord blood BPA was accepted as representative of maternal exposure based on results of previous studies.[5,6] Therefore, we aimed to show the association between the cord blood BPA and cytokine levels including IFN-γ, TGF-β, IL10, IL4, IL5, IL17A, and IL22. This question was prompted by the fact that placental exposure may alter immune system development and may promote the risk of development of certain immune system disorders in later life.[10]

In the current study, cord blood BPA measurements were divided into percentiles 25th, 50th, and 75th to detect the dose-response effect on the cytokine secretion. IFN-γ is produced by a variety of immune cells including ILC1 and Th1 cells playing an essential role in the inflammatory reactions and viral infections. TGF-β and IL10 are anti-inflammatory cytokines that have regulatory functions, aswell. IL4 and IL5 play an important role in the development of certain immune disorders, particularly allergies. IL17A and IL22 are proinflammatory cytokines that are produced mainly by activated ILC3 and Th17 cells.[18] The proinflammatory activity of Th17 cells can be beneficial to the host during infection. However, uncontrolled Th17 activation has been linked to several autoimmune and autoinflammatory pathologies such as multiple sclerosis, arthritis, and lupus.[19]

The in vitro experiments using human cord blood cells have shown that neonates have a shallow frequency or complete absence of Th17 cells due to the lower expression of RORC mRNA.[20] Meanwhile, several experimental murine studies evaluated the impact of various concentrations of BPA exposure on the differentiation of T helper cells into functional subpopulations of Th1, Th2, Th17, and Treg. The preferential development into those subgroups varied between studies, and results were conflicting.[15] It has been reported that gestational exposure of mice to BPA results in increased numbers of Th17 cells and IL17 mRNA expression.[1] BPA induced enhanced Th17 cell activity could provide a mechanism through which BPA might induce inflammatory responses. In a recent murine study prenatal BPA exposure of mothers resulted in intestinal and systemic increased release of IL17 and Th17 - Th1 responses besides decreased Treg responses in the offsprings. In addition the offsprings demonstrated a decreased frequency of IL22 producing ILC3 cells in the small intestinal lamina propria.[21]

Currently, the immunological effect of BPA on human immune responses is unclear. In the current study, cord blood IL22 levels, and the ratio of IL22/TGFβ with high BPA exposure (> 75th percentile) were significantly higher. IL22 is a member of IL10 superfamily, which is the class of potent mediators of inflammatory responses. High IL22 levels generally correlate with the increased ILC3 and Th17 cell activity. ILC3 population is enriched at the mucosal sites during lynphoid tissue organegenesis in fetal life and regulates intestinal hemostasis in the long term through the secretion of IL22.[22]

Also, in the current study ratio of IL5 which is secreted mainly from ILC2 and Th2 cells to the regulatory cytokine TGFβ17 was demonstrated to be low with borderline significance (p = 0.05) in those with higher exposure to BPA (> 75th percentile). The role of BPA exposure in the development of allergy and allergic diseases is conflicting.[10,12,15,23-25]

The major limitation of the current study is that only cord blood cytokine measurements were performed which does not reveal the source of IL22 secretion. Determination of the source cell, such as T helper or ILC, of any cytokine needs further testing by flow cytometric analysis. Another limitation is the absence of long-term neonatal follow-up monitorization of the serum cytokine levels.

In conclusion, the results of this in vivo real-life study demonstrated that very high levels of cord blood (more than 75th percentile) BPA might enhance the proinflammatory responses through the induction IL22 release. The impact of neonatal cytokine shifts on the development of neonatal immune responses may lead to immune system disorders such as allergies and autoimmune diseases later in life. Those patients should be followed up in the long run, and possible adverse effects of BPA exposure should be further investigated.

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