

# High Prenatal Exposure to Bisphenol A Reduces Anogenital Distance in Healthy Male Newborns

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## What is already known on this topic?

Bisphenol A (BPA) is suspected to alter genital development. Several animal studies have shown significant results regarding this issue. At the same time, several human studies have reported significant results on the relationship between BPA and genital development.

## What this study adds?

To our knowledge, this is the first study investigating the relationship between cord blood BPA levels and anogenital measurements in healthy newborns.

## Abstract

**Objective:** To estimate the relationship between cord blood bisphenol A (BPA) levels and anogenital measurements in healthy newborns.

**Methods:** Pregnancy and birth history, together with body mass and length data, anogenital measurements, penile measurements and cord blood samples were obtained from healthy newborns. Cord blood concentration of BPA was analyzed by sandwich enzyme-linked immunosorbent assays kit.

**Results:** Among 130 healthy newborns (72 boys, 58 girls), mean anopenile distance was  $45.2 \pm 6$  mm and anoscrotal distance was  $21.9 \pm 5.4$  mm in boys; mean anoclitral distance was  $33.8 \pm 6.6$  mm and mean anofourchette distance was  $12.2 \pm 4.9$  mm in girls. Mean cord blood BPA level was  $4.75 \pm 2.18$  ng/mL. 90<sup>th</sup> percentile value for cord blood BPA was 8.26 ng/mL and the analysis showed a statistically significant correlation between anoscrotal distance and cord blood BPA levels above the 90<sup>th</sup> percentile ( $p = 0.047$ ) in boys. The changes in anogenital distance in girls were not statistically significant.

**Conclusion:** We showed a significant association between high cord blood BPA levels and shortened anoscrotal distance in male newborns. However, this result should be interpreted with caution since there were no significant external genital abnormalities in our study group.

**Keywords:** Anogenital distance, bisphenol A, cord blood, newborn

## Introduction

Being first produced as a synthetic estrogen, bisphenol A (BPA) was later widely used in the plastic and resin production industry as a plasticizer due to its cross-linking qualities. Exposure of products containing BPA to increased temperatures was shown to cause leakage of this molecule into food and beverages (1,2). BPA is detectable in many body fluids including blood, urine, amniotic fluid, breast milk and cord blood. The anti-androgenic effect of this

chemical has been widely researched and is suspected to be related to several disorders, both in childhood and adulthood (3). The main emphases of current studies are the effect of BPA on obesity, gonadal abnormalities, infertility, thyroid function and malignancy (4,5,6,7,8,9,10,11).

Anogenital distance (AGD) is a relatively new measurement parameter showing the distance from anus to the genitals. While it was used for sex determination in animals for a long time (12,13), the effort to implement this measurement



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as an epidemiological marker of genital development in humans is quite recent. Animal studies have shown a shortened AGD in male offspring reflecting decreased *in utero* androgen exposure and conversely a longer distance in females reflecting increased *in utero* androgen exposure (14).

In this study, we aimed to investigate the relationship between cord blood BPA levels and anogenital measurements in healthy newborns.

## Methods

Near East University Local Ethics Committee approval was obtained prior to the study (approval number: YDU/2015/32-215) and informed parental consent was obtained for each participant. One hundred and fifty healthy newborns up to three days of age who were born in the period of May-August 2016 were included in the study population. Infants who had congenital anomalies, perinatal asphyxia, major surgical operation and those who were hospitalized in the neonatal intensive care unit were excluded from the study. Pregnancy and birth history together with body mass and length data were obtained from the patients' hospital records.

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. Total serum concentration of BPA was analyzed by sandwich enzyme-linked immunosorbent assays (ELISA) kit (General Bisphenol A ELISA, MyBioSource, Inc., San Diego, California, USA) with a Spectramax M5 Series Multi-Mode Microplate Reader (Molecular Devices, Sunnyvale, California, USA). The kit is characterized by a limit of detection for BPA of 0.6 ng/mL.

The anogenital measurement technique for the study was standardized as follows. The infants were placed in supine position, with flexed hips and knees to provide a "frog leg" posture. After marking the center of the anus with a pencil, the distances from the anus to the anterior base of the penis; anopenile distance ( $AGD_{AP}$ ) and to the base of the scrotum; anoscrotal distance ( $AGD_{AS}$ ) were measured

in boys. In girls, the distance from anus to the anterior tip of the clitoral hood; anoclitoral distance ( $AGD_{AC}$ ) and the posterior fourchette of labia majora; anofourchette distance ( $AGD_{AF}$ ) were measured. Two blinded (digital screen turned away from the researcher) measurements per patient with digital Vernier caliper were obtained from five newborns by two different researchers as a pilot study to assure the right measurement technique. The study proceeded after the measurement consistency was ensured. All measurements were performed by one blinded researcher and the results were analyzed and interpreted by a second blinded researcher.

## Statistical Analysis

Statistical analysis was performed using SPSS version 22 for Macintosh (SPSS Inc., Chicago, Illinois, USA). The results are expressed as mean and standard deviation of the mean. 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 less than 0.05 was considered statistically significant.

## Results

Twenty newborns were excluded due to the exclusion criteria, leaving 130 patients in the study group. This consisted of 72 (55%) boys and 58 (45%) girls. The mean birth weight of the group was  $3172 \pm 492$  grams and mean birth length was  $48.3 \pm 2$  cm. In boys, the mean  $AGD_{AP}$  was  $45.2 \pm 6$  mm and  $AGD_{AS}$  was  $21.9 \pm 5.4$  mm. In girls, the mean  $AGD_{AC}$  was  $33.8 \pm 6.6$  mm and mean  $AGD_{AF}$  was  $12.2 \pm 4.9$  mm. Mean cord blood BPA level was  $4.75 \pm 2.18$  ng/mL (Table 1). The 90<sup>th</sup> percentile value of the cord blood BPA was 8.26 ng/mL. None of the patients had any obvious genital development abnormality.

In general, anogenital measurements did not show statistically significant correlations with the cord blood BPA levels ( $p > 0.05$ ). However, a significant negative correlation was found between  $AGD_{AS}$  and cord blood BPA levels above the 90<sup>th</sup> percentile ( $p = 0.047$ ) in boys.  $AGD_{AS}$  mean value was significantly lower in the group with cord blood BPA levels above 90<sup>th</sup> percentile (higher than 8.26 ng/mL). In contrast, an apparent but not statistically significant increase was noted in  $AGD_{AP}$  with increased levels of BPA. In girls, a statistically nonsignificant increase in the  $AGD_{AC}$  and a similar decrease in the  $AGD_{AF}$  was found in the group with high cord blood BPA levels (Table 2).

**Table 1. Anthropometric measurements (anopenile distance, anoscrotal distance, anoclitral distance, anofourchette distance) and cord blood bisphenol A levels**

Measurement	Mean ± SD	Range
Birth weight (g)	3172 ± 492	1540-4525
Birth length (cm)	48.3 ± 2	40-52
AGD <sub>AP</sub> (mm)	45.2 ± 6	10.3-57.9
AGD <sub>AS</sub> (mm)	21.9 ± 5.4	7.9-36.5
AGD <sub>AC</sub> (mm)	33.8 ± 6.6	12.3-45.4
AGD <sub>AF</sub> (mm)	12.2 ± 4.9	7.2-36.5
Cord blood BPA level (ng/mL)	4.75 ± 2.18	1.58-10.8

SD: standard deviation, AGD: anogenital distance, AGD<sub>AP</sub>: anopenile distance, AGD<sub>AS</sub>: anoscrotal distance, AGD<sub>AC</sub>: anoclitral distance, AGD<sub>AF</sub>: anofourchette distance, BPA: bisphenol A

**Table 2. The relationship between anogenital (anogenital distance, anopenile distance, anoscrotal distance, anoclitral distance, anofourchette distance) measurements and 90<sup>th</sup> percentile of cord bisphenol A values**

	Mean value in group under 90 <sup>th</sup> percentile for BPA	Mean value in group above 90 <sup>th</sup> percentile for BPA	p
AGD <sub>AP</sub> (mm)	45.2	45.7	0.956
AGD <sub>AS</sub> (mm)	22.3	17.7	0.047
AGD <sub>AC</sub> (mm)	33.5	35.4	0.471
AGD <sub>AF</sub> (mm)	12.2	12	0.652

AGD: anogenital distance, AGD<sub>AP</sub>: anopenile distance, AGD<sub>AS</sub>: anoscrotal distance, AGD<sub>AC</sub>: anoclitral distance, AGD<sub>AF</sub>: anofourchette distance, BPA: bisphenol A

## Discussion

BPA, a well-known endocrine disruptor has been investigated for more than a decade. Leaching of this molecule from daily used plastic products is highly dependent on heat, on contact with chemicals and deterioration of the product itself (15,16). Drinking water carries risk of pollution by BPA as plastic bottles used for household water dispensers are being reused and exposed to high temperatures during the cleaning process. BPA is suspected to have an anti-androgenic effect on genital development *in utero*. Studies regarding the prenatal effect of BPA on fetal development have mostly been performed in rodents and confirm adverse effects of increased BPA exposure on the growth and genital development of the offspring (14,17). The mechanism of action of BPA is thought to be through its binding to estrogen receptors thus triggering their activation. However, some authors do not attribute the anti-androgenic action solely to estrogen receptor activation as BPA has the ability

to interact with other receptors such as aryl hydrocarbon receptor (the androgen receptor behaving as an antagonist) and the seven transmembrane domain estrogen receptor (G protein-coupled receptor 30) (18,19).

Several human studies have reported significant results regarding the relationship between BPA and genital development. Liu et al (20), in a study that compared maternal, urinary BPA and cord blood sex hormones, maternal urinary BPA was found to be negatively associated with cord blood testosterone levels. The authors proposed that BPA might decrease testosterone levels by affecting both the testes and the pituitary system or by inhibiting the testosterone surge *in utero*. The hypothesis that BPA may reduce testosterone acting as estradiol was also suggested by Nakamura et al (21) in a different study. The effects of endocrine disrupting molecules on androgens are thought to be more profound in the masculinization programming window (8-14 gestational weeks) during intrauterine life, especially in male offspring (22). The findings in our study are coherent with this postulate.

AGD measurement, as an epidemiological marker of sexual development, is still controversial due to conflicting results from different studies. Miao et al (23) showed a significant relationship between AGD in boys and their parents' occupational BPA exposure. In a study in adults, Eisenberg et al (24) found a significant association between serum testosterone levels and AGD. However, Parra et al (25) reported no significant relationship between anogenital measurements, reproductive hormone levels and semen quality.

To our knowledge, this is the first study investigating the relationship between cord blood BPA levels and anogenital measurements in neonatal human subjects. In our study, BPA values in cord blood were found to be higher than those reported in the literature (20,26,27,28). This may be due to poor governmental regulation of water supply companies in our country and the continuous reuse of plastic containers beyond their lifespan. We know that BPA is not the only phenolic endocrine disrupting substance that may be implicated in altering the reproductive development of the fetus, as prenatal exposure to other phenolic molecules and phthalates was also shown to alter AGDs (29). As other environmental pollutants were not measured during our investigation, we can neither confirm nor dismiss their potential adverse effects in our study group.

AGD is measured in different ways in different studies. AGD<sub>AS</sub> in boys and AGD<sub>AF</sub> in girls are mostly accepted as AGDs.

We measured the distances from anus to the anterior base of the penis ( $AGD_{AP}$ ) and the base of the scrotum ( $AGD_{AS}$ ) in boys; the distance from anus to the anterior tip of the clitoral hood ( $AGD_{AC}$ ) and the posterior fourchette of labia majora ( $AGD_{AF}$ ) in girls, as described by Sathyanarayana et al (30). The most important finding in our study population was that male newborns in the group with a cord blood BPA level over 90<sup>th</sup> percentile (8.26 ng/mL) had significantly shorter  $AGD_{AS}$  values compared to the group with lower cord blood BPA levels and this finding may reflect the anti-androgenic effect of BPA on fetal male genital development *in utero*.  $AGD_{AC}$  was longer in the group with high cord blood BPA levels, although this finding was not statistically significant. The  $AGD_{AF}$  did not show any major change with increased levels of BPA. Despite variations in AGDs, none of the patients in our study population had any external genital abnormality which suggests that these changes did not have a major impact on the development of the external genitalia. However, other adverse effects of BPA need to be further investigated.

### Study Limitations

One of the major limitations of our study was the relatively small study sample. Larger series are needed to confirm our results. Another limitation is the high coefficient of variability for which we do not have a clear explanation.

### Conclusion

The results of our study showed a significant association between high cord blood BPA levels and shortened  $AGD_{AS}$  in healthy male newborns. Based on our findings, we suggest that even if BPA has any effect on genital development *in utero*, this effect is subtle at low dose exposure.

### Ethics

**Ethics Committee Approval:** The study was approved by the Near East University. Local Ethics Committee (approval number: YDU/2015/32-215).

**Informed Consent:** Informed parental consent was obtained for each participant.

**Peer Review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Emil Mammadov, Ceyhun Dalkan, Concept: Emil Mammadov, Ceyhun Dalkan, Design: Emil Mammadov, Ceyhun Dalkan, Data Collection or Processing: Emil Mammadov, Ceyhun Dalkan, Murat Uncu, Analysis or Interpretation: Emil Mammadov, Ceyhun

Dalkan, Murat Uncu, Literature Search: Emil Mammadov, Writing: Emil Mammadov, Ceyhun Dalkan.

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