RESEARCH LETTER

# Bisphenol A levels are negatively correlated with serum vitamin D–binding protein and sex hormone–binding globulin levels in women with polycystic ovary syndrome: a pilot study

Diana Jędrzejuk[1], Justyna Kuliczkowska-Płaksej[1], Andrzej Milewicz[1,2], Kamila Wilczewska[3], Jacek Namieśnik[3], Aleksandra Rutkowska[4]

1 Department of Endocrinology, Diabetology and Isotopes Therapy, Wroclaw Medical University, Wrocław, Poland
2 Faculty of Natural Sciences and Technology, Karkonosze College in Jelenia Góra, Jelenia Góra, Poland
3 Department of Analytical Chemistry, Gdańsk University of Technology, Gdańsk, Poland
4 Department of Nursing Management, Medical University of Gdańsk, Gdańsk, Poland

**Introduction** Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age and is characterized by hormonal, biochemical, and metabolic abnormalities.[1] Recently, bisphenol A (BPA), a monomer widely used in the production of polycarbonate and epoxy resins and as an additive in polyvinyl chloride,[2] was linked to the pathogenesis of PCOS.[3] In terms of its mechanism of action, BPA can interact with both estrogen and androgen receptors.[3]

Vitamin D deficiency has also been shown to be highly prevalent in women with PCOS[4] and to contribute to the etiology of the syndrome; however, the results are controversial.[1] To evaluate vitamin D metabolism and deficiency, serum vitamin D–binding protein (VDBP) levels should be determined, because this parameter affects free and bioavailable 25-hydroxyvitamin D [25(OH)D] levels.[5] VDBP is secreted by the liver along with other proteins (eg, sex hormone–binding globulin [SHBG]). It has also been suggested that VDBP is a biochemical predictor of liver fibrosis.[6]

Recently, negative correlations have been found between serum levels of 25(OH)D and BPA in humans,[7] but the effect of serum BPA levels on VDBP concentrations in women with PCOS has not been assessed. In this pilot study, we aimed to determine the relationships between serum VDBP, vitamin D, SHBG, and BPA levels in women with PCOS.

**Patients and methods** A total of 63 women were recruited from among patients of the Department of Endocrinology, Diabetology and Isotope Therapy at Wroclaw Medical University (Wrocław, Poland) during the years 2011 to 2013. The mean (SD) age was 25.6 (5.7) years. PCOS was diagnosed in 27 women according to the European Society of Human Reproduction and Embryology and American Society for Reproductive Medicine (ASRM) consensus on diagnostic Rotterdam criteria. The control group was recruited from healthy volunteers (doctors, nurses, or students) with regular and ovulatory menstrual cycles. The exclusion criteria in both groups were diabetes, parathyroid function disorders, hyperlipidemia, special diet, intense physical exercise, smoking, and the use of oral contraceptives, calcium, and vitamin D supplements. None of the patients with PCOS or controls suffered from a serious and chronic illness.

Blood samples were collected from the cubital vein in the morning after an overnight fast at the follicular phase, between May and September. Hormonal and biochemical parameters included: 1) serum total testosterone, androstenedione, and SHBG measured by a chemiluminescent method (IMMULITE 2000; Siemens Healthcare, Erlangen, Germany); 2) serum 25(OH)D levels measured by a radioimmunoassay kit (RIA ImmunoAssays, Nivelles, Belgium); 3) serum VDBP measured using a human VDBP ELISA kit (Quantikine ELISA kit, R&D Systems, Minneapolis, Minnesota, United States); 4) serum bilirubin, γ-glutamyltransferase (GGT), alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) measured by commercial kits using Architect

Correspondence to:
Diana Jędrzejuk, MD, PhD,
Department of Endocrinology,
Diabetology and Isotopes Therapy,
Wroclaw Medical University,
ul. Wybrzeże L. Pasteura 4,
50-367 Wrocław, Poland,
phone: +48 71 784 25 65,
email: diana.jedrzejuk@gmail.com
Received: November 22, 2018.
Revision accepted: January 11, 2019.
Published online: January 16, 2019.
Conflict of interest: none declared.
Pol Arch Intern Med. 2019;
129 (2): 133-136
doi:10.20452/pamw.4419

**TABLE 1** Comparison of demographic, clinical, and laboratory characteristics between women with polycystic ovary syndrome and controls

| Parameters | PCOS group (n = 27) | Control group (n = 36) | *P* value |
|---|---|---|---|
| Age, y | 24.6 (5.0) | 26.9 (6.7) | 0.07 |
| BMI, kg/m$^2$ | 22.0 (1.5) | 22.0 (1.3) | NS |
| Waist circumference, cm | 73.0 (4.8) | 74.3 (4.5) | NS |
| BPA, ng/ml | 18.8 (16.4) | 15.9 (12.0) | NS |
| Total testosterone, ng/ml | 0.5 (0.2) | 0.4 (0.1) | <0.001 |
| SHBG, nmol/l | 53.3 (20.0) | 63.4 (20.5) | 0.03 |
| FAI | 3.7 (1.8) | 2.6 (1.4) | 0.003 |
| Androstenedione, nmol/l | 4.6 (2.0) | 3.0 (1.1) | <0.001 |
| 25(OH)D, ng/ml | 24.7 (9.8) | 26.0 (7.7) | NS |
| Vitamin D deficiency, n (%) | 7 (26) | 7 (19) | NS |
| VDBP, ng/ml | 38.3 (7.9) | 40.4 (7.6) | NS |
| Alkaline phosphatase, IU/l | 66.2 (13.7) | 61.9 (13.3) | NS |
| AST, IU/l | 21.3 (7.1) | 20.5 (4.8) | NS |
| ALT, IU/l | 16.6 (6.5) | 18.9 (9.6) | NS |
| AST/ALT ratio | 1.4 (0.4) | 1.2 (0.3) | 0.04 |
| GGT, IU/l | 20.0 (10.1) | 18.0 (6.3) | NS |
| Bilirubin, mg/dl | 0.7 (0.3) | 0.6 (0.2) | 0.01 |

Data are expressed as mean (SD) unless otherwise indicated. A *P* value of less than 0.05 was considered significant.

SI conversion factors: to convert bilirubin to µmol/l, multiply by 17.1; testosterone to nmol/l, by 3.467; 25(OH)D to nmol/l, by 2.496.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; BPA, bisphenol A; FAI, free androgen index; GGT, γ-glutamyltransferase; PCOS, polycystic ovary syndrome; SHBG, sex hormone–binding globulin; NS, nonsignificant; VDBP, vitamin D–binding protein; 25(OH)D, 25-hydroxyvitamin D

Plus ci4100 (Abbott Diagnostics, Lake Forrest, Illinois, United States).

Free androgen index (FAI) was calculated using the following formula: FAI = serum total testosterone concentration (nmol/l) × 100/SHBG (nmol/l). The AST/ALT ratio was also calculated.

Serum BPA concentrations were assessed using high-performance liquid chromatography with tandem mass spectrometry at the Department of Analytical Chemistry, Gdańsk University of Technology, Poland. A Shimadzu LCMS-8050 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization source working in the negative multiple reaction monitoring mode was used for the analysis. The method was described in detail elsewhere.[8]

The study was performed in accordance with the guidelines of the 1964 Declaration of Helsinki on human experimentation and with approval of the local ethics committee. Informed consent was obtained from all participants.

The data were analyzed using the STATISTICA 12.0 package (StatSoft Polska, Kraków, Poland). The Shapiro–Wilk test was used for distribution analysis. Differences in clinical parameters between the groups were assessed with the unpaired *t* test or the nonparametric Mann–Whitney test. Associations between serum BPA levels and VDBP and 25(OH)D levels, as well as between serum BPA and liver biomarkers, were analyzed using the Pearson product-moment correlation (for data with normal distribution) or the Spearman rank-order correlation (for data that did not pass the normality test). The results were considered significant at a *P* value of less than 0.05.

**Results** Both groups were similar in terms of age, body mass index, and waist circumference (TABLE 1). Women with PCOS presented significantly higher mean serum total testosterone and androstenedione levels as well as FAI value, but lower serum SHBG levels, than controls. Serum BPA, VDBP, and 25(OH)D levels as well as the frequency of vitamin D deficiency (<20 ng/ml) were comparable between groups. In contrast to the control group, woman with PCOS showed a negative correlation between serum BPA and VDBP levels ($r$ = –0.46; $P$ = 0.02) and SHBG levels ($r$ = –0.46; $P$ = 0.02). We found no correlation between serum BPA and 25(OH)D levels in either group.

To explain the differences in the association between serum BPA concentrations and liver proteins (VDBP and SHBG) in women with PCOS and the control group, we also measured the concentrations of liver biomarkers (AST, ALT, ALP, and GGT) and bilirubin. Serum bilirubin levels and the AST/ALT ratio were significantly higher in women with PCOS than in controls (TABLE 1). Also, bilirubin levels correlated positively with BPA in the PCOS group ($r$ =

0.45, $P = 0.02$), controls ($r = 0.35$, $P = 0.046$), and the whole study group ($r = 0.42$, $P = 0.001$). There were no correlations between VDBP and SHBG concentrations and any liver biomarkers.

**Discussion** The data on serum BPA levels in women with PCOS are limited and controversial. In our study, we found comparable serum BPA levels in women with PCOS and healthy controls matched for age and body mass index. Our data are in contrast with the findings of another study, which showed a higher serum BPA concentration in adolescents and adult women with PCOS compared with nonobese healthy controls.[9] Nonetheless, it is difficult to compare the results of these studies because of the different patient ethnicities, study protocols, biological material collected, and methods used to assess BPA levels.

The role of vitamin D in PCOS is still controversial. Some studies have shown a close association between 25(OH)D deficiency and negative metabolic consequences in women with PCOS, but others have reported similar serum levels of vitamin D in women with PCOS and controls.[4,10] In our study, we found comparable levels of serum 25(OH)D in women with PCOS and healthy controls and a relatively large number of cases with vitamin D deficiency (26% of women with PCOS vs 19% of women in the control group). Our observation is supported by recent data from the Netherlands.[4]

Serum VDBP levels in both groups were comparable, but we found a significant negative correlation between serum VDBP, SHBG, and BPA levels only in women with PCOS.

Because VDBP was discovered as a novel biomarker in liver fibrosis (hepatic metabolism),[6] we explored the relationship between serum BPA concentrations and the levels of liver biomarkers and bilirubin. In our study, serum BPA did not correlate with liver enzymes, but there was a significant correlation between serum BPA and bilirubin levels in women with PCOS and the control group. Our results are not in line with previous observations in women with PCOS by Tarantino et al,[11] who reported that serum BPA levels correlated with liver enzymes; bilirubin levels were not determined in their study.

In our preliminary study, we found for the first time a significant relationship between serum BPA and bilirubin levels in women with PCOS. Until now, the cumulative hepatotoxicity of BPA was presented only in rats by dose-dependent increases in serum biochemical markers, ALT, ALP, and bilirubin.[12] We postulate that bilirubin levels can be the most sensitive marker of early liver dysfunction associated with exposure to BPA. The higher AST/ALT ratio in women with PCOS supported this concept, which requires further investigations. On this basis, we hypothesize that the different relationships between VDBP and BPA in women with PCOS in comparison with controls can be explained by liver dysfunction.

Our preliminary study has some limitations, including the small number of participants and no information about duration of exposure to BPA in either group. Another limitation is that all studied women were of normal weight, which prevented our assessment of the effect of BPA on obesity. The strengths of our pilot study include the very precise and sensitive method for BPA estimation.[8] Moreover, this is the first study to show the associations between serum VDBP and BPA levels in women with PCOS, as well as bilirubin and BPA in women with PCOS and healthy controls.

In summary, to our knowledge, this is the first study to show the association between serum VDBP, SHBG, and BPA levels in nonobese women with PCOS compared with healthy controls. Our findings require further research in a larger cohort of women with PCOS and different body weights.

**ACKNOWLEDGMENTS** Editorial support was provided by Proper Medical Writing Sp. z.o.o., Warsaw, Poland. This work was supported by grant from the Polish Ministry of Science and Higher Education (no. NN402 318 839; to AM).



## REFERENCES

1 Bellver J, Rodriguez-Tabernero L, Robles A, et al. Polycystic ovary syndrome throughout a woman's life. J Assist Reprod Genet. 2018; 35: 25-39.

2 Szybiak A, Rutkowska A, Wilczewska K, et al. Daily diet containing canned products significantly increases serum concentrations of endocrine disruptor bisphenol A in young women. Pol Arch Intern Med. 2017; 28: 278-280.

3 Akin L, Kendirci M, Narin F, et al. The endocrine disruptor bisphenol A may play a role in the aetiopathogenesis of polycystic ovary syndrome in adolescent girls. Acta Paediatr. 2015; 104: e171-e177.

4 Krul-Poel YHM, Koenders PP, Steegers-Theunissen RP, et al. Vitamin D and metabolic disturbances in polycystic ovary syndrome (PCOS): A cross--sectional study. PLoS One. 2018; 4: e0 204748.

5 Bikle DD, Gee E, Halloran B, et al. Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. J Clin Endocrinol Metab. 1986; 63: 954-959.

6 Ho AS, Cheng CC, Lee SC, et al. Novel biomarkers predict liver fibrosis in hepatitis C patients: alpha 2 macroglobulin, vitamin D binding protein and apolipoprotein AI. J Biomed Sci. 2010; 17: 58-64.

7 Erden ES, Genc S, Motor S, et al. Investigation of serum bisphenol A, vitamin D, and parathyroid hormone levels in patients with obstructive sleep apnea syndrome. Endocrine. 2014; 45: 311-318.

8 Wilczewska K, Namiesnik J, Wasik A. Troubleshooting of the determination of bisphenol A at ultra-trace levels by liquid chromatography and tandem mass spectrometry. Anal Bioanal Chem. 2016; 408: 1009-1013.

9 Takeuchi T, Tsutsumi O, Ikezuki Y, et al. Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction. Endocr J. 2004; 51: 165-169.

10 Bostanci EI, Ozler S, Yilmaz NK, Yesilyurt H. Serum 25-hydroxy vitamin D levels in Turkish adolescent girls with polycystic ovary syndrome and

the correlation with clinical/biochemical parameters. J Pediatr Adolesc Gynecol. 2017; 31: 270-273.

**11** Tarantino G, Valentino R, Di Somma C, et al. Bisphenol A in polycystic ovary syndrome and its association with liver-spleen axis. Clin Endocrinol (Oxf). 2013; 78: 447-453.

**12** Hassan ZK, Elobeid MA, Virk P, et al. Bisphenol A induces hepatotoxicity through oxidative stress in rat model. Oxid Med Cell Longev. 2012; 2012: 194829.