# CORRELATION STUDY OF URINARY BISPHENOL-A IN A SAMPLE OF EGYPTIAN WOMEN WITH POLYCYSTIC OVARY SYNDROME

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### ABSTRACT

Background: Polycystic Ovary Syndrome (PCOS) is a typical Endocrine Syndrome in women. It has 5 to 10% prevalence. **Objective:** This study aims to investigate the Bisphenol-A (BPA) as a marker for PCOS with a high level in women with PCO, and to evaluate the ability of urinary noninvasive method for the detection of BPA. The secondary outcomes from our study were to correlate the BPA level with fasting insulin levels, and free testosterone in women with PCO, and if there is association with infertility. **Participants and methods:** A cross sectional study was made in 2 groups (group 1; women with PCOS (60 women) and group 2; non PCOS women (60 women). Detection of urinary Bisphenol-A was made by using HPLC. The measurement of serum FSH, LH, fasting insulin and free testosterone levels was performed. **Results:** Women with PCO had a significant high urinary Bisphenol A level and more than the control group ( $3439.05 \pm 2118.25 \ \mu g \ /mL$  and  $813.84 \pm 584.78 \ \mu g \ /mL$ , P<0.001, respectively). A positive correlation between the urinary Bisphenol-A levels and the serum LH was determined as well as the free testosterone and fasting insulin (P<0.001). On contrary, there is a negative correlation between the urinary Bisphenol-A and the levels of the serum FSH. By using logistic regression analysis, BPA was the main independent variable and significantly associated with PCOS with adjusted Odds Ratio (OR) equal to 1.3 (95% CI: 0.25-6.73, P<0.001). Conclusion: The urinary method for Bisphenol-A level detection can be used for women and is taken as an easy noninvasive method. There is a positive correlation between elevated Bisphenol-A levels and PCO and infertility. The results of this study indicated that BPA might play a major role in the PCOS pathogenesis. Thus, our advisement is limiting usage of these substances which including BPA, especially in food products and other related plastic products.

Keywords: Bisphenol-A, concentration, PCO, urinary, reproductive health, Fasting insulin

### **INTRODUCTION**

Polycystic Ovary Syndrome (PCOS) is a typical to Endocrine Syndrome in women. It has 5 to 10% prevalence (**Rostamtabar et al 2021**).

Ladies having PCOS complain from menstrual abnormalities and infertility, hyperandrogenism causing hirsutism, and acne (Gilbert ET AL, 2018; Neven et al, 2018; Pan et al, 2021). This syndrome is associated with insulin resistance which causes central obesity beside its metabolic sequels. Thus, sometimes women having PCOS suffer from impaired glucose tolerance which is a cause of developing type 2 diabetes mellitus (T2DM); atherogenic dyslipidemia, high serum triglycerides and elevated blood pressure (**Jin & Xie, 2018**).

In spite of the causes of polycystic ovarian syndrome have not been understood yet clearly, the PCOS may be developing from multiple factors such as; genetic which is responsible for resistance to insulin with hyperinsulinemia and hyperandrogenism. However, in recent decades, there are researches declare that role of ethnicity, geographic place and environmental that influences alongside the lifestyle as a predisposing factor for PCOS (Jin & Xie, 2018).

Endocrine disruptors (EDs) comprise a diversity of chemicals, as pesticides, plasticizers, industrial by-products, and pollutants. Thus, EDs can be a reason of many metabolic syndromes or even hormone-dependent tumors (i.e., uterine or breast tumors). The most common contaminant Bisphenol-A pollutants are (BPA). polychlorinated biphenyls (PCB), phthalates and dioxins. They have a lipophilic construction and have ability to be bio accumulated in the fatty tissue of all living organisms (Jin & Xie, 2018). Bisphenol-A is composed of phenol and acetone; it is used in excretion of epoxy, polyester and polycarbonate resins which are used in plastics, adhesives, and structural compounds. Therefore, the Bisphenol-A acts as endocrine disruptor via interfering with transcription of proteins and hormones. Regarding to its phenolic nature, BPA has been proved to interact with estrogenic receptors (Khan et al., 2019).

Hence, human beings are at the end of the food category sequence, so that they are liable to the high amount of those composites. Often, EDs are detected in human adipose tissue and biological fluids (milk, serum, urine, amniotic fluid) (Rowdhwal, & Chen, 2018; Mahemuti, et al., 2018; Jalal, et al., 2018; Zhou, et al., 2019).

BPA can influence androgen synthesis in ovarian theca cells. Also, there is a relation between high levels of serum testosterone detected in women having PCOS and BPA serum levels (**Akgül, et al., 2019; Lu, et al., 2020**).

#### **AIM OF THE STUDY**

This study aims to investigate the Bisphenol-A (BPA) as a marker for PCOS with a high level in women with PCO, and to evaluate the ability of urinary noninvasive method for the detection of BPA.

The secondary outcomes from our study were to correlate the BPA level with fasting insulin levels, and free testosterone in women with PCO, and if there is association with infertility.

#### PARTICIPANTS AND METHODS

### • Study Design

This an observational design, a case-control study.

• Study Setting and Location

The study was conducted in Algezeera Hospital, Egypt from December 2018 till January 2020.

### • Study Population

Case group includes women having polycystic ovary syndrome PCOS and are diagnosed by Ultrasound and biochemical tests. The Control group includes women without the diagnostic criteria of PCO. In both groups the age was in between 18 and 40 years.

Each woman was subjected to a transvaginal US after a full history was taken.

## Eligibility Criteria

### **Inclusion Criteria**

Egyptian Women with age in between (18-40) who were diagnosed of having polycystic ovary syndrome PCOs were enclosed in case group, while women without PCO diagnostic criteria were enclosed in control group. According to the European Society of Human Reproduction and Embryology, criteria for PCO women selection were determined as well as to the American Society for Reproductive Medicine Rotterdam (ESHRE/ASRM). Criteria for PCO diagnosis was defined by finding 2 out of 3 criteria: anovulation or oligo-ovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovarian morphology (PCOM) via US. Additionally, the Androgen Excess Society defined PCOS has as hyperandrogenism with ovarian dysfunction or polycystic ovaries (Zhang, et al., 2018).

Ultrasound (US) was made for each woman by Voluson E8 transvaginal probe; US criteria of PCO were detecting the following: increased follicle number per ovary (FNPO), peripherally arranged follicles with their diameter measured size 2-9 mm, ovarian enlargement volume more than 10 mL, and central stromal brightness. Only one ovary has these criteria is considered enough to be described PCO via using the US (Nylander, et al., 2017).

### **Exclusion Criteria:**

Any Egyptian woman with current smoking, chronic diseases (DM), hypertension (140/85mm), heart disease, tumors and renal impairment were excluded.

#### **Bisphenol-A detection method:**

Detection of urinary Bisphenol-A from a morning urine sample via HPLC was performed. A high-pressure isocratic system was used consisting of a Dionex UltiMate 3000 UHPLC; RS pump, auto sampler, column compartment, and DIODE ARRAY detector. A chromatographic column reversed phase 150 mm $\times$  4.6 mm Hypersil BDS with an attached C<sub>18</sub> particle 5u in size. The method of detection included both conjugated and unconjugated BPA.

Urine (500  $\mu$ L) was buffered with 30  $\mu$ L of 2.0 M sodium acetate buffer (pH 5.0) and was enzymatically hydrolyzed by ßglucuronidase/sulfatase (4,414/168 U/µL) for 3 hrs at 37°C in a shaking water bath. After centrifugation, 4 mL of the produced supernatant was transferred to a new tube and evaporated. The residue was dissolved with 200  $\mu$ L of 60% acetonitrile in water, and then 40  $\mu$ L of the solution was injected into the highperformance liquid chromatography (HPLC) system. The measurement of FSH, LH, fasting insulin and free testosterone levels was made for every woman.

### Sample Size:

To compare between women with PCOS in the case group and women without PCOS in the control groups concerning to the amount of urinary Bisphenol, the sample size calculation would be done using PASS software version 11 for MS-Windows. Our calculation of the minimum proper sample size is 53 patients for each group and a difference of 197.7 between the null- and alternative hypotheses was detected. Since, the null hypothesis group mean is 941.9 while the alternative hypothesis mean of group 2 is 1139.7 and the estimated standard deviations of groups are 428.0 and 266.6 respectively. The power is 80% with ( $\beta$ =0.2) and 95% significance level ( $\alpha = 0.05$ ) using a two-sided two-sample t-test. The Sample size was increased 10% for drop out allowance to give us a value of **60** patients for each group.

### Statistical Analysis

The Statistical analyses were performed using the SPSS software (SPSS, version 25, PSS. Inc., IL, USA). Shapiro test was used to determine the distribution of measured variables which are presented as mean value  $\pm$ For normally distributed variables, SD. student's t-test has been used to estimate the statistical significance of the differences for the numerical data: also. categorical data differences were compared using the Chisquare test. Pearson Correlation was used to analyze numerical variables. ROC curve analysis was also done for estimating the sensitivity and specificity of the obtained cutoff values of the biochemical markers of the

PCOS diagnosis. For all statistical tests, the statistical significance would be accredited, if the p-values were less than 0.05. Logistic regression was done to detect independent predictors of PCOS.

### **Consent:**

Before participation in this research, informed consents were obtained from participants which conform to the Declaration of Helsinki provisions. The study was conducted after the approval of the ethical committee of Aljazeera Hospital (Approval Number 0021).

### <u>RESULTS</u>

The presented study was conducted on 60 women having PCOS (cases group) as well as another 60 women without having PCO (control group). Women in the PCO group had shown ultrasound diagnostics of PCOS, while all women in the control group had shown nothing of those features.

Table 1 compares the demographic, clinical, and hormonal characteristics of women in both groups. A comparison between both groups do not reveal any significant differences in age (P=0.845). Whereas the body mass index BMI in the PCOS group was significantly higher than the control group (P 0.01) as expected.

Moreover, the infertility, hirsutism, and acne were being found more significantly higher in the PCO group with more BPA levels (P<0.001) than the control group too. Women having PCO had also a remarkable high levels of serum LH, free testosterone, and fasting insulin (P<0.001) with a significant low levels of serum FSH (P<0.001) than the control group of women without PCO.

Similarly, women having PCO had expressively higher urinary Bisphenol A level more than the control group  $(3439.05 \pm 2118.25 \ \mu g /mL vs. 813.84 \pm 584.78 \ \mu g /mL, P<0.001)$  (Fig. 1).

Regarding infertility study in relation with level of BPA, number of infertile females with high level (above 90th percentile) of BPA was 18 while number of non-infertile women having same BPA level was 8 (with diagnostic rate 69.2%). Number of infertile females with low level (below 90th percentile) of BPA was 31 while number of non-infertile women having same BPA level was 63 (with diagnostic rate 69.2%). (**Table. 2**)

Correlation analysis model between the biochemical markers represented a significant positive association of urinary **BPA** concentration against serum LH, free testosterone, and fasting insulin (P<0.001). Also, LH was positively correlated with BPA concentration (0.439) as shown in table 2. On contrary, there Also, LH was positively correlated with BPA concentration (0.439)as shown in table 2. (Table 3).

The urinary **Bisphenol A cut off value** at 1803.62 had a reliable sensitivity and specificity

(75.5% and 94.5% respectively), if it was compared to the serum LH, FSH, free testosterone, and fasting insulin for diagnosis of PCO (Table **4**, Fig. **2**).

Using logistic regression analysis, BPA as the main dependent variable was significantly associated with PCOS (**Table 5**) with adjusted Odds Ratio (OR) equal to 1.3 (95% CI: 0.25-6.73, P<0.001) (**Table 6**).

### List of tables

 Table (1): Demographic, clinical, and hormonal characteristics of women of both groups

	PCO group (n=60)	Control group (n=60)	P-value	95% Confidence Interval
Age (year)	$28.36 \pm 5.11$	$28.49 \pm 4.9$	0.845 (NS)	(-1.46, 1.20)
Body mass index BMI	$25.97 \pm 4.26$	$24.48 \pm 4.25$	0.01 *	(0.36, 2.62)
Infertility • Yes • No	42 (70 %) 18 (30 %)	7 (11.82%) 53 (88.18%)	< 0.001 *	17.41 (OR) (8.58, 35.35)
Hirsutism • Yes • No	32 (53.64%) 28 (46.36%)	4 (6.36%) 56 (93.64%)	< 0.001 *	17.02 (OR) (7.26, 39.92)
Acne • Yes • No	29 (48.18%) 31 (51.82%)	5 (8.18%) 55 (91.82%)	< 0.001 *	10.43 (OR) (4.79, 22.71)
Bisphenol A (μg/mL)	$3439.05 \pm 2118.25$	$813.84 \pm 584.78$	< 0.001 *	(2210.56, 3039.87)
FSH (mIU/mL)	$5.14 \pm 1.88$	$6.77 \pm 1.52$	< 0.001 *	(-2.09, -1.18)
LH (mIU/mL)	$6.98\pm2.14$	$3.61 \pm 1.36$	< 0.001 *	(2.89, 3.84)
Free testosterone(pg/mL)	$4.71\pm0.98$	$2.23\pm0.65$	< 0.001 *	(2.26, 2.70)
Fasting insulin (mIU/L)	$19.34\pm3.27$	$14.39 \pm 1.75$	< 0.001 *	(4.25, 5.65)

Normally distributed variables are represented as mean  $\pm$  SD and unpaired Student's t-test was used; Categorical variables are represented as number (%) and Pearson Chi square test was used;

\*: Statistically significant (p<0.05); NS: Statistically not significant (P>0.05)

 Table (2): The association of Bisphenol A and infertility

		Infertility					
		% Present	% No	Diagnostic rate (%)	P value		
Bisphenol A	< 90 percentiles	31	63	32.9%	0.01		
	≥ 90 percentiles	18	8	69.2%	0.01		
	_	49	71				

\*: Statistically significant (p<0.05).

Bisphenol A 90 percentile is  $3015 \ \mu g/mL$ 

	Bisphenol level (ug/mL)	FSH (mIU/mL)	LH (mIU/mL)	Free testosterone (pg/mL)	Fasting insulin (mU/L)
Bisphenol level	1	-0.248**	0.439**	0.473**	0.384**
(ug/mL)		(<0.05)	(<0.001)	(<0.001)	(<0.001)
FSH	-0.248**	1	0.106	-0.340**	-0.284**
(mIU/mL)	(<0.05)		(0.116)	(<0.001)	(<0.05)
LH	0.439**	0.106	1	0.623**	0.504**
(mIU/mL)	(<0.001)	(0.116)		(<0.001)	(<0.001)
Free testosterone (pg/mL)	0.473** (<0.001)	-0.340** (<0.001)	0.623** (<0.001)	1	0.640** (<0.001)
Fasting insulin	0.384**	-0.284**	0.504**	0.640**	1
(mU/L)	(<0.001)	(<0.05)	(<0.001)	(<0.001)	

Table (3): Correlation matrix between the biochemical markers

Data are represented as Pearson's r correlation coefficient and (p-value); \*\* Correlation is significant at the 0.01 level (2-tailed);

Correlation is significant at the 0.01 level (2-tailed),

\* Correlation is significant at the 0.05 level (2-tailed)

Table (4): Sensitivity and	specificity of urinary	Bisphenol	A and	serum	LH,	FSH,	free
testosterone and fasting	nsulin for diagnosis o	f PCOS					

	Cut off Value	Sensitivity	Specificity	AUC	p-Value	95% Confidence Interval
Bisphenol level (ug/mL)	1803.62	75.5%	94.5%	0.898	< 0.001 *	(0.853, 0.943)
FSH (mIU/mL)	5.85	76.4%	64.5%	0.750	< 0.001 *	(0.685, 0.815)
LH (mIU/mL)	4.60	88.2%	83.6%	0.905	< 0.001 *	(0.865, 0.945)
Free testosterone (pg/mL)	3.50	98.2%	100.0%	0.987	< 0.001 *	(0.968, 1.000)
Fasting insulin (mU/L)	16.35	88.2%	89.1%	0.905	< 0.001 *	(0.862, 0.949)

AUC: area under the curve;

\*: Statistically significant (p<0.05)

		В	S.E.	Wald	df	Sig.	Exp(B)		C.I.for TP(B)
								Lower	Upper
Stop 10	BPA	4.875	0.833	34.212	1	0.000	130.991	25.573	670.973
Step 1 <sup>a</sup>	Constant	-16.581	2.863	33.551	1	0.000	0.000		
Step 2 <sup>b</sup>	Free testosterone	0.008	0.007	1.314	1	0.252	1.008	0.994	1.021
	BPA	18.523	16.543	1.254	1	0.263	110818851.088	0.000	#######
	Constant	-70.852	62.909	1.268	1	0.260	0.000		
	Hirsutism	39.855	2326.216	0.000	1	0.986	#######################################	0.000	
Step 3 <sup>c</sup>	Free testosterone	0.038	1.212	0.001	1	0.975	1.039	0.097	11.173
	BPA	74.246	2299.286	0.001	1	0.974	#######################################	0.000	
	Constant	-310.566	9467.890	0.001	1	0.974	0.000		
a Variable(s) optared on stan 1: BPA									

### Table (5): Multivariate logistic regression to detect independent predictors of PCOS

a. Variable(s) entered on step 1: BPA.

b. Variable(s) entered on step 2: Free testosterone

c. Variable(s) entered on step 3: Hirsutism.

### Table 6: Odds ratio between PCOS and BPA level

		D voluo	P value OR	95% C.I.		
		1 value		Lower	Upper	
РСО	BPA	<0.001*	1.30.991	0.25	6.73	
*: Statistically si	gnificant					
OR: Odds ratio						

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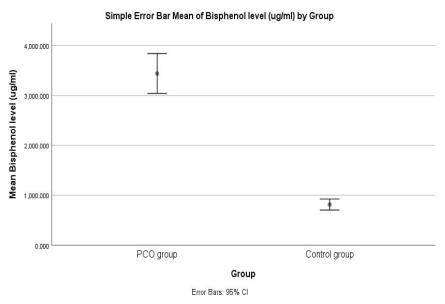


Figure (1): Mean urinary Bisphenol A level in both groups

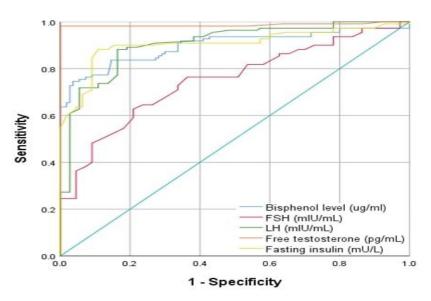


Figure (2): Roc curve of urinary Bisphenol A and serum LH, FSH, free testosterone and fasting insulin for diagnosis of PCO

#### **DISCUSSION**

The worldwide mass production of BPA as well as the increase in its usages, the BPA becomes a ubiquitous pollutant in the environment. Human exposure occurs in a direct and chronic ways when the Bisphenol-A permeated from plastic materials to humans by heating those plastics bottles or by exposure them to acidic media (Jalal, et al, 2018; Kumar, et al., 2018).

The present study investigated the relation between BPA and PCOS, Women who were diagnosed of having polycystic ovary syndrome PCOS were enclosed in case group, while women without PCO diagnostic criteria were enclosed in control group.

In the present study, there is no statistically significant differences between the age of women with PCOS and whom in the control group (mean  $\pm$  SD of both groups are 28.36  $\pm$  5.11 vs. 28.49  $\pm$  4.9 respectively, P=0. 845).

**Regarding BMI**, women with PCOS had significantly higher Body Mass Index BMI more than the control group (mean  $\pm$  SD of both groups are 25.97  $\pm$  4.26 vs. 24.48  $\pm$  4.25 respectively, P=0.01). However, in **Konieczna et al. (2018)** research, there was a nonsignificant correlation between serum BPA and BMI (**Konieczna, et al., 2018**).

Regarding infertility study in relation with level of BPA, number of infertile females with high level (above 90<sup>th</sup> percentile) of BPA was 18 while number of non-infertile women having same BPA level was 8 (with diagnostic rate 69.2%). Number of infertile females with low level (below 90<sup>th</sup> percentile) of BPA was 31 while number of non-infertile women having same BPA level was 63 (with diagnostic rate 69.2%).

The Infertility was found to be significantly higher in PCOS group (P<0.001), as 70% of women within PCO group against only 11.82% of women in the control group

Similarly, Hirsutism was significantly determined high in PCOS group (P<0.001), which was 53.64% of women within PCOS group versus 6.36% of women only in the control group. Acne was found to be significantly higher in PCO group (P<0.001). Acne occurrence percentage was 48.18% of women within PCO group against 8.18% of women in the control group.

The previous studies were carried out on different ethnic groups rather than our study. They were carried out in China and with different detection methods (serum level of BPA was detected using ELISA systems as well as the detection of BPA in follicular fluid) (**Hu**, et al., 2018). However, in the current research, women with PCO had significantly higher urinary Bisphenol A level if they compared to women in the control group (mean  $\pm$  SD of both groups 3439.05  $\pm$  2118.25 µg/mL vs. 813.84  $\pm$  584.787 µg/mL respectively, P<0.001).

It was reported that once Bisphenol-A had been in blood, a quick metabolization was done by the liver and for this reason, and the previous research assumed that urine could be a superior way to detect the actual exposure of the hazardous BPA (Calafat, et al, 2013; Miao, et al, 2015).

In the present study, women having PCOS had significantly lower level of serum FSH than those women in the control group (mean  $\pm$  SD of both groups are  $5.14 \pm 1.88$  mIU/mL vs.  $6.77 \pm 1.52$  mIU/mL respectively, P<0.001). Moreover, the women having the PCOS had also a significant high level of serum LH, if they were compared to those women in the control group (mean  $\pm$  SD of both groups are  $6.98 \pm 2.14$  mIU/mL vs.  $3.61 \pm 1.36$  mIU/mL respectively, P<0.001).

According to Zhou et al. who reported that the exposure to BPA has a negative association with third day FSH levels in infertile women who are diagnosed as polycystic ovary syndrome (**Zhou, et al., 2017**). Thus, the BPA is able to act as an endogenous ligand of estrogen receptor (ER) and make a negative effect on the pituitary gland, leading to a decrease in FSH secretion (**Chen, et al., 2018**).

Outstandingly, free testosterone level and sex hormone binding globulin SHBG is very crucial in assessment of androgen excess in women with subfertility with ovulation dysfunction and the supposed PCOS, because it contributes to the metabolic and ovarian disturbances (Antonio, et al., 2018).

In the most recent review article about BPA which was conducted by **Pivonello** et al. (2020), the BPA had been detected in infertile females, this might resultant in the assumption of probable effect of BPA on normal conception. Additionally, the BPA exposure during ICSI is established to be related negatively to peak serum estradiol results during gonadotropin stimulation, number of retrieved oocytes, number of fertilized oocytes and implantation. Therefore, the BPA harmful properties are more serious during perinatal exposure and producing deregulation of hypothalamic-pituitary-ovarian axis in pups and adults, with an early maturation of the axis through an injury of GnRH pulsatility, gonadotropin signaling and sex steroid hormone secretion. Furthermore, exposure to BPA could cause PCOS -like abnormalities through hindering secretion of sex hormones, disturbing ovarian morphology, and functions, especially folliculogenesis (Pivonello, et al., 2020).

In the present study, the serum free testosterone level of women within PCO group

was more higher than women's level in the control group (mean  $\pm$  SD of both groups are 4.71  $\pm$  0.98 pg/mL vs. 2.23  $\pm$  0.65 pg/mL respectively, P<0.001), women having PCOS had also a significant high level of serum fasting insulin if compared with women in the control group (mean  $\pm$  SD of both groups are 19.34  $\pm$  3.27 mU/L vs. 14.39  $\pm$  1.75 mU/mL respectively, P<0.001).

In a recent review on BPA and PCOS, the collected evidence proposes a correlation between high levels of Bisphenol-A and the presence of PCOS. Controverting results from animals and human research made it hard to settle the exact role of Bisphenol-A in pathogenesis of PCOS. BPA might resemble a consequence of the syndrome rather than a cause, but more research are important to give more declarations (Kechagias, et al., 2020). The current study is in agreement with a systematic review and meta-analysis which stated that the Bisphenol-A could be positively correlated with women with PCOS and BPA, it might be also associated in hyperandrogenism and insulin-resistance at PCOS (Hu, et al., 2018).

In the present study by using logistic regression analysis, BPA was the main independent variable and significantly associated with PCOS with adjusted Odds Ratio (OR) equal to 1.3 (95% CI: 0.25-6.73, P<0.001). The results of this study indicated that BPA might play a major role in the PCOS pathogenesis.

### **STUDY LIMITATIONS**

The limitation of the present study is the number of the women included, future studies with larger number of women are needed to support the obtained findings of this study with other previous studies. In additional to prove either BPA is having a role in the pathogenesis of PCOS, or BPA is a result of the association with PCOS.

### **CONCLUSION**

Comparative method via determines the BPA levels in differentiation among women who suffer PCOS versus normal women in finding cut off values for PCOS women.

The urinary method of Bisphenol-A level detection in women is a reliable and an easy noninvasive method. As it is not yet established whether the observed association suggests an etiological factor or whether the endocrine pattern associated with PCOS alters the storage and clearance patterns of BPA and causing elevated BPA, So the present study reported that there is a significant positive association between BPA and PCOS.

Also, the correlation analysis model among the biochemical markers showed a significant positive association of urinary BPA concentration with serum LH, free testosterone, and fasting insulin.

The supposed influences of BPA on reproductive health of women and increase infertile level in women had been determined. Percentage of infertile women was higher in BPA measures above 90<sup>th</sup> percentile of total number.

In the present study by using logistic regression analysis, BPA was the main independent variable and significantly associated with PCOS

The BPA could be well used as a marker for PCO in women. Beside that BPA was significantly associated with PCOS, it was the significant independent variable of PCOS. Thus, our advisement is limiting usage of these substances which including BPA, especially in food products and other related plastic products.

### **Ethical Considerations and Consent**

The study was conducted after taking the approval of the ethical committee at Aljazeera hospital, Egypt with approval number 0021. An informed consent was obtained from the participants of the study in which the work was undertaken and that it conforms to the provisions of the Declaration of Helsinki.

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### **Disclosure statement**

All authors declare that there are not any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

The first author (Mokhtar Fathy) is an associate editor in the Egyptian journal of forensic sciences and applied toxicology.

### **REFERENCES**

Akgül, S., Sur, Ü., Düzçeker, Y., Balcı, A.,
Kızılkan, M. P., Kanbur, N., ... &
Derman, O. (2019): Bisphenol A and phthalate levels in adolescents with polycystic ovary syndrome. Gynecological Endocrinology.

- Antonio L., Pauwels S., Laurent M. R., D. Vanschoubroeck D., Jans I, Billen J., Claessens F., Decallonne, B. Diane De Neubourg, Р. Vermeersch, and D. Vanderschueren. (2018): Free Testosterone Reflects Metabolic as well as Ovarian Disturbances in Subfertile Oligomenorrheic Women. Volume 2018 |Article ID 7956951; https://doi.org/10.1155/2018/7956951
- Calafat, A. M., Koch, H. M., Swan, S. H., Hauser, R., Goldman, L. R. Lanphear, B. P., ... & Wolff, M. S. (2013): Misuse of blood serum to assess exposure to bisphenol A and phthalates. *Breast Cancer Research*, 15(5), 403.
- Chen, Y., Wang, Y., Ding, G., Tian, Y., Zhou, Z., Wang, X., & Huang, H. (2018): Association between bisphenol a exposure and idiopathic central precocious puberty (ICPP) among school-aged girls in Shanghai, China. *Environment international*, 115, 410-416.
- Gilbert, E. W., Tay, C. T., Hiam, D. S., Teede, H. J., & Moran, L. J. (2018): Comorbidities and complications of polycystic ovary syndrome: An overview of systematic reviews. Clinical endocrinology, 89(6), 683-699. 5-Amowitz LL, Sobel BE. Cardiovascular consequences of polycystic ovarv syndrome. Endocrinol Metab Clin North Am 1999 . 28:439-58, viii.
- Hu Y, Wen S, Yuan D, Peng L, Zeng R, Yang Z, Liu Q, Xu L, Kang D. (2018):. The association between the environmental endocrine disruptor bisphenol A and polycystic ovary syndrome: a systematic review and meta-analysis. Gynecol Endocrinol. 2018 May;34(5):370-377. doi: 10.1080/09513590.2017.1405931. Epub 2017 Nov 30. PMID: 29191127.
- Jalal, N., Surendranath, A. R., Pathak, J. L., Yu, S., & Chung, C. Y. (2018): Bisphenol A (BPA) the mighty and the mutagenic. Toxicology reports, 5, 76-84..
- Jin, P., & Xie, Y. (2018): Treatment strategies for women with polycystic ovary syndrome. Gynecological Endocrinology, 34(4), 272-277.
- Kechagias KS, Semertzidou A, Athanasiou A, Paraskevaidi M, Kyrgiou M. Bisphenol-A and polycystic ovary syndrome: a review of the literature (2020): Rev Environ Health. 2020 Jul

14;35(4):323-331. doi: 10.1515/reveh-2020-0032. PMID: 32663175.

- Khan, M. J., Ullah, A., & Basit, S. (2019): Genetic basis of polycystic ovary syndrome (PCOS): current perspectives. The application of clinical genetics, 12, 249.
- Konieczna, A., Rachoń, D., Owczarek, K., Kubica, P., Kowalewska, A., Kudłak, B., ... & Namieśnik, J. (2018): Serum bisphenol A concentrations correlate with serum testosterone levels in women with polycystic ovary syndrome. Reproductive Toxicology, 82, 32-37.
- Kumar, A., Verma, R., & Agrawal, N. K. (2018): A study of urinary bisphenol A levels in endocrine disorders. International Journal of Research in Medical Sciences, 6(2), 696-700.
- Lu, X., Bao, H., Cui, L., Zhu, W., Zhang, L., Xu, Z., ... & Zhang, H. (2020): hUMSC transplantation restores ovarian function in POI rats by inhibiting autophagy of thecainterstitial cells via the AMPK/mTOR signaling pathway. Stem Cell Research & Therapy, 11(1), 1-13.
- Mahemuti, L., Chen, Q., Coughlan, M. C., Qiao, C., Chepelev, N. L., Florian, M., ... & Willmore, W. G. (2018): Bisphenol A induces DSB-ATM-p53 signaling leading to cell cycle arrest, senescence, autophagy, stress response, and estrogen release inhuman fetal lung fibroblasts. Archives of toxicology, 92(4), 1453-1469.
- Miao M, Yuan W, Yang F, Liang H, Zhou Z, Li R, et al. (2015): Associations between bisphenol A exposure and reproductive hormones among female workers. Int J Environ Res Public Health 2015: 12:13240–50.
- Neven, A. C. H., Laven, J., Teede, H. J., & Boyle, J. A. (2018): A summary on polycystic ovary syndrome: diagnostic criteria, prevalence, clinical manifestations, and management according to the latest international guidelines. In Seminars in reproductive medicine (Vol. 36, No. 01, pp. 005-012). Thieme Medical Publishers.
- Nylander, M., Frøssing, S., Bjerre, A. H., Chabanova, E., Clausen, H. V., Faber, J., & Skouby, S. O. (2017): Ovarian morphology in polycystic ovary syndrome: estimates from 2D and 3D ultrasound and magnetic resonance imaging and their

correlation to anti-Müllerian hormone. Acta Radiologica, 58(8), 997-1004.

- Pan, M. L., Chen, L. R., & Chen, K. H. (2021): The Risk of Subsequent Miscarriage in Pregnant Women with Prior Polycystic Ovarian Syndrome: Α Nationwide Population-Based Study. International Journal of Environmental Research and Public Health, 18(16), 8253.3- Vutyavanich T, Khaniyao V, Wongtra-Ngan S, et al. Clinical, endocrine and ultrasonographic features of polycystic ovary syndrome in Thai women. J Obstet Gynaecol Res 2007. 33:677-80.
- Pivonello, C., Muscogiuri, G., Nardone, A., Garifalos, F., Provvisiero, D. P., Verde, N., & Pivonello, R. (2020): Bisphenol A: an emerging threat to female fertility. Reproductive Biology and Endocrinology, 18(1), 1-33. https://doi.org/10.1186/s12958-019-0558-8
- Rostamtabar, M., Esmaeilzadeh, S., Tourani, M., Rahmani, A., Baee, M., Shirafkan, F., ... & Nouri, H. R. (2021): Pathophysiological roles of chronic lowgrade inflammation mediators in polycystic ovary syndrome. Journal of Cellular Physiology, 236(2), 824-838.
- Rowdhwal, S. & Chen, J. (2018): Toxic effects of di-2-ethylhexyl phthalate: an overview. BioMed research international, 2018.
- Zhang, Y., Luo, S., Gong, Z., Feng, X., Wang, Z., Zhu, H., & Wang, Y. (2018): Improvement of hyperandrogenism, oligoovulation, and ovarian morphology in a patient with polycystic ovary syndrome: possible role of ovarian wedge resection. Gynecological Endocrinology, 34(6), 460-463.
- Zhou, W., Fang, F., Zhu, W.T., Chen, Z.J., Du, Y.Z., Zhang, J., (2017): Bisphenol A and ovarian reserve among infertile women with polycystic ovarian syndrome. Int. J.Environ. Res. Public Health 14, 18. http://dx.doi.org/10.3390/ijerph14010018.
- Zhou, Z., Lei, Y., Wei, W., Zhao, Y., Jiang,
  Y., Wang, N., ... & Chen, X. (2019):
  Association between prenatal exposure to bisphenol a and birth outcomes: A systematic review with meta-analysis. Medicine, 98(44).

الملخص العربي دراسة الارتباط بين تركيز ثنائي الفينول أ في البول لدى عينة من النساء المصريات والإصابة بمتلازمة تكيس المبايض

**خلفية**: متلازمة المبيض المتعدد الكيسات (PCOS) هي متلازمة الغدد الصماء النموذجية لدى النساء. لديها انتشار 5 إلى 10٪.

مع مستويات Bisphenol-A (BPA) مع مستويات Bisphenol-A (BPA) كعلامة ل PCOS مع مستويات عالية في النساء المصابات بهذه المتلازمة، وتقييم قدرة الطريقة البولية غير الباضعة للكشف عن BPA. كانت النتائج الثانوية لدر استنا هي ربط مستوى BPA بمستويات الأنسولين الصائم، والتستوستيرون الحر لدى النساء المصابات بمتلازمة المبيض المتلازمة وتقييم قدرة الطريقة البولية عبر الباضعة للكشف عن BPA. كانت النتائج الثانوية لدر استنا هي ربط مستوى المصابات بهذه المتلازمة، وتقييم قدرة الطريقة البولية عبر الباضعة للكشف عن BPA. كانت النتائج الثانوية الدر استنا هي ربط مستوى BPA بمستويات الأنسولين الصائم، والتستوستيرون الحر لدى النساء المصابات بمتلازمة المتلازمة المبيض المتعاد المصابات بمتلازمة المبيض المتعاد التستوستيرون الحر لدى النساء المصابات بمتلازمة المبيض المتعاد المتنا والنستوستيرون الحر لدى النساء المصابات بمتلازمة المبيض المتعاد المنا والن المتنا في معام المتعاد المبيض المتعاد المتعاد التعام المتعاد المبيض المتعاد المتعاد المتعاد المستوى المتعاد المتعاد المبيض ما مستوى المتعاد المبيض المتعاد المبيض المتعاد المتعاد المتعاد المبيض المتعاد المبيض المتعاد المبيض المتعاد المبيض المتعاد المبيض المتعاد الكيسات وإذا كان هناك ارتباط بالعقم.

**المشاركون والأساليب**: تم إجراء دراسة بالملاحظة "الحالة والمرجع" في مجموعتين (المجموعة 1؛ النساء المصابات بمتلازمة تكيس المبايض (60 نساء) والمجموعة 2؛ النساء غير المصابات بمتلازمة تكيس المبايض (60 نساء)

LH وFSH المداخلة: تم الكشف عن A-Bisphenol البولي باستخدام جهاز HPLC. تم إجراء قياس مصل FSH و والأنسولين الصائم ومستويات هرمون التستوستيرون الحرة. النتائج: كان لدى النساء المصابات بمتلازمة تكيس المبايض مستوى مرتفع كبير من ثنائي الفينول A البولي وأكثر من المجموعة الضابطة (3439.05 ± 2118.25 ميكروغرام / مل و. 813.84 ± 843.78 ميكروغرام / مل، 20.001، على التوالي). تم تحديد علاقة إيجابية بين مستويات -Bisphenol A البولية وHL المصل. على العكس من ذلك، هناك علاقة سلبية بين A-Bisphenol البولي ومستويات A

النتائج: أشارت نتائج هذه الدراسة إلى أنه يمكن استخدام الطريقة البولية للكشف عن مستوى ثنائي الفينول -أ كطريقة سهلة غير باضعة. وأن هناك علاقة إيجابية بين مستويات ثنائي الفينول –أ ومتلازمة تكيس المبايض وقد تلعب دورًا رئيسيًا في التسبب في هذه المتلازمة.

وبالتالي، فإن نصيحتنا هو الحد من استخدام هذه المواد التي تشمل ثاني الفينول-أ ، وخاصة في المنتجات الغذائية والمنتجات الغذائية والمنتجات البلاستيكية الأخرى ذات الصلة