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RESEARCH ARTICLE

ENVIRONMENTAL EXPOSURE OF BISPHENOL A AND ITS ASSOCIATION WITH POLYCYSTIC OVARY SYNDROME (PCOS) AND DEPLETION OF OVARIAN RESERVE AMONG INFERTILE WOMEN

Mona E. Elkafrawy[1], Hanaa Farouk Ahmed[1], Doaa M. Effat[1,*], Amal A.M. Ahmed[2], Samia S.M. Barghash[2] and Gamil Elbrahim[3]

[1]Obstetrics and Gynecology, Faculty of Medicine for Girls, Al-Azhar University
[2]Fronsic medicine and clinical toxicology, Faculty of Medicine for Girls, Al-Azhar University, Cairo,Egypt.
[3]Chemistry of Aroma and Flavor Dept, National Research Center, Cairo, Egypt

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*Corresponding author:
Doaa M. Effat

ABSTRACT

Background: The main cause of polycystic ovary disease has not been established, there are only hypotheses dictate its etiopathogenesis, environmental factor is one of these theories. **Objective of research:** To test the relation between bisphenol A (BPA) and poly- cystic ovarian syndrome (PCOs) and to evaluate the impact of BPA exposure on ovarian reserve of infertile women. **Patients and methods:** One hundred and sixty infertile women, 80 of whom were non PCOs and the other 80 women were PCOs, selected from outpatient's clinic, after they met inclusion and exclusion criteria. PCOs cases were selected according to Rotterdam criteria (2003). All the participants, at 3 day of cycle were subjected to 1-Venous blood sample for hormonal assay to FSH, LH, TSH, E2, AMH, and serum prolactin. 2- Urine sample to detect BPA and 3- Transvaginal U/S to detect the size of the uterus, ovarian volume and number of AFCs. **Results**: There was a highly significant increase in ovarian volume, FSH, LH and E2 hormones and PBA levels in PCOs group in comparison to non PCOs group, p<0.001.But there was no significant difference between the studied groups in AFCn, serum prolactin, TSH, and serum AMH hormone,p >0.05. In PCOs group, there was a direct correlation between BPA and age, r= 0.276, p =0.013* .But there was an inverse correlation between BPA and each of AFCn and AMH in PCOs & non PCOs groups as follows, r= -0.245, p=0.029*and r= -0.521, p =0.017* & r=-0.251, p=0.024*,r=-1.000,p=0.000* respectively. The crude model, a unit increase in BPA was associated with a significant lower of 0.656 in AFCn (_ = -0.656, 95% CI = (0.518, – 0.794; p = 0.026*). Similar relation was found in the adjusted model (_ = - 0.660, 95% CI = -0.509 – 0.760); p = 0.031*). While adjusting other characteristic data, there was significant decrease of --0.790 in AMH hormone (= --0.790), 95% CI = (--0.624 – 0.957), p = <0.001**. **Conclusion:** Urinary BPA is increased in infertile women, PCOs and non PCOs groups, implying that BPA may influence the hormonal profile of infertile women. As result of a higher significant increase of PBA level in PCOs group in comparison to non PCOs group, this indicates that BPA may be an environmental issue or cofactor implicated in the pathogenesis of PCOs. Furthermore, the association between BPA exposure and lower of antral follicles count and AMH level in infertile women, suggests that BPA may impair human ovarian function, reserve, and fecundity.

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INTRODUCTION

Human reproduction indices have declined in recent decades due to changes in lifestyle factors that lead to delayed childbearing (Crain, 2008). Endocrine disrupting compounds (EDC) can diminish the function of sex hormones or antagonize the steroid signaling, resulting in adverse outcomes (Crain, 2008). One of the most common endocrine disruptors is bisphenol A (Diamanti-Kandarakis, 2009).

It is an industrial estrogen-mimic compound that's one of the most widely used synthetic chemicals in the world, with applications in drink and food packaging, consumer plastics, and materials used in dentistry (Healy, 2015). Ingestion, inhalation, and skin absorption are all ways to be exposed to BPA (Laven *et al*., 2002). Evidence of ovarian steroid synthesis interference, folliculogenesis, and ovarian biology affection, indicate that the female gonad is an extremely sensitive BPA disruption target (Laven, 2002). BPA changes the production of androgens in the liver and displaces androgens, serving as a sex

hormone-binding globulin (SHBG) binder, leading to elevated serum-free androgen levels (Bornehag *et al*., 2015) At reproductive age, PCOs have a variety of symptoms including, Hirsutism, acne, oligo-ovulation or anovulation, insulin resistance, and increased luteinizing hormone (LH) levels (Zhou *et al*., 2008; Kandaraki, 2011). The etiology of this condition is still uncertain (Carwile, 2011; Le, 2008**).** PCO is diagnosed using, any two of the three Rotterdam criteria with exclusion of similar eitiology, as, adrenal hyperplasia, Cushing syndrome, etc., (Rotterdam, 2003; Monash Centre for Health Research and Implementation, 2018), ovulatory dysfunction (oligo, or anovulation), clinical and/ or, biochemical increased androgen level, and PCO picture on ultrasound, i.e. ovary with more than12 follicles averaging 2-9mm in diameter and ovarian volume>10ml. Although a U/S picture (PCO) is not required for diagnosis if both ovulatory dysfunction and hyperandrogenism are present, it will show the full PCOs phenotype. Le et al. (2008) stated that repeated human exposure to BPA may harm oocyte viability, follicular metabolism, follicle proliferation, ovarian reserve, and overall fertility (Le, 2008; Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2003; Monash Centre for Health Research and Implementation (MCHRI), 2018; Hunt, 2003). This study aimed To test the association between the exposure of BPA and PCOs and to evaluate the impact of BPA on ovarian reserve among infertile women.

PATIENTS AND METHODS

This a cross sectional study was studied at Al zahraa University Hospital, Cairo Egypt, from January 2017 to July 2018, on 160 infertile women, 80 of them were PCOS and the other 80 women were non PCOS. The participants were selected from outpatient clinic. Patients with; infertility due to male factor, due to ovarian failure, with history of previous contraception or hormonal treatment for induction before the research was excluded. PCOs cases were selected according to Rotterdam criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2003). This study approved as ethical committee of Al-Azhar University faculty of medicine for Girls. Consent was taken from all the patients after explaining the purpose and procedure of the study, All women underwent to the following, full details history, complete examinations. Investigations which in the form of 1- Venous blood samples for estimation of FSH, LH, TSH, E2, AMH, and serum prolactin at day 3 of unstimulated cycle using an automated electro chemiluminescence immunoassay. 2-Urine sample for detection of Bisphenol A was also done at day 3 of the same cycle.

Extraction of BPA: *Liao and Kannan* (Liao, 2014) used a liquid-liquid extraction technique to remove BPA from urine. A 2 mL aliquot of thawed urine was moved to a 10 mL glass tube with a lid in just a few minutes. 1.5 mL NaAC-HAC buffer solution and 10 mL -glucuronidase/arylsulfatase were applied to the sample in a sequential order and incubated at 37 °C for 8 hours while shaking. 3 mL ethyl acetate was added after that.The resulting solution was then hand-shaken for 1 minute before being centrifuged at 6000 g for 3 minutes. The supernatant was extracted, and the extraction procedure was performed two more times. Under nitrogen gas, the combined extract was condensed to near dryness, reconstituted in 1 mL methanol, screened through a 0.22 m screen membrane, and processed at 20 °C before instrumental analysis. On the third day of an unstimulated cycle, a transvaginal ultrasound was performed to determine the size of the uterus and ovaries in order to rule out any organic pathology (fibroid, ovarian cyst), ovarian volume, and AFCn, this done by LOGIQ V5 2D ultrasound scanner. The ovarian volume was calculated by the equation for prolate ellipsoid using the longest longitudinal (d1), anteroposteriore (d2).and transverse diameter (d3) (Rosendahl, 2010). All data were collected and statistically analyzed.

Sample size calculation: The sample size was determined using the Open Epi software version 13 in accordance with a previous analysis by *Ye et al.* (2018**),** who found that LH/FSH in the PCOS group was 1.65 1.05, while in the non PCOS group was 1.22 0.85, with a p-value of 0.009 and a margin of error of 5% by changing the confidence interval to 95%. The test's power is set to 80%, and the group ratio is set to 1:1. This study's overall sample size was determined to be 156 patients (78 patients in each group).

RESULTS

No significant differences were found between the studied groups regarding, age, BMI, smoking, education % and caffeine consumption % (table 1). Regarding representation of Rotterdam criteria in the PCOs group, we found that, 47women (58.57%) experienced all three criteria i,e, clinical, biochemical and PCO picture, while the rest of women, 33(41.25%) were only experienced clinical and biochemical parameters without PCO picture. The main symptom in all patients was infertility, while the associated, symptoms were only found in PCOS group in form of Hairsutism & acne, in 35 women (43.75%), oligomenorrhea or menorrhea in 6 women (7.5%) and polymenorrhea in 25 women (31.25%) (Table 1). Comparison between the studied groups regarding the ultrasound and biochemical investigations showed that a highly significant difference in ovarian volume , FSH, LH and E2 hormones and Bisphenol A levels on day 3 of cycle, but no significant differences were found between the studied groups as regard to serum prolactin, TSH, and AMH hormone (table 2). Although regarding AFC difference between the study groups is tolerant nearly significant but it was still statistically insignificant (Table 2). Roc curve analysis revealed, BPA at the cutoff value of >9.68, AUC of 0.81with sensitivity of 80%and specificity of 90% decreased the ovarian reserve (Table 3, Fig 1). There was a positive correlation between BPA and age in PCOs group r=0.276, p=0.013.But there was a negative correlation between BPA and each of AFCn, r=-0.251, p=0.024 and r=-0.245, p=0.029 and AMH r=-1.000,p=0.000 and r=-0.521, p=0.017 in non PCOs and PCOs groups respectively (table 4).

DISSCUSION

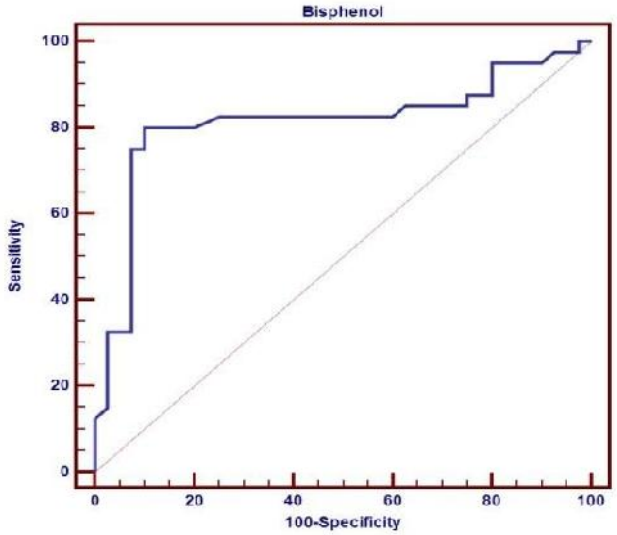
The prevalence of PCOS is one amongst the disadvantages of civilization. Even with the advancement of manufacture industry the utilization of existence plasticization. The treatise that environmental factors play an important role in pathological process of PCOs as endocrine distance was endorsed by several data (Bozdag, 2016). External endocrine disruptors are substances or combinations that modify the function(s) of the endocrine system and trigger detrimental consequences in an intact organism, its progenitor, or (sub) populations.

Table 1. Comparison between the studied groups as regard the demographic data and symptoms

		Non PCO	PCO	Test value	P -value	Sig.
		No. = 80	No. = 80			
Age (years)	Mean±SD Range	27.01 ± 4.23 21-35	25.95 ± 4.66 19-37	1.511-	0.133	NS
Length (cm)	Mean±SD Range	161. 55±5.79 150-178	162.68 ± 6.27 150-180	-1.180-	0.240	NS
Weight (kg)	Mean±SD Range	83.25 ±17.81 54-118	79.80 ± 18.20 52 – 150	1.212-	0.227	NS
BMI (kgm2)	Mean±SD Range	31.94 ±6.62 19.57-46.09	30.06 ± 6.06 20.31-51.9	1.868-	0.064	NS
Caffeine%	Negative Positive	58 (72.5%) 22 (27.5%)	64 (80.0%) 16 (20.0%)	1.242*	0.265	NS
Smoking %	Negative Positive	78 (97.5%) 2 (2.5%)	79 (98.8%) 1 (1.3%)	0.340*	0.559	NS
Education %	Primary College Not educated	3 (3.8%) 42 (52.5%) 35(43.8%)	6 (7.5%) 29 (36.3%) 45 (56.3%)	4.630*	0.099	NS

Table 2. Comparisons between the studied groups as regard the biochemical investigations and U/S findings

		Non PCO	PCO	Test value	P – value	Sig.
		No. = 80	No. = 80			
Ovarian volume	Median (IQR)Range	10 (10 - 12) 812	22 (20 - 24) 1426	-11.119*	<0.001	HS
AFC (n)	Median (IQR)Range	6 (6 - 8) 48	7 (6 - 9) 514	1.667	0.098	NS
AMH (ng/mL)	Median (IQR)Range	3.3 (2.59 - 5.25) 1.89-7.19	4.26 (2.05 - 6.91) 1.7-7.7	1.550'	0.123	NS
FSH(IU/I)	Median (IQR)Range	7.3 (6.1 - 8.15) 4.2 – 13	6.35 (4.7 - 7.2) 3.3-10.1	4.365*	<0.001	HS
LH(IU/I)	Median (IQR)Range	4.3(3.4-5.8) 356	7.7 (6.2 - 10.6) 1.4-20.7	-6.126*	<0.001	HS
LH/FSH	Median (IQR)Range	0.61(0.53-0.79) 0.42 - 6.83	1.36 (1.03 - 1.76) 0.19-3.83	7.168	<0.001	HS
TSH (miu/L)	Median (IQR)Range	2.3(1.7-3.15) 1.07-9.35	2.2(1.7-2.6) 0.93-3.5	-0.787*	0.431	NS
Prolactin(ng/ml)	Median (IQR) Range	11(8.17-12) 0.4-40.1	11(10-12) 5.5-22	-1.494*	0.135	NS
E2(pg/ml)	Median (IQR) Range	50 (48 - 55) 30.3 – 66	66 (66 - 88) 55116	-9.267*	<0.001	HS
Bisphenol (ng/ml)	Median (IQR) Range	5.63 (4.2 - 6.85) 1.84-30.45	24.49(12.97-28.66) 2.18-34.95	-6.806*	<0.001	HS



Cut off value	AUC	Sensitivity	Specificity	+PV	-PV
>9.68	0.812	80.00	90.00	88.9	81.8

Figure 1, table (3): Roc curve analysis of BPA level in PCOS and non PCOS groups with diminish Ovarian reserve.

Table 4. Correlations between BPA, demographic, U/S and biochemical investigations, in the studied groups

	Bisphenol			
	Non PCO		PCO	
	r	p-value	r	p-value
Age (years)	0.029	0.800	0.276*	0.013
BMI (kg/m2)	0.064	0.576	-0.139	0.217
Ovarian volume	-0.119	0.294	0.131	0.247
AFC (n)	-0.251*	0.024	-0.245	0.029*
AMH (ng/mL)	-1.000**	0.000	-0.521	0.017*
FSH (IU/L)	-0.022	0.847	0.165	0.143
LH (IU/L)	-0.025	0.321	0.095	0.402
LH/FSH	0.168	0.217	0.054	0.628
TSH (IU/L)	0.048	0.670	0.047	0.680
Prolactin (ng/ml)	-0.026	0.819	0.196	0.082
E2 (pg/ml)	0.117	0.299	0.121	0.286

Table 5. The association of urinary BPA levels with age, AFCn and AMH levels in the studied groups

	Crude (95.0% CI)	P-value	Multivariate (95.0 % CI)	P-value
Age (years)	0.606 (0.132 – 1.081)	0.013	0.058 (-0.048 – 0.164)	0.219
AMH((ng/mL)	-0.790 (0.624 – 0.957)	<0.001**	-0.795 (0.632 – 0.957)	<0.001**
AFC(n)	-0.656 (0.518 – 0.794)	0.026*	-0.660 (0.509 – 0.760)	0.031*

In the crude model, a unit increase in BPA was significantly linked with a diminish in AFC (_ = -0.656, 95% CI = (0.518, (– 0.794; p = 0.026*). The correlation was similar within the adjusted model (_ = - 0.660, 95% CI = -0.509 – 0.760); p = 0.031*), when adjusting for other demographic data, there also significant decrease of --0.790 in AMH hormone (= --0.790), 95% CI = (--0.624 – 0.957), p =<0.001**),table 5

Exposure to BPA which is one of this substances (Richter, 2007). In terms of demographic data, the mean age in PCOS groups was 25.95 ±4.66 years and in non PCOS groups was 27.01 ±4.23 years, BMI in PCOS was 30.06 ±6.06 and in non PCOs was 31.94± 6.62, caffeine consumption percent in PCOS was (20.0%) and in non PCOS was (27.5%), and smoking percent were 1 (1.3%) vs (2.5%) in PCOs vs no PCOS groups, respectively (Table 1). Regarding the ultrasound findings, it revealed that high significant differences between PCOS and non PCOS regarding the ovarian volume, on the other hand, although the AFCn was higher in PCOs group and was nearly relative significant when compared to non PCOs group but it was statistically insignificant, p=0.098 (Table 2). It was stated that,PCOS is characterized by a high AFCn and a considerably large ovarian volume (Rosenfield, 2016). The current study showed that a high significant increase in LH and LH/FSH ratio in PCOs group versus non PCOs with p value (0.001) (Table 2).this finding was in agreement with the study which concluded that the Increase of LH pulse frequency, LH pulse amplitude, and the increase of LH/FSH ratios were described in women with PCOS (Hampton, 2013). Similarly, this agree with (Chun, 2014) ***s***tudy which revealed that, the elevated LH concentrations and LH/FSH ratio were common clinical manifestation of patients with PCOS. Our study revealed that increased of AMH in PCOs compared to non PCOs groups, but this was statistically insignificant = 0.123 (Table 2). The failure of our result to reach a statistical significance in AMHdid not contradict some researches that have reached a statistical significance value such as; *Maas et al.* (2015), who reported that the total AFCn (in both ovaries) significantly higher in women with PCOS (58.2 ± 6.2) than those non PCOS women (31.8 ± 2.9). Also the authors found that AMH levels were also signficantly higher in PCOS patients (11.9 ±1.6 ng/mL) than in non PCOS group (5.3±1.0 ng/mL) and; *Pigny et al.* (2003) who reported that in women with PCOS, AMH levels are often significantly elevated than those non PCOS. Because, the diagnosis of PCOs was only based on 2 parameters of the three parameter of Roterrdam criteria, hence, some participants in PCOS group of our study were not experienced U/s PCO picture, therefore they didn't have neither high AFCn nor high level of AMH. Our research investigated the BPA level in both of PCOS and non PCOS groups, it showed that increased the level of BSA in PCOS group compared to non PCOS with highly significant difference, p= 0.001 (Table 2). A number of human and animal studies looked into the possible connection between PCOs and BPA exposure. Also, *Kandaraki et al.* (2017), BPA levels were found to be substantially higher in the PCOs community than in the controls (1.050.56 vs. 0.720.37 ng/ml, P 0.001). In the same line, in a meta-analysis which performed by *Ying Hu et al.* (2018) to show the relationship between PCOS and PBA, the authors discovered that PCOS patients had substantially higher levels of BPA than controls, p.001.

It was found that BPA could disrupt the hormonal profile and influence the phenotype of PCOS via different pathways. This can be proposed by BPA has the potential to disrupt the hormonal profile and affect the phenotype of PCOS through a variety of mechanisms. Exposure to hypothalamic BPA may activate the pulse generator for GnRH, resulting in increased LH and decreased pituitary secretion of FSH, as well as promoting ovarian hyperandrogenism. In addition, BPA may be involved in direct stimulation, synapse formation, and ovarian hyperandrogenism, which can in turn lead to increased theca cells leads to subsequence hyperestrogenemia (Richter, 2007). Moreover, *Richter et al.* (2007) reported that BPA is an endocrine disrupting chemical that can affect a wide range of physiological receptors, comprising genomic estrogen receptors 1 and 2, membrane bound estrogen receptors, androgen receptors, peroxisome proliferator-activated gamma receptors, and thyroid hormone receptors. On the other hand

Li *et al* .(2011) disagree with our findings, they performed a research on 60 patients with PCOS, 23 of them had insulin resistance (PCOS-IR) and 37 without insulin resistance , as well as 29 non-PCOS women were asking the treatment advice for infertility or menstrual irregularities (control group)". All of their participant's serum levels of six phthalic acid esters (PAEs), PBA, and octylphenol (OP) were measured. The authors reported that while 6 phthalic acid esters (PAEs) and BPA had no discernible impact on PCOS pathogenesis or insulin resistance, octylphenol (OP) can play a role in PCOS patients' insulin resistance. Ovarian reserve is known as oocytes quantity (oocytes number) left in the ovarian pool. Diminishing ovarian reserve seems to be the reason for declining fecundity and approaching of menopause. Ovrian reserve measure usually consists of estimation of an AMH blood test and an antral follicle counts by U/S (Hansen *et al*., 2012). The second part of our objective was to answer the question: Does exposure of PBA can affect the ovarian reserve?, in order to evaluate the potential effects of exposure of BPA on ovarian reserve, we tested BPA in all participants i.e., infertile women with or without PCOS.

Really we imposed obstacle in case of patients with PCOs because, the 2 parameters that tested the ovarian reserve i.e., AFCn, AMH were already increased in many patients of this group, so we considered how to conduct research into the potential effects of BPA on ovarian reserve in PCO patients, thus we created a ROC curve to determine the individual cutoff value of BPA level that may affect ovarian reserve. We also conducted a crude model to show the link between increased exposure and, as a result, increased BPA levels in the urine and ovarian depletion. ROC Curve analysis was established to predict the level of BPA at which may affecting ovarian reserve, our study suggested that in infertile women groups, either with or without PCOS, the BPA in Excess at the cut level >9.68, with AUC of 0.812, specificity of 90% and sensitivity of 80%, can damage ovarian follicles and potentially decrease its number, thus decrease the ovarian reserve (Table 3, Fig. 1). These results indicate that BPA in Excess can have a detrimental effect on ovarian function, this was supported by Pearson correlation test which in turn revealed that, in both study groups, there was a negative correlation between BPA and each of AFCn and AMH (Table 4). This was in line with the results of *Souter et al.* (2013), who used regression models to determine the relationship between BPA and AFC in a prospective cohort of women receiving infertility care. Higher urinary BPA concentrations were related to lower AFC, day3 serum F.S.H., and ovarian volume in those patients.

Moreover, our study revealed in the crude model, a unit increase in BPA was related with a significant diminish of 0.656 in AFC (_-0.656, 95% CI (0.518, (– 0.794; p = 0.026). The same relation was found in the adjusted model (_ = -0.660, 95% CI = -0.509 – 0.760); p = 0.031) when adjusting the other characteristic data, we found that there was significant decrease of --0.790 in AMH hormone (= --0.790), 95% CI = (--0.624 – 0.957), p = <0.001). The association was similar in the adjusted model (_ = - 0.795, 95% CI = (0.632 – 0.957)-; p =<0.001 (Table 5).Also this was consistent with finding of *Wei Zhou et al.* (2017) when they illustrated the correlation between BPA and the follicular reserve parameters. The authors found an inverse relationship between urinary BPA concentration & AFC and reported, a unit increase in BPA was correlated with a significant decrease in AFC in the crude model. BPA was also decreased AMH and day3 FSH levels, but none of these associations were statistically significant. Furthermore, our study revealed, no significant association was observed between BSA, FSH, and ovarian volume , this was consistent with previous findings of *Souter et al.*(2013), who reported that there was no significant association between urinary BPA quartiles and day-3 FSH blood levels (p=0.64) or ovarian volume (p= 0.8).

CONCLUSION

Urinary BPA is increased in infertile women, PCOS and non PCOS groups, implying that BPA may influence the hormonal profile of infertile women. But with higher significant increase of PBA level in PCOS group compared to non PCOS group, this indicate that BPA may be an environmental issue or cofactor implicated in the pathogenesis of PCOS. Furthermore, the connection between BPA exposure and decrease of antral folicle count and AMH level in infertile women, suggests that BPA may impair human ovarian function, reserve, and fecundity. Since temperature variations favor BPA transfer into food, so we recommend some safety precautions are required to prevent this transfer and the associated risk of human exposure specially during storage, freezing, and heating food in plastic containers. It is also important to enact legislation prohibiting the use of BPA in containers and food packaging intended for children. In addition, we encourage using containers without PBA like, glass which seems the best option. This study was conducted according to the guideline of declaration of Helsinki 2013,and approved by the ethical committee of the faculty of medicine for Girls, Cairo, Al Azhar University ,informed written consent was taken from all participants before enrolling this study.

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Availability of data and material: Not applicable.

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Authors' contribution: M E , D E and H F: Project development, data management & analysis and manuscript writing, A A and SB: Data analysis and data management while G I: Participated in biochemical part of research

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