

Effect of Bisphenol A as an Environmental Obesogen on Antioxidant Activity

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ABSTRACT

Background: The synthetic chemical bisphenol A (BPA) is a common endocrine –disruptor. Its widespread use has resulted in extensive worldwide exposure. It has been linked to obesity and many other health effects. **Objectives:** The aim of the work is to study the impact of BPA exposure on oxidative stress and antioxidant status in a group of females. The study was conducted on 33 obese female matched in their age with another 26 overweight females and 31 referent females employed in administrative tasks. Results revealed that the obese females had higher mean values of BPA levels (1.83±1.1 ng/ml) when compared to their matched referent (1.4±0.4 ng/ml) and overweight females (1.5± 0.48 ng/ml). The difference between the mean values of BPA in the studied groups was statistically insignificant (p-value>0.05). However, significant direct correlation was observed between the body mass index of studied groups and their serum levels of the environmental pollutant BPA (r=0.2& p-value=0.038). The body mass index of the studied groups showed a statistically significant direct correlation with the oxidative stress indicator Malondialdehyde. Moreover, according to the regression model, it could be predicted that each 1ng/ml increase in serum BPA level will lead to an increase in the serum level of Malondialdehyde by 0.25nmol/ml. The tested antioxidant enzymes activities were markedly lower in overweight and obese groups relative to the referent one. **Conclusion:** That rise in oxidative stress marker and decrease in antioxidant enzymes activities threaten human health and increase risk of different diseases.

Keywords: Bisphenol A, Oxidative stress, Obesity, Antioxidant enzymes.

INTRODUCTION

The industrial chemical bisphenol A (BPA) is a well-known endocrine –disruptor in human products and environments^{1,2}. BPA, 2,2-bis(4-hydroxyphenyl) propane, is an organic compound composed of 2 phenol rings attached by a methyl bridge, with two methyl functional groups attached to the bridge. It is a monomer that was first developed as a synthetic estrogen in the 1890s³. It is a constituent of polycarbonate plastics, epoxy resins, and dental sealants⁴. The massive use of BPA-containing products resulted in worldwide human exposure. Humans are exposed to BPA through ingestion, inhalation of household dust, and dermal exposure⁵. BPA can leach out of variable products, and high levels of the monomer was detected in human and animal samples⁶. It seems that increased temperature leaches BPA into food and water products as does acidic pH of fluids². Besides, dermal contact with sales receipts and printer paper containing BPA compounds leads to BPA exposure⁷. Nowadays, there is raising public concerns about low-level exposure to BPA in the general population. Environmental protection agency's current safe dose for BPA is 50 ug/kg-day. Numerous published articles described the adverse health outcomes of BPA in mammalian and non-mammalian laboratory, wildlife, and in vitro models⁸⁻¹⁰.

Among the many health effects associated with BPA exposure, this chemical has been linked with oxidative stress and excessive production of reactive oxygen species¹¹. Reactive oxygen species (ROS) are cytotoxic agents that cause oxidative damage by attacking cell membrane and DNA¹². Oxidative stress results from an imbalance between anti-oxidant defence mechanisms and excessive generation of oxidants, leading to cell and tissue injury. To prevent the harmful effects of ROS, anti-oxidant systems, both enzymatic and non-enzymatic, are naturally present and counteract free radicals. At a certain dose, BPA exposure causes oxidative toxicity and carcinogenic effects¹³. The aim of the work is to study the oxidative stress and antioxidant status in a group of females and its relation to the BPA level as a well documented endocrine-disruptor.

SUBJECTS AND METHODS

This work was conducted on a total of 90 females. All are working in administrative tasks at the National Research Centre. Weight was measured using a digital Toledo scale (Mettler-Toledo International, Inc., Columbus, OH). Height was measured using a fixed stadiometer. Body weight, height, waist circumference were measured following standard procedures. Calibrated instruments

Table 1: Characteristics of the studied groups.

Variable	Obese(n=33)	Overweight(n=26)	Normal(n=31)	p-value
Age (years) (mean±SD)	36.21±10.9	37.46±12.1	32.84±9.1	0.24
Range	(16-58)	(17-58)	(18-58)	
waist circumference(cm) (mean±SD)	99.9±13.0	83.2±7.3	72.2±8.4	<0.0001*
Range	(76-143)	(70-98)	(56-86)	
Body Mass Index(cm/m ²) (mean±SD)	36.8±5.4	26.6±1.6	21.5±2.0	<0.0001*
Range	(31-51)	(25-29)	(18-24)	

Table 2: Comparison between the studied groups regarding their antioxidant activity:

Serum antioxidant enzyme	Referent (n=31)	Overweight (n=26)	Obese (n=33)	p-value
Glutathione peroxidase(ng/ml)	53± 17.5 ^a (56.8) ^b	45.9±12.2 ^a (47.4) ^b	38.2±14.4 ^a (33.3) ^b	0.001*
Catalase (IU/ml)	27.3±7.6 ^a (62.7) ^b	23.5±6.4 ^a (47.8) ^b	18.1±4.7 ^a (27.5) ^b	<0.0001*
Reduced Glutathione(mg/dl)	1.5±1.7 ^a (54.9) ^b	0.9±0.5 ^a (42.2) ^b	0.85±0.4 ^a (39.2) ^b	0.042*
Glutathione transferase(μmol/dl)	14.1±2.7 ^a (61.2) ^b	12.9±3.0 ^a (52.0) ^b	9.4 ±2.7 ^a (25.7) ^b	<0.0001*
Total antioxidant capacity(mmol/l)	3.6±1.7 ^a (59.5) ^b	2.8 ±1.1 ^a (43.1) ^b	2.4±0.9 ^a (34.1) ^b	<0.0001*

*P-value of Kruskal-Wallis test.

^aMean ±SD^b(Mean Rank)

Table 3: Correlations between antioxidant enzymes and both body mass index and Bisphenol A levels of the studied groups

Antioxidant enzyme		Body mass index	Bisphenol A(ng/ml)
Glutathione peroxidase(ng/ml)	r	-0.33	-0.24
	p-value	0.001*	0.1
Catalase(IU/ml)	r	-0.5	-0.01
	p-value	<0.0001*	0.9
Reduced Glutathione(mg/dl)	r	-0.25	-0.08
	p-value	0.02*	0.4
Glutathione transferase(μmol/dl)	r	-0.45	-0.17
	p-value	<0.0001*	0.1
Total antioxidant capacity(mmol/l)	r	-0.37	-0.06
	p-value	<0.0001*	0.58

r=Pearson correlation coefficient

*correlation is significant at ≤ 0.05 level.

Table 4: Regression coefficient table to predict the effect of bisphenol A on Malondialdehyde as an oxidative stress indicator after controlling the effect of BMI:

Oxidative Stress Marker	Unstandardized Coefficients(95%CI)		Standardized Coefficients	t-value	P-value
	B	S. E	Beta		
Malondialdehyde (nmol/ml)	0.25	0.1	0.2	2.2	0.03*

(e.g., scales and height measuring meters) were used. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m²), and standard categories were applied to classify the participants into three groups¹⁴. The first one included thirty- one referent subjects with normal weight (18.5-24.9 kg/m²). The second group consisted of twenty –six overweight females (25–29.9 kg/m²). The third group comprised the obese group with body mass index ≥ 30 kg/m² and included thirty- three subjects. Waist circumference was measured to the nearest 0.1 cm at the level of the iliac crest. Standard cutpoints for abdominal

obesity in women (>88cm) was used, as outlined by the Adult Treatment Panel (ATP) III report. The studied groups were matched for age and socioeconomic status. None of the included females were exposed to toxic substances. Also, smokers were excluded from the study. A written consent was obtained from all participants prior to the study. Approval of the Ethical committee at the National Research Center was taken. Especially designed questionnaire was filled by all subjects covering personal and medical history. Full clinical examination was performed. About 10 ml of venous blood were collected from all participants by sterile disposable syringes. The

samples were divided into 2 portions, the first one was left to clot and centrifuged; the separated serum was used for estimation of Malondialdehyde (MDA) and total antioxidant capacity (TAC) calorimetrically according to Satoh¹⁵ and Koracevic et al.,¹⁶ respectively. BPA was detected in serum by Elisa using Kits purchased from Glory Science Company, USA

The second portion of the blood sample was collected in heparin tubes and dealt with as follows:

- Apart of the whole heparinized blood was used for estimation of reduced glutathione (GSH) by Colorimetric Method¹⁷.

- The rest was centrifuged and the separated plasma was used for estimation of catalase and Glutathione transferase according to Aebi¹⁸ and Habig et al.¹⁹ respectively. Packed RBCs were used for the estimation of Glutathione peroxidase (GPx) by UV method²⁰. All kits were purchased from Bio-Diagnostic company (Egypt).

Statistical analysis

The collected data and the clinical results have been computerized and coded using SPSS version 20.0 IBM software and statistically analyzed. Quantitative data were expressed as mean values \pm standard deviation (SD). Ranges, frequency of distributions were estimated for quantitative variables. Normally distributed data were compared using ANOVA test for more than 2 groups. On the other hand, non-parametric Kruskal-Wallis test has been used in case of skewed data. Differences were considered significant with P value ≤ 0.05 . The correlations between individual variables were tested using Pearson correlation coefficient (r). Values ≤ 0.05 were considered statistically significant. Linear Regression analysis has been used to predict the changes in Malondialdehyde levels as an oxidative stress indicator based on serum Bisphenol values. Confounding factors were controlled with 95% confidence interval.

RESULTS

The general characteristics of the studied groups are presented in table 1. It is clear that the three groups were matched in their age (p -value >0.05) while, their body mass indices as well as their waist circumferences were significantly different (P -value <0.0001). The median level of BPA of the studied groups is 1.6ng/ml. It is observed that 54.5% ($n=18/33$) of obese subjects have had Bisphenol level higher than the median. Consequently, 53.8% ($n=14/26$) of overweight subjects versus 32.2% ($n=10/31$) of normal weight subjects have had BPA levels higher than the median. Differences among the three groups were statistically insignificant (P -value >0.05) (Fig.1). Moreover, the statistical comparison between the mean ranks of BPA levels was also insignificant (p -value >0.05) with higher mean ranks, among obese (48.3ng/ml) and overweight subjects (42.4ng/ml), than normal weight subjects (38.1ng/ml). However, statistically significant positive correlation was observed between the body mass index of studied groups and their serum levels of the environmental pollutant BPA ($r=0.2$ & p -value $=0.038$) as demonstrated in figure

(2). Studying the oxidative stress in the three groups revealed that the obese females had higher levels of mean values of serum MDA (2.8 ± 1.1 nmol/ml) when compared to the referent (2.4 ± 0.7 nmol/ml) and the overweight (2.5 ± 1.0 nmol/ml) groups. However, the difference between the three groups was statistically insignificant (p -value >0.05). But, as illustrated in figure (3) statistically significant positive correlation between the body mass index of the studied groups and the oxidative stress indicator serum MDA was observed ($r=0.2$ & p -value $=0.028$). It is clear from table (2) that there were statistically significant differences in mean values as well as in mean ranks of studied antioxidant enzymes in the three groups under study. Markedly observed lower values were detected among the overweight and obese females respectively when compared to normal weight group. When we studied the correlation between serum levels of Bisphenol A and serum levels of studied antioxidant enzymes after controlling the effect of BMI we noticed absence of correlations between Bisphenol A and all antioxidant enzymes (p -value >0.05). Strong inverse correlations between BMI and all antioxidant enzymes are obvious (P -value <0.05) (table 3). After controlling the effect of BMI among the studied groups, a positive significant correlation between BPA and level of oxidative stress marker MDA ($r=0.2$ & p -value $=0.03$) had illustrated in figure (4). Table (4) shows that the regression model significantly predicted the effect of Bisphenol A on the serum level of malondialdehyde as an oxidative stress indicator after controlling the effect of BMI. It is predicted that each 1ng/ml increase in Bisphenol serum level could lead to an increase in the serum level of Malondialdehyde by 0.25nmol/ml.

DISCUSSION

Recently, an alarming rise in the prevalence of obesity was observed in developing as well as developed countries²¹. This emerging worldwide obesity epidemic has been related to increased exposures to environmental endocrine disruptors, collectively known as "environmental obesogens"²². Bisphenol A is an important potential obesogen with worldwide human exposure. In the present study, we depended on the BMI as an indicator for obesity according to WHO. BPA was present in serum of all examined subjects. The level was higher in the category of obese females ($BMI \geq 30$ Kg/m²). This finding was expected as this compound is a building block of polycarbonate plastics often used for food and beverage storage²². It is also a component of epoxy resins that are used to line food and beverage containers. Numerous studies have shown that BPA can leach from those and other products in contact with food and drink, and as a result, routine intake of BPA is expected^{2,10,23}. Several National Health and Nutrition Examination Survey (NHANES) studies found associations between urinary BPA and BMI/obesity. Carwile and Michels²⁴ examined data from NHANES 2003–2006 participants, and found that higher urinary BPA was significantly associated with higher BMI and waist circumference, indicating associations with both general and central

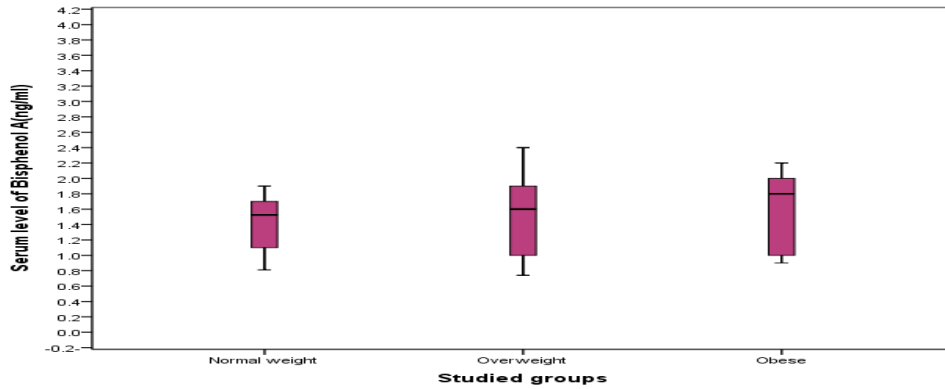


Figure 1: Box plot shows serum levels of Bisphenol A among the three studied groups.

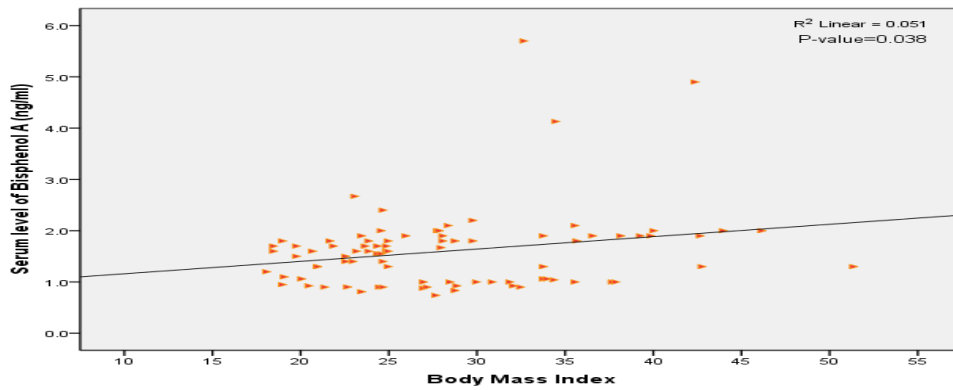


Figure 2: Scatter plot shows the correlation between serum level of Bisphenol A and BMI.



Figure 3: Scatter plot shows the correlation between serum level of Malondialdehyde and BMI.

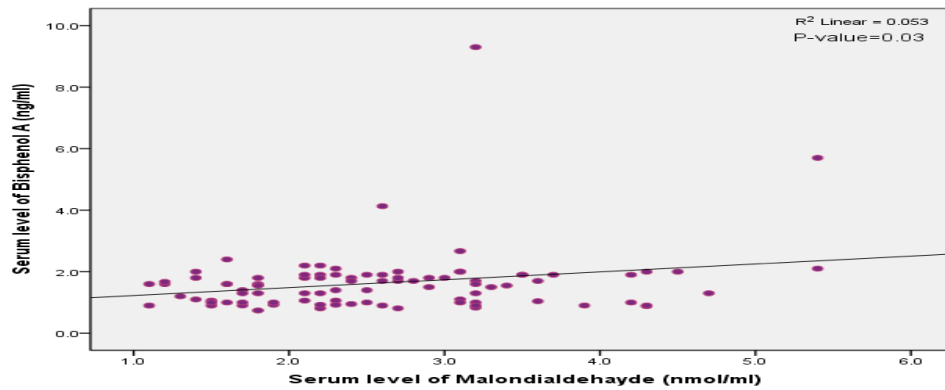


Figure 4: Scatter plot shows the correlation between serum levels of Bisphenol A and Malondialdehyde enzyme.

obesity. Shankar et al.²⁵ studied NHANES 2003–2008 participants greater than 20 years old. In that study,

higher urinary BPA was strongly correlated with higher BMI and waist circumference as a whole. Trasande et

al.²⁶ studied childhood obesity in the NHANES 2003–2004 population, examining children aged 6–19 years. Again, higher urinary BPA was associated with obesity. Although positive associations between BPA exposure and BMI/obesity are well established, yet their interpretation still needs further studies. In an in vitro study of human adipose tissue, there was no association between BMI and adipose tissue BPA concentration. This indicates that increased urinary or serum BPA is not due to increased adipose stores of BPA²⁷. It is also possible that individuals with increased BMI have higher caloric intake and may be exposed to higher concentrations of BPA through food packaging or other lifestyle factors. Alteration of adiponectin release from adipose tissue²⁸, insulin resistance^{29,30} and increased inflammatory cytokines³¹ are suggested mechanisms by which BPA increases BMI. Although the BPA level was insignificant (p value > 0.05), yet this level induced oxidative stress as reflected by the higher level of MDA in the overweight and obese groups compared to the referent group. The results of the current study points to the deleterious effect of BPA on the measured antioxidant defense enzymes namely (glutathione peroxidase, catalase, reduced glutathione, glutathione transferase and total antioxidant capacity). Obese subjects who reported a higher level of BPA compared to the referent and overweight group showed more decline in those enzymes. Moreover, according to the regression model it could be predicted that each 1ng/ml increase in the level of serum BPA will lead to a decrease in the serum level of glutathione peroxidase by 0.01ng/ml. Hong et al.³² suggested a positive association (p < 0.01) between total urinary BPA and measures of oxidative stress, (MDA) and 8-hydroxydeoxyguanosine (8-OHdG). This study was conducted on a large population of Korean adults. It proved that environmental exposure to this chemical is associated with oxidative stress in urban adult populations. The authors concluded that exposure to certain environmental chemicals as BPA might contribute to insulin resistance and to obesity. In our study, a significant positive correlation was evident between BPA level and MDA. An interesting finding was observed in the regression model. Accordingly we predicted that a 1ng/ml rise in Bisphenol serum level might lead to an increase in the serum level of Malondialdehyde by 0.25nmol/ml. Hence continuous exposure to BPA even at a low level might ultimately lead to marked cumulative oxidative stress and consequently hazardous effect on human health.

CONCLUSION

More studies at a larger scale are needed as exposure to BPA became ubiquitous in humans. The safe level of BPA should be reconsidered as health effects may occur at much lower doses than that are applied in traditional toxicology studies. The global obesity epidemic is increasing worldwide and needs more attention. The environmental exposure to obesogenic chemicals has brought about an urgency to clarify their role in such threatening epidemic.

CONFLICT OF INTEREST

The Authors declare that there are no conflicts of interest.

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