### URINARY BISPHENOL A CONCENTRATIONS IN CHILDREN WITH ATTENTION-DEFICIT/HYPERACTIVITY DISORDER: A PILOT STUDY

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### ABSTRACT

Background: Bisphenol A (BPA) is an industrial chemical associated with several health effects and exposure to BPA remains poorly characterized in the developing countries. In this pilot study, we evaluated BPA exposure, assessed some sources of exposure and its health effects among Egyptian children. Methods: Total BPA concentration was measured in two groups of spot urinary samples of ADHD (N = 24) and apparently healthy control (N = 24) children. Questionnaire data regarding food storage behaviors to assess potential sources of exposure was collected. Results: ADHD children exhibited higher urinary BPA concentrations; with median value of 24.5  $\mu$ g/L. Reported consumption of canned food was a significant predictor of increasing concentrations of urinary BPA. Conclusion: The reported relatively high urinary BPA concentration between the canned food consumption and the increased urinary BPA concentration highlights the need to modify food and consumer product patterns to prevent potential adverse health outcomes in the future.

Keywords: ADHD, Egyptian, Children, Bisphenol A, Canned food

### **INTRODUCTION**

Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder in children. Manyenvironmental risk factors e.g. chemical exposures, psychosocial factors and nutrition hada role in its etiology and pathophysiology (**Grandjean and** Landrigan, 2014; Adesman et al., 2017).

Bisphenol A (BPA) is an exogenous toxin that is endocrine disrupting. It is used as a monomer for producing polycarbonated plastics. As well as being a precursor of epoxy resins. (**Bosch-Panadero**, 2018)

Ubiquitous worldwide use of BPA containing products results in increase in the number of exposed humans everyday especially young children (Marcelle et al., 2016).

Bisphenol A (BPA) is a weak estrogenic chemical used in the production of some plastics and resins (in food and drink containers), flame retardants, dental sealants, and in the recycling of thermal paper (Vandenberg et al. 2007). According to the National Health and Nutrition Examination Survey, the geometric mean urinary concentration of BPA in the general U.S. population sampled was 2.6 µg/L in concentrations 2003-2004; were significantly higher in women than in men, in children than in adults, and in individuals with low household income compared with individuals with higher income (Calafat et al. 2008).

Regarding human exposure to BPA, food intake can be considered the most

serious among all the routes, not only because it potentially reaches more people in different age groups (including infants, an especially vulnerable group), but also because it inadvertently occurs over long time periods. **Aris (2014).** 

An association was reported between BPA exposure, childhood behavior and cognitive development disorders (Harley et al., 2013; Hong et al., 2013; Evans et al., 2014; Roen et al., 2015; Adesman et al., 2017), as BPA had been detected as an organic developmental neurotoxic agent (Grandjean and Landrigan, 2014).

Neuroendocrinal effects of bisphenol A are linked to the pathogenesis of ADHD. Perinatal exposure to bisphenol A modulates neurotransmitters: dopaminergic it accelerates turnover of dopamine (Honma et al., 2006), reduces functional dopamine 3 forebrain receptors in and causes abnormalities in expression of dopamine transporter gene expression in neuronal membrane (Mizuo et al., 2004). Any imbalance in dopamine neurotransmition underlies the pathophysiology of ADHD (Ishido et al., 2004).

### Aim of the study

This study is aimed to explore environmental Bisphenol A exposure at

vulnerable childhood stages of development, including Egyptian children aged 6-12 years old with ADH

### MATERIAL AND METHODS

### Study participants

After obtaining Princess NourahBint Abdulrahman University institutional ethical review board approval (18-0216). We recruited 24 children between ages of 6-12 years (**Tewar et al., 2016**); attending Abu-Elreesh child psychiatry outpatient clinic, Egypt. The children were diagnosed as Attention-deficit/hyperactivity disorder (ADHD) by psychiatric consultant adhered to the Diagnostic and Statistical Manual of Mental Disorders, 5<sup>th</sup>edition (ADHD-DSM-V) (American Psychiatric Association, 2013).

The Arabic version of Conner's Parent Rating score (CPRS) was completed by parents to assess behavioral problems. CPRS with scores up to 60 was considered mild, scores from 61 up to 70 was considered moderate and scores above 70 was considered severe (Casas et al., 2015). IQ assessment was done using Stanford Binet test (Bain and Allin, 2005).

Children with a diagnosis of one or more of the following conditions were excluded from the study: IQ level below 70 (which denotes intellectual disability) (AAIDD, 2010), organic brain disease, chronic medical condition, psychotic, anxiety; or bipolar disorders

Information on child age, residence, medical history, type of feeding, potential exposure to bisphenol A sources e.g. food/water storage and canned food consumption was obtained by mother selfreported questionnaire (**Dey et al., 2011**; **Nahar et al., 2012**).

Apparently healthy 24 children, matched for age and sex, were recruited as a control group.

## Urine sample collection and bisphenol A measurements

Mothers of participants gave an informed consent prior to urine samples collection. One spot urine sample was collected from each participant in asterile polypropylene container and stored at -20°C (**Casa et al., 2015**). Specific gravity was measured by refractometry to detect sample dilution. In the analytical toxicology laboratory, forensic medicine and clinical toxicology department, Cairo University, Egypt, the total urinary bisphenol A concentration was quantified by high performance liquid chromatography (HPLC) with fluorescence detectorfollowing (Matsumoto et al. (2003).

HPLC high-pressure The system isocratic system consisted of а DionexUltiMate 3000 UHPLC, RS pump, auto-sampler, column c18 (6 mm inner diameter  $\times$  150 mm length). The mobile phases were prepared by mixing acetonitrile, tetrahydrofuran, and water (35:35:130, 70:35:95) in the gradient mode. The auto sampler injected 40 µL of the processed sample into the system at 1.0 mL/min flow rate.

For quality assessment, analytical procedure linearity was tested by using series of concentrations (10, 25, 50, 100, 200, 400, 1000 ug/ml). It was proved to be linear (R2 =0.99). The limit of detection (LOD) was 0.04  $\mu$ g/L and it wascalculated as 3.3\*St deviations/slope. In addition to the calibration standards, two blanks, two lowconcentration quality control materials, and two high-concentration quality control materials were anlalyzed, along with the samples. Bisphenol study А was undetectable in the two blanks.

### Statistical analysis

The data were entered and analyzed using Statistical Package Program (SPSS version, 17) (SPSS, Inc., Chicago, Illinois). Quantitative data were summarized as mean ± standard deviation. Qualitative data were summarized as number and percentage. Chisquare tests were used to compare sex, medical history, type of feeding, food storage behavior and canned/tinned food consumption and a t-test was used to compare urinary BPA concentrations between ADHD and control groups. Nonnormally distributed numeric variables were compared by Mann-Whitney test. P-value  $\leq$ 0.05 was considered statistically significant.

Regression models were conducted to identify the factors that may have contributed to the concentration of urinary BPA. Age and sex were included in the regression model as continuous biologically variables, while feeding type, and canned food consumption were included as categorical variables. Regression coefficients with p < 0.05 were considered statistically significant

### **RESULTS**

Participant's age ranged between 6-12 years with average age of 8 and 7.2 years for ADHD and control groups respectively. Overall, there was no significant differences in age, sex, residency and medical history between the two groups. Learning disability was significantly higher among ADHD group table (1). Most of ADHD group were combined inattentive hyperactive subtype (58%) and their Conner's Parent Rating score was moderate (75%) figure (1, 2).

Analysis of feeding type, food storage behaviors, canned food/drinks consumption indicated significantly different reports of feeding type and canned food/drinks consumption (p-value=0.05 and .001), but similar reports of food storage in plastics between the two groups. 100% of ADHD group compared to 33% of the control group reported consuming canned food/drinks. In general, all studied Egyptian children stored food in plastics (table 2).

Both groups had significantly different detectable urinary BPA concentrations (p-value= .024). ADHD group had a higher median bisphenol A concentration of  $24.5\mu g/L$  (range <LOD-246), while control group had a median bisphenol A concentration of 1.6  $\mu g/L$  (range <LOD-12) (table 2).

The limit of detection (LOD), according to the regression models, canned food consumption was associated with higher urinary BPA concentrations among studied school-age Egyptian children (p-value=.044 with  $\beta$  =83.8) table (3).

Variable	ADHD	Control	P-value				
	group	group					
Demographic data of the studied sample							
Age (mean±SD)	8±2	7.2±1.2	.26	95% confidence			
				interval (66-2.3)			
Sex							
Boy (N/%)	14(58)	18(75)	0.4				
Girl (N/%)	10(42)	6(25)					
Residency in urban areas	24(100)	24(100)					
Pre-natal, natal and postnatal history							
Positive parent	4(16.7)	-	.14				
consanguinity							
Negative pregnancy	24(100)	24(100)					
complication							
Delivery			0.6				
Vaginal	18(75)	20(83.3)					
Caesarian section	6(25)	4(16.7)					
Positive postnatal	4(16.7)	-	0.14				
complication							
Learning disability and intelligence Quotient							
Positive learning disability	24(100)	2(8.3)	0.003*				
IQ	81.6±3.4	91.8±5.2	0.000*	95% confidence			
				interval (-13.96.4)			

# Table (1): Demographic data, medical history, learning disability and intelligence quotient among studied cases



Figure (1): Percent of ADHD subtype among studied cases



Figure (2): percent of ADHD score among studied cases using Conner's Parent Rating score

Variable	ADHD	Control	P-value			
	group	group				
Potential exposure to Bisphenol A						
Type of Feeding						
Breast	12(50)	24(100)	0.05*			
Breast and bottle	12(50)	-				
Food/water storage in plastic	24(100)	24(100)				
Canned food/drinks	24(100)	8(33)	.001*			
consumption						
Urinary bisphenol A concentrations						
Bisphenol level (µg/L)						
Mean, median	63.9, 24.5	2.2, 1.6	0.024*			
Minimum, maximum	LOD, 246	LOD, 12				

Table (2): Feeding type, food storage behavior, canned food/drinks consumption and urinary
bisphenol A concentrations among studied cases

Table (3): Regression analysis model between individual covariates and urinaryBPA concentrations ( $\mu g/L$ ) the used individual covariants were age,sex,feeding and cannedfood/drinks

TOOd/drinks								
Model summary								
R	<b>R</b> <sup>2</sup>	F	p-value					
.63	.4	2.4	.05*					
Coefficients								
β	t	p-value	95% confident					
			interval					
9.7	1.5	.14	(-4 to 23.5)					
35.8	1.3	.18	(-20.2 to 91)					
-3.4	235	.8	(-33 to 26.9)					
83.8	1.9	.044*	(7.8 to 175.3)					
	Model sum         R         .63         Coefficient         β         9.7         35.8         -3.4         83.8	Model summary           R         R <sup>2</sup> .63         .4           Coefficients         β           β         t           9.7         1.5           35.8         1.3           -3.4        235           83.8         1.9	Model summary           R         R <sup>2</sup> F           .63         .4         2.4           Coefficients         p-value           β         t         p-value           9.7         1.5         .14           35.8         1.3         .18           -3.4        235         .8           83.8         1.9         .044*					

### DISCUSSION

This pilot study explored childhood BPA exposure and its urinary concentration in school- age Egyptian children.

Current study findings showed that school-age boys suffered from ADHD more than girls, this was consistent with (Ghanizadeh, 2008; Tewar et al., 2016). Learning disability and lower IQ were significantly higher among ADHD group similar to what reported byAboul-ata and Amin (2018) that the common aspects of ADHD were disturbances in functioning and academic achievement failure.

The combined ADHD was the highest detected subtype, that was in the same line with Jordanian study (Al Azzam et al., 2017), but the current study had higher rates than Turkish (Zurlo et al., 2015) and Nigerian studies (Ministry of education, 2014). These differences could be due to the differencein the study methodology, ADHD diagnostic criteria, studied populations and the sample size (Mustieles et al., 2015; Al Azzam et al., 2017).

This study showed higher total urinary BPA concentrations among studied Egyptian children compared to several local and international studies done in Gharbiah province, Egypt (Nahar et al., 2012), Japan (Ouchi and Watanabe, 2002), and Mexic (Cantonwine et al., 2010). This difference may be confounded by the analysis method, timing of urine collection, as well as age, sex, and genetic differences (Yang et al., 2003; Volkel et al., 2005). However, the differences can also be attributed to the country specific lifestyle practices including food storage, canned food consumption, consumer product use and plastic wastes elimination.

The National Food Consumption Survey reported the highly increased number of the Egyptian household consumed ready-made foods and carbonated soft drinks with the substantial differences in food consumption patterns over many decades (Galal, 2002). The expansion of the Egyptian economy showed a potential increase in various environmental exposures so appropriate actions should be considered to prevent its potential adverse health outcomes in the future. In addition, Fu and Kawamura(2010) stated that open burning of plastic wastes in developing countries was associated with high atmospheric BPA levels.

Total urinary BPA was significantly higher in ADHD group indicating an underlying variability in the individual health, and/or lifestyle factors within the two groups. This was inconsistent with many previous studies which reported anincreased behavioral problems in children who had high BPA levels**Braun et al.**, 2011; Hong et al., 2013; Casas et al., 2015; Mustieles et al.,2015; Roen et al., 2015; Tewar et al., 2016.

Tewar et al. (2016) reported that alloin children's fold increase BPA concentrations was associated with two-fold increased odds of ADHD. Moreover, Hong et al. (2013) stated that BPA concentration was negatively associated with learning disability. This could be explained by that BPA exposure could change the expression of genes in some tissues, including brain, and these changes may be persisted into adulthood and perhaps in the next generations(Kundakovic et al., 2013; Wolstenholme et al., 2013).

Egyptian children had been exposed to BPA from many sources during their childhood (**Nahar et al., 2012**); however, these exposures had been the same for both studied groups with no significant difference except for the type of feeding and canned food consumption. This was in the same line with **Casas et al. (2013**), who reported that the detected urinary BPA was due to dietary exposure, as the diet was the primary source of BPA exposure (**Morgan et al., 2011**). Adesman et al. (2017) found a significant association between formula-feeding and ADHD, as non-breast-fed (formula fed) children had 5 fold increased risk of ADHD. However, the same association in 2011not found; 2 to 3 years after removal of BPA from infant formula cans and baby bottles. It was concluded that ADHD could be a neurotoxic consequence of BPA as an indirect food additive.

Carwile et al. (2011) reported a relevant BPA exposure from canned food use. Current results indicated a direct significant positive association between canned food consumption and urinary BPA and it acted as a potential confounder. Another Egyptian study noted that food in plastic was significantly storage associated with higher urinary BPA concentrations (Nahar et al., 2012), suggesting a higher use of BPA-containing storage products.

To the best of our knowledge, this study is one of the earliest efforts to assess food storage practices among ADHD children from a developing country and urinary BPA concentrations using HPLC.

### **CONCLUSION**

This study showed that ADHD children were significantly different from control group as they had high prevalence of canned food consumption with high urinary bisphenol level. Furthermore, the current study showed a significant association between canned food consumption and BPA concentrations and suggested that the diet could be an important route for BPA exposure in this population. Current findings could be used as a guide for further investigations of ADHD in Egypt with a closer look at changing food patterns and the consumer market in developing countries.

Current study had some limitations. First, our sample was convenience sample with small size. Second, only one spot urine sample from each participant was collected. However, this study is a preliminary pilot study and spot urine samples reflect recent BPA exposure. Third, our data relied on parent report for infant history, which may not be accurate. However, it was the only source to obtain the children's information. For these reasons, we recommend future researches with large sample size and analysis of multiple spot urine testing to account for variation in exposure with specific analyses of behavioral and dietary habits.

### DISCLAIMER

All authors declared that the abstract had not been previously presented or published in a conference, and the manuscript was not a part of a research, PhD or thesis project, or any other relevant information.

### **CONFLICT OF INTEREST**

There are not any financial, personal, or professional interests that could be construed to have influenced the work.

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### RECOMMENDATIONS

1. Decrease use of plastic appliances and replace with glass or stainless steel, or if possible with BPA free plastics

2. Use of fresh instead of canned food

3. Further studies showing difference in BPA levels between children in ueban and rural areas and between children in different socioeconomic levels

4. Futher studies on the effect of BPA on learning disabilities and other childhood disorders

5. Legalizations for the use of bisphenol in plastics and canned food

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### الملخص العربى

تركيز مادة بيسفينول أفى البول لدى الاطفال المصابون بفرط حركه و تشتت انتباه: در اسه تجريبيه

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ان اضطراب نقص الانتباه وفرط الحركه لدى الاطفال ، هو عباره عن اضئطراب فى النمو العصبى لدى الاطفال. قد يحدث هذا نتيجة لمسببات فى البيئه المحيطه مثل التعرض لكيماويات ، تناول انواع معينه من الغذاء او لاسباب نفسيه. تعتبر مادة بيسفينول أ من المواد المعيقه لعمل الغدد الصماء فى جسم الانسان. و تستخدم هذه المادة فى تصنيع البلاستيك كما انها تستخدم كماده لحفظ الغذاء من الفساد.

يتعرض الانسان لهذه الماده من خلال استخدام الأغذيه المعلبه ، علب حفظ الطعام و قنينات الماء المصنوعه من البلاستيك و ايضا من خلال الاجهزه الطبيه المصنوعه من البلاستيك .

وتشير الابحاث الى وجودعلاقه بين زيادة نسبة مادة البيسفينول فى الدم وبين التطور المعرفى و النمو العقللى لدى الاطفال و هو بالتبعيه يؤثر فى سلوك الاطفال فيكثر فرط الحركه، التوتر، الاكتئاب و ايضا تشتت الانتباه و الاندفاعيه الزائده لدى الاطفال

اجريت هذه الدراسه على الاطفال المصريين من سن السادسه و حتى الثانيه عشر لبيان نسبة البيسفينول أ و مدى تاثير ها على الاصابه بمرض فرط الحركه و تشتت الانتباه لدى هؤلاء الاطفال