May detectable urinary Bisphenol A among children be associated with cardiovascular risk factor?

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Bisphenol A (BPA) is suspected to be associated with several chronic metabolic diseases. The aim of the present study was to examine the associations of urinary BPA concentrations, with cardio metabolic risk factors including: age, gender, lipid profile, High-sensitivity C-reactive protein (hs-CRP) levels among children and adolescents. It's a cross-sectional study enrolling 167 children; 95boys and 72girls. They aged from 6 to 16 years, randomly selected from primary, and preparatory schools in Giza, Egypt. All participants were subjected to thorough clinical examination, Anthropometric measurements. Urinary Bisphenol A was detected by high performance liquid chromatography-tandem mass spectrometry (HPLC) then total, conjugated, unconjugated, log total, BPA/creatinine and log BPA/creatinine were estimated. Serum samples were assayed for total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and High-sensitivity C-reactive protein (hs-CRP).Results: Participants mean age was 11.70 $\pm$ 3.08,while mean weight and height, was 53.63± 22.47, 149.09 ±17.99, respectively. Serum total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and hs-CRP) showed no statistically significant differences as regard to age and gender (P> 0.05).No significant association between BPA and the age, gender was found(P>0.05).Total BPA/creatinine ratio was significantly positively correlated with the serum total cholesterol and LDL-cholesterol (P<0.05).We did not find significant associations between BPA and any other cardiovascular risk factors. Our work provides additional evidence of associations between urinary BPA and cardiovascular risk factors among Egyptian children and adolescents.Future policy regulating children's consumer products is mandatory.

Keywords: bisphenol A, lipid profile, children, adolescents.

INTRODUCTION

Bisphenol A (BPA) has been found to be the highest volume chemicals produced in the world as it is used in polycarbonate (PC) plastics and epoxy resins production, which in turn are used as internal coating of food and beverage cans to protect food and drinks from direct contact with metals(Vandenberg et al ., 2010). PC plastics and epoxy resins are also found in many products as reusable bottles, electronic equipment, medical devices (e.g., dental sealants) as well as plastic containers. Because unbound monomers remain after BPA polymerization, BPA molecules can be leached from those products into environment and food. That human exposure to BPA is extensive through food intake which has been canned, this made BPA well detected in human urine, blood, milk and even human tissue as well as soil,

sediment, air, municipal waste and food(Huang et al.,2012). Human major route of exposure to BPA is the dietary pathway (Geens et al., 2012) .BPA is efficiently absorbed (>95%) once ingested, it is mainly excreted in urine as BPA conjugates: The urinary concentration of total BPA equals free BPA plus conjugated BPA (Kang et al.,2006)(Vandenberg et al.,2007)

As Endocrine disrupting chemicals bisphenol A (BPA) and its products have been implicated in obesity-related metabolic disturbances (Grun and Blumberg 2009)

Animal models have shown alteration in the circulating lipid profile after exposure to BPA(Moghaddam et al.,2015).Human exposure to BPA results in various side effect starting from reproductive abnormality, metabolic diseases up to chronic diseases.(Rubin2011)(Brody et al.,2007) (Lang et al.,2008)**.**Behavioral abnormalities have been reported when exposure to BPA occurs during critical phases of development (Braun et al.,2011).Concerns about the adverse health effects of BPA on children have increased. In adults high urinary BPA was related and linked to risk of obesity, type2 diabetes, and cardiovascular disease based on cross-sectional studies (Shankar and Teppala 2011)(Wang et al.,2012)**.** Moreover, studies have shown higher urinary concentrations of BPA in children and adolescents compared with adults (Calafat et al., 2008).

Inflammation as a primary causative factor in many chronic diseases and the inflammation hypothesis has resurfaced today in the pathogenesis of diabetes, heart disease, cancer and degenerative brain disease (Danesh et al., 2000).Cardiovascular diseases were commonly assessed by the standard lipid profile. Meanwhile high sensitive c reactive protein (hs-CRP) level has a better prospective value than known risk factors, such as low density lipoprotein (LDL) cholesterol levels and is now used in daily practice for risk stratification of coronary heart diseases in adult population(Vikram et al., 2003)(Kavey et al., 2003).

The objective of this study was to examine the relationship between urinary BPA levels and cardio metabolic risk factors including: age, gender, lipid profile, High-sensitivity C-reactive protein (hs-CRP) levels among Egyptian children and adolescents.

MATERIALS AND METHODS

Study population

This study included 167 Children,95 boys and 72 girls, randomly selected from primary, and preparatory schools in Giza, Egypt. Their age range was 6-16 years old.
Exclusion criteria: Children with liver diseases, renal diseases, and thyroid disorders, endocrinal and genetic obesity.
Children whose parents or guardians refuse to participate.

Each child enrolled in this study was subjected to the following:
1- Thorough clinical examination that included chest, heart, abdominal, and central nervous system examination.
2- Anthropometric measurements include: Height was measured to the nearest 0.1 cm using a Holtain portable anthropometer, while weight was determined to the nearest 0.01kg using a Seca scale Balance with the subject dressed in minimal clothes and without shoes.
3-Laboratory investigations:
a-Each child gave a urine sample for
Determination of urinary creatinine using kinetic kit according to the method of Bartel, 1972.

Estimation of urinary BPA concentration:
i- Total urinary BPA was determined using high performance liquid chromatography-tandem mass spectrometry HPLC, Agilent technologies 1100 series, equipped with a quaternary pump (G131A) according to the method described by Alkaranfilly et al., (2015).
ii-Free BPA (unconjugated BPA) was measured by buffering 500 µL of urine with 30 µL of 2.0 M sodium acetate buffer (pH 5.0) and hydrolyzed enzymatically with β-glucuronidase/sulfatase (4,414/168 U/µL) for 3 hr at 37°C in a shaking water bath. After hydrolysis, 100 µL of 2N HCl was added, and the hydrolysate was extracted once with 5 mL of ethyl acetate with 10 µg/L bisphenol B (internal standard). After centrifugation, 4 mL of supernatant was transferred to a new tube and evaporated with N2 gas. The residue was dissolved with 200 µL of 60% acetonitrile in water, and 40 µL of the solution was injected onto the high-performance liquid chromatography (HPLC) system (Agilant 1100 series).
iii- Conjugated BPA was calculated by subtracting the amount of free BPA from the total BPA.
b- Fasting venous blood samples were collected from each child following an overnight fast (12 to 14 hours).

Sera were separated by centrifugation and stored at -80 until assays.

i- Lipid profile was determined, total cholesterol (TC) and triglycerides (TG) were measured by quantitative enzymatic calorimetric technique (Titez 1982).

Serum High density lipoprotein - cholesterol (HDL) was measured by the phosphotungstate precipitation method (Lopez et al., 1977). Serum low density lipoprotein (LDL) was calculated using the Friedewald formula

ii- Serum High -Sensitivity C-Reactive Protein (hs-CRP) was estimated by enzyme-linked immunosorbant assay (ELISA), the kit was purchased from Monobind Inc, USA according to the method of (Kimberly et al., 2003).

Statistical analysis:

The BPA concentration was adjusted to the urinary creatinine concentration to correct for the urine volume The log total BPA, BPA/creatinine and log BPA/creatinine were estimated. Urinary BPA and BPA/Creatinine levels were log-transformed to improve normality of the distribution. The data were expressed as mean ± standard deviation and were compared by use of student's t-test. Pearson's correlation analysis was conducted to evaluate the correlation between the different variables normally distributed, while Spearman's correlation was conducted for the variables not normally distributed. SPSS version 21 (SSPS Inc, Pennsylvania, USA) was used for all analyses. Two-sided P-values <0.05 were considered statistically significant.

RESULTS

Hundred and sixty seven children were enrolled in this study with mean age 11.70 ± 3.08; they were 95 boys (56.1%) and 72girls (43.9%). Their mean weight was 53.63± 22.47, while their mean height was 149.09 ±17.99, respectively.

The children were sub grouped twice first according to gender (boys versus girls) and other according to age "group 1" less than or equals 12 years old while "group 2" older than 12 years old. In this study we used the American Academy of Pediatrics'2011proposed definition of dyslipidemia in pediatric populations to parameterized lipid levels in which abnormal total cholesterol ≥200 mg/dL , triglycerides ≥100 for mg/dL for children <9 years; ≥130 mg/dL for children 10–19 years, abnormal LDL-C ≥130 mg/dL and abnormal HDL-C <40 mg/dL. Meanwhile cut off point for serum hs-CRP level 2 mg/ml for defining an increased risk of CVD as analyses from large-scale clinical trials have used (Roberts2004) (Kamath et al.,2015)

Lipid profile of all participant did not reach the levels of dyslipidemia among the studied age group in spite of presence of some high results .On Comparing the means of the biochemical variables(lipid profile and hsCRP) as regard to age and gender, no statistically significant differences were detected (P> 0.05) (Table 1).

Overall serum BPA levels is described as total BPA , conjugated BPA, unconjugated BPA , log total BPA, BPA/creatinine ratio and log BPA/creatinine are shown in table 2.

The BPA/creatinine ratio showed positive significant correlation with the serum cholesterol and LDL, r=0.04, 0.01 respectively (figures 1, 2)

Positive correlations were also found between BPA (total, conjugated, unconjugated) and both Total cholesterol and serum LDL level but did not reach a statistically significant level.

Table 1:Serum lipid profile , high-senstive C-reactive protien and anthropometric characteristics of the studied children and adolescents

|	Boys(n=95)	Girls(n=72)	p-value	Age <12 n=85	Age ≥ 12 n=82	p-value
Weight(Kg)	50.04±18.63	51.39±17.87	0.661	44.06±16.28	57.58±17.68	0.00
Height(cm)	147.5±13.25	152.16±13.28	0.040	141.29±11.61	158.5±8.7	0.00
BMI-z score	1.38±1.5	1.82±1.4	0.026*	1.39±1.47	0.81±1.54	0.022
Serum cholesterol (mg/dl)	166.36±42.96	181.87±40.4	0.062	181.35±48.9	170.16±38.02	0.186
Serum triglyceride (mg/dl)	79.31±46.99	79.66±37.28	0.968	82.70±43.03	75.93±44.09	0.447
Serum HDL(mg/dl)	39.60±7.95	40.07±8.81	0.774	40.56±8.6	39.81±8.1	0.65
Serum LDL (mg/dl)	110.91±40.02	125.87±40.11	0.067	122.84±49.60	115.26±34.77	0.35
Hs-CRP (mg/ml)	5.17±7.01	4.13±5.75	0.529	6.50±7.8	6.50±5.4	0.1

Data are expressed in Mean±SD

Table 2: Overall Urinary BPA levels in the studied groups

|	Boys (n=95)	Girls (n=72)	p-value	Age <12 n=85	Age ≥ 12 n=82	p-value
Total BPA in ng/ml (Median – IR)	0.600-1.065	0.670-0.845	0.789	0.602-0.895	0.640-1.106	0.459
Conjugated BPA in ng/ml (Median – IR)	0.250-0.664	0.330-0.534	0.530	0.227-0.358	0.340-0.765	0.291
Unconjugated BPA in ng/ml (Median – IR)	0.200-0.513	0.210-0.525	0.743	0.210-0.553	0.180-0.465	0.490
log total BPA (Mean±SD)	-0.21 ± 0.49	-0.17 ± 0.43	0.57	-0.18 ± 0.43	-0.15 ± 0.51	0.795
BPA/creatinine ratio (Median – IR)	399-905	455-627	0.802	404-612	352-681	0.408
log BPA/creatinine (Mean±SD)	2.56 ± 0.54	2.62 ± 0.49	0.51	2.72 ± 0.56	2.55 ± 0.52	0.06

P < 0.05 is significant   IR = interquartile range
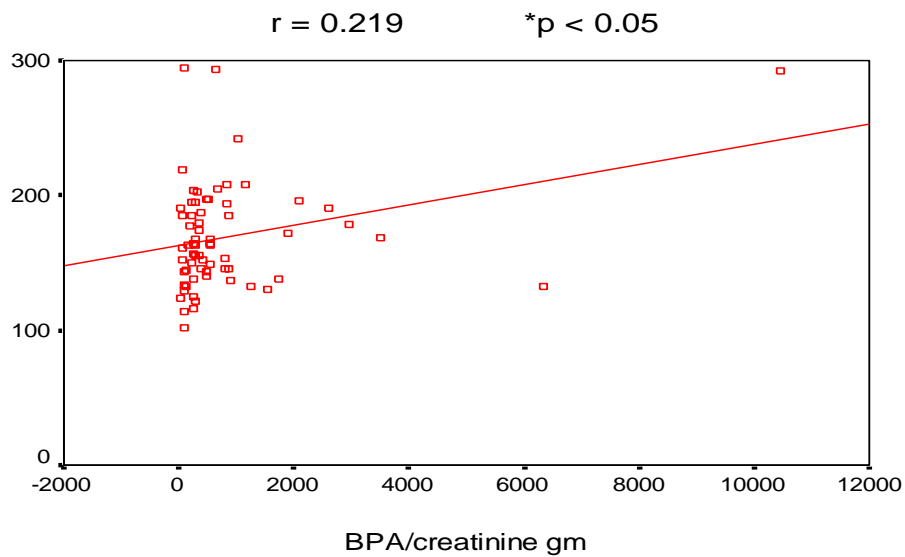
Correlation between BPA/creatinine and serum cholesterol

Figure 1: Correlation between total BPA\Creatinine ratio and serum cholesterol
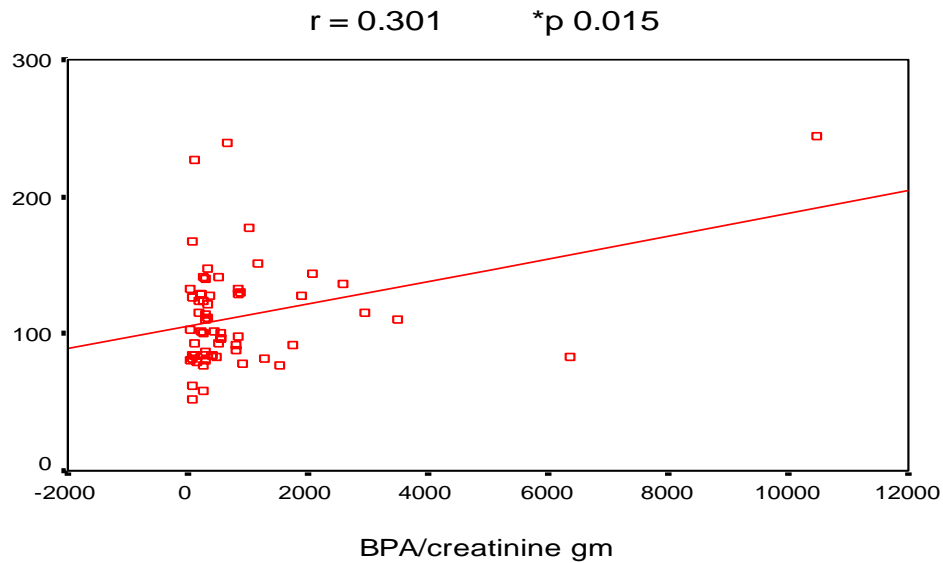
Correlation between BPA/creatinine gm and serum LDL

Figure 2: Correlation between total BPA \Creatinine ratio and serum LDL

DISCUSSION

To the best of our knowledge, this study is one of the first to assess Urinary BPA levels among Egyptian children and adolescents and to correlate this to lipid profile and hs-CRP. The present study revealed the presence of BPA in the urine samples of all participants which goes with old data from the 2003–2004 US National Health and Nutrition Examination Survey (NHANES) that reported 90% detectable levels of BPA in urine among population ≥6 years of age.

In this study the girls showed significant high BMI Z-score than boys, as many studies that showed the environmental risk factors more likely impacting girls 'weight than boys' weight but the present study didn't show difference in urinary BPA levels between boys and girls opposite to human studies that found gender difference in the BPA effect on other outcomes. In the same aspect Trasande et al 2012 published that urine BPA was associated with obesity in children and adolescents with no gender difference same study also reported ethnic differences with an absence of any association with Hispanic children .The present study didn't find significant difference between urine BPA level in different age group although significant higher BMI z-score was reported between group1 (less than 12years) and group 2 , Participants of this study were all 6 years old or above which may explain the

absence of age effect. .Other studies mentioned a relation between BPA and age. One of the estimated daily urinary BPA excretion ranged from 26.2 to 18,200 ng/kg-d for children, and from 20.1 to 165 ng/kg-d for adults (Heffernan et al 2013). On the other hand, they recorded inversely associated urinary concentrations and estimated excretion rates with age in infants and young children compared to adults (geometric mean: 107 and 47.0 ng/kg-d, respectively). This was explained by children higher food consumption relative to body weight compared to adults' and may also reflect alternative exposure pathways and sources. In this study lipid profile was not related to urinary BPA level, in consistent with a study enrolling diabetic adults that reported that higher BPA levels were positively associated with diabetes mellitus independent of confounding cholesterol levels and other factors Anoop and Srinivas 2011. One of the factors which restricted judgment on the potential risk of BPA is the normal lipid profile of all participants. However, this study can't exclude this risk as there is positive correlation between BPA/creatinine ratio and both serum cholesterol and LDL .No associations between BPA and other laboratory measures of cardiovascular was found, contrasting previous adult studies that have linked BPA levels with cardiovascular disease and diabetes (Lang et al. ,2008) in additional to mouse

researches that associate high cholesterol levels among mice with perinatal and postnatal exposure to BPA (Miyawaki et al.,2007)

CONCLUSION
`Based on these results this work provides additional consideration about the possibility of the use of   BPA and its adverse effects on cardiovascular system. However, the cross-sectional design of this study limited our findings. Respecting the cumulative adverse effects of BPA compound over time, Future policy regulating children's consumer products is mandatory to avoid health effects that may manifest later in adulthood

CONFLICT OF INTEREST
The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

AUTHOR CONTRIBUTIONS
This work was carried out in collaboration between all authors. MMY and EMSE designed and formulated the study. MAM, STZ, MMY, EMSE, IRE, KA and SFS interviewed children and collected clinical and anthropometric data. MAM, STZ, and SFS managed the literature searches and wrote the draft of the manuscript. JH and SM performed the laboratory procedures. MMA performed statistical data analysis. SFS designed results of the manuscript. All authors read, reviewed and approved the final version.

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